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New Concept

Regioselectivity in the Homolytic Cleavage of S-Adenosylmethionine

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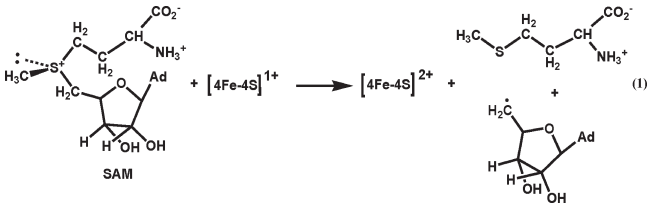
ABSTRACT: The observed regioselectivity of the homolytic cleavage of S-adenosylmethionine (SAM) by the radical SAM enzymes is modeled by free radical displacement reactions at sulfoxide centers. These displacements are also regioselective, in direct consequence of the reaction mechanism. The selectivity in the radical SAM reactions is explained by the geometry of the free radical displacement mechanism, required by the chemical reaction and arranged in the active site by the radical SAM proteins.

An excitng development in free radical chemistry and biology is the discovery of a "superfamily" of enzymes that require S-adenosylmethionine (SAM) and the reduced form of an iron sulfur cluster as cofactors (*1*, *2*). The usual experimental observation is that SAM is selectively cleaved to give methionine and the primary 5′-deoxyadenosyl radical, which subsequently reacts with substrate to abstract an hydrogen atom and initiate a variety of free radical reactions. The cleavage of SAM has been described as a reductive cleavage of the sulfonium ion, initiated by a one electron transfer from $[4Fe4S]^{1+}$ to SAM that facilitates homolytic cleavage of the S-5′-deoxyadenosyl bond (eq 1). While this working hypothesis is stoichiometrically correct, it is mechanistically misleading and stimulated considerable discussion and experimentation (*3*, *4*).



The most significant mechanistic challenge (*5*) was the apparent mismatch between the half-wave reduction potential of trialkylsulfonium ions in solution (ca. −1.8 V) and the redox potential of the $[4Fe\text{-}4S]^{2+/1+}$ couple (ca. −0.4 V). This issue is resolved by the insight that the reaction of $[4Fe\text{-}4S]^{1+}$ with SAM is not an electron transfer from the cluster to the lowest unoccupied molecular orbital (LUMO) of the S-5′-deoxyadenosyl bond. Instead, the extensive studies by Frey of lysine 2,3-aminomutase show (*2*−*6*) that the cleavage of SAM by the $[4Fe\text{-}4S]^{1+}$ cluster is an iron−sulfur bond-forming reaction. More recently, the results of crystallographic studies of HydE, related computational modeling, and a review of the three-dimensional structures of several other radical SAM enzymes make a strong case for the generality of the iron−sulfur bond-forming mechanism (*7*).

A second problem arises from the observed specificity of the cleavage of SAM in the radical SAM enzymes (*8*, *9*). In a competition among the three possible bond-breaking pathways, fragmentation to give primary radicals rather than the less stable methyl radical should be preferred. However, the choice between the two primary radicals, 5′-deoxyadenosyl radical and 3-amino-3-carboxypropyl radical (ACP) is not apparent.

We know that a radical displacement reaction at the sulfur of a sulfoxide requires a colinear arrangement of the bond-forming atom, the sulfur atom, and the bond-breaking atom and inversion of configuration at the sulfur (*10*, *11*). The alternative displacement of the endocyclic CH_2R radical, which is not observed, corresponds to a nonlinear front side displacement reaction with retention of configuration. The observed specificity is easily rationalized and visualized in Figure 1 by a requirement for apical entering and leaving groups in a three-center, three-electron transition state. The hypervalent (9-S-4) bonding in transition state (X) is appropriately described (*12*) by a filled three-center bonding molecular orbital and a singly occupied nonbonding molecular orbital with electron density at the terminal atoms, the entering and departing groups in the displacement reaction. In the conversion from reactants to products, the reactive carbon atom of the aromatic ring is formally oxidized and the carbon atom of the departing methyl radical is formally reduced.

