Schomburg · Schomburg
Editors

SPRINGER Handbook of Enzymes

Second Edition

SUPPLEMENT VOLUME S9

CLASS 2-3.2

Transferases, Hydrolases

EC 2-3.2

Springer Handbook of Enzymes
Supplement Volume S9

Dietmar Schomburg and
Ida Schomburg (Eds.)

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coedited by Antje Chang

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Preface

Today, as the full information about the genome is becoming available for a rapidly increasing number of organisms and transcriptome and proteome analyses are beginning to provide us with a much wider image of protein regulation and function, it is obvious that there are limitations to our ability to access functional data for the gene products – the proteins and, in particular, for enzymes. Those data are inherently very difficult to collect, interpret and standardize as they are widely distributed among journals from different fields and are often subject to experimental conditions. Nevertheless a systematic collection is essential for our interpretation of genome information and more so for applications of this knowledge in the fields of medicine, agriculture, etc. Progress on enzyme immobilisation, enzyme production, enzyme inhibition, coenzyme regeneration and enzyme engineering has opened up fascinating new fields for the potential application of enzymes in a wide range of different areas.

The development of the enzyme data information system BRENDA was started in 1987 at the German National Research Centre for Biotechnology in Braunschweig (GBF), continued at the University of Cologne from 1996 to 2007, and then returned to Braunschweig, to the Technical University, Institute of Bioinformatics & Systems Biology. The present book "Springer Handbook of Enzymes" represents the printed version of this data bank. The information system has been developed into a full metabolic database.

The enzymes in this Handbook are arranged according to the Enzyme Commission list of enzymes. Some 5,000 "different" enzymes are covered. Frequently enzymes with very different properties are included under the same EC-number. Although we intend to give a representative overview on the characteristics and variability of each enzyme, the Handbook is not a compendium. The reader will have to go to the primary literature for more detailed information. Naturally it is not possible to cover all the numerous literature references for each enzyme (for some enzymes up to 40,000) if the data representation is to be concise as is intended.

It should be mentioned here that the data have been extracted from the literature and critically evaluated by qualified scientists. On the other hand, the original authors' nomenclature for enzyme forms and subunits is retained. In order to keep the tables concise, redundant information is avoided as far as possible (e.g. if K_m values are measured in the presence of an obvious cosubstrate, only the name of the cosubstrate is given in parentheses as a commentary without reference to its specific role).

The authors are grateful to the following biologists and chemists for invaluable help in the compilation of data: Cornelia Munaretto and Dr. Antje Chang.

Braunschweig
Autumn 2012

Dietmar Schomburg, Ida Schomburg

List of Abbreviations

A	adenine
Ac	acetyl
ADP	adenosine 5'-diphosphate
Ala	alanine
All	allose
Alt	altrose
AMP	adenosine 5'-monophosphate
Ara	arabinose
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine 5'-triphosphate
Bicine	N,N'-bis(2-hydroxyethyl)glycine
C	cytosine
cal	calorie
CDP	cytidine 5'-diphosphate
CDTA	trans-1,2-diaminocyclohexane-N,N,N,N-tetraacetic acid
CMP	cytidine 5'-monophosphate
CoA	coenzyme A
CTP	cytidine 5'-triphosphate
Cys	cysteine
d	deoxy-
D-	(and L-) prefixes indicating configuration
DFP	diisopropyl fluorophosphate
DNA	deoxyribonucleic acid
DPN	diphosphopyridinium nucleotide (now NAD^+)
DTNB	5,5'-dithiobis(2-nitrobenzoate)
DTT	dithiothreitol (i.e. Cleland's reagent)
EC	number of enzyme in Enzyme Commission's system
E. coli	Escherichia coli
EDTA	ethylene diaminetetraacetate
EGTA	ethylene glycol bis(-aminoethyl ether) tetraacetate
ER	endoplasmic reticulum
Et	ethyl
EXAFS	extended X-ray absorption fine structure
FAD	flavin-adenine dinucleotide
FMN	flavin mononucleotide (riboflavin 5'-monophosphate)
Fru	fructose
Fuc	fucose
G	guanine
Gal	galactose

GDP	guanosine 5'-diphosphate
Glc	glucose
GlcN	glucosamine
GlcNAc	N-acetylglucosamine
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GMP	guanosine 5'-monophosphate
GSH	glutathione
GSSG	oxidized glutathione
GTP	guanosine 5'-triphosphate
Gul	gulose
h	hour
H4	tetrahydro
HEPES	4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid
His	histidine
HPLC	high performance liquid chromatography
Hyl	hydroxylysine
Hyp	hydroxyproline
IAA	iodoacetamide
IC 50	50% inhibitory concentration
Ig	immunoglobulin
Ile	isoleucine
Ido	idose
IDP	inosine 5'-diphosphate
IMP	inosine 5'-monophosphate
ITP	inosine 5'-triphosphate
K_m	Michaelis constant
L-	(and D-) prefixes indicating configuration
Leu	leucine
Lys	lysine
Lyx	lyxose
M	mol/l
mM	millimol/l
m-	*meta-*
Man	mannose
MES	2-(N-morpholino)ethane sulfonate
Met	methionine
min	minute
MOPS	3-(N-morpholino)propane sulfonate
Mur	muramic acid
MW	molecular weight
NAD^+	nicotinamide-adenine dinucleotide
NADH	reduced NAD
$NADP^+$	NAD phosphate
NADPH	reduced NADP
NAD(P)H	indicates either NADH or NADPH

NBS	N-bromosuccinimide
NDP	nucleoside 5'-diphosphate
NEM	N-ethylmaleimide
Neu	neuraminic acid
NMN	nicotinamide mononucleotide
NMP	nucleoside 5'-monophosphate
NTP	nucleoside 5'-triphosphate
o-	*ortho-*
Orn	ornithine
p-	*para-*
PBS	phosphate-buffered saline
PCMB	*p*-chloromercuribenzoate
PEP	phosphoenolpyruvate
pH	$-\log 10[H^+]$
Ph	phenyl
Phe	phenylalanine
PHMB	*p*-hydroxymercuribenzoate
PIXE	proton-induced X-ray emission
PMSF	phenylmethane-sulfonylfluoride
p-NPP	*p*-nitrophenyl phosphate
Pro	proline
Q_{10}	factor for the change in reaction rate for a 10°C temperature increase
Rha	rhamnose
Rib	ribose
RNA	ribonucleic acid
mRNA	messenger RNA
rRNA	ribosomal RNA
tRNA	transfer RNA
Sar	N-methylglycine (sarcosine)
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser	serine
T	thymine
t_H	time for half-completion of reaction
Tal	talose
TDP	thymidine 5'-diphosphate
TEA	triethanolamine
Thr	threonine
TLCK	N^α-*p*-tosyl-L-lysine chloromethyl ketone
T_m	melting temperature
TMP	thymidine 5'-monophosphate
Tos-	tosyl- (*p*-toluenesulfonyl-)
TPN	triphosphopyridinium nucleotide (now $NADP^+$)
Tris	tris(hydroxymethyl)-aminomethane
Trp	tryptophan
TTP	thymidine 5'-triphosphate
Tyr	tyrosine
U	uridine

U/mg	μmol/(mg*min)
UDP	uridine 5'-diphosphate
UMP	uridine 5'-monophosphate
UTP	uridine 5'-triphosphate
Val	valine
Xaa	symbol for an amino acid of unknown constitution in peptide formula
XAS	X-ray absorption spectroscopy
Xyl	xylose

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Description of Data Fields

All information except the nomenclature of the enzymes (which is based on the recommendations of the Nomenclature Committee of IUBMB (International Union of Biochemistry and Molecular Biology) and IUPAC (International Union of Pure and Applied Chemistry) is extracted from original literature (or reviews for very well characterized enzymes). The quality and reliability of the data depends on the method of determination, and for older literature on the techniques available at that time. This is especially true for the fields *Molecular Weight* and *Subunits*.
The general structure of the fields is: **Information – Organism – Commentary – Literature**
The information can be found in the form of numerical values (temperature, pH, K_m etc.) or as text (cofactors, inhibitors etc.).
Sometimes data are classified as *Additional Information*. Here you may find data that cannot be recalculated to the units required for a field or also general information being valid for all values. For example, for *Inhibitors*, *Additional Information* may contain a list of compounds that are not inhibitory.
The detailed structure and contents of each field is described below. If one of these fields is missing for a particular enzyme, this means that for this field, no data are available.

1 Nomenclature

EC number

The number is as given by the IUBMB, classes of enzymes and subclasses defined according to the reaction catalyzed.

Systematic name

This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Recommended name

This is the name as given by the IUBMB/IUPAC Nomenclature Committee

Synonyms

Synonyms which are found in other databases or in the literature, abbreviations, names of commercially available products. If identical names are frequently used for different enzymes, these will be mentioned here, cross references are given. If another EC number has been included in this entry, it is mentioned here.

CAS registry number

The majority of enzymes have a single chemical abstract (CAS) number. Some have no number at all, some have two or more numbers. Sometimes

two enzymes share a common number. When this occurs, it is mentioned in the commentary.

2 Source Organism

In this data field the organism in which the enzymes has been detected are listed. The systematic names according to the NCBI Taxonomy are preferred. If the scientific name is missing, the synonym or the names from the respective literature references are used. In addition, organism are listed for which a specific protein sequence or nucleotide sequence has been allocated. The accession number and the respective data source, e.g, UNIPROT is given in the commentary.

3 Reaction and Specificity

Catalyzed reaction

The reaction as defined by the IUBMB. The commentary gives information on the mechanism, the stereochemistry, or on thermodynamic data of the reaction.

Reaction type

According to the enzyme class a type can be attributed. These can be oxidation, reduction, elimination, addition, or a name (e.g. Knorr reaction)

Natural substrates and products

These are substrates and products which are metabolized in vivo. A natural substrate is only given if it is mentioned in the literature. The commentary gives information on the pathways for which this enzyme is important. If the enzyme is induced by a specific compound or growth conditions, this will be included in the commentary. In *Additional information* you will find comments on the metabolic role, sometimes only assumptions can be found in the references or the natural substrates are unknown.
In the listings, each natural substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included only if the respective authors were able to demonstrate the formation of the specific product. If only the disappearance of the substrate was observed, the product is included without organisms of references. In cases with unclear product formation only a ? as a dummy is given.

Substrates and products

All natural or synthetic substrates are listed (not in stoichiometric quantities). The commentary gives information on the reversibility of the reaction, on isomers accepted as substrates and it compares the efficiency of substrates. If a specific substrate is accepted by only one of several isozymes, this will be stated here.

The field *Additional Information* summarizes compounds that are not accepted as substrates or general comments which are valid for all substrates. In the listings, each substrate (indicated by a bold **S**) is followed by its respective product (indicated by a bold **P**). Products are given with organisms and references included if the respective authors demonstrated the formation of the specific product. If only the disappearance of the substrate was observed, the product will be included without organisms or references. In cases with unclear product formation only a **?** as a dummy is given.

Inhibitors

Compounds found to be inhibitory are listed. The commentary may explain experimental conditions, the concentration yielding a specific degree of inhibition or the inhibition constant. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Cofactors, prosthetic groups

This field contains cofactors which participate in the reaction but are not bound to the enzyme, and prosthetic groups being tightly bound. The commentary explains the function or, if known, the stereochemistry, or whether the cofactor can be replaced by a similar compound with higher or lower efficiency.

Activating Compounds

This field lists compounds with a positive effect on the activity. The enzyme may be inactive in the absence of certain compounds or may require activating molecules like sulfhydryl compounds, chelating agents, or lipids. If a substance is activating at a specific concentration but inhibiting at a higher or lower value, the commentary will explain this.

Metals, ions

This field lists all metals or ions that have activating effects. The commentary explains the role each of the cited metal has, being either bound e.g. as Fe-S centers or being required in solution. If an ion plays a dual role, activating at a certain concentration but inhibiting at a higher or lower concentration, this will be given in the commentary.

Turnover number (s^{-1})

The k_{cat} is given in the unit s^{-1}. The commentary lists the names of the substrates, sometimes with information on the reaction conditions or the type of reaction if the enzyme is capable of catalyzing different reactions with a single substrate. For cases where it is impossible to give the turnover number in the defined unit (e.g., substrates without a defined molecular weight, or an undefined amount of protein) this is summarized in *Additional Information*.

Specific activity (U/mg)

The unit is micromol/minute/milligram of protein. The commentary may contain information on specific assay conditions or if another than the natur-

al substrate was used in the assay. Entries in *Additional Information* are included if the units of the activity are missing in the literature or are not calculable to the obligatory unit. Information on literature with a detailed description of the assay method may also be found.

K_m-Value (mM)

The unit is mM. Each value is connected to a substrate name. The commentary gives, if available, information on specific reaction condition, isozymes or presence of activators. The references for values which cannot be expressed in mM (e.g. for macromolecular, not precisely defined substrates) are given in *Additional Information*. In this field we also cite literature with detailed kinetic analyses.

K_i-Value (mM)

The unit of the inhibition constant is mM. Each value is connected to an inhibitor name. The commentary gives, if available, the type of inhibition (e.g. competitive, non-competitive) and the reaction conditions (pH-value and the temperature). Values which cannot be expressed in the requested unit and references for detailed inhibition studies are summerized under *Additional information*.

pH-Optimum

The value is given to one decimal place. The commentary may contain information on specific assay conditions, such as temperature, presence of activators or if this optimum is valid for only one of several isozymes. If the enzyme has a second optimum, this will be mentioned here.

pH-Range

Mostly given as a range e.g. 4.0–7.0 with an added commentary explaining the activity in this range. Sometimes, not a range but a single value indicating the upper or lower limit of enzyme activity is given. In this case, the commentary is obligatory.

pI-Value

The isoelectric point (IEP) of an enzyme is the pH-value at which the protein molecule has no net electric charge, carrying the equal number of positively and negatively ions. In the commentary the method of determination is given, if it is provided by the literature.

