Enzymatic C-Methylation

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Dual Role of *S*-Adenosylmethionine (SAM^+) in the Methylation of sp^2-Hybridized Electrophilic Carbons

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active sites · radicals · reaction mechanisms · transferases

Many biomolecules contain C-bound methyl groups that are formed from C_1 units; examples include thymidine and ubiquinone. In these compounds the methyl groups are introduced through S_N2 methylene- and methyl-group-transfer reactions from methylenetetrahydrofolate (methylene-H_4F)[1] and *S*-adenosylmethionine (SAM^+),[2] respectively. Other cases of C-methyl groups generated by C_1 transfer to nucleophilic carbon atoms are the SAM^+-derived C-methyl groups in 5-methylcytosine[3] and menaquinone (methylnaphthoquinone)[2] as well as the two methyl groups in precorrin-2, an intermediate in the biosyntheses of vitamin B_{12}, siroheme, heme D, and factor 430 (F_{430}) of the methanogenic Archaea.[4]

There are, however, many compounds that are C-methylated with SAM^+ without a nucleophilic carbon for methyl-group acceptance. Such a compound is adenosine 2503 in the 23S rRNA of bacterial ribosomes, whose posttranscriptional methylation at C-2 has a housekeeping function important in translational fidelity and whose methylation at C-8 confers resistance of bacteria to several classes of antibiotics that target the large subunit of the ribosome. C-2 and C-8 of adenosine are electrophilic rather than nucleophilic sp^2-hybridized carbon atoms with poor C–H acidity, but despite this they are methylated by the SAM^+-dependent methyltransferases RlmN from *Escherichia coli* and Cfr from *Staphylococcus aureus*, respectively.[5]

The first clue to the mechanism came from the finding that the methyltransferases catalyzing C-2 and C-8 methylations belong to the radical SAM^+ family of iron–sulfur proteins[5a] and by that differ from those enzymes involved in S_N2 methylation reactions. The radical SAM^+ enzymes generally catalyze reactions triggered by the 5′-deoxyadenolyl radical (5′-dA•) that is generated by one-electron reduction of SAM^+ which is bound to a [4Fe-4S] cluster.[6] The second clue was the observation that the C-2 and C-8 methyl groups are derived from the methyl group of SAM^+ and that in the methyl-transfer reaction *S*-adenosylhomocysteine (SAH) and 5′-deoxyadenosine (dA-H) plus methionine are formed.[5a] And finally, the third clue was the observation that methyl-group transfer to C-2 and C-8 from methyl *S*-adenosyl[*methyl*-$^2H_3$]methionine takes place with loss of one deuterium atom, which is replaced by the hydrogen of the respective amidine carbon of the adenosine (Figure 1).[5b,7] In contrast, the SAM^+-dependent methyl transferases, which work by an S_N2 mechanism, catalyze the methyl-group transfer without exchange of any of the three hydrogen atoms.

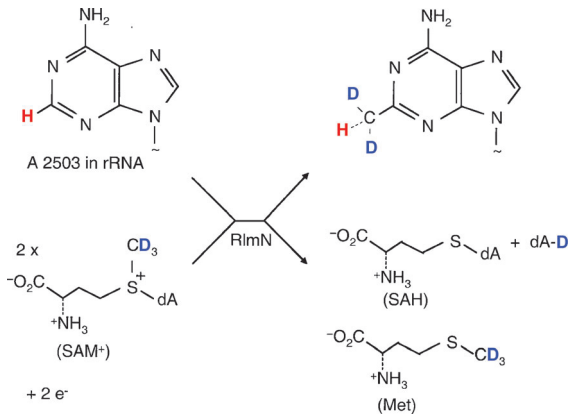

Figure 1. Formation of *C-2*-methyladenosine in 23S rRNA at position 2503 from SAM^+ and 23S rRNA as catalyzed by the radical SAM^+ methyltransferase RlmN from *E. coli*. Formation of *C-8*-methyl-A2503 is catalyzed analogously by the radical SAM^+ methyltransferase Cfr from *Staphylococcus aureus*. RlmN and Cfr are phylogenetically closely related (33% sequence identity). dA-D = 5′-deoxyadenosine containing one deuterium in the methyl group.

These and other experimental findings indicate that methylation of C-2 of adenosine proceeds in two steps consuming two molecules of SAM^+ per methyl group transferred. In step 1, one of the two essential cysteine thiol groups in the active site of the methyltransferase (HS-MT-SH) is methylated by SAM^+ in an S_N2 reaction yielding HS-MT-S-CH_3 and SAH (reaction 1).[7] In step 2, a second SAM^+ molecule which is bound to the [4Fe-4S] cluster, is reduced by one electron to give methionine (Met) and 5′-dA•, which subsequently abstracts a hydrogen from the protein-bound methyl group to yield 5′-dA-H and a neutral, carbon-centered methylene radical. Attack of this radical at the sp^2-hybridized C-2 of adenosine 2503 (A2503) in rRNA, removal of an electron, and deprotonation of the respective amidine carbon yields a thioether that is reduced to C-2 methyl-A2503 by an adjacent cysteine (reaction 2).[7] The catalytic cycle is closed by reduction of the disulfide bond (reaction 3) lastly provid-