Analogy to the sulfoxide reaction indicates that the reaction of the $[4Fe\text{-}4S]^{1+}$ cluster with SAM is best described as a radical displacement reaction at the sulfonium center. In particular, the displacement mechanism requires that the reactive iron atom of the cluster, the sulfonium atom in SAM, and the 5′-deoxyadenosyl group are approximately colinear, placing the iron and the 5′-deoxyadenosyl groups in apical positions with respect to the sulfur. The carboxylate and amino groups of the methionine are ligated to the reacting iron atom (*2*) requiring an apical-equatorial arrangement of the iron and the ACP substituent, respectively. The methyl substituent of SAM is in the same equatorial plane as the methylene of the ACP group. The resulting transition state (Z) shown in Figure 2 is isoelectronic and isomorphic at the sulfur atom with the sulfoxide transition state (X). Computational studies of the HydE active site and simplified models also reveal transition state (Z) and the central role of the S-p_z orbital in the displacement reaction (*7*).

As shown in Figure 2, the reactants in the radical displacement reaction are the $[4Fe\text{-}4S]^{1+}$cluster and SAM. The entering group is the unique iron of the cluster, and an iron−sulfur bond is formed in the reaction. The leaving group is the 5′-deoxyadenosyl radical, and the sulfur-5′-deoxyadenosyl bond is broken in the reaction. The products of the radical displacement reaction are

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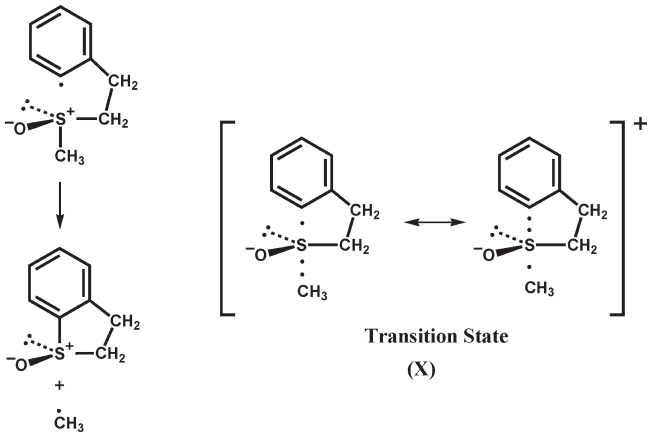


FIGURE 1: The observed stereochemistry of a radical displacement reaction at a sulfoxide center (*10*, *11*).

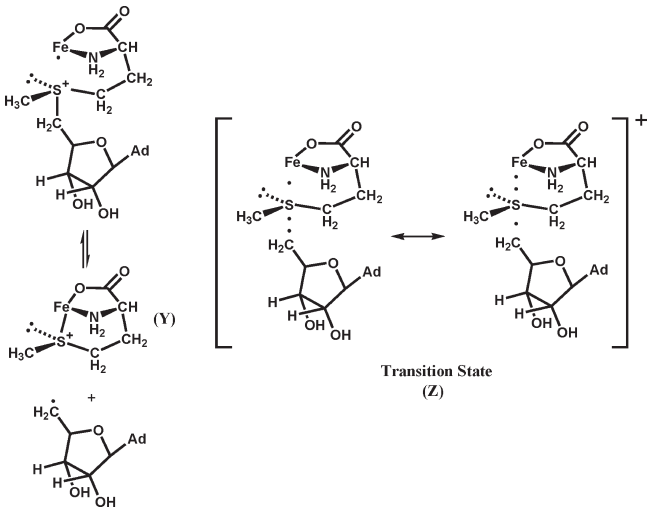
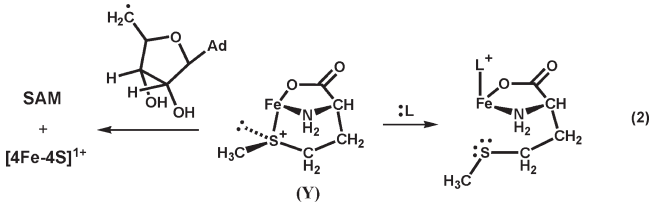


FIGURE 2: The proposed stereochemistry of a radical displacement reaction at the sulfonium center of SAM.

