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S-adenosylmethionine and radical-based catalysis

Review Article

M. A. Grillo and **S. Colombatto**

Dipartimento di Medicina e Oncologia Sperimentale, Sezione di Biochimica, Università di Torino, Torino, Italy

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Summary. S-adenosylmthionine is the major methyl donor in all living organisms, but it is also involved in many other reactions occurring through radical-based catalysis. The structure and function of some of these enzymes, including those involved in the synthesis of the molybdenum cofactors, biotin, lipoate, will be discussed.

Keywords: Molybdenum cofactors – Biotin – Lipoate

Introduction

S-adenosylmethionine (SAM) is the major methyl donor in all living organisms, and is also involved in the formation of the 5′-deoxyadenosyl radicals that start radical catalysis. A large superfamily composed of ''radical SAM proteins'' has now been discovered. They catalyze a variety of reactions, including methylation, isomerization, sulfur insertion, ring formation, anaerobic oxidation and protein radical formation, and are involved in the synthesis of DNA precursors, vitamins, cofactors, antibiotics, etc. (Sofia et al., 2001). The role of SAM, however, is not always the same. In some enzymes it acts as a cofactor, i.e. it is restored and reused, in others it is a co-substrate, i.e. the radical is used to oxidize the substrate and therefore consumed. Iron–sulfur clusters function as a source of the electrons necessary for the reduction of SAM and the formation of radicals. Enzymes are characterized both by the use of SAM for radical generation and by a highly conserved motif CX_3CX_2C (C, cysteine, X, any amino acid) that coordinates an FeS cluster (Fontecave et al., 2001; Jarrett, 2003).

Their structure and the mechanism of action, however, are different. Some enzymes are composed of a two-protein system, i.e. an FeS protein, which is the activating protein catalyzing the reductive cleavage of SAM into methionine and the radical, which can then generate a glycyl radical on the catalytic protein and so create a thiyl radical; others are a single-protein system combining activating and catalytic activities by direct substrate radical formation.

However, due to the dissimilar modes of SAM-binding in individual enzymes, SAM plays a slightly different role in each reaction. The sequence analysis performed by Sofia et al. (2001) has been used to identify and characterize some enzymes that have attracted particular attention. These include those involved in molybdenum cofactor synthesis, and in thiolation and methylation of tRNA, biotin synthase, lipoate synthase, coproporphyrinogen III oxidase and anaerobic ribonucleotide reductase. The structure and function of these enzymes will be discussed.

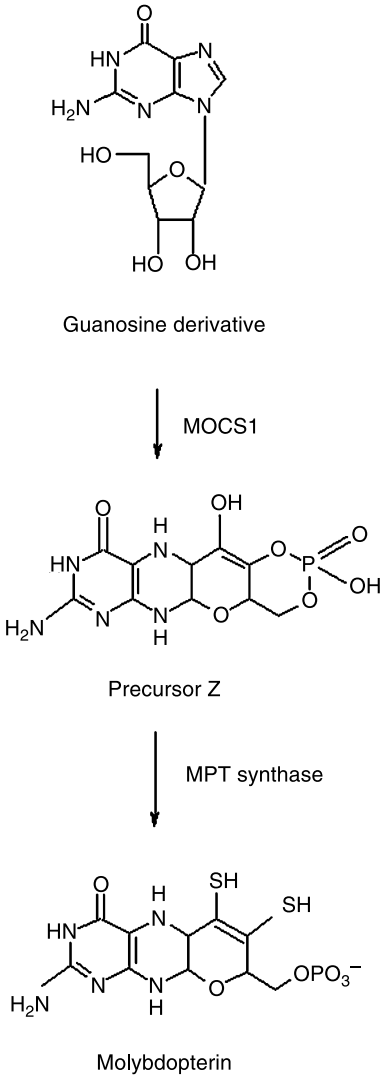
Ring formation

Synthesis of precursor Z of molybdenum cofactors

All molybdenum cofactors (Moco), with the exception of nitrogenase, consist of a mononuclear molybdenum coordinated by the dithiolene moiety of one or two of a family of tricyclic pyranopterins, the simplest of which is called molybdopterin (MPT). In humans, defects in Moco synthesis lead to loss of activity of sulfite oxidase, aldehyde oxidase and xanthine dehydrogenase (Reiss, 2000) and serious neurological disorders.

