Surprise! A hidden B_{12} cofactor catalyzes a radical methylation

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Radical *S*-adenosylmethionine (SAM) (RS) methylases perform methylation reactions at unactivated carbon and phosphorus atoms. RS enzymes typically abstract a hydrogen from their substrates, generating a substrate-centered radical; class B RS methylases catalyze methyl transfer from SAM to cobalamin and then to a substrate-centered carbon or phosphorus radical. Radle *et al.* now show that Mmp10, an RS enzyme implicated in the methylation of Arg-285 in methyl coenzyme M reductase, binds a methylcobalamin cofactor required for methyl transfer from SAM to a peptide substrate. However, Mmp10 has little sequence homology to known methylases, suggesting this enzyme belongs to a new subclass of B_{12}-dependent RS methylases.

Biochemical methylation reactions are seemingly some of the most straightforward and simple of all enzyme-catalyzed reactions (1). *S*-Adenosyl-L-methionine (SAM)[2] is the methyl-donating substrate for most methyltransferase enzymes, with a methyl group that is appended to an electron-withdrawing sulfonium center that makes the methyl group reactive toward strong nucleophiles. Traditional methyltransferases use general base catalysis to generate reactive $R\text{-}O^-$, $R\text{-}NH_2$, and $R\text{-}S^-$ nucleophiles that readily attack the methyl group of SAM in a prototypical S_N2 nucleophilic substitution reaction, producing the methylated product and *S*-adenosyl-L-homocysteine. Methyltransferases can also methylate carbon atoms in aromatic heterocycles, such as nucleotides, imidazoles, or indoles, that can be rendered nucleophilic by intramolecular tautomerization or by nucleophilic catalysis.

Methyl groups are also found at unactivated carbons as post-translational protein modifications, post-transcriptional ribonucleotide modifications, and decorations on many natural products (2, 3) at positions that could not be deprotonated by a typical protein-derived base. Examples of *C*-methylated molecules include the following: polytheonamide, a ribosomal peptide-derived natural product in which methylation of several valine residues generates *t*-butyl side chains (4); nosiheptide, a nonribosomal peptide natural product whose biosynthesis incorporates carboxymethylation of an aromatic ring (5); and methyl coenzyme M reductase (MMR), a post-translationally–modified enzyme in which methanogenesis marker protein 10 (Mmp10) catalyzes the methylation of Arg-285 at C5 adjacent to the guanidino functional group (6). Methylation reactions at unactivated carbons are carried out by enzymes from the radical SAM (RS) superfamily.

The RS superfamily includes over 113,000 largely uncharacterized enzymes with a semiconserved core domain that binds SAM and a catalytic iron–sulfur cluster. The RS superfamily has evolved to catalyze diverse reactions through the addition of N- and C-terminal domains that bind substrates and/or additional cofactors that augment the basic RS chemistry (7). As now reported by Radle *et al.* (8), Mmp10 is an RS enzyme that incorporates a C-terminal domain annotated as DUF512 (domain of unknown function). Radle *et al.* (8) demonstrate that Mmp10 catalyzes the SAM- and B_{12}-dependendent methylation of arginine in a peptide substrate and suggest that DUF512 may be a novel B_{12}-binding domain.

Approximately 10% of enzymes in the RS superfamily are assigned to one of four methylase classes (7). The shared chemistry catalyzed by all RS enzymes involves a catalytic [4Fe-4S] cluster that binds SAM and catalyzes a one-electron reductive cleavage of one of the sulfonium carbon–sulfur bonds. This reaction generates a high-energy 5′-deoxyadenosyl carbon radical (5′-dA•) that typically activates the substrate by abstracting a hydrogen atom from a C–H bond, generating 5′-deoxyadenosine (5′-dAH) and a substrate radical. Class A, B, and C methylases all utilize two SAM equivalents per enzyme turnover: 1 eq donates a methyl or methylene group, and the other generates 5′-dA• to activate the substrate or the methyl group (2). Class D enzymes are proposed to use 5,10-methylenetetrahydrofolate (or similar pterin analogs) as a methylene group donor (2).