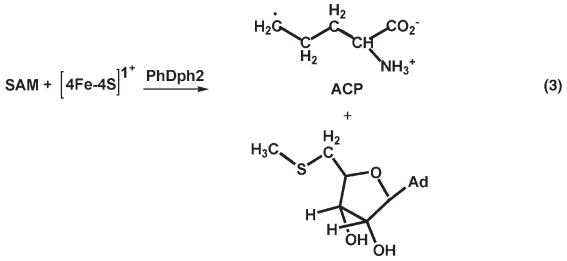
the 5′-deoxyadenosyl radical and a new sulfonium ion (Y) with the iron bonded to the sulfur; the configuration at sulfur is inverted. As required by the stoichiometry of the reaction, the reactive iron atom of the cluster is formally oxidized from [4Fe-4S]$^{1+}$ to[4Fe-4S]$^{2+}$ in the formation of the iron−sulfur bond, and the 5′-carbon of SAM is formally reduced in the cleavage of the sulfur−carbon bond to give the 5′-deoxyadenosyl radical.

Accordingly, the distinction between the two primary radicals, 5′-deoxyadenosyl radical and the ACP radical, is a result of the stereochemical requirements of the chemical reaction, constraints that are imposed by the displacement reaction mechanism. Crystallographic studies of several radical SAM enzymes bound to the cofactors show that the geometric assignments derived from the reaction mechanism are fully realized in the active site structures (*7*, *13*−*17*).

SAM and the [4Fe-4S]$^{1+}$ cluster are used catalytically as cofactors in the reactions of lysine 2,3-aminomutase and photoproduct lyase. In contrast, SAM is used stoichiometrically as a substrate in several other enzyme systems such as the glycyl radical activases and the sulfur inserting enzymes (*2*). As shown in Figure 2, the product of the displacement reaction is an intermediate (Y) with the iron bonded to the sulfur atom of methionine. The competitive partitioning of this intermediate shown in eq 2 can explain the distinction between catalytic and stoichiometric utilization of SAM. In the back reaction of Y, the iron is displaced from the sulfur by a 5′-deoxyadenosyl radical; SAM and the [4Fe-4S]$^{1+}$cluster are regenerated, the configuration at S is reverted, and the process is catalytic. In the forward reaction of Y, the sulfur is displaced from the iron by a nucleophilic ligand (preceded or followed by displacement of the carboxyl and amino groups) to release the stoichiometric products, methionine, and the [4Fe-4S]$^{2+}$ cluster; neither SAM nor [4Fe-4S]$^{1+}$ is regenerated, and the process is not catalytic.



Most recently, a study of the biosynthesis of diphthamide revealed a radical SAM reaction that does not form the 5′-deoxyadenosyl radical (*18*). The enzyme in question, PhDph2, requires SAM and the [4Fe-4S]$^{2+}$ cluster and is activated by dithionite, characteristics shared with other radical SAM enzymes. However, in this case, SAM is cleaved to give the other primary radical, ACP, and 5′-deoxy-5′-methylthioadenosine (eq 3).



While the direction of cleavage of SAM by PhDph2 is biologically unprecedented, it is not chemically surprising. As noted above, there is no reason to expect specificity in the homolytic cleavage products from SAM. In the case of PhDph2, the observed chemical reaction requires a colinear diapical arrangement of the reactive iron atom, the sulfur atom, and the ACP group. The structure with SAM bound to active site is not known, but we can predict with confidence that SAM will bind as required by the chemical mechanism with the entering iron and the departing ACP group in apical positions and the methyl and the 5′-deoxyadenosyl groups in the equatorial plane[2]. A corollary prediction is that the diapical arrangement of the iron and the ACP group will preclude the ligation of the amino and carboxylate of the ACP group to the reacting iron that is observed in the intimate active site structure of the 5′-deoxyadenosyl-radical SAM enzymes[3].

