New Light on Methylthiolation Reactions

M. Fontecave,[1,*] E. Mulliez,[1] and M. Atta[1]
[1]Laboratoire de Chimie et Biologie des Métaux, UMR 5249, Université Joseph Fourier, CEA, CNRS, 17 Avenue des Martyrs, 38054 Grenoble Cedex 9, France
*Correspondence: M.Fontecave.mfontecave@cea.fr
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A novel enzyme, named RimO for *ri*bosomal *mo*dification (Anton et al., 2008) catalyzes the methylthiolation of aspartate 88 of the S12 ribosomal protein in *Escherichia coli* and shows a strong similarity with the iron-sulfur enzyme MiaB involved in the methylthiolation of tRNAs.

One of the most challenging problems in chemical biology concerns the biosynthesis of sulfur-containing biomolecules. Whereas a great variety of such essential compounds (biotin, lipoic acid, thiamine pyrophosphate, thio- and methylthionucleosides, to name a few) is present in all living organisms, the current understanding of the mechanisms of sulfur insertion into substrates is quite limited (Fontecave et al., 2003). This issue obviously requires the identification, purification, and characterization of the enzymes responsible for these reactions. Surprisingly, in many cases this has not been achieved yet.

Biotin synthase (BioB), lipoate synthase (LipA), and the methylthiotransferase (MTTase) MiaB, which catalyzes the introduction of a methylthio group at position 2 of N^6-isopentenyladenosine in tRNAs, constitute an important class of sulfur-inserting enzymes. Recent studies of the structure and reactivity of these fascinating systems have provided new insights into the understanding of the mechanisms of C-H to C-S bond conversion reactions (Fontecave et al., 2003; Booker et al., 2007). In particular, it is now well established that substrate activation, as a prerequisite for S insertion, involves hydrogen atom abstraction. This is achieved through the action of a very reactive 5′-deoxyadenosyl radical which derives from S-adenosylmethionine (SAM; Figure 1). Indeed, the three proteins belong to the large family of iron-sulfur enzymes called ''Radical-SAM,'' characterized by the presence of a [4Fe-4S] cluster chelated by three cysteines of a conserved CysXXXCysXXCys sequence and by the ability of this cluster to bind and reductively cleave SAM into the 5′-deoxyadenosyl radical (Sofia et al., 2001; Frey and Magnusson, 2003). Furthermore, the discovery that these enzymes have in common the presence of a second conserved iron-sulfur cluster (either a [2Fe-2S] or a [4Fe-4S] cluster) has led to the novel and intriguing hypothesis that the latter is the ultimate sulfur atom donor (Booker et al., 2007; Hernandez et al., 2007). On the other hand, a number of very important chemical issues remain unclear. In particular it is still unknown how the sulfur atoms are continuously provided to the enzymes for multiple turnovers and from which source, thus explaining why no one has succeeded in getting a catalytically functional system in vitro yet.

The paper by Anton et al. (2008) provides a new exciting tool to study this reaction. Furthermore it shows that the same radical chemistry is used by living organisms to specifically introduce a sulfur atom into a small molecule (BioB, LipA), a tRNA (MiaB), or a protein (RimO). The presence of a methylthio group at the β-carbon of the universally conserved residue D88 of the ribosomal protein S12 in *Escherichia coli* was already established and this modification was suggested to serve an essential function (Kowalak and Walsh, 1996). However, the enzyme responsible for this modification was unknown. Reasoning that such a methylthiolation should proceed by a similar mechanism to the MiaB reaction, the authors looked for a protein with sequence similarity to MiaB in the *E. coli* genome. This led to the unambiguous identification of the product of the *yliG*