Temperature optimum (°C)

Sometimes, if no temperature optimum is found in the literature, the temperature of the assay is given instead. This is always mentioned in the commentary.

Temperature range (°C)

This is the range over which the enzyme is active. The commentary may give the percentage of activity at the outer limits. Also commentaries on specific assay conditions, additives etc.

4 Enzyme Structure

Molecular weight

This field gives the molecular weight of the holoenzyme. For monomeric enzymes it is identical to the value given for subunits. As the accuracy depends on the method of determination this is given in the commentary if provided in the literature. Some enzymes are only active as multienzyme complexes for which the names and/or EC numbers of all participating enzymes are given in the commentary.

Subunits

The tertiary structure of the active species is described. The enzyme can be active as a monomer a dimer, trimer and so on. The stoichiometry of subunit composition is given. Some enzymes can be active in more than one state of complexation with differing effectivities. The analytical method is included.

Posttranslational modifications

The main entries in this field may be proteolytic modification, or side-chain modification, or no modification. The commentary will give details of the modifications e.g.:

- proteolytic modification <1> (<1>, propeptide Name) [1];
- side-chain modification <2> (<2>, N-glycosylated, 12% mannose) [2];
- no modification [3]

5 Isolation / Preparation / Mutation / Application

Source / tissue

For multicellular organisms, the tissue used for isolation of the enzyme or the tissue in which the enzyme is present is given. Cell-lines may also be a source of enzymes.

Localization

The subcellular localization is described. Typical entries are: cytoplasm, nucleus, extracellular, membrane.

Purification

The field consists of an organism and a reference. Only references with a detailed description of the purification procedure are cited.

Renaturation

Commentary on denaturant or renaturation procedure.

Crystallization

The literature is cited which describes the procedure of crystallization, or the X-ray structure.

Cloning

Lists of organisms and references, sometimes a commentary about expression or gene structure.

Engineering

The properties of modified proteins are described.

Application

Actual or possible applications in the fields of pharmacology, medicine, synthesis, analysis, agriculture, nutrition are described.

6 Stability

pH-Stability

This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Temperature stability

This field can either give a range in which the enzyme is stable or a single value. In the latter case the commentary is obligatory and explains the conditions and stability at this value.

Oxidation stability

Stability in the presence of oxidizing agents, e.g. O_2, H_2O_2, especially important for enzymes which are only active under anaerobic conditions.

Organic solvent stability

The stability in the presence of organic solvents is described.

General stability information

This field summarizes general information on stability, e.g., increased stability of immobilized enzymes, stabilization by SH-reagents, detergents, glycerol or albumins etc.

Storage stability

Storage conditions and reported stability or loss of activity during storage.

References

Authors, Title, Journal, Volume, Pages, Year.

demethylmenaquinone methyltransferase 2.1.1.163

1 Nomenclature

EC number
2.1.1.163

Systematic name
S-adenosyl-L-methione:demethylmenaquinone methyltransferase

Recommended name
demethylmenaquinone methyltransferase

Synonyms
2-heptaprenyl-1,4-naphthoquinone methyltransferase <4> [4]
Dmkt1 <2> [5]
UbiE <3> [3]
menaquinone biosynthesis methyltransferase <4> [4]

CAS registry number
37259-80-6

2 Source Organism

<1> *Escherichia coli* [1,2]
<2> *Oryza sativa* [5]
<3> *Escherichia coli* (UNIPROT accession number: P0A887) [3]
<4> *Geobacillus stearothermophilus* (UNIPROT accession number: O86169) [4]

3 Reaction and Specificity

Catalyzed reaction
a demethylmenaquinol + S-adenosyl-L-methionine = a menaquinol + S-adenosyl-L-homocysteine

Substrates and products
S 2-polyprenyl-6-methoxy-1,4-benzoquinol + S-adenosyl-L-methionine <3> (Reversibility: ?) [3]
P 2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinol + S-adenosyl-L-homocysteine
S 2-polyprenyl-6-methoxy-1,4-benzoquinone + S-adenosyl-L-methionine <4> (Reversibility: ?) [4]

D. Schomburg and I. Schomburg (eds.), *Class 2–3.2 Transferases, Hydrolases: EC 2–3.2*,
Springer Handbook of Enzymes S9, DOI 10.1007/978-3-642-36240-8_1,
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P 2-polyprenyl-3-methyl-6-methoxy-1,4-benzoquinone + S-adenosyl-L-homocysteine
S demethylmenaquinol + S-adenosyl-L-methionine <3> (Reversibility: ?) [3]
P menaquinol + S-adenosyl-L-homocysteine

5 Isolation/Preparation/Mutation/Application

Source/tissue

stem <2> (<2> the transcript level increases after 3 h of low-temperature exposure, reaching a maximum at 12 h [5]) [5]

Cloning

<4> (expression in Escherichia coli) [4]

Engineering

G142D <3> (<3> mutant accumulates 2-octaprenyl-6-methoxy-1,4-benzoquinone and demethylmenaquinone as predominant intermediates [3]) [3]
Additional information <1,4> (<4> expression in Escherichia coli complements mutation AN70 deficient of a specific methyltransferase in the ubiquinone biosynthetic pathway [4]; <1> mutant defective in the demthylmenaquinone methyltransferase activity expresses the same formate-dependent nitrite reduction activity as the parental strain [2]; <1> mutant ubiE lacks ubiquinone due to defect in a specific methylation step of ubiquinone synthesis. Synthesis of menaquinone from demethylmenaquinone depends on the same gene ubiE. Mutant contains only demethylmenaquinone, but not menaquinone. Strain is able to grow with fumarate, trimethylamine N-oxide and dimethylsulfoxide, but not with nitrate as electron acceptor. Anaerobic respiration with fumarate and trimethylamine are catalyzed at 69% and 74% of wild-type rates, respectively. Dimethylsulfoxide respiration is reduced to 38% of wild-type, and nitrate respiration is below 8% [1]) [1,2,4]

References

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[2] Tyson, K.; Metheringham, R.; Griffiths, L.; Cole, J.: Characterisation of Escherichia coli K-12 mutants defective in formate-dependent nitrite reduction: essential roles for hemN and the menFDBCE operon. Arch. Microbiol., **168**, 403-411 (1997)
[3] Lee, P.T.; Hsu, A.Y.; Ha, H.T.; Clarke, C.F.: A C-methyltransferase involved in both ubiquinone and menaquinone biosynthesis: isolation and identification of the Escherichia coli ubiE gene. J. Bacteriol., **179**, 1748-1754 (1997)

[4] Koike-Takeshita, A.; Koyama, T.; Ogura, K.: Identification of a novel gene cluster participating in menaquinone (vitamin K_2) biosynthesis. Cloning and sequence determination of the 2-heptaprenyl-1,4-naphthoquinone methyltransferase gene of Bacillus stearothermophilus. J. Biol. Chem., **272**, 12380-12383 (1997)
[5] Lee, S.; Kim, J.; Kim, S.; Kim, S.; Lee, K.; Han, S.; Choi, H.; Jeong, D.; An, G.; Kim, S.: Trapping and characterization of cold-responsive genes from T-DNA tagging lines in rice. Plant Sci., **166**, 69-79 (2004)

demethylrebeccamycin-D-glucose O-methyltransferase 2.1.1.164

1 Nomenclature

EC number
2.1.1.164

Systematic name
S-adenosyl-L-methionine:demethylrebeccamycin-D-glucose O-methyltransferase

Recommended name
demethylrebeccamycin-D-glucose O-methyltransferase

Synonyms
RebM <1> (<1> gene name [1,3]) [1,2,3]

2 Source Organism

<1> *Lechevalieria aerocolonigenes* (UNIPROT accession number: Q8KZ94) [1,2,3]

3 Reaction and Specificity

Catalyzed reaction
4'-demethylrebeccamycin + S-adenosyl-L-methionine = rebeccamycin + S-adenosyl-L-homocysteine

Natural substrates and products
S 4'-demethylrebeccamycin + S-adenosyl-L-methionine <1> (<1> last step in the biosynthesis of rebeccamycin [1]) (Reversibility: ?) [1,2,3]
P rebeccamycin + S-adenosyl-L-homocysteine

Substrates and products
S 3-bromo-11-β-D-glucopyranosyl-11,12-dihydroindolo[2,3-a]carbazole + S-adenosyl-L-methionine <1> (Reversibility: ?) [1]
P ? + S-adenosyl-L-homocysteine
S 3-bromo-12-β-D-glucopyranosyl-11,12-dihydroindolo[2,3-a]carbazole + S-adenosyl-L-methionine <1> (Reversibility: ?) [1]
P ? + S-adenosyl-L-homocysteine
S 4'-demethylrebeccamycin + S-adenosyl-L-methionine <1> (<1> last step in the biosynthesis of rebeccamycin [1]) (Reversibility: ?) [1,2,3]
P rebeccamycin + S-adenosyl-L-homocysteine

D. Schomburg and I. Schomburg (eds.), *Class 2–3.2 Transferases, Hydrolases: EC 2–3.2*,
Springer Handbook of Enzymes S9, DOI 10.1007/978-3-642-36240-8_2,
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S dechlorinated 4'-demethyl-rebeccamycin + 5'-[[(3S)-3-amino-3-carboxypropyl](2-iodoethyl)ammonio]-5'-deoxyadenosine <1> (Reversibility: ?) [2]
P dechlorinated rebeccamycin + ?
S dechlorinated 4'-demethyl-rebeccamycin + S-adenosyl-L-methionine <1> (Reversibility: ?) [1,2]
P dechlorinated rebeccamycin + S-adenosyl-L-homocysteine
S dechlorinated 5-deoxo-4'-demethyl-rebeccamycin + S-adenosyl-L-methionine <1> (Reversibility: ?) [1]
P ? + S-adenosyl-L-homocysteine
S dechlorinated 6-[3-(1H-imidazol-1-yl)propyl]-4'-demethyl-rebeccamycin + S-adenosyl-L-methionine <1> (Reversibility: ?) [1]
P ? + S-adenosyl-L-homocysteine
S dechlorinated 6-methyl-2'-deoxy-4'-demethyl-α-D-Glc-rebeccamycin + S-adenosyl-L-methionine <1> (Reversibility: ?) [1]
P ? + S-adenosyl-L-homocysteine
S dechlorinated 6-methyl-2'-deoxy-4'-demethyl-rebeccamycin + S-adenosyl-L-methionine <1> (Reversibility: ?) [1]
P ? + S-adenosyl-L-homocysteine
S dechlorinated 7-deoxo-4'-demethyl-rebeccamycin + S-adenosyl-L-methionine <1> (Reversibility: ?) [1]
P ? + S-adenosyl-L-homocysteine
S Additional information <1> (<1> RebM accepts a wide range of alternate substrates. Specifically, variation on the imide heterocycle by removal or addition of a bulky group is tolerated by RebM. Deoxygenation of the sugar moiety only slightly decreases RebM activity. RebM displays flexibility toward anomers and is able to process both α and β-glycosidic analogues [1]) [1]
P ?

Inhibitors

Ca^{2+} <1> (<1> 1 mM, 8% inhibition [1]) [1]
Co^{2+} <1> (<1> 1 mM, 87% inhibition [1]) [1]
Cu^{2+} <1> (<1> 1 mM, 93% inhibition [1]) [1]
EDTA <1> (<1> 1 mM, 5% inhibition [1]) [1]
Fe^{2+} <1> (<1> 1 mM, 17% inhibition [1]) [1]
Mg^{2+} <1> (<1> 1 mM, 3% inhibition [1]) [1]
Mn^{2+} <1> (<1> 1 mM, 48% inhibition [1]) [1]
Ni^{2+} <1> (<1> 1 mM, complete inhibition [1]) [1]
Zn^{2+} <1> (<1> 1 mM, complete inhibition [1]) [1]

Metals, ions

Additional information <1> (<1> RebM activity is not enhanced by divalent metals [1]) [1]

Turnover number (s^{-1})

0.0023 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme H140A [3]) [3]

0.005 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme D166A [3]) [3]
0.0063 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme P75S [3]) [3]
0.01 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme H141A [3]) [3]
0.017 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme W134Y [3]) [3]
0.023 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme S138A [3]) [3]
0.025 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme L136V [3]) [3]
0.047 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, wild-type enzyme [3]; <1> pH 8.0, 30°C, cosubstrate: dechlorinated 4-demethyl-rebeccamycin [1]) [1,3]
0.047 <1> (dechlorinated 4'-demethyl-rebeccamycin, <1> pH 8.0, 30°C [1]) [1]
0.048 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme C70A [3]) [3]
0.072 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme C70S [3]) [3]

K_m-Value (mM)

0.0021 <1> (dechlorinated 4'-demethyl-rebeccamycin, <1> pH 8.0, 30°C [1]) [1]
0.0112 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme H140A [3]) [3]
0.012 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, wild-type enzyme [3]; <1> pH 8.0, 30°C, cosubstrate: dechlorinated 4-demethyl-rebeccamycin [1]) [1,3]
0.016 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme W134Y [3]) [3]
0.017 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme C70S [3]) [3]
0.017 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme C70A [3]) [3]
0.018 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme S138A [3]) [3]
0.021 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme S138A [3]) [3]
0.0215 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme L136V [3]) [3]
0.0225 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme C70A [3]) [3]
0.0311 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme H140A [3]) [3]