Class A, B, and C methylases differ in how they handle the SAM methyl group and use 5′-dA•. Class C methylases are proposed to use 5′-dA• to abstract a hydrogen from the methyl group on a second SAM molecule, generating a SAM-bound methylene radical that attacks a π bond in the substrate; electron and proton transfers generate a product with a methylene group in a cyclopropane ring or a methyl group containing one solvent-derived hydrogen. Class A methylases appear to be a more sophisticated elaboration of this mechanism: the SAM methyl group is first transferred to a cysteine residue on the enzyme, followed by hydrogen atom abstraction from the methyl group, generating a cysteine-bound methylene radical; electron and proton transfers generate a methylated product in

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[2] The abbreviations used are: SAM, *S*-adenosyl-L-methionine; MMR, methyl coenzyme M reductase.

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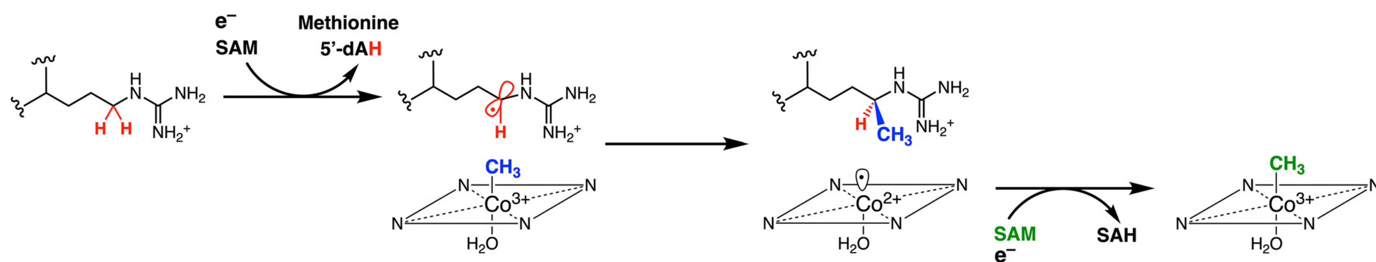

Figure 1. Proposed mechanism for the B_{12}-dependent methylase Mmp10 (Ref. 8). Reductive cleavage of the SAM sulfonium generates a 5′-dA• that abstracts a hydrogen atom from C5 of Arg-285 in MMR. Methylcobalamin transfers a methyl group to the arginine C5 radical via an S_R2 radical substitution reaction, leaving behind cob(II)alamin with an unpaired electron. Cob(II)alamin is reduced to cob(I)alamin, and a methyl group is transferred from a second SAM equivalent via an S_N2 nucleophilic substitution reaction, regenerating the active methylcobalamin enzyme.

which the methyl group contains one hydrogen derived from the substrate. Because class A and C methylases use 5′-dA• to activate the methyl group and not the substrate, they are limited to methylation of substrates with π electrons in double bonds or aromatic rings.

Class B methylases appear to employ a completely different mechanism involving a cobalamin (B_{12}) cofactor. In these enzymes, 5′-dA• activates the substrate by abstracting a hydrogen from an aliphatic C–H bond (Fig. 1). The substrate radical then attacks methylcobalamin in an S_R2 radical substitution reaction, generating the methylated product and cob(II)alamin. Reactivation of the enzyme requires a strong reductant that generates cob(I)alamin, a supernucleophile that readily reacts with a second SAM equivalent to regenerate methylcobalamin. All characterized class B methylases contain an N-terminal domain with weak sequence homology to methylcorrinoid proteins from methanogenic archaea that likely contains the cobalamin-binding site.

Radle *et al.* (8) observed that Mmp10, heterologously expressed in *Escherichia coli* and purified under anaerobic conditions, exhibits a broad UV-visible absorbance expected for the catalytic $[4Fe\text{-}4S]^{2+}$ cluster found in RS enzymes, but they found that the enzyme has very low activity. Suspecting that cobalamin could play an important role in the methylation reaction, the authors noted the presence of a trace mixture of tightly-bound cobalamins whose presence was hidden by the iron–sulfur cluster, and they show that reconstitution of the Mmp10 with either methylcobalamin or hydroxocobalamin dramatically increases activity.