Moco biosynthesis in humans occurs in 3 major steps. In step 1, *mocs1* (molybdenum cofactor syntheses-step 1) gives rise to two enzymes (MOCS1A and MOCS1B)

within a bicistronic transcript with two consecutive ORFs (Reiss et al., 1998). These two enzymes catalyze the synthesis of precursor Z, an oxygen-sensitive 6-alkyl pterin with a cyclic phosphate, from a guanosine derivative, GTP; in step 2, precursor Z is converted into MPT by MPT synthase formed of two subunits (MOCS2A and MOCS2B) encoded by a single gene comprising two ORFs, where MOCS2A is apparently thiocarboxylated to catalyze the transfer of sulfur to precursor Z and give rise to MPT dithiolene (Pitterle and Rajagopalan, 1993), though the in vivo sulfur source remains to be elucidated (Matthies et al., 2004); in step 3, molybdenum is incorporated into MPT by the two-domain protein gephyrin (Stallmeyer et al., 1999). Moco deficiency may be due to defective *mocs1*, *mocs2* or *gephyrin* genes (Reiss et al., 2001) (see Scheme 1).



Scheme 1

MOCS1A belongs to the superfamily of ''radical SAM proteins''. It contains two highly conserved cysteine motifs thought to be involved in iron–sulfur cluster binding, one located near the N-terminus (consensus sequence CX_3CX_2C) and one near the C terminus (consensus sequence $CX_2CX_{13}C$). SAM serves as the free radical initiator and undergoes cleavage to methionine and a 5′-deoxyadenosyl radical that in turn propagates radical formation by abstracting hydrogen atoms from substrate molecules or from glycyl residues of enzymes to activate them for radical-based biochemistry (Jarrett, 2003; Frey and Magnusson, 2003). The source of the electron required for the cleavage of SAM is a reduced form of an FS cluster.

All six cysteines are necessary for activity. The anaerobically purified MOCS1A is a monomeric protein containing two FeS clusters, each coordinated by three cysteine residues. A redox-active $[4Fe–4S]^{2+}$ cluster is ligated to the N-terminal CX_3CX_2C motif, as in the case of all other SAM radical enzymes. The C terminal $CX_2CX_{13}C$ motif, unique to MOCS1A, ligates a $[3Fe–4S]^{\circ}$ cluster. However, it can be reconstituted in vitro to yield a form containing two $[4Fe–4S]^{2+}$ clusters (Hänzelmann et al., 2004).

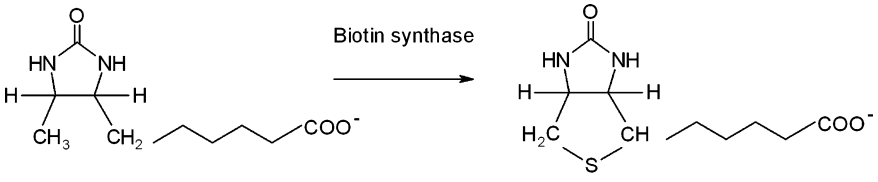
The catalytic activity of MOCS1A requires an accessible C-terminal, double-glycine motif that may be necessary for interaction with MOCS1B, or may be involved in a radical-based reaction catalyzed by the putative radical SAM protein MOCS1A. The exact function of MOCS1B is not known. It is suggested (Hänzelmann et al., 2002) that it serves as a scaffold for the formation of precursor Z by facilitating the rearrangement reaction catalyzed by MOCS1A, or that it is the protein that delivers oxygen-sensitive precursor Z to MPT synthase for the formation of MPT.

The reason why MOCS1A requires two $[4Fe–4S]^{2+,+}$ clusters has not been discovered. According to Hänzelmann et al. (2004), the C-terminal cluster either facilitates catalysis by binding and activating the substrate, or is involved in the reductive cleavage of SAM. The authors believe it is unlikely that this cluster functions by providing one sulfur atom to form the dithiolene group of molybdopterin, as suggested previously for molybdopterin synthesis by MoaD in *E. coli* (Pitterle et al., 1993).

Sulfur insertion

Biotin synthase

Biotin synthase catalyzes the final step of biotin synthesis, i.e. insertion of a sulfur atom into dethiobiotin (see Scheme 2).