0.032 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme D166A [3]) [3]
0.032 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme W134Y [3]) [3]
0.0345 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme C70S [3]) [3]
0.0355 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, wild-type enzyme [3]) [3]
0.0535 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme P75S [3]) [3]
0.056 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme L136V [3]) [3]
0.0798 <1> (4'-demethylrebeccamycin, <1> pH 8.0, 25°C, mutant enzyme H141A [3]) [3]
0.0943 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme H141A [3]) [3]
0.162 <1> (S-adenosyl-L-methionine, <1> pH 8.0, 25°C, mutant enzyme D166A [3]; <1> pH 8.0, 25°C, mutant enzyme P75S [3]) [3]

pH-Optimum

6.5-8 <1> (<1> functional over a broader pH range from pH 6.5 to above 8.0 [1]) [1]

4 Enzyme Structure

Subunits

monomer <1> (<1> 1 * 32000, SDS-PAGE [1]; <1> 1 * 31400, calculated from sequence [1]) [1]

5 Isolation/Preparation/Mutation/Application

Crystallization

<1> (hanging drop vapor diffusion method at 20°C. The 2.65 A crystal structure of the rebeccamycin 4'-O-methyltransferase RebM in complex with S-adenosyl-L-homocysteine reveals RebM to adopt a typical S-adenosyl methionine binding fold of small molecule O-methyltransferases and display a weak dimerization domain unique to methyltransferases) [3]

Engineering

C70A <1> (<1> k_{cat} for S-adenosyl-L-methionine is nearly identical to wild-type value [3]) [3]
C70S <1> (<1> k_{cat} for S-adenosyl-L-methionine is 1.53fold higher than wild-type value [3]) [3]
D166A <1> (<1> k_{cat} for S-adenosyl-L-methionine is 10% of wild-type value [3]) [3]

H140A <1> (<1> k_{cat} for S-adenosyl-L-methionine is 5% of wild-type value [3]) [3]
H141A <1> (<1> k_{cat} for S-adenosyl-L-methionine is 21% of wild-type value [3]) [3]
H149A/H141A <1> (<1> activity is below the detection limit [3]) [3]
L136V <1> (<1> k_{cat} for S-adenosyl-L-methionine is 54% of wild-type value [3]) [3]
P75S <1> (<1> although properly folded based upon CD spectroscopy, the mutant displays a substantially reduced affinity for AdoMet (about 10fold increase in K_m). k_{cat} for S-adenosyl-L-methionine is 13% of wild-type value [3]) [3]
S138A <1> (<1> k_{cat} for S-adenosyl-L-methionine is 49% of wild-type value [3]) [3]
W134Y <1> (<1> k_{cat} for S-adenosyl-L-methionine is 35% of wild-type value [3]) [3]

References

[1] Zhang, C.; Albermann, C.; Fu, X.; Peters, N.R.; Chisholm, J.D.; Zhang, G.; Gilbert, E.J.; Wang, P.G.; van Vranken, D.L.; Thorson, J.S.: RebG- and RebM-catalyzed indolocarbazole diversification. Chembiochem, **7**, 795-804 (2006)
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methyl halide transferase 2.1.1.165

1 Nomenclature

EC number
2.1.1.165

Systematic name
S-adenosylmethionine:iodide methyltransferase

Recommended name
methyl halide transferase

Synonyms
AtHOL1 <12> [9,11]
$AtHOL_2$ <20> [11]
AtHOL3 <19> [11]
HARMLESS TO OZONE LAYER <12> (<12> gene name [2,9]) [2,9]
HMT <14> [6]
HMT/HTMT <13> [7]
HOL <12> (<12> gene name [2]) [2]
HTMT <13> [7]
MCT <10> [4]
MHT <2,4,5,6,7,8,9,21> [8]
S-adenosyl-L-methionine: halide ion methyltransferase <14> [6]
S-adenosyl-L-methionine:halide/bisulfide methyltransferase <1> (<1> bifunctional enzyme also shows activity of EC 2.1.1.9 [3]) [3]
S-adenosylmethionine-dependent halide/thiol methyltransferase <13> (<13> bifunctional enzyme [7]) [7]
SAM:halide ion methyltransferase <15,16,17> [10]
halide/bisulfide methyltransferase <1> (<1> bifunctional enzyme also shows activity of EC 2.1.1.9 [3]) [3]
methyl chloride transferase <10,18> [4,13,14]
methyl halide transferase <2,4,5,6,7,8,9,21> [8]

CAS registry number
129877-08-3

2 Source Organism

<1> *Brassica oleracea* [3,12]
<2> *Oryza sativa* [8]
<3> *Rhizobium sp.* [5]

D. Schomburg and I. Schomburg (eds.), *Class 2–3.2 Transferases, Hydrolases: EC 2–3.2*,
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<4> *Brassica rapa* [8]
<5> *Vitis vinifera* [8]
<6> *Burkholderia pseudomallei* [8]
<7> *Burkholderia xenovorans* [8]
<8> *Burkholderia thailandensis* [8]
<9> *Burkholderia phytofirmans* [8]
<10> *Batis maritima* (UNIPROT accession number: Q9ZSZ7) [4,13]
<11> *Phellinus pomaceus* [1]
<12> *Arabidopsis thaliana* (UNIPROT accession number: Q0WP12) [2,9,11]
<13> *Raphanus sativus* (UNIPROT accession number: C6L2E7) [7]
<14> *Pavlova pinguis* [6]
<15> *Pavlova gyrans* [10]
<16> *Papenfussiella kuromo* [10]
<17> *Sargassum horneri* [10]
<18> *Endocladia muricata* [14]
<19> *Arabidopsis thaliana* (UNIPROT accession number: Q6AWU6) [11]
<20> *Arabidopsis thaliana* (UNIPROT accession number: O80562) [11]
<21> *Batis maritima* [8]

3 Reaction and Specificity

Catalyzed reaction

S-adenosyl-L-methionine + iodide = S-adenosyl-L-homocysteine + methyl iodide

Natural substrates and products

S S-adenosyl-L-methionine + chloride <10,11> (<10> an obvious function for a halophytic methylase would be the maintenance of homeostatic levels of cytoplasmic chloride ion. The secretion of excess chloride into the soil could not greatly benefit a halophytic plant. On the other hand, the synthesis and distillation of a volatile gas, methyl chloride, into the atmosphere could be a useful mechanism for disposing of excess chloride [4]; <11> the enzyme is responsible for the massive amounts of CH_3Cl produced by this fungus [1]; <10> this enzyme possibly functions in the control and regulation of the internal concentration of chloride ions in halophytic plant cells [13]) (Reversibility: ?) [1,4,13]

P S-adenosyl-L-homocysteine + methyl chloride

S Additional information <3,12,13,15,16,17,19,20> (<12> a phylogenetic analysis with the HOL gene suggests that the ability to produce methyl halides is widespread among vascular plants. All wild-type plants strongly favor the methylation of I^- to Br^- to Cl^-. Adult plants show a relative methylation preference ratio for I:Br:Cl of roughly 10000:50:1. Juvenile plants showed a ratio of roughly 40000:9:1 [2]; <12> AtHOL1 is involved in glucosinolate metabolism and defense against phytopathogens. CH_3Cl synthesized by AtHOL1 could be considered a byproduct of NCS- metabolism [9]; <3> bacteria contribute to iodine transfer from the terrestrial

and marine ecosystems into the atmosphere [5]; <16,17> marine microalgae are the main oceanic source of methyl bromide. The monohalomethanes produced by marine microalgae are probably important in the global cycling of gaseous organohalogen species, especially bromine and iodine [10]; <15> marine microalgae are the main oceanic source of methyl bromide. The monohalomethanes produced by marine microalgae are probably important in the global cycling of gaseous organohalogen species, especially bromine and iodine. From the viewpoint of stratospheric ozone depletion, methyl bromide is the most destructive compound because it has a high ozone depletion potential [10]; <12,19,20> the activation of AtHOL1, AtHOL2 and AtHOL3 genes contributes to the methyl halide emissions from Arabidopsis [11]; <13> the enzyme may be involved in the detoxification of sulfur compounds produced by the degradation of glucosinolates to release them as volatile compounds. The volatile sulfur compounds, including CH_3SH and CH_3SCN and methyl halides, are believed to act as insecticidal or anti-pathogenic agents. Therefore, it is speculated that the enzyme plays a role in controlling the levels of anions that can inhibit metabolic enzymes in the leaves and also to protect them from damage caused by insects or pathogens [7]) [2,5,7,9,10,11]

P ?

Substrates and products

S S-adenosyl-L-methionine + bromide <11> (<11> V_{max}/K_m for bromide is 17fold lower than V_{max}/K_m for iodide [1]) (Reversibility: ?) [1]

P S-adenosyl-L-homocysteine + methyl bromiode

S S-adenosyl-L-methionine + bromide <1,2,4,5,6,7,8,9,10,13,14,15,18,21> (<4,6,8> very low activity [8]; <1> k_{cat}/K_M for bromide is 12529fold lower than k_{cat}/K_m for iodide [3]; <13> production rate of bromomethane is 24fold lower than production rate of iodomethane [7]; <18> the rate of production of methyl bromide is 135fold lower than production of methyl iodide [14]) (Reversibility: ?) [3,6,7,8,10,12,13,14]

P S-adenosyl-L-homocysteine + methyl bromide

S S-adenosyl-L-methionine + chloride <1,4,5,7,9,10,11,12,13,14,18,19,20,21> (<5,7> very low activity [8]; <10> an obvious function for a halophytic methylase would be the maintenance of homeostatic levels of cytoplasmic chloride ion. The secretion of excess chloride into the soil could not greatly benefit a halophytic plant. On the other hand, the synthesis and distillation of a volatile gas, methyl chloride, into the atmosphere could be a useful mechanism for disposing of excess chloride [4]; <11> the enzyme is responsible for the massive amounts of CH_3Cl produced by this fungus [1]; <10> this enzyme possibly functions in the control and regulation of the internal concentration of chloride ions in halophytic plant cells [13]; <1> k_{cat}/K_M for chloride is 19065fold lower than k_{cat}/K_m for iodide [3]; <13> production rate of chloromethane is 925fold lower than production rate of iodomethane [7]; <18> the rate of production of methyl chloride is 270fold lower than production of methyl iodide [14]; <11> V_{max}/K_m for

chloride is 709fold lower than V_{max}/K_m for iodide [1]) (Reversibility: ?) [1,3,4,6,7,8,11,12,13,14]

P S-adenosyl-L-homocysteine + methyl chloride

S S-adenosyl-L-methionine + iodide <1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21> (<1,2,4,5,6,7,8,9,10,11,13,18,21> iodide is the preferred substrate [1,3,4,7,8,12,14]; <12> recombinant protein methylates iodide with greater efficiency than chloride [11]; <19,20> recombinant proteins methylate iodide with greater efficiency than chloride [11]; <14> the enzyme is strictly dependent on S-adenosyl-L-methionine as a methyl donor [6]) (Reversibility: ?) [1,3,4,5,6,7,8,10,11,12,13,14]

P S-adenosyl-L-homocysteine + methyl iodide

S Additional information <1,3,10,12,13,14,15,16,17,18,19,20> (<12> a phylogenetic analysis with the HOL gene suggests that the ability to produce methyl halides is widespread among vascular plants. All wild-type plants strongly favor the methylation of I^- to Br^- to Cl^-. Adult plants show a relative methylation preference ratio for I:Br:Cl of roughly 10000:50:1. Juvenile plants showed a ratio of roughly 40000:9:1 [2]; <12> AtHOL1 is involved in glucosinolate metabolism and defense against phytopathogens. CH_3Cl synthesized by AtHOL1 could be considered a byproduct of NCS- metabolism [9]; <3> bacteria contribute to iodine transfer from the terrestrial and marine ecosystems into the atmosphere [5]; <16,17> marine microalgae are the main oceanic source of methyl bromide. The monohalomethanes produced by marine microalgae are probably important in the global cycling of gaseous organohalogen species, especially bromine and iodine [10]; <15> marine microalgae are the main oceanic source of methyl bromide. The monohalomethanes produced by marine microalgae are probably important in the global cycling of gaseous organohalogen species, especially bromine and iodine. From the viewpoint of stratospheric ozone depletion, methyl bromide is the most destructive compound because it has a high ozone depletion potential [10]; <12,19,20> the activation of AtHOL1, AtHOL2 and AtHOL3 genes contributes to the methyl halide emissions from Arabidopsis [11]; <13> the enzyme may be involved in the detoxification of sulfur compounds produced by the degradation of glucosinolates to release them as volatile compounds. The volatile sulfur compounds, including CH_3SH and CH_3SCN and methyl halides, are believed to act as insecticidal or anti-pathogenic agents. Therefore, it is speculated that the enzyme plays a role in controlling the levels of anions that can inhibit metabolic enzymes in the leaves and also to protect them from damage caused by insects or pathogens [7]; <1> also methylates HS to CH_3SH (EC 2.1.1.9) at a rate comparable to that for iodide [12]; <18> fluoride is not a substrate [14]; <15> no activity with chloride, no activity with L-methionine, S-methyl methionine or dimethylsulfoniopropionate [10]; <10> purified enzyme is unable to use bisulfide (HS-) as an acceptor [4]; <1> the bifunctional enzyme also shows activity of EC 2.1.1.9 [3]; <14> the enzyme also catalyzes the methylation of HS- to methyl mercaptan (EC 2.1.1.9) [6]; <13> the enzyme also shows

thiol methyltransferase activity (EC 2.1.1.9), high activity towards SCN^- [7]) [2,3,4,5,6,7,9,10,11,12,14]

P ?