The authors then used d_3-methyl-SAM to explore the methylation sequence. First, they reconstituted the enzyme with hydroxocobalamin, followed by incubation with d_3-SAM and a strong reductant in the absence of substrate, and observed formation of bound d_3-methylcobalamin. Next, they reconstituted the enzyme with unlabeled methylcobalamin and incubated with d_3-SAM together with a peptide substrate and a strong reductant. These conditions promoted rapid formation of a peptide product containing a methylcobalamin-derived unlabeled methyl group, followed by multiple equivalents of product containing an intact d_3-methyl group, derived from d_3-SAM presumably via a d_3-methylcobalamin intermediate. Although these data are consistent with a class B RS methylase mechanism (Fig. 1), the low sequence homology with known class B methylases led the authors to conclude that Mmp10 represents the first example of a new subclass of class B RS methylases (8).

Bioinformatics has become a staple of the enzymologist's toolkit. Following a relatively straightforward BLAST analysis, gene annotations provide a repetitive starting point for deciding which experimental approaches might be most useful. Particularly in studying natural product biosynthetic pathways, it is sometimes tempting to take a shortcut and use the gene annotation as a definitive proclamation of its function and likely enzymatic mechanism. The study by Radle *et al.* (8) is a strong reminder that these annotations depend on a few well-characterized homologs and that the presence of an additional domain may result in altered substrate specificity, binding of an unexpected cofactor (as in Mmp10), or perhaps catalysis of a completely new reaction. This is particularly true for the RS superfamily, where many thousands of yet-to-be discovered enzymes have functions and catalyze chemistry that we cannot even begin to imagine today.

References

1. Boriack-Sjodin, P. A., and Swinger, K. K. (2016) Protein methyltransferases: a distinct, diverse, and dynamic family of enzymes. *Biochemistry* **55,** 1557–1569 CrossRef Medline
2. Bauerle, M. R., Schwalm, E. L., and Booker, S. J. (2015) Mechanistic diversity of radical *S*-adenosylmethionine (SAM)-dependent methylation. *J. Biol. Chem.* **290,** 3995–4002 CrossRef Medline
3. Zhang, Q., van der Donk, W. A., and Liu, W. (2012) Radical-mediated enzymatic methylation: a tale of two SAMs. *Acc. Chem. Res.* **45,** 555–564 CrossRef Medline
4. Parent, A., Guillot, A., Benjdia, A., Chartier, G., Leprince, J., and Berteau, O. (2016) The B_{12}-radical SAM enzyme PoyC catalyzes valine Cβ-methylation during polytheonamide biosynthesis. *J. Am. Chem. Soc.* **138,** 15515–15518 CrossRef Medline
5. Wang, B., LaMattina, J. W., Marshall, S. L., and Booker, S. J. (2019) Capturing intermediates in the reaction catalyzed by NosN, a class C radical *S*-adenosylmethionine methylase involved in the biosynthesis of the nosiheptide side-ring system. *J. Am. Chem. Soc.* **141,** 5788–5797 CrossRef Medline
6. Deobald, D., Adrian, L., Schöne, C., Rother, M., and Layer, G. (2018) Identification of a unique radical SAM methyltransferase required for the sp^3-C-methylation of an arginine residue of methyl-coenzyme M reductase. *Sci. Rep.* **8,** 7404 CrossRef Medline
7. Holliday, G. L., Akiva, E., Meng, E. C., Brown, S. D., Calhoun, S., Pieper, U., Sali, A., Booker, S. J., and Babbitt, P. C. (2018) Atlas of the radical SAM superfamily: divergent evolution of function using a "plug and play" domain. *Methods Enzymol.* **606,** 1–71 CrossRef Medline
8. Radle, M. I., Miller, D. V., Laremore, T. N., and Booker, S. J. (2019) Methanogenesis marker protein 10 (Mmp10) from *Methanosarcina acetivorans* is a radical *S*-adenosylmethionine methylase that unexpectedly requires cobalamin. *J. Biol. Chem.* **294,** 11712–11725 CrossRef Medline