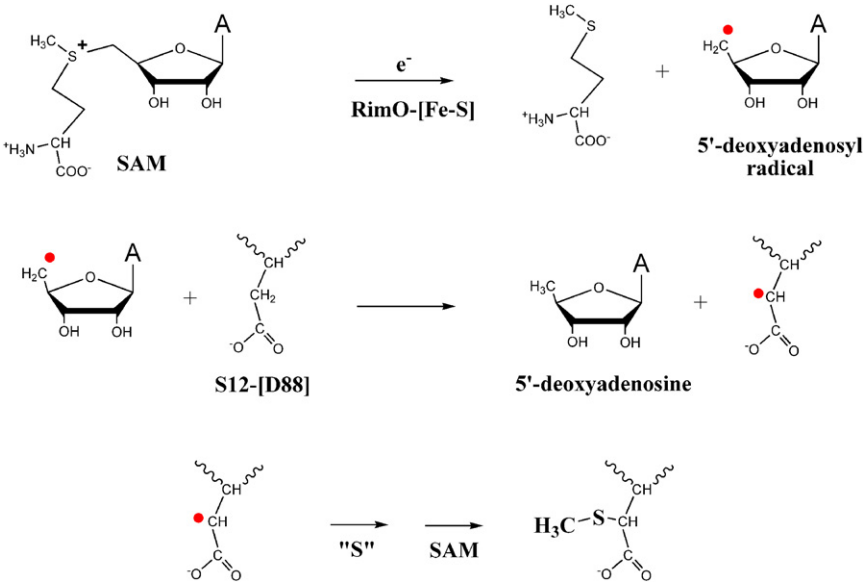

Figure 1. Postulated Reaction Mechanism Leading to the Posttranslational Modification of Ribosomal Protein S12 in *E. coli*, Methylthiolation of D88
The enzyme involved in this reaction is RimO. SAM, S-adenosylmethionine serves both as a source of radical in the initial part of the reaction and as a methylating agent; S, sulfur atom donor; A, adenine.

gene as the enzyme responsible for S12 specific modification. In *E. coli* only two methylthiolation reactions are known so far. Thus YliG (now RimO) and MiaB are the two MTTases of this microorganism. However, examination of all completely sequenced genomes available reveal the presence of MTTases in other organisms (bacteria, archaea, and eukaryotes) probably involved in the biosynthesis of the three other methythiolated nucleosides known so far, 2-methylthio-N^6-threonylcarbamoyladenosine, 2-methylthio-N^6-hydroxynorvalyl-carbamoyladenosine, and 2-methylthio-N^6-methyladenosine. These enzymes remain to be characterized. The very high sequence homology between MiaB and RimO, in particular the presence of the CysXXXCysXXCys motif characteristic for "Radical-SAM" enzymes and of three additional conserved cysteines, strongly suggests that the latter binds two iron-sulfur clusters and that the reaction proceeds via H atom abstraction at the β-carbon of D88 of S12 by a 5′-deoxyadenosyl radical derived from SAM followed by S insertion (Figure 1). However, this remains to be confirmed in future biochemical studies of RimO.

One of the most intriguing aspects of this study is that the similarity of RimO and MiaB also extends to the C-terminal part of the proteins. This portion in MiaB is a TRAM domain which is predicted to be involved in RNA binding, in agreement with tRNA being the substrate of MiaB (Anantharaman et al., 2001). This has been recently confirmed by structural studies of the 23S rRNA methylase RumA (Lee et al., 2005). That such a domain has been largely conserved in RimO is surprising considering that the substrate is a protein and not a tRNA. A very interesting working hypothesis, awaiting experimental confirmation, is that a S12-rRNA complex, rather than free S12, is the substrate of RimO and that the C terminus of RimO recognizes a RNA stem-loop in contact with S12. The MiaB-RimO similarity provides an illustration of Nature's thriftiness, when same molecules are used for either different functions or for similar functions applied to very different substrates. Future structural and functional characterization of MiaB-RimO couple should provide new insights into how substrate specificity can be tuned to allow either a protein or a tRNA modification.

The reaction catalyzed by RimO and MiaB involves the methylation of the sulfur atom introduced in the initial step (Figure 1). It is likely that the overall reaction is sequential, with methylation following sulfuration, but this has not been firmly demonstrated. In the case of methylthiolation of N^6-isopentenyladenosine it has been clearly established that both the sulfuration and the methylation are catalyzed by the same enzyme, MiaB, and that SAM is the methylating agent, as shown by labeling experiments using ^{3}H-*methyl*-SAM (Pierrel et al., 2004). This means that MiaB binds two molecules of SAM, one chelating the iron-sulfur cluster for radical initiation and the second as the methyl donor. It is very likely that the same occurs in the case of RimO and further studies are required to better structurally and functionally characterize this unique protein-SAM interaction. RimO and MiaB seem also to be unique catalysts for methylation since they lack any of the widely conserved motifs present in SAM-dependent methyltransferases and specifically involved in SAM binding. Thus, these proteins may provide a novel class of SAM-dependent methyltransferases and illuminate new aspects of methylation reactions.

Obviously the paper by B.P. Anton et al. and the discovery of RimO, the enzyme responsible for protein S12 modification, are opening new fascinating questions on the mechanisms of both sulfuration and methylation reactions and on the chemical strategies used by living organisms for an economical exploitation of enzymes in order to achieve the same chemical modification on very different substrates.

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