Inhibitors

2-mercaptoethanol <14> (<14> 1 mM, 16% inhibition, production of methyl iodide [6]) [6]
CN^- <14> (<14> 100 mM, complete inhibition, production of methyl iodide [6]) [6]
$CoCl_2$ <14> (<14> 1 mM, 18% inhibition, production of methyl iodide [6]) [6]
EDTA <14> (<14> 5 mM, 32% inhibition, production of methyl iodide [6]) [6]
HS^- <14> (<14> 10 mM, 84% inhibition, production of methyl iodide [6]) [6]
$MgSO_4$ <14> (<14> 1 mM, 25% inhibition, production of methyl iodide [6]) [6]
NaN_3 <14> (<14> 5 mM, 32% inhibition, production of methyl iodide [6]) [6]
$NiCl_2$ <14> (<14> 1 mM, 38% inhibition, production of methyl iodide [6]) [6]
PCMB <14> (<14> 0.5 mM, 30% inhibition, production of methyl iodide [6]) [6]
S-adenosyl-L-homocysteine <1,3,11> (<11> 50% inhibition at equimolar concentrations of S-adenosyl-L-homocysteine and S-adenosyl-L-methionine [1]; <1> competitive with respect to S-adenosyl-L-methionine, noncompetitive to iodide [3]; <3> inhibition is approximately 65% at equimolar concentrations of S-adenosyl-L-homocysteine and S-adenosyl-L-methionine [5]) [1,3,5]
SCN^- <14> (<14> 10 mM, 60% inhibition, production of methyl iodide [6]) [6]
$ZnCl_2$ <14> (<14> 1 mM, 14% inhibition, production of methyl iodide [6]) [6]
bisulfide <1> [3]
chloride <10> (<10> competitive inhibition of methyl iodide formation [13]) [13]
dithiothreitol <14> (<14> 5 mM, 28% inhibition, production of methyl iodide [6]) [6]
iodide <15> (<15> above 25 mM [10]) [10]
monoiodoacetate <14> (<14> 5 mM, 28% inhibition, production of methyl iodide [6]) [6]
Additional information <14> (<14> activity is not inhibited by high iodide concentrations [6]) [6]

Cofactors/prosthetic groups

S-adenosyl-L-methionine <10,11> (<11> specific for, natural methyl donor [1]) [1,4]

Activating compounds

urea <10> (<10> activity in 1, 2, and 3 M urea is about 1.75-, 2-, and 1.5-fold higher than in the absence of urea, respectively [13]) [13]

Metals, ions

ammonium sulfate <10> (<10> the activity of the recombinant methylase in 0.5 M and 1.0 M ammonium sulfate is about 1.25fold higher than in the absence of ammonium sulfate [13]) [13]

Additional information <14> (<14> various metal ions have no significant effect on methyl iodide production, suggesting that the enzyme does not require metal ions [6]) [6]

Specific activity (U/mg)

0.000000547 <3> [5]
0.0000058 <20> (<20> production of methyl iodide [11]) [11]
0.00009 <19> (<19> production of methyl iodide [11]) [11]
0.000555 <18> (<18> partially purified enzyme, synthesis of methyl bromide [14]) [14]
0.0018 <14> [6]
0.3 <10> (<10> purified enzyme, substrate iodide [4]) [4]
205 <12> (<12> production of methyl iodide [11]) [11]

K_m-Value (mM)

0.0045 <11> (S-adenosyl-L-methionine, <11> pH 6.8, 22°C [1]) [1]
0.012 <14> (S-adenosyl-L-methionine, <14> 30°C [6]) [6]
0.016 <18> (S-adenosyl-L-methionine, <18> cosubstrate: bromide [14]) [14]
0.024 <3,15> (S-adenosyl-L-methionine, <3,15> pH 7.0, 30°C [5,10]) [5,10]
0.0294 <10> (S-adenosyl-L-methionine, <10> pH 6.8, 22°C, native enzyme [13]) [13]
0.03 <1> (S-adenosyl-L-methionine, <1> pH 7.5, 22°C, cosubstrate: iodide [3]) [3]
0.19 <13> (S-adenosyl-L-methionine, <13> pH 7.0, 30°C, purified recombinant enzyme [7]) [7]
0.23 <10> (S-adenosyl-L-methionine, <10> pH 6.8, 22°C, recombinant enzyme [13]) [13]
0.25 <11> (iodide, <11> pH 6.8, 22°C [1]) [1]
0.26 <3> (iodide, <3> pH 7.0, 30°C [5]) [5]
1.3 <1> (iodide, <1> pH 7.5, 22°C [3]) [3]
4.47 <13> (iodide, <13> pH 7.0, 30°C, purified recombinant enzyme [7]) [7]
5 <18> (bromide) [14]
6.5 <10> (iodide, <10> pH 6.8, 22°C, recombinant enzyme [13]) [13]
8.5 <10> (iodide, <10> pH 6.8, 22°C, native enzyme [13]) [13]
10 <11> (bromide, <11> pH 6.8, 22°C [1]) [1]
12 <14> (iodide, <14> 30°C [6]) [6]
18.5 <10> (bromide, <10> pH 6.8, 22°C, native enzyme [13]) [13]
25 <10> (bromide, <10> pH 6.8, 22°C, recombinant enzyme [13]) [13]
29 <1> (bromide, <1> pH 7.5, 22°C [3]) [3]
63 <15> (iodide, <15> pH 7.0, 30°C [10]) [10]
85 <1> (chloride, <1> pH 7.5, 22°C [3]) [3]
100 <10> (chloride, <10> pH 6.8, 22°C, recombinant enzyme [13]) [13]
155 <10> (chloride, <10> pH 6.8, 22°C, native enzyme [13]) [13]
177.3 <13> (bromide, <13> pH 7.0, 30°C, purified recombinant enzyme [7]) [7]
300 <11> (chloride, <11> pH 6.8, 22°C [1]) [1]
1657 <13> (chloride, <13> pH 7.0, 30°C, purified recombinant enzyme [7]) [7]

K_i-Value (mM)

0.032 <1> (S-adenosyl-L-homocysteine, <1> pH 7.5, 22°C [3]) [3]
1.35 <1> (bisulfide, <1> pH 7.5, 22°C [3]) [3]

pH-Optimum

5.5-7 <1> (<1> methylation of iodide [3]) [3]
6.2 <10> (<10> methyl bromide production [13]) [13]
6.2-6.8 <10> (<10> methyl chloride production [13]) [13]
6.8 <10> (<10> assay at [4]) [4]
6.8-7.5 <10> (<10> methyl iodide production [13]) [13]
7 <3> (<3> assay at [5]) [5]
7-7.2 <11> [1]
7-7.5 <15> [10]
7.5-7.6 <18> (<18> synthesis of methyl bromide [14]) [14]
8 <14> [6]

pH-Range

5.4-10 <14> (<14> pH 5.4: about 50% of maximal activity, pH 10.0: about 80% of maximal activity [6]) [6]
6-8 <11,15> (<15> about 60% of maximal activity at pH 6.0 and 8.0 [10]; <11> pH 6.0: about 35% of maximal activity, pH 8.0: about 75% of maximal activity [1]) [1,10]
6-9 <18> (<18> pH 6.0: about 40% of maximal activity, pH 9.0: about 35% of maximal activity, at pH greater than 9.2 no methyl bromide synthesis detected [14]) [14]

pi-Value

4.8 <1> (<1> chromatofocusing [3]) [3]
5.1 <10> (<10> calculated from sequence [4]) [4]
5.3 <14> [6]

Temperature optimum (°C)

22 <1,10> (<1> assay at [3]; <10> assay at room temperature [4]) [3,4]
30 <3,15> (<3,15> assay at [5,10]) [5,10]

4 Enzyme Structure

Molecular weight

20000-25000 <18> (<18> gel filtration [14]) [14]
29000 <14> (<14> gel filtration [6]) [6]
29500 <1> (<1> gel filtration [3]) [3]

Subunits

? <10,12,13,19,20> (<13> x * 29000, SDS-PAGE [7]; <12,20> x * 53500, SDS-PAGE [11]; <10> x * 22474, the methylase can function in either a monomeric or oligomeric form, mass spectrometry [4]; <10> x * 22500, the methylase can function in either a monomeric or oligomeric form, SDS-PAGE [4]; <10> x * 25761, the methylase can function in either a monomeric or oligo-

meric form, calculated from sequence [4]; <13> x * 27500, calculated from sequence [7]; <19> x * 53300, SDS-PAGE [11]) [4,7,11]
monomer <1,14> (<14> 1 * 29000, SDS-PAGE [6]; <1> 1 * 28000, SDS-PAFE [3]) [3,6]

5 Isolation/Preparation/Mutation/Application

Source/tissue

leaf <1,10,13> (<1> leaf disc and leaf extract [12]) [3,4,7,12]
mycelium <11> (<11> the fungus is cultured in undisturbed glucose mycological peptone liquid medium [1]) [1]

Localization

membrane <11> (<11> bound to [1]) [1]

Purification

<1> (gel filtration, anion exchange chromatography, and affinity chromatography on adenosine-agarose) [3]
<10> (native enzyme 2700fold to homogeneity by ammonium sulfate fractionation, gel filtration, adenosine affinity chromatography, and a second step of gel filtration) [4]
<11> (the difficulty of solubilization of this membrane-bound labile enzyme is the greatest obstacle to its purification) [1]
<13> (partial) [7]
<14> [6]
<18> (the purification is achieved by an 80 to 100% ammonium sulfate precipitation step followed by a high-performance liquid chromatography gel filtration step on a 60 cm by 2.15 cm preparative Bio-Sil SEC-250 column. The column is eluted with 10 mM phosphate buffer, pH 7.0) [14]

Cloning

<2> (synthesis of all putative methyl halide transferase from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]
<4> (synthesis of all putative methyl halide transferases from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]
<5> (synthesis of all putative methyl halide transferases from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]
<6> (synthesis of all putative methyl halide transferases from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]
<7> (synthesis of all putative methyl halide transferase from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]
<8> (synthesis of all putative methyl halide transferases from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]
<9> (synthesis of all putative methyl halide transferases from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]
<10> [4]
<10> (expression in Escherichia coli) [13]

<12> [2,11]
<13> (expressed in Escherichia coli) [7]
<19> [11]
<20> [11]
<21> (synthesis of all putative methyl halide transferases from the NCBI sequence database and assay of methyl halide production in Escherichia coli) [8]

Engineering

Additional information <10> (<10> the disparity between the observed (22500 Da) and calculated molecular mass (25761 Da) suggests that the methylase undergoes posttranslational cleavage, possibly during purification [4]) [4]

Application

synthesis <2,4,5,6,7,8,9,21> (<2,4,5,6,7,8,9,21> producing methyl halides from non-food agricultural resources by using a symbiotic co-culture of an engineered yeast and the cellulolytic bacterium Actinotalea fermentans, methyl halide production from unprocessed switchgrass (Panicum virgatum), corn stover, sugar cane bagasse, and poplar (Populus sp.). Methyl halides are used as agricultural fumigants and are precursor molecules that can be catalytically converted to chemicals and fuels [8]) [8]

6 Stability

Storage stability

<1>, - 80°C, enzyme after the first gel filtration purification step, in 25 mM Tris acetate, pH 7.4, 10% glycerol, and 14 mM 2-mercaptoethanol, stable for over 2 months [3]
<1>, -80°C, after affinity chromatography, the halide/bisulfide methyltransferase becomes extremely labile losing all activity after overnight storage [3]
<1>, 20°C, enzyme after the affinity chromatography purification step, 25 mM Tris acetate, pH 7.4, 14 mM 2-mercaptoethanol, and 30% glycerol, 12% remaining activity after 48 h [3]
<1>, 4°C, enzyme after anion exchange purification step, in 25 mM Tris acetate, pH 7.4, 14 mM 2-mercaptoethanol, and 175 mM NaCl, more than 70% remaining activity after 24 h and 55% after 48 h [3]
<10>, -20°C, enzyme forms an aggregate with molecular mass of approximately 500000 Da [4]
<11>, -20°C to 4°C, partially purified enzyme, complete loss of activity overnight, also in the presence of protease inhibitors [1]
<12>, -20°C, purified protein stored in a buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 1 mM DTT, 30% glycerol), after 15 days, the recombinant protein AtHOL1 retains 60% of the iodide methyltransferase activity [11]
<19>, -20°C, purified protein stored in a buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 1 mM DTT, 30% glycerol), after 15 days, the recombinant protein AtHOL1 retains 90% of the iodide methyltransferase activity [11]

<20>, -20°C, purified protein stored in a buffer (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 1 mM DTT, 30% glycerol), after 15 days, the recombinant protein AtHOL1 retains 40% of the iodide methyltransferase activity [11]
<16,17>, -20°C, enzyme in cell extract is unstable and loses activities almost completely upon storage even if dithioerythritol, EDTA, protease inhibitor or glycerol are added to the extracts [10]
<16,17>, 4°C, enzyme in cell extract is unstable and loses activities almost completely upon storage even if dithioerythritol, EDTA, protease inhibitor or glycerol are added to the extracts [10]

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23S rRNA (uridine^{2552}-2′-O-)-methyltransferase 2.1.1.166

1 Nomenclature

EC number
2.1.1.166

Systematic name
S-adenosyl-L-methionine:23S rRNA (uridine^{2552}-2’-O-)-methyltransferase

Recommended name
23S rRNA (uridine^{2552}-2’-O-)-methyltransferase

Synonyms
23 S ribosomal RNA methyltransferase <3> [6]
23S rRNA methyltransferase <3> [5]
FTSJ <1,3> [1,2,5,6,7,8]
FtsJ/RrmJ heat shock protein <3> [6]
FtsJ2 <4> [4]
Mj0697 <2> [2]
RrmJ <1,3> [1,2,3,5,6]
Um(2552) 23S ribosomal RNA methyltransferase <3> [1]
Um2552 methyltransferase <3> [1,3]
heat shock protein RrmJ <3> [7]