Scheme 2

The enzyme most studied is that obtained from *E. coli*. It is a homodimer comprising 39 kD subunits; when prepared, each subunit contains a $[2Fe–2S]^{2+}$ cluster. However, when the enzyme is reduced, the clusters dimerize and form $[4Fe–4S]^{2+}$ clusters (Duin et al., 1997). According to Ugulava et al. (2003), a $[4Fe–4S]^{2+}$ cluster and a $[2Fe–2S]^{2+}$ cluster are needed for the activity. The reaction starts with the transfer of an electron from flavodoxin to the first cluster to promote the reduction cleavage of SAM to methionine and 5′-deoxyadenosyl radical. This promotes the abstraction of hydrogen from dethiobiotin. The second cluster seems to serve as the sulfur source for the formation of biotin (Tse Sum Bui et al., 2003).

According to Begley et al. (1999), two SAM molecules are required for the production of one biotin, whereas Ollagnier et al. (2002a) maintain that only one is required and that the discrepancy is due to the fact that the 5′-deoxyadenosine produced by the reaction is a strong inhibitor and slows it down.

The crystal structure of the enzyme has recently been determined (Berkovitch et al., 2004). A unique observation is that the ligands for the [2Fe–2S] cluster are three cysteines and one arginine. The unusual presence of arginine suggests that this residue modulates the properties of the cluster or facilitates catalysis. One possibility is that, when S is transferred into biotin, arginine rearranges to bridge the two Fe atoms and facilitate the S transfer. Berkovitch and coworkers also found that the 5′-deoxyadenosyl radical accomplishes the second hydrogen bond abstraction, so that only one SAM would be needed for one biotin.

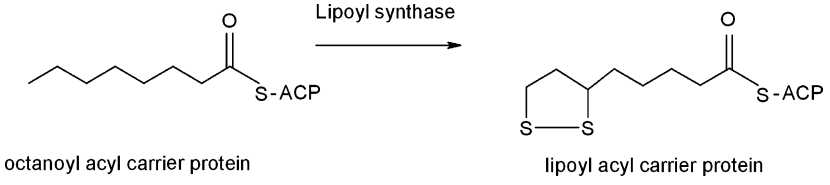
The origin of the sulphur has also been a matter of controversy. Cysteine has long been considered the most likely source (DeMoll and Shive, 1983), though the mechanism involved is not known. According to Ollagnier et al. (2002b), biotin synthase itself has cysteine desulfurase activity dependent on pyridoxal phosphate (PLP). This allows mobilization of the sulfur atom from free cysteine. Two conserved residues of cysteine, Cys^{97} and Cys^{128}, are critical for desulfuration and have been proposed as sites for a persulfide.

According to Tse Sum Bui et al. (2004), however, PLP is not involved in the reaction, and the enzyme does not possess desulfurase activity. Moreover, experiments by Jameson et al. (2004) induced the authors to suggest that the [2Fe–2S] cluster generates a protein-bound polysulfide or persulfide that acts as a immediate donor for biotin production. Once again, however, the conditions of the study were different. Tse Sum Bui and coworkers thus continue to maintain that the $[2Fe–2S]^{2+}$ cluster is the ultimate sulfur donor.