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ing the two electrons required for the reduction of SAM^+ to Met and 5′-dA-H (reaction 4 = reactions 1 + 2 + 3).[7] The methylation at C-8 proceeds by the same mechanism.

$$SAM^+ + HS\text{-}MT\text{-}SH \rightarrow SAH + HS\text{-}MT\text{-}S\text{-}CH_3 + H^+ \quad (1)$$

$$SAM^+ + HS\text{-}MT\text{-}S\text{-}CH_3 + A2503 \rightarrow dA\text{-}H + Met + (S\text{-}MT\text{-}S)_{disulfide} + CH_3\text{-}A2503 + H^+ \quad (2)$$

$$(S\text{-}MT\text{-}S)_{disulfide} + 2\,e^- + 2\,H^+ = HS\text{-}MT\text{-}SH \quad (3)$$

$$2\,SAM^+ + 2\,e^- + A2503 = SAH + 5'\text{-}AH + Met + CH_3\text{-}A2503 \quad (4)$$

It has not yet been possible to show that the methyltransferase can catalyze reaction 3. In the experiments the Cys355-methylated enzyme was incubated with SAM^+, an rRNA fragment, and an electron donor, yielding methyladenosine and the disulfide (S-MT-S) as products, which corresponds to only half of the catalytic cycle.

The crystal structures of RlmN and RlmN with SAM^+ reveal that a single molecule of SAM^+ coordinates the [4Fe-4S] cluster. Residue Cys355 is S-methylated and located proximal to the SAM^+ methyl group, suggesting that SAM^+ involved in the initial methyl transfer (reaction 1) binds to the same site.[8] Thus, RlmN takes advantage of the two faces of SAM^+[9] in one reaction with structural economy.

C-2 and C-8 methylations of adenosine in rRNA are not the only examples of methylations of sp^2-hybridized electrophilic carbons with SAM^+. Others include the methylation of C-2 of tryptophan in thiostrepton[10] and the synthesis of cyclopropane fatty acids from mono-unsaturated fatty acids and SAM^+.[11] However, there is evidence that these two methylations do not follow the mechanism involved in the formation of *C-2*- and *C-8*-methyladenosine in rRNA. In the case of thiostrepton, which may also employ radical chemistry, it was shown that the methyl group in 2-methyltryptophan is incorporated from SAM^+ with retention of configuration at the methyl carbon.[10] This excludes mechanisms proceeding either by an S_N2 methyl-group transfer with inversion of configuration or via a methylene radical with hydrogen exchange. In the case of the cyclopropane fatty acids, the analysis of the responsible genes revealed the absence of the characteristic [4Fe-4S] binding motif of radical SAM^+ enzymes (CX_3CX_2C, whereby C = cysteine and X = any amino acid).[11a]

There are also sp^3-hybridized electrophilic carbons that can be methylated,[12] for example C-8^2 and C-12^1 in bacteriochlorophyll c (Figure 2), whose methylations involve radical SAM^+ enzymes.[13] Other examples are the synthesis of the C-alkyl groups in pactamycin,[14] thienamycin, sitosterol, stigmasterol, and related 24-ethyl steroids, the synthesis of *C-5*-methylarginine and *C-2*-methylglutamine in methyl-coenzyme M reductase (MCR) from methanogenic Archaea,[15] the synthesis of the two C-methyl groups in tetrahydromethanopterin,[16] and the synthesis of the C-methyl group in fosfomycin.[17] In the case of the two amino acids in MCR[15] and of tetrahydromethanopterin[16] it was shown that the methyl groups are introduced from [*methyl*-$^2H_3$]methionine without D/H exchange by enzymes that still remain to be identified.[18]

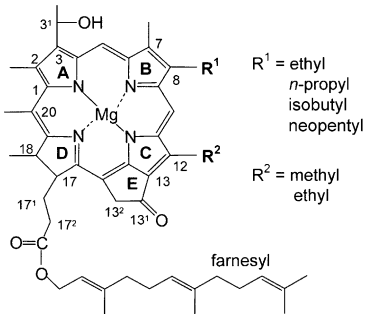

Figure 2. Structure of bacteriochlorophyll c from *Chlorobaculum tepidum*. The side chains R^1 and R^2 arise from successive methylations at the unreactive C-8^2 and C-12^1 methyl groups with SAM^+ as the donor.[13]

A mechanistic proposal for the methylation of the sp^3-hybridized carbon atom has been put forward for the methylation reaction in fosfomycin.[12b] It is based on the finding that the gene *fom3* involved encodes for a protein with two domains. The conserved N-terminal domain is annotated as B_{12}-cofactor-binding, whereas the C-terminal domain shows a high degree of sequence similarity to the enzyme of the radical SAM^+ family, including the diagnostic [4Fe-4S] cluster binding motif. The hypothesis is that a 5′-deoxyadenosyl radical generates a carbon-centered substrate radical, which can subsequently react with the methyl cobalamin to form the methylated product. This would be the first example of a radical methyl transfer from methylcobalamin (methyl-B_{12}) as the donor. Thus, much remains much to be discovered in this area.

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