2 Source Organism

<1> *Escherichia coli* [2]
<2> *Methanocaldococcus jannaschii* [2]
<3> *Escherichia coli* (UNIPROT accession number: C5W9C6) [1,3,5,6,7,8]
<4> *Homo sapiens* (UNIPROT accession number: Q9UI43) [4]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + uridine^{2552} in 23S rRNA = S-adenosyl-L-homocysteine + 2’-O-methyluridine^{2552} in 23S rRNA (<3> a reaction mechanism for the methyltransfer activity of RrmJ is proposed [7]; <3> mechanism, based on modeling studies and the structure of the 50S ribosome, a two-step model is proposed where the A loop undocks from the tightly packed 50S ribosomal subunit, allowing RrmJ to gain access to the substrate nucleotide

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U2552, and where U2552 undergoes base flipping, allowing the enzyme to methylate the 2-O position of the ribose [5])

Natural substrates and products

S S-adenosyl-L-methionine + uridine^{2552} in 23S rRNA <3> (<3> in vivo methylation of 23S rRNA by FtsJ goes to near completion [8]; <3> the 2-O-ribose methylation of the universally conserved base U2552 in the A-loop of the 23 S rRNA [7]; <3> the enzyme is responsible for the 2-O methylation of the universally conserved U2552 in the A loop of 23S rRNA [5]; <3> U2552 is an ubiquitously methylated residue [3]) (Reversibility: ?) [3,5,7,8]

P S-adenosyl-L-homocysteine + 2'-O-methyluridine^{2552} in 23S rRNA

Substrates and products

S S-adenosyl-L-methionine + uridine^{2552} in 23S rRNA <3> (<3> in vivo methylation of 23S rRNA by FtsJ goes to near completion [8]; <3> the 2-O-ribose methylation of the universally conserved base U2552 in the A-loop of the 23 S rRNA [7]; <3> the enzyme is responsible for the 2-O methylation of the universally conserved U2552 in the A loop of 23S rRNA [5]; <3> U2552 is an ubiquitously methylated residue [3]; <3> identification of the methylated nucleotide as 2-O-methyluridine 2552, by reverse phase high performance liquid chromatography analysis, boronate affinity chromatography, and hybridization-protection experiments. In vitro, FtsJ does not efficiently methylate ribosomes purified from a strain producing FtsJ, suggesting that these ribosomes are already methylated in vivo by FtsJ. FtsJ is active on ribosomes and on the 50 S ribosomal subunit, but is inactive on free rRNA, suggesting that its natural substrate is ribosomes or a pre-ribosomal ribonucleoprotein particle [6]; <3> the 2-O-ribose methylation of the universally conserved base U2552 in the A-loop of the 23 S rRNA. The active site of RrmJ appears to be formed by a catalytic triad consisting of two lysine residues, Lys-38 and Lys-164, and the negatively charged residue Asp-124. Another highly conserved residue, Glu-199, that is present in the active site of RrmJ and VP39 appears to play only a minor role in the methyltransfer reaction in vivo. A reaction mechanism for the methyltransfer activity of RrmJ is proposed [7]; <3> the isolated unmodified A loop serves as the minimal methylation substrate of wild-type RrmJ in vitro. 50S ribosomal subunits prepared from the rrmJ deletion strain appear to serve as substrates for RrmJ in vitro, while naked 23S rRNA or 40S ribosomal particles that are prepared from the rrmJ deletion strain are not methylated by purified RrmJ. This finding suggests that either the correct folding of the 23S rRNA or additional protein-protein interactions are necessary for the substrate recognition. A positively charged, highly conserved ridge in RrmJ appears to play a significant role in 23S rRNA binding and methylation. A structural model is provided of how the A loop of the 23S rRNA binds to RrmJ. Based on modeling studies and the structure of the 50S ribosome, a two-step model is proposed where the A loop undocks from the tightly packed 50S ribosomal subunit, allowing RrmJ to gain access to the substrate nucleotide U2552, and where

U2552 undergoes base flipping, allowing the enzyme to methylate the 2-O position of the ribose [5]) (Reversibility: ?) [1,3,5,6,7,8]

P S-adenosyl-L-homocysteine + 2'-O-methyluridine^{2552} in 23S rRNA

Cofactors/prosthetic groups

S-adenosyl-L-methionine <3> (<3> tightly bound [8]) [1,3,5,6,7,8]

Turnover number (s^{-1})

0.001 <3> (S-adenosyl-L-methionine, <3> pH 7.5, 37°C [7]) [7]
Additional information <3> (<3> changes in apparent k_{cat}-values for 50S subunit binding in RrmJ mutant proteins [5]; <3> the apparent k_{cat} value for 23 S rRNA within 50S ribosomal subunits is 0.001/s * mM at 37°C [7]) [5,7]

K_m-Value (mM)

0.0037 <3> (S-adenosyl-L-methionine, <3> pH 7.5, 37°C [7]) [7]
Additional information <3> (<3> apparent K_m values for 50S ribosomal subunits. The apparent K_m value for 23S rRNA within 50S ribosomal subunits is 0.0008 mM at 37°C [7]; <3> changes in apparent K_m-values for 50S subunit binding in RrmJ mutant proteins [5]) [5,7]

pH-Optimum

7.5 <3> (<3> assay at [8]) [8]

Temperature optimum (°C)

37 <3> (<3> assay at [8]) [8]
55 <3> [7]

Temperature range (°C)

50-60 <3> (<3> 50°C: about 75% of maximal activity, 60°C: about 95% of maximal activity [7]) [7]

4 Enzyme Structure

Subunits

? <4> (<4> x * 24700, calculated [4]) [4]

5 Isolation/Preparation/Mutation/Application

Source/tissue

carcinoma cell <4> (<4> transcripts are abundant [4]) [4]
heart <4> (<4> transcripts are abundant [4]) [4]
placenta <4> (<4> transcripts are abundant [4]) [4]
skeletal muscle <4> (<4> transcripts are abundant [4]) [4]

Localization

nucleolus <4> [4]

Purification

<3> [7,8]

<3> (a chimera protein of RrmJ fused to a chitin-binding domain was purified by affinity chromatography on a chitin affinity column) [6]

Crystallization

<1> (identification of a conserved tetrad K-D-K-H in the family of small nucleolar RNA-guided ribose 2'-O-methyltransferases related to fibrillarin. The corresponding functional groups of putative catalytic tetrads of Escherichia coli RrmJ and Methanococcus jannaschii Mj0697 may be superimposed in space. The invariant residues K164 in RrmJ and K179 in Mj0697 are observed in two distinct locations in the primary sequence, suggesting an interesting case of migration of the conserved side chain within the framework of the active site) [2]

<2> (identification of a conserved tetrad K-D-K-H in the family of small nucleolar RNA-guided ribose 2'-O-methyltransferases related to fibrillarin. The corresponding functional groups of putative catalytic tetrads of Escherichia coli RrmJ and Methanococcus jannaschii Mj0697 may be superimposed in space. The invariant residues K164 in RrmJ and K179 in Mj0697 are observed in two distinct locations in the primary sequence, suggesting an interesting case of migration of the conserved side chain within the framework of the active site) [2]

<3> (crystal structure of the FtsJ protein at 1.5 A resolution in complex with its cofactor S-adenosyl-L-methionine) [8]

Cloning

<3> [8]

Engineering

D124A <3> (<3> the mutant D124A is unable to rescue the growth defect of the rrmJ deletion strain, indicating that this mutation causes the inactivation of RrmJ in vivo [7]) [7]

D136N <3> (<3> D136N mutant strain accumulates larger amounts of 30S and 50S ribosomal subunits than wild-type strains under nonstringent salt conditions, and has a significant amount of 40S ribosomal particles under stringent salt conditions [5]; <3> mutation leads to slight decrease in k_{cat} value [5]) [5]

D20A <3> (<3> mutation leads to slight decrease in k_{cat} value [5]) [5]

D83A <3> (<3> the mutant D83A is unable to rescue the growth defect of the rrmJ deletion strain, indicating that this mutation causes the inactivation of RrmJ in vivo [7]) [7]

E199A <3> (<3> the RrmJ deletion strains expressing the E199A variant protein shows only slight growth defects, indicating that the residue is not as important in the catalytic mechanism [7]) [7]

F166A <3> (<3> decrease in S-adenosyl-L-methionine binding affinity and/or the presence of a certain amount of an inactive yet stably folded RrmJ mutant species [5]) [5]

F37A/L39A <3> (<3> mutant strain shows ribosome profiles that are indistinguishable from wild-type ribosome profile [5]) [5]
K164A <3> (<3> the mutant D83A is unable to rescue the growth defect of the rrmJ deletion strain, indicating that this mutation causes the inactivation of RrmJ in vivo [7]) [7]
K38A <3> (<3> the mutant D83A is unable to rescue the growth defect of the rrmJ deletion strain, indicating that this mutation causes the inactivation of RrmJ in vivo [7]) [7]
Q67A/Y68A <3> (<3> mutant strain shows ribosome profiles that are indistinguishable from wild-type ribosome profile [5]) [5]
R32A/R34A <3> (<3> R32A/R34A mutant strain accumulates larger amounts of 30S and 50S ribosomal subunits than wild-type strains under nonstringent salt conditions, and has a significant amount of 40S ribosomal particles under stringent salt conditions [5]) [5]
Y201A <3> (<3> the RrmJ deletion strains expressing the Y201A variant protein shows only slight growth defects, indicating that the residue is not as important in the catalytic mechanism [7]) [7]
Additional information <3> (<3> extensive site-directed mutagenesis of the residues conserved in RrmJ and characterization of the mutant proteins both in vivo and in vitro [5]; <3> lack of U2552 methylation, obtained in rrmJ-deficient mutants, results in a decrease in programmed +1 and -1 translational frameshifing and a decrease in readthrough of UAA and UGA stop codons. The increased translational accuracy of rrmJ-deficient strains suggests that the interaction between aminoacyl-tRNA and U2552 is important for selection of the correct tRNA at the ribosomal A site, and supports the idea that translational accuracy in vivo is optimal rather than maximal, thus pointing to the participation of recoding events in the normal cell physiology [3]; <3> rrmJ-deficient strain exhibit growth and translational defects compared to the wild-type strain. Growth rates of the rrmJ mutant are decreased at both low and high temperatures. Protein synthesis activity is reduced up to 65% when S30 rrmJ mutant extracts are tested in a coupled in vitro transcription/translation assay. In vitro methylation of these extracts by RrmJ partially restores protein synthesis activity [1]) [1,3,5]

6 Stability

Temperature stability

Additional information <3> (<3> RrmJ is a thermostable heat shock protein [7]) [7]

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27S pre-rRNA (guanosine^{2922}-2′-O)-methyltransferase 2.1.1.167

1 Nomenclature

EC number
2.1.1.167

Systematic name
S-adenosyl-L-methionine:27S pre-rRNA (guanosine^{2922}-2'-O)-methyltransferase

Recommended name
27S pre-rRNA (guanosine^{2922}-2'-O)-methyltransferase

Synonyms
Spb1p <1,2> (<1> gene name [3]) [1,3]

2 Source Organism

<1> *Saccharomyces cerevisiae* [3]
<2> *Saccharomyces cerevisiae* (UNIPROT accession number: P25582) [1,2,4]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + guanosine^{2922} in 27S pre-rRNA = S-adenosyl-L-homocysteine + 2'-O-methylguanosine^{2922} in 27S pre-rRNA

Natural substrates and products

S S-adenosyl-L-methionine + guanosine^{2922} in 27S pre-rRNA <1> (<1> Spb1p is a site-specific 2-O-ribose RNA MTase that catalyzes the formation of Gm2922, a universally conserved position of the catalytic center of the ribosome that is essential for translation. The enzyme is required for proper assembly of pre-ribosomal particles during the biogenesis of the 60S ribosomal subunit. Specifically methylates the guanosine in position 2922 of the 25S rRNA at the stage of 27S pre-rRNA maturation. Methylates also the uridine in position 2921 in the absence of methylation of this residue guided by snoRNA snR52 at the stage of 35S pre-rRNA maturation. 2-O-methylguanosine^{2922} appears at a late processing stage, during the maturation of the 27S pre-rRNA [3]) (Reversibility: ?) [3]

P S-adenosyl-L-homocysteine + 2'-O-methylguanosine^{2922} in 27S pre-rRNA

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S S-adenosyl-L-methionine + uridine^{2918} in 25S rRNA <1,2> (<1,2> the enzyme is required for proper assembly of pre-ribosomal particles during the biogenesis of the 60S ribosomal subunit. Specifically methylates the guanosine in position 2922 of the 25S rRNA at the stage of 27S pre-rRNA maturation. Methylates also the uridine in position 2921 in the absence of methylation of this residue guided by snoRNA snR52 at the stage of 35S pre-rRNA maturation [2,3]) (Reversibility: ?) [2,3]
P S-adenosyl-L-homocysteine + 2'-O-methyluridine^{2918} in 25S rRNA
S Additional information <2> (<2> Spb1p is required for proper assembly of pre-ribosomal particles during the biogenesis of 60S ribosomal subunits [4]) [4]
P ?