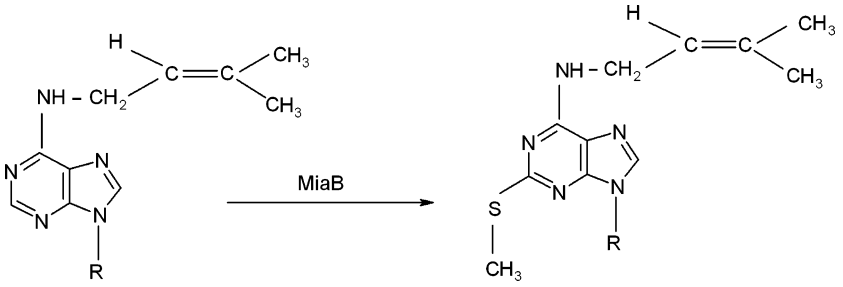
Lipoyl synthase

Lipoyl synthase, another enzyme that catalyzes the insertion of sulfur atom(s), has been mainly studied in *E. coli*, where two genes, *lipA* and *lipB*, are both necessary. The role of LipB has not been established, whereas LipA is regarded as the protein necessary for the insertion of a sulfur atom in the octanoic acid backbone (Reed and Cronan, 1993) and has since been shown to be one of the radical SAM proteins (Miller et al., 2000). The reaction occurs by insertion of sulfur atoms on octanoyl-residues bound to the acyl carrier protein (ACP). The lipoyl-ACP thus formed is then used to donate lipoic acid to the subunits of the pyruvate dehydrogenase complex (PDC), namely the α-ketodehydrogenase complex and the glycine cleavage enzyme (Jordan and Cronan, 1997) (see Scheme 3).

This synthesis is now known to occur in mitochondria of mammalian cells as well (Morikawa et al., 2001), while its presence in plant cell mitochondria had suggested that the function of the fatty acid synthesized in mitochondria was the synthesis of lipoic acid (Wada et al., 1997).



Scheme 3



Scheme 4

As to the mechanism of the reaction, it has been suggested that SAM and octanoyl-ACP bind the protein (probably reduced by flavodoxin and flavodoxin reductase). The $[4Fe–4S]^{1+}$ cluster cleaves SAM. The radical formed may abstract a hydrogen atom from the alkyl chain of octanoyl-ACP and generate an alkyl radical that may be supposed to recombine with a sulfide cluster to form a carbon–sulfur bond. Repetition of the process could generate a lipoyl-ACP-iron–sulfur cluster. LipB would then remove the lipoyl moiety and transfer it to an unlipoylated apoprotein (e.g., apo-PDC).

According to Cicchillo et al. (2004) lipoyl synthase contains two FeS clusters. By contrast with biotin synthase, they are both [4Fe–4S] clusters. Even in this case, the second cluster residing in a CX_4CX_5C motif would supply the sulfur atoms incorporated in the substrate. Moreover, these authors suggest that formation of one lipoyl group is catalyzed by two molecules of the enzyme, in other words, each enzyme molecule provides one sulfur atom. The reaction by LipA also occurs on octanoyl moieties bound to a pyruvate dehydrogenase subunit (E2) and forms lipoyl-E2 (Zhao et al., 2003).

The exact identity of the sulfur donor is not known. To our knowledge, the demonstration that cysteine is the precursor of lipoic acid in mammals (Dupre et al., 1983) has not been followed by the publication of other molecular details of the reaction involved.

Since SAM is synthesised in the cytoplasm, it must be transported into the mitochondria. This can be done by Saccharomyces cerevisiae (Marobbio et al., 2003), where biotin synthesis also occurs in mitochondria. A human mitochondrial SAM carrier has now been demonstrated (Agrimi et al., 2004), though it appears to be involved in exchange of SAM with SAO, not with 5-deoxyadenosine, and thus has a different role.

MiaB

Another enzyme is involved in the insertion of sulfur to form 2-methylthio-N^6-isopentenyl-adenosine (ms^2i^6A), one of the thiolated nucleosides occurring in almost all eukaryotic and bacterial tRNAs. The reaction has been mainly studied in microorganisms, particularly *E. coli*. After introduction of the isopentenyl group into the N^6 nitrogen of adenosine, by catalysis of a transferase encoded by a *miaA* gene both sulfur and a methyl are introduced at position 2 of the base. This is promoted by a MiaB protein, the product of *miaB* gene (Pierrel et al., 2002). The enzyme purified by another bacterium, Thermotoga maritima, is a monomer of 443 residues and molecular mass 50,710. It contains the sequence CX_3CX_2C and a $[4Fe–4S]^{+2,+1}$ cluster (Pierrel et al., 2003) (see Scheme 4).

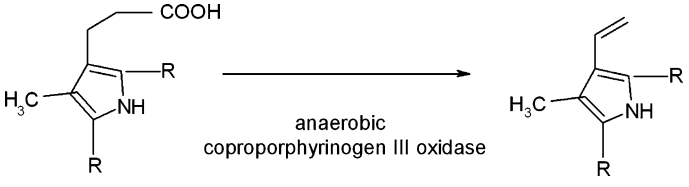
Surprisingly, however, MiaB is bifunctional and catalyzes both sulfur insertion and methylation. Two SAM molecules are therefore required (Pierrel et al., 2004).