There are other differences between the 5-deoxyadenosyl-radical SAM enzymes and PhDph2 (*18*). The ACP radical subsequently adds to the imidazole ring of a histidine residue,

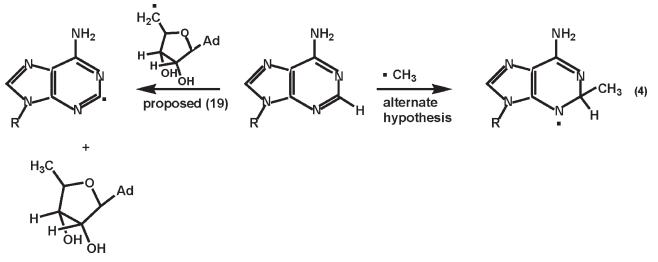
[2]Hening Lin and co-workers (*18*) noted: "It is likely that the different reaction outcome, that is, the cleavage of the C5′-S bond in the radical SAM enzymes versus the cleavage of the (ACP-S) bond in PhDph2, is controlled by SAM having different orientations relative to the [4Fe-4S] cluster. Future studies are required to investigate how SAM is bound at the active site of PhDph2".

[3]The editor, P. A. Frey, noted that the equatorial disposition of the 5′-deoxyadenosyl group might allow "ligation of SAM to Fe through the cis-vicinal hydroxyl groups of ribose."

in contrast to the ubiquitous hydrogen atom abstraction reactions of the 5′-deoxyadenosyl radicals. The three-dimensional structure of PhDph2 is also different; three cysteines are conserved, but they are widely separated in the sequence and do not follow the usual CX_3CX_2C motif that characterizes the 5′-deoxyadenosyl radical SAM enzymes. PhDph2 may be the first example of a new family of ACP-radical SAM enzymes, with unique chemistry and active site structure.

Finally, we can speculate that there may be a set of CH_3-radical SAMs, with yet a third type of enzyme and active site structure that positions the sulfur-methyl bond of SAM colinear with the reactive iron of the cluster, as required to produce methyl radicals by the displacement mechanism. We can also predict that the carboxylate and amino groups of the methionine of SAM will be positioned to potentially ligate the reactive iron of the cluster.

The methyltransferases, RlmN and Cfr, are radical SAM enzymes that methylate the C2 or C8 position of an adenosine nucleotide in rRNA. The initial step is proposed to be an "high activation energy" abstraction of an hydrogen atom from an sp^2 hybridized carbon (C2 or C8) of adenosine by a SAM derived 5′-deoxyadenosyl radical, followed by a methyl transfer from a second SAM by an unknown mechanism (*19*). However, the experimental data also allow the alternate hypothesis that RlmN and Cfr are CH_3-radical SAM enzymes and that the initial step in the methylation sequence is the energetically more favorable addition of a methyl radical to the adenosine ring to give a resonance stabilized adduct (eq 4). Subsequent rearomatization of this adduct by abstraction of the labile hydrogen atom (or the stoichiometric equivalent of oxidation and deprotonation) gives methylated adenosine. The first hypothesis (*19*) requires that tritium at C2 or C8 in the substrate will be transferred to the 5′-position of deoxyadenosine. In the alternate hypothesis, labeled deoxyadensine will be observed *only* if a 5′-deoxyadenosyl radical abstracts the labile tritium of the adduct. All other mechanisms of rearomatization will give unlabeled deoxyadenosine.



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REFERENCES

1. Sofia, H. J., Chen, G., Hetzler, B. G., Reyes-Spindola, J. F., and Miller, N. E. (2001) Radical SAM, a novel protein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. *Nucleic Acids Res. 29*, 1097–1106.
2. Frey, P. A., Hegeman, A. D., and Ruzicka, F. J. (2008) The radical SAM superfamily. *Crit. Rev. Biochem. Mol. Biol. 43*, 63–88.
3. Frey, P. A., and Reed, G. H. (2000) Radical mechanisms in adenosylmethionine- and adenosylcobalamin-dependent enzymatic reactions. *Arch. Biochem. Biophys. 382*, 6–14.
4. Cosper, N. J., Booker, S. J., Ruzicka, F., Frey, P. A., and Scott, R. A