Substrates and products

S S-adenosyl-L-methionine + guanosine^{2922} in 27S pre-rRNA <1> (<1> Spb1p is a site-specific 2-O-ribose RNA MTase that catalyzes the formation of Gm2922, a universally conserved position of the catalytic center of the ribosome that is essential for translation. The enzyme is required for proper assembly of pre-ribosomal particles during the biogenesis of the 60S ribosomal subunit. Specifically methylates the guanosine in position 2922 of the 25S rRNA at the stage of 27S pre-rRNA maturation. Methylates also the uridine in position 2921 in the absence of methylation of this residue guided by snoRNA snR52 at the stage of 35S pre-rRNA maturation. 2-O-methylguanosine2922 appears at a late processing stage, during the maturation of the 27S pre-rRNA [3]) (Reversibility: ?) [3]
P S-adenosyl-L-homocysteine + 2'-O-methylguanosine^{2922} in 27S pre-rRNA
S S-adenosyl-L-methionine + uridine^{2918} in 25S rRNA <1,2> (<1,2> the enzyme is required for proper assembly of pre-ribosomal particles during the biogenesis of the 60S ribosomal subunit. Specifically methylates the guanosine in position 2922 of the 25S rRNA at the stage of 27S pre-rRNA maturation. Methylates also the uridine in position 2921 in the absence of methylation of this residue guided by snoRNA snR52 at the stage of 35S pre-rRNA maturation [2,3]; <2> no methylation of uridine2791 [2]) (Reversibility: ?) [2,3]
P S-adenosyl-L-homocysteine + 2'-O-methyluridine^{2918} in 25S rRNA
S Additional information <2> (<2> Spb1p is required for proper assembly of pre-ribosomal particles during the biogenesis of 60S ribosomal subunits [4]; <2> a point mutation in the Ado-Met binding site of Spb1p affects cell growth but does not abolish methylation of U2918. When this mutation is combined with disruption of snR52 cell growth is severely impaired and U2918 is no longer methylated. In vitro, Spb1p is able to methylate U2918 on 60S subunits. For this methylation two mechanisms coexist: a site-specific methyltransferase (Spb1p) and a snoRNA-dependent mechanism [2]) [2,4]
P ?

Cofactors/prosthetic groups

S-adenosyl-L-methionine <2> (<2> Spb1p possesses a putative S-adenosyl-L-methionine-binding domain, which is common to the S-adenosyl-L-methionine-dependent methyltransferases [1]) [1]

4 Enzyme Structure

Molecular weight

96500 <2> (<2> calculated from sequence [1]) [1]

Subunits

? <2> (<2> x * 96500, calculated from sequence [1]) [1]

5 Isolation/Preparation/Mutation/Application

Localization

nucleolus <2> [1,4]

Engineering

D52A <1> (<1> when the mutant protein is the sole source of Spb1p in the cell (strain YBL4637), it leads to a severe growth defect with a generation time of about 300 min. No formation of 2-O-methylguanosine2922 [3]) [3]

Additional information <2> (<2> a point mutation in the Ado-Met binding site of Spb1p affects cell growth but does not abolish methylation of U2918. When this mutation is combined with disruption of snR52 cell growth is severely impaired and U2918 is no longer methylated. In vitro, Spb1p is able to methylate U2918 on 60S subunits. For this methylation two mechanisms coexist: a site-specific methyltransferase (Spb1p) and a snoRNA-dependent mechanism [2]) [2]

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21S rRNA (uridine^{2791}-2′-O)-methyltransferase 2.1.1.168

1 Nomenclature

EC number
2.1.1.168

Systematic name
S-adenosyl-L-methionine:21S rRNA (uridine^{2791}-2'-O)-methyltransferase

Recommended name
21S rRNA (uridine^{2791}-2'-O)-methyltransferase

Synonyms
MRM2 (gene name) <1> [1]
mitochondrial 21S rRNA methyltransferase <1> [1]
mitochondrial rRNA MTase 2 <1> [1]

2 Source Organism

<1> *Saccharomyces cerevisiae* (UNIPROT accession number: P53123) [1]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + uridine^{2791} in 21S rRNA = S-adenosyl-L-homocysteine + 2'-O-methyluridine2791 in 21S rRNA

Natural substrates and products
S S-adenosyl-L-methionine + uridine^{2791} in 21S rRNA <1> (<1> mitochondria of the yeast Saccharomyces cerevisiae assemble their ribosomes from ribosomal proteins, encoded by the nuclear genome (with one exception), and rRNAs of 15S and 21S, encoded by the mitochondrial genome. Unlike cytoplasmic rRNA, which is highly modified, mitochondrial rRNA contains only three modified nucleotides: a pseudouridine and two 2-O-methylated riboses (Gm2270 and Um2791) located at the peptidyl transferase centre of 21S rRNA. Mrm2 is required for methylating U2791 of 21S rRNA [1]) (Reversibility: ?) [1]
P S-adenosyl-L-homocysteine + 2'-O-methyluridine^{2791} in 21S rRNA

Substrates and products
S S-adenosyl-L-methionine + uridine^{2791} in 21S rRNA <1> (<1> mitochondria of the yeast Saccharomyces cerevisiae assemble their ribosomes from

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ribosomal proteins, encoded by the nuclear genome (with one exception), and rRNAs of 15S and 21S, encoded by the mitochondrial genome. Unlike cytoplasmic rRNA, which is highly modified, mitochondrial rRNA contains only three modified nucleotides: a pseudouridine and two 2-O-methylated riboses (Gm2270 and Um2791) located at the peptidyl transferase centre of 21S rRNA. Mrm2 is required for methylating U2791 of 21S rRNA [1]; <1> Mrm2p methylates the 21S rRNA at position U2791 in vitro, when it is assembled with proteins into the ribosomal large subunit ir the precursor of the ribosomal large subunit. NO methylation is observed with deproteinized rRNA [1]) (Reversibility: ?) [1]

P S-adenosyl-L-homocysteine + 2'-O-methyluridine^{2791} in 21S rRNA

Cofactors/prosthetic groups

S-adenosyl-L-methionine <1> [1]

Temperature optimum (°C)

37 <1> (<1> assay at [1]) [1]

5 Isolation/Preparation/Mutation/Application

Localization

mitochondrion <1> [1]

Cloning

<1> [1]

References

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tricetin 3′,4′,5′-O-trimethyltransferase 2.1.1.169

1 Nomenclature

EC number
2.1.1.169

Systematic name
S-adenosyl-L-methionine:tricetin 3',4',5'-O-trimethyltransferase

Recommended name
tricetin 3',4',5'-O-trimethyltransferase

Synonyms
FOMT <1> [2]
TaCOMT1 <2> [3]
TaOMT <2> [3]
TaOMT2 <1> [1]

2 Source Organism

<1> *Triticum aestivum* (UNIPROT accession number: Q38J50) [1,2]
<2> *Triticum aestivum* (UNIPROT accession number: Q84N28) [3]

3 Reaction and Specificity

Catalyzed reaction
3 S-adenosyl-L-methionine + tricetin = 3 S-adenosyl-L-homocysteine + 3',4',5'-O-trimethyltricetin (overall reaction)
S-adenosyl-L-methionine + tricetin = S-adenosyl-L-homocysteine + 3'-O-methyltricetin
S-adenosyl-L-methionine + 3'-O-methyltricetin = S-adenosyl-L-homocysteine + 3',5'-O-dimethyltricetin
S-adenosyl-L-methionine + 3',5'-O-dimethyltricetin = S-adenosyl-L-homocysteine + 3',4',5'-O-trimethyltricetin

Substrates and products
S 3 S-adenosyl-L-methionine + tricetin <1,2> (<1> overall reaction [2]; <2> TaOMT1 (TaCOMT1) catalyzes the sequential methylation of tricetin to its 3-O-monomethyl-derivative (selgin), 3,5-O-dimethyl-derivative (tricin) and 3,4,5-O-trimethyl derivative. 3,5-O-dimethyltricetin appears to be the major reaction product. The enzyme also catalyzes methylation of

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luteolin, quercetin and eriodictyol [3]; <1> the enzyme catalyzes three sequential O-methylations, forming 3-O-methyltricetin, 3,5-dimethyltricetin and 3,4,5-O-trimethyltricetin. Tricetin is the preferred substrate. Also catalyzes the methylation of 5-hydroxyferulic acid, luteolin, quercetin, eriodyctiol, quercetagetin, taxifolin, gossypetin and myricetin. TaOMT2 is a flavonoid, B-ring-specific O-methyltransferase with a preference for flavones > dihydroflavones > flavonols that possess at least two, B-ring hydroxyl groups [1]) (Reversibility: ?) [1,2,3]

P 3 S-adenosyl-L-homocysteine + 3',4',5'-O-trimethyltricetin

S S-adenosyl-L-methionine + 3',5'-O-dimethyltricetin <1,2> (<1> the enzyme catalyzes three sequential O-methylations, forming 3-O-methyltricetin, 3,5-dimethyltricetin and 3,4,5-O-trimethyltricetin. Tricetin is the preferred substrate [1]) (Reversibility: ?) [1,2,3]

P S-adenosyl-L-homocysteine + 3',4',5'-O-trimethyltricetin

S S-adenosyl-L-methionine + 3'-O-methyltricetin <1,2> (<1> the enzyme catalyzes three sequential O-methylations, forming 3-O-methyltricetin, 3,5-dimethyltricetin and 3,4,5-O-trimethyltricetin. Tricetin is the preferred substrate [1]) (Reversibility: ?) [1,2,3]

P S-adenosyl-L-homocysteine + 3',5'-O-dimethyltricetin

S S-adenosyl-L-methionine + tricetin <1,2> (<1> the enzyme catalyzes three sequential O-methylations, forming 3-O-methyltricetin, 3,5-dimethyltricetin and 3,4,5-O-trimethyltricetin. Tricetin is the preferred substrate [1]) (Reversibility: ?) [1,2,3]

P S-adenosyl-L-homocysteine + 3'-O-methyltricetin

Inhibitors

Additional information <1> (<1> no loss of activity up to 0.4 M perchlorate [2]) [2]

Cofactors/prosthetic groups

S-adenosyl-L-methionine <1> [1]

Metals, ions

Additional information <2> (<2> no requirement for Mg^{2+} [3]) [3]

pi-Value

5.75 <2> (<2> calculated from sequence [3]) [3]

Temperature optimum (°C)

30 <1> (<1> assay at [2]) [2]

4 Enzyme Structure

Subunits

? <2> (<2> x * 38900, calculated from sequence, SDS-PAGE [3]) [3]
dimer <1> (<1> 2 * 42616, characterisation of the dimer/monomer equilibrium, calculated from sequence [2]) [2]

monomer <1> (<1> 1 * 42616, characterisation of the dimer/monomer equilibrium, calculated from sequence [2]) [2]

5 Isolation/Preparation/Mutation/Application

Source/tissue

leaf <1> [1]

Purification

<1> [1]
<2> [3]

Cloning

<1> (expression in Escherichia coli) [1]
<2> (expression in Escherichia coli) [3]

6 Stability

Storage stability

<1>, 4°C, the activity of the affinity-purified TaOMT2 is quite stable for up to 2 months when stored in buffer containing 10% glycerol, with a half-life of 10 to 11 weeks [1]
<2>, 4°C, almost no loss of catalytic activity when stored for 3-4 weeks [3]

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16S rRNA (guanine^{527}-N^{7})-methyltransferase 2.1.1.170

1 Nomenclature

EC number
2.1.1.170

Systematic name
S-adenosyl-L-methionine:16S rRNA (guanine^{527}-N^{7})-methyltransferase

Recommended name
16S rRNA (guanine^{527}-N7)-methyltransferase

Synonyms
16S rRNA methyltransferase <5> [2]
16S rRNA methyltransferase RsmG <4> [6]
gidB <2,4,6> (<2,4> gene name [4,6]) [4,5,6]
glucose-inhibited division protein B <6> [5]
ribosomal RNA small subunit methyltransferase G <4> [6]
rsmG <4,5> (<4,5> gene name [2,6]) [2,6]

2 Source Organism

<1> *Bacillus subtilis* (MsPDH$_2$ [3]) [3]
<2> *Escherichia coli* [4]
<3> *Streptomyces griseus* [1]
<4> *Thermus thermophilus* (UNIPROT accession number: Q9LCY2) (extracellular isozyme [6]) [6]
<5> *Streptomyces coelicolor* (UNIPROT accession number: O54571) (testis-specific serine/threonine protein kinase 5 variant α [2]) [2]
<6> *Escherichia coli* (UNIPROT accession number: P0A6U5) [5]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + guanine^{527} in 16S rRNA = S-adenosyl-L-homocysteine + N^{7}-methylguanine^{527} in 16S rRNA

Natural substrates and products
S S-adenosyl-L-methionine + guanine^{527} in 16S rRNA <1,4> (<4> methylations concentrated in the decoding site of the 30S ribosomal subunit may act to fine tune codon recognition in a manner similar to tRNA modifica-

D. Schomburg and I. Schomburg (eds.), *Class 2–3.2 Transferases, Hydrolases: EC 2–3.2*, Springer Handbook of Enzymes S9, DOI 10.1007/978-3-642-36240-8_8,
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tions. The intact 30S subunit is very unlikely to be the natural substrate for Thermus thermophilus RsmG in vivo. This interpretation is consistent with the position of G527 in the intact 30S subunit, where it is buried and would be inaccessible for methylation without substantial unfolding of the local subunit structure. Deproteinized 16S rRNA is the most active substrate in vitro. In vivo, several ribosomal proteins probably begin binding to the nascent 16S rRNA transcript prior to its completion, making an early assembly intermediate a plausible candidate for the biological substrate of RsmG [6]; <1> the methyltransferase RsmG methylates the N^7 position of nucleotide G535 in 16S rRNA of Bacillus subtilis (corresponding to G527 in Escherichia coli). Nucleotide G527 is situated within a hairpin loop (the socalled 530 loop) that is one of the most highly conserved features of 16S rRNA [3]) (Reversibility: ?) [3,6]