The origin of sulphur remains to be established. According to Pierrel et al. (2003) it would seem more probable that it derives from persulfide, as a second FeS cluster does not seem to be present. However, the other possibility is not completely ruled out.

Anaerobic oxidation

Coproporphyrinogen III oxidase

For the biosynthesis of the tetrapyrrole ring of hemes, the oxidative decarboxylation of coproporphyrinogen-III is



Scheme 5

required. This is catalyzed by coproporphyrinogen III oxidase. In this reaction, two propionate side chains are converted to the corresponding vinyl group under either aerobic or anaerobic conditions, which means that two enzymes are involved, one for the oxygen-dependent and one for the oxygen-independent reaction (see Scheme 5).

Oxygen-independent conversion occurs in several microorganisms. The enzyme involved, coproporphyrinogen III oxidase (HemN), is one of the radical SAM proteins. Purified from *E. coli*, it is a monomeric protein of 52 kD (Layer et al., 2002). Formation of the 5′-deoxyadenosyl radical through the action of the FeS cluster has been postulated. This abstracts a hydrogen atom (the pro-S-hydrogen) from the β-C atom of the propionate side-chain of the substrate and generates the corresponding substrate radical. During the final step, the vinyl group of protoporphyrinogen-IX is formed and CO_2 is released. This step requires an acceptor of an electron for the remaining electron of the substrate. However, the physiological acceptor has not yet been identified. Layer et al. (2003) have determined the crystal structure of the enzyme. Its catalytic domain is unique and unrelated to that of all the methyltransferases and the other known 4Fe–4S binding domains. Moreover, it has been shown that HemN binds two SAM. It is thought that the first electron transferred from the FeS cluster to (S) SAM1 is passed to SAM2, perhaps after conversion of configuration to (R)-SAM1 This would induce radical formation in SAM2 and decarboxylation in one propionate side-chain. Reduction of the cluster and a second electron transfer to SAM1 would induce radical formation in SAM1, possibly relayed to the second propionate side chain causing the second decarboxylation. In this way, each SAM may catalyze the oxidative decarboxylation of one propionate side-chain. Layer and coworkers have therefore suggested that inhibitors with antibacterial function due to the unique bacterial occurrence of the enzyme could be developed.

Anaerobic ribonucleotide reductase

Ribonucleotide reductases are necessary to reduce ribonucleotides to deoxyribonucleotides for the synthesis of DNA in all organisms. Three classes have been identified. Class III is found in anaerobically growing microorganisms. The *E. coli* enzyme catalyzes the reduction of the four common ribonucleotides. Reduction is stimulated by an appropriate modulator (dGTP for ATP reduction, ATP for CTP and UTP reduction, dTTP for GTP reduction). In this way, a single enzyme provides a balanced supply of the four deoxyribonucleotides required (Eliasson et al., 1994). The enzyme is a dimer, α_2, and contains the active site. However, as isolated it is not active. It is activated with a reducing system and protein β. For the reaction to occur, therefore, a system formed of NADPH, flavodoxin oxidoreductase and flavodoxin reduces SAM to methionine and S-deoxyadenosyl radical; this radical reacts with a glycine residue to generate a glycyl radical on protein α. This is formed by effect of protein β or ''activase'', which contains an oxygen-sensitive $[4Fe–4S]^{2+/1+}$ centre catalyzing electron transfer from flavodoxin to SAM in reducing conditions. It has since been shown that the thioredoxin system efficiently replaces other reducing agents (Padovani et al., 2001). The 4Fe–4S cluster has three cysteine ligands. The fourth has not been identified (Tamarit et al., 2000). Four cysteines (662, 665, 644, 647) in protein α participate in the formation of the glycyl radical located at the Gly^{681} residue of the dimeric protein α (Sun et al., 1996) necessary for the activity. They provide a metal-binding site, probably with a structural function (Logan et al., 2003).

An enzyme obtained from *Lactococcus lactis* resembles the *E. coli* enzyme in many respects and has much the same allosteric regulation (Torrents et al., 2000).

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Authors' address: M. A. Grillo, Dipartimento di Medicina e Oncologia Sperimentale, Sezione di Biochimica, Università di Torino, Torino, Italy, Fax: +39-011-6705311, E-mail: mariaangelica.grillo@unito.it