P S-adenosyl-L-homocysteine + N^7-methylguanine^{527} in 16S rRNA

Substrates and products

S S-adenosyl-L-methionine + guanine^{527} in 16S rRNA <1,2,3,4> (<4> methylations concentrated in the decoding site of the 30S ribosomal subunit may act to fine tune codon recognition in a manner similar to tRNA modifications. The intact 30S subunit is very unlikely to be the natural substrate for Thermus thermophilus RsmG in vivo. This interpretation is consistent with the position of G527 in the intact 30S subunit, where it is buried and would be inaccessible for methylation without substantial unfolding of the local subunit structure. Deproteinized 16S rRNA is the most active substrate in vitro. In vivo, several ribosomal proteins probably begin binding to the nascent 16S rRNA transcript prior to its completion, making an early assembly intermediate a plausible candidate for the biological substrate of RsmG [6]; <1> the methyltransferase RsmG methylates the N7 position of nucleotide G535 in 16S rRNA of Bacillus subtilis (corresponding to G527 in Escherichia coli). Nucleotide G527 is situated within a hairpin loop (the socalled 530 loop) that is one of the most highly conserved features of 16S rRNA [3]; <2> GidB is a m^7G methyltransferase specific for 16S rRNA, identification of methylated nucleotide [4]; <3> the methyltransferase RsmG methylates the N^7 position of nucleotide G535 in 16S rRNA of Bacillus subtilis (corresponding to G527 in Escherichia coli) [1]; <1> the methyltransferase RsmG methylates the N^7 position of nucleotide G535 in 16S rRNA of Bacillus subtilis (corresponding to G527 in Escherichia coli), identification of the exact target site of RsmG methylation [3]; <4> the most active substrate for Thermus thermophilus RsmG in vitro is deproteinized 16S rRNA. 30S subunits in their native conformation are not a proper substrate, removal of Mg^{2+} ions from the subunit is required to open the structure sufficiently to expose elements involved in enzyme binding. Identification of methylated nucleotide [6]) (Reversibility: ?) [1,3,4,6]

P S-adenosyl-L-homocysteine + N^7-methylguanine^{527} in 16S rRNA

Cofactors/prosthetic groups

S-adenosyl-L-methionine <4> (<4> S-adenosyl-L-methionine is bound in a canonical conformation above the β-sheet and close to the conserved GxGxG methyltransferase signature motif (residues 88-92 between strand β_1 and helix α_4). The AdoMet cofactor is tightly bound in RsmG and copurifies with the recombinant protein [6]) [6]

Metals, ions

Mg^{2+} <4> (<4> the 30S subunits in their native conformation are not a proper substrate and removal of Mg^{2+} ions from the subunit is required to open the structure sufficiently to expose elements involved in enzyme binding [6]) [6]

pH-Optimum

7.5 <2> (<2> assay at [4]) [4]

Temperature optimum (°C)

37 <2> (<2> assay at [4]) [4]

4 Enzyme Structure

Molecular weight

23840 <6> (<6> MALDI-MS [5]) [5]

Subunits

monomer <6> (<6> 1 * 23842, MALDI-MS [5]) [5]

5 Isolation/Preparation/Mutation/Application

Purification

<2> [4]
<4> (the AdoMet cofactor is tightly bound in RsmG and copurifies with the recombinant protein) [6]

Crystallization

<4> (microbatch technique under oil at 4°C. Determination of the structure of RsmG (249 amino acids) in three different crystal forms: the enzyme in complex with the cofactor S-adensosyl-L-methionine, the enzyme in complex with S-adenosyl-L-homocysteine, the enzyme in complex with adenosine monophosphate and S-adenosyl-L-methionine. RsmG X-ray crystal structures at up to 1.5 A resolution. Cofactor-bound crystal structures of RsmG reveals a positively charged surface area remote from the active site that binds an adenosine monophosphate molecule) [6]
<6> (sitting-drop vapor diffusion at 4°C against a reservoir containing 0.1 M of sodium citrate pH 6.5, 15% polyethylene glycol 4000, and 10% isopropanol) [5]

Cloning

<2> [4]
<4> [6]
<6> (expression in Escherichia coli BL21) [5]

Engineering

Additional information <2> (<2> mutations within the gene gidB confer low-level streptomycin resistance. gidB mutations emerge spontaneously at a high frequency of 0.000001 and, once emerged, result in vigorous emergence of high-level streptomycin-resistant mutants at a frequency more than 2000 times greater than that seen in wild-type strains [4]) [4]

References

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16S rRNA (guanine^{966}-N^2)-methyltransferase 2.1.1.171

1 Nomenclature

EC number
2.1.1.171

Systematic name
S-adenosyl-L-methionine:16S rRNA (guanine^{966}-N^2)-methyltransferase

Recommended name
16S rRNA (guanine^{966}-N^2)-methyltransferase

Synonyms
16S rRNA-specific methyltransferases m^{2}GMT <1> [4]
RNA:(guanine-N^2) methyltransferase RsmD <1> [3]
RsmD <1> (<1> gene name [1,2]) [1,2]
m^{2}G966 methyltransferase <1> [1,4]
yhhF <1> (<1> gene name [1,2]) [1,2]

CAS registry number
50812-26-5

2 Source Organism

<1> *Escherichia coli* (UNIPROT accession number: P0ADX9) [1,2,3,4]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + guanine^{966} in 16S rRNA = S-adenosyl-L-homocysteine + N^2-methylguanine966 in 16S rRNA (<1> proposed model of rRNA/RsmD interactions in the active site [1])

Natural substrates and products
S S-adenosyl-L-methionine + guanine^{966} in 16S rRNA <1> (<1> the enzyme uses unmethylated 30S subunits as a substrate, but not free unmethylated 16S rRNA. Binding of ribosomal proteins S7, S9, and S19 to unmodified 16S rRNA individually and in all possible combinations shows that S7 plus S19 are sufficient to block methylation by the m^{5}C967 methyltransferase, while simultaneously inducing methylation by the m^{2}G966 methyltransferase. A purified complex containing stoichiometric amounts of proteins S7, S9, and S19 bound to 16S rRNA is isolated and shown to possess the

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same methylation properties as 30S subunits, that is, the ability to be methylated by the m^2G966 methyltransferase but not by the m^5C967 methyltransferase. Since binding of S19 requires prior binding of S7, which has no effect on methylation when bound alone, the switch in methylase specificity is attributed solely to the presence of RNA-bound S19. Single-omission reconstitution of 30S subunits deficient in S19 results in particles that could not be efficiently methylated by either enzyme. Thus while binding of S19 is both necessary and sufficient to convert 16S rRNA into a substrate of the m^2G966 methyltransferase, binding of either S19 alone or some other protein or combination of proteins to the 16S rRNA can abolish activity of the m^5C967 methyltransferase. Binding of S19 to 16S rRNA is known to cause local conformational changes in the 960-975 stem-loop structure surrounding the two methylated nucleotides [4]) (Reversibility: ?) [1,2,4]

P S-adenosyl-L-homocysteine + N^2-methylguanine966 in 16S rRNA

Substrates and products

S S-adenosyl-L-methionine + guanine966 in 16S rRNA <1> (<1> the enzyme uses unmethylated 30S subunits as a substrate, but not free unmethylated 16S rRNA. Binding of ribosomal proteins S7, S9, and S19 to unmodified 16S rRNA individually and in all possible combinations shows that S7 plus S19 are sufficient to block methylation by the m5C967 methyltransferase, while simultaneously inducing methylation by the m^2G966 methyltransferase. A purified complex containing stoichiometric amounts of proteins S7, S9, and S19 bound to 16S rRNA is isolated and shown to possess the same methylation properties as 30S subunits, that is, the ability to be methylated by the m^2G966 methyltransferase but not by the m^5C967 methyltransferase. Since binding of S19 requires prior binding of S7, which has no effect on methylation when bound alone, the switch in methylase specificity is attributed solely to the presence of RNA-bound S19. Single-omission reconstitution of 30S subunits deficient in S19 results in particles that could not be efficiently methylated by either enzyme. Thus while binding of S19 is both necessary and sufficient to convert 16S rRNA into a substrate of the m^2G966 methyltransferase, binding of either S19 alone or some other protein or combination of proteins to the 16S rRNA can abolish activity of the m^5C967 methyltransferase. Binding of S19 to 16S rRNA is known to cause local conformational changes in the 960-975 stem-loop structure surrounding the two methylated nucleotides [4]; <1> RsmD methyltransferase utilizes assembled small subunits or its late assembly intermediates as a substrate. It is likely that the latter class of proteins uses the decoding cleft of the small subunit as the binding site [2]; <1> RsmD protein efficiently methylates guanine^{966} of the assembled 30S subunits (purified from yhhF knock-out strain in vitro) in vitro in the presence of AdoMet. Protein-free 16 S rRNA was not a substrate for RsmD. The methylation is specific for guanine^{966} of 16S rRNA [1]) (Reversibility: ?) [1,2,4]

P S-adenosyl-L-homocysteine + N^2-methylguanine^{966} in 16S rRNA

Cofactors/prosthetic groups

S-adenosyl-L-methionine <1> [1,4]

4 Enzyme Structure

Subunits

dimer <1> (<1> based on a comprehensive bioinformatic analysis of m2G methyltransferases it is inferred that the prokaryotic RsmC and RsmD methyltransferases are pseudodimers. The C-terminal catalytic domain is closely related to the structurally characterized Mj0882 protein, while the N-terminal domain lacks the cofactor-binding and catalytic side-chains [3]) [3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1]

Crystallization

<1> (vapor diffusion method in hanging drops, structure determined and refined to 2.05 A) [1]

Cloning

<1> [1]

References

[1] Lesnyak, D.V.; Osipiuk, J.; Skarina, T.; Sergiev, P.V.; Bogdanov, A.A.; Edwards, A.; Savchenko, A.; Joachimiak, A.; Dontsova, O.A.: Methyltransferase that modifies guanine 966 of the 16 S rRNA: functional identification and tertiary structure. J. Biol. Chem., **282**, 5880-5887 (2007)

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16S rRNA (guanine^{1207}-N^{2})-methyltransferase 2.1.1.172

1 Nomenclature

EC number
2.1.1.172

Systematic name
S-adenosyl-L-methionine:16S rRNA (guanine^{1207}-N^{2})-methyltransferase

Recommended name
16S rRNA (guanine^{1207}-N^{2})-methyltransferase

Synonyms
16 S RNA m^{2}G1207 methyltransferase <1> [1]
RNA:(guanine-N^{2}) methyltransferase RsmC <1> [3]
RsmC <1> (<1> gene name [1,2,4]) [1,2,4]
ribosomal RNA small subunit methyltransferase C <1> [1]
yjjT <1> (<1> gene name [1,2]) [1,2]

CAS registry number
50812-26-5

2 Source Organism

<1> *Escherichia coli* (UNIPROT accession number: P39406) [1,2,3,4]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + guanine^{1207} in 16S rRNA = S-adenosyl-L-homocysteine + N^{2}-methylguanine^{1207} in 16S rRNA

Natural substrates and products
S S-adenosyl-L-methionine + guanine^{1207} in 16S rRNA <1> (<1> because the m^{2}G1207 methyltransferase reacts with 30S particles but barely at all with 16 S RNA, it seems likely that methylation of the guanine residue occurs after the 16S RNA has associated with some ribosomal proteins [1]; <1> RsmD methyltransferase utilizes assembled ribosomal small subunit or its late assembly intermediates as a substrate. It is likely it uses the decoding cleft of the small subunit as the binding site. Nucleotide N^{2}-methylguanine^{1207} is located in the helix 34 of the 16S rRNA. This functionally important helix is involved in the formation of the binding pocket

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for A-site bound tRNA and translocation. Despite location in the very functionally important region of the 30S subunit, N^2-methylguanine^{1207} is not involved in the contact with tRNA. Methylation of G1207 may be necessary to freeze secondary structure in the functional conformation [2]) (Reversibility: ?) [1,2,4]

P S-adenosyl-L-homocysteine + N^2-methylguanine^{1207} in 16S rRNA

Substrates and products

S S-adenosyl-L-methionine + guanine^{1207} in 16S rRNA <1> (<1> because the m^2G1207 methyltransferase reacts with 30S particles but barely at all with 16S RNA, it seems likely that methylation of the guanine residue occurs after the 16S RNA has associated with some ribosomal proteins [1]; <1> RsmD methyltransferase utilizes assembled ribosomal small subunit or its late assembly intermediates as a substrate. It is likely is uses the decoding cleft of the small subunit as the binding site. Nucleotide N^2-methylguanine^{1207} is located in the helix 34 of the 16S rRNA. This functionally important helix is involved in the formation of the binding pocket for A-site bound tRNA and translocation. Despite location in the very functionally important region of the 30S subunit, N^2-methylguanine^{1207} is not involved in the contact with tRNA. Methylation of G1207 may be necessary to freeze secondary structure in the functional conformation [2]; <1> the enzyme reacts well with 30S subunits reconstituted from 16S RNA transcripts and 30S proteins but is almost inactive with the corresponding free RNA. Of the three naturally occurring N^2-methylguanine residues, only N^2-methylguanine^{1207} is formed. It is suggested that the optimal substrate may be a ribonucleoprotein particle less structured than a 30S ribosome but more so than free RNA. Localization of the site of methylation by hybridization-protection studies using deoxyoligomers that are complementary to the RNA sequence spanning each of the N^2-methylguanine sites [1]) (Reversibility: ?) [1,2,3,4]

P S-adenosyl-L-homocysteine + N^2-methylguanine^{1207} in 16S rRNA

Inhibitors

EDTA <1> (<1> 2 mM, markedly reduces the level of methylation [1]) [1]
Mg^{2+} <1> (<1> 6 mM, markedly reduces the level of methylation [1]) [1]

Metals, ions

Mg^{2+} <1> (<1> close to unit stoichiometry of methylation can be achieved at 0.9 mM Mg^{2+} [1]) [1]

pH-Optimum

7.5 <1> (<1> assay at [1]) [1]

Temperature optimum (°C)

37 <1> (<1> assay at [1]) [1]

4 Enzyme Structure

Molecular weight

37000 <1> (<1> gel filtration [1]) [1]

Subunits

dimer <1> (<1> based on a comprehensive bioinformatic analysis of m^2G methyltransferases it is inferred that the prokaryotic RsmC and RsmD methyltransferases are pseudodimers. The C-terminal catalytic domain is closely related to the structurally characterized Mj0882 protein, while the N-terminal domain lacks the cofactor-binding and catalytic side-chains [3]) [3]
monomer <1> (<1> 1 * 37600, calculated from sequence [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1]
<1> (purification and refolding of C-RsmC from inclusion bodies) [4]

Crystallization

<1> (hanging drop vapor diffusion method, crystal structure of RsmC refined to 2.1 A resolution, reveals two homologous domains tandemly duplicated within a single polypeptide. Characterization of the function of the individual domains and identification of key residues involved in binding of rRNA and S-adenosyl-L-methionine, and in catalysis. It is discovered that one of the domains is important for the folding of the other. RsmC can be regarded as a model system for functional streamlining of domains accompanied by the development of dependencies concerning folding and stability) [4]

Cloning

<1> [1]
<1> (the rsmC gene, cloned into pCA24N vector with a noncleavable N-terminal His6 tag) [4]

References

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23S rRNA (guanine^{2445}-N^2)-methyltransferase 2.1.1.173

1 Nomenclature

EC number
2.1.1.173

Systematic name
S-adenosyl-L-methionine:23S rRNA (guanine^{2445}-N^2)-methyltransferase

Recommended name
23S rRNA (guanine^{2445}-N^2)-methyltransferase

Synonyms
RlmL <1> (<1> gene name [1,2]) [1,2]
ycbY <1> (<1> gene name [1,2,3]) [1,2,3]

CAS registry number
50812-26-5

2 Source Organism

<1> *Escherichia coli* (UNIPROT accession number: P75864) [1,2,3]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + guanine^{2445} in 23S rRNA = S-adenosyl-L-homocysteine + N^2-methylguanine^{2445} in 23S rRNA

Natural substrates and products
S S-adenosyl-L-methionine + guanine^{2445} in 23S rRNA <1> (<1> N^2-methylguanosine^{2445} of the 23S rRNA is located in a cluster of modified nucleotides concentrated at the peptidyl transferase center of the ribosome. It is likely that the G2445 modification is necessary for prevention of nonfunctional secondary or tertiary structure formation at the peptidyl transferase center [1]) (Reversibility: ?) [1,2]
P S-adenosyl-L-homocysteine + N^2-methylguanine2445 in 23S rRNA

Substrates and products
S S-adenosyl-L-methionine + guanine^{2445} in 23S rRNA <1> (<1> N^2-methylguanosine^{2445} of the 23S rRNA is located in a cluster of modified nucleotides concentrated at the peptidyl transferase center of the ribo-

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some. It is likely that the G2445 modification is necessary for prevention of nonfunctional secondary or tertiary structure formation at the peptidyl transferase center [1]; <1> recombinant YcbY protein is able to methylate 23S rRNA purified from the ycbY knock-out strain in vitro, assembled 50S subunits are not a substrate for the methylase [1]; <1> RlmL possessing an additional RNA-binding domain acts on naked ribosomal RNA or early assembly intermediates in the cell [2]) (Reversibility: ?) [1,2]

P S-adenosyl-L-homocysteine + N^2-methylguanine^{2445} in 23S rRNA

4 Enzyme Structure

Subunits

? <1> (<1> x * 79000, SDS-PAGE [1]; <1> x * 78854, calculated from sequence [3]) [1,3]

5 Isolation/Preparation/Mutation/Application

Purification

<1> (recombinant enzyme) [1]

Cloning

<1> (YcbY protein carrying a C-terminal His6 tag is expressed in Escherichia coli) [1]

References

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23S rRNA (guanine^{1835}-N^{2})-methyltransferase 2.1.1.174

1 Nomenclature

EC number
2.1.1.174

Systematic name
S-adenosyl-L-methionine:23S rRNA (guanine^{1835}-N^{2})-methyltransferase

Recommended name
23S rRNA (guanine^{1835}-N^{2})-methyltransferase

Synonyms
RlmG <1> (<1> gene name [1,2]) [1,2]
ribosomal RNA large subunit methyltransferase G <1> [1,2]
ygjO <1> (<1> gene name [1,2]) [1,2]

CAS registry number
50812-26-5

2 Source Organism

<1> *Escherichia coli* (UNIPROT accession number: Q0T0I4) [1,2]

3 Reaction and Specificity

Catalyzed reaction
S-adenosyl-L-methionine + guanine^{1835} in 23S rRNA = S-adenosyl-L-homocysteine + N^{2}-methylguanine^{1835} in 23S rRNA

Natural substrates and products
S S-adenosyl-L-methionine + guanine^{1835} in 23S rRNA <1> (<1> the nucleotide m2G1835 is located in a functionally extremely important region of the ribosome, being located within intersubunit bridges of group B2 [1]) (Reversibility: ?) [1,2]
P S-adenosyl-L-homocysteine + N^{2}-methylguanine^{1835} in 23S rRNA

Substrates and products
S S-adenosyl-L-methionine + guanine^{1835} in 23S rRNA <1> (<1> the nucleotide m^{2}G1835 is located in a functionally extremely important region of the ribosome, being located within intersubunit bridges of group B2 [1]; <1> recombinant YgjO protein is able to methylate in vitro protein-

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free 23S rRNA, but not assembled 50S subunits purified from the ygjO knock-out strain [1]; <1> RlmG possessing an additional RNA-binding domain act on naked ribosomal RNA or early assembly intermediates in the cell [2]) (Reversibility: ?) [1,2]

P S-adenosyl-L-homocysteine + N^2-methylguanine1835 in 23S rRNA

4 Enzyme Structure

Subunits

? <1> (<1> x * 71000, SDS-PAGE [1]) [1]

5 Isolation/Preparation/Mutation/Application

Purification

<1> [1]

Cloning

<1> (recombinant YgjO protein carrying a C-terminal GFP-His6-tag is expressed in Escherichia coli) [1]

References

[1] Sergiev, P.V.; Lesnyak, D.V.; Bogdanov, A.A.; Dontsova, O.A.: Identification of Escherichia coli m2G methyltransferases: II. The ygjO gene encodes a methyltransferase specific for G1835 of the 23 S rRNA. J. Mol. Biol., **364**, 26-31 (2006)

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tricin synthase 2.1.1.175

1 Nomenclature

EC number
2.1.1.175

Systematic name
S-adenosyl-L-methionine:tricetin 3’,5’-O-dimethyltransferase

Recommended name
tricin synthase

Synonyms
HvOMT1 <1> (<1> gene name [2]) [2]
ROMT-15 <4> (<4> gene name [1]) [1]
ROMT-17 <3> (<3> gene name [1]) [1]
ZmOMT1 <2> (<2> gene name [2]) [2]

2 Source Organism

<1> *Hordeum vulgare* [2]
<2> *Zea mays* [2]
<3> *Oryza sativa* [1]
<4> *Oryza sativa* (UNIPROT accession number: Q9XGP7) [1]

3 Reaction and Specificity

Catalyzed reaction
2 S-adenosyl-L-methionine + tricetin = 2 S-adenosyl-L-homocysteine + 3’,5’-O-dimethyltricetin
S-adenosyl-L-methionine + tricetin = S-adenosyl-L-homocysteine + 3’-O-methyltricetin
S-adenosyl-L-methionine + 3’-O-methyltricetin = S-adenosyl-L-homocysteine + 3’,5’-O-dimethyltricetin

Substrates and products
S 2 S-adenosyl-L-methionine + tricetin <1,2,3> (<1> stepwise methylation of tricetin to its 3-mono- and 3,5-dimethyl ethers (major product). The enzyme also catalyzes the 3-O-methylation of luteolin (EC 2.1.1.42) and the methylation of 5-hydroxyferulic acid and quercetin, prefers tricetin over luteolin [2]; <2> the enzyme also catalyzes the 3-O-methylation of

D. Schomburg and I. Schomburg (eds.), *Class 2–3.2 Transferases, Hydrolases: EC 2–3.2*,
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luteolin (EC 2.1.1.42) and the methylation of 5-hydroxyferulic acid and quercetin, prefers tricetin over luteolin [2]; <3> the enzyme catalyzes the stepwise methylation of tricetin to its 3-mono- and 3,5-dimethyl ethers. In contrast with the wheat enzyme, tricetin dimethyl ether is not converted to its 3,4,5-trimethylated ester derivatives. The enzyme also catalyzes the methylation of luteolin, myricetin (formation of the 3,5-dimethyl ether of myricetin, EC 2.1.1.149) and caffeoyl-CoA. ROMT-17 prefers tricetin. ROMT-15 can not utilize naringenin, apigenin, or kaempferol. The 2,3-double bond and the O-dihydroxyl group are both required for catalytic activity [1]) (Reversibility: ?) [1,2]

P 2 S-adenosyl-L-homocysteine + 3',5'-O-dimethyltricetin

S 2 S-adenosyl-L-methionine + tricetin <4> (<4> the enzyme catalyzes the stepwise methylation of tricetin to its 3-mono- and 3,5-dimethyl ethers. In contrast with the wheat enzyme, tricetin dimethyl ether is not converted to its 3,4,5-trimethylated ester derivatives. The enzyme also catalyzes the methylation of luteolin, myricetin (formation of the 3,5-dimethyl ether of myricetin, EC 2.1.1.149) and caffeoyl-CoA. ROMT-15 exhibits similar $Kcat/K_m$ values for the four substrates. ROMT-15 can not utilize naringenin, apigenin, or kaempferol. The 2,3-double bond and the O-dihydroxyl group are both required for catalytic activity [1]) (Reversibility: ?) [1]

P 2 S-adenosyl-L-homocysteine + 3',5'-O-methyltricetin

S S-adenosyl-L-methionine + 3'-O-methyltricetin <1,2,3,4> (<1,2> the enzyme catalyzes the stepwise methylation of tricetin to its 3-mono- and 3,5-dimethyl ethers (major product). In contrast with the wheat enzyme, tricetin dimethyl ether is not converted to its 3,4,5-trimethylated ester derivatives [2]; <3,4> the enzyme catalyzes the stepwise methylation of tricetin to its 3-mono- and 3,5-dimethyl ethers. In contrast with the wheat enzyme, tricetin dimethyl ether is not converted to its 3,4,5-trimethylated ester derivatives [1]) (Reversibility: ?) [1,2]

P S-adenosyl-L-homocysteine + 3',5'-O-dimethyltricetin

S S-adenosyl-L-methionine + tricetin <1,2,3,4> (<1,2> the enzyme catalyzes the stepwise methylation of tricetin to its 3-mono- and 3,5-dimethyl ethers (major product). In contrast with the wheat enzyme, tricetin dimethyl ether is not converted to its 3,4,5-trimethylated ester derivatives [2]; <3,4> the enzyme catalyzes the stepwise methylation of tricetin to its 3-mono- and 3,5-dimethyl ethers. In contrast with the wheat enzyme, tricetin dimethyl ether is not converted to its 3,4,5-trimethylated ester derivatives [1]) (Reversibility: ?) [1,2]

P S-adenosyl-L-homocysteine + 3'-O-methyltricetin

Cofactors/prosthetic groups

S-adenosyl-L-methionine <3,4> [1]

Metals, ions

Co^{2+} <3,4> (<3> the enzyme is metal-dependent, Mg^{2+} is the best cation for catalytic activity, Co^{2+} shows 41% of the activity with Mg^{2+} (with quercetin as substrate) [1]; <4> the enzyme is metal-dependent, Mg^{2+} is the best cation

for catalytic activity, Co^{2+} shows 82% of the activity with Mg^{2+} (with quercetin as substrate) [1]) [1]
Mg^{2+} <3,4> (<3,4> the enzyme is metal-dependent, Mg^{2+} is the best cation for catalytic activity (with quercetin as substrate) [1]) [1]
Mn^{2+} <3,4> (<3> the enzyme is metal-dependent, Mg^{2+} is the best cation for catalytic activity, Mn^{2+} shows 69% of the activity with Mg^{2+} (with quercetin as substrate) [1]; <4> the enzyme is metal-dependent, Mg^{2+} is the best cation for catalytic activity, Mn^{2+} shows 74% of the activity with Mg^{2+} (with quercetin as substrate) [1]) [1]
Additional information <1,2> (<1,2> requires no Mg^{2+} [2]) [2]

K_m-Value (mM)
0.00248 <1> (tricetin, <1> 30°C [2]) [2]
0.036 <3> (tricetin, <3> pH 7.5, 37°C [1]) [1]
0.0417 <2> (tricetin, <2> 37°C [2]) [2]
0.072 <4> (tricetin, <4> pH 7.5, 37°C [1]) [1]

pH-Optimum
7.5 <1,2,3,4> (<3,4> assay at [1]) [1,2]

pi-Value
5.48 <2> (<2> calculated from sequence [2]) [2]
5.64 <1> (<1> calculated from sequence [2]) [2]

Temperature optimum (°C)
30 <1,2> (<1,2> assay at [2]) [2]
37 <3,4> (<3,4> assay at [1]) [1]

4 Enzyme Structure

Subunits
? <1,2,3,4> (<3> x * 30600, SDS-PAGE, glutathione S-transferase fusion protein [1]; <4> x * 31100, SDS-PAGE, glutathione S-transferase fusion protein [1]; <1> x * 38700, calculated from sequence [2]; <2> x * 39600, calculated from sequence [2]) [1,2]

5 Isolation/Preparation/Mutation/Application

Source/tissue
root <4> [1]
stem <3,4> (<3> expressed in stems only [1]) [1]

Purification
<1> [2]
<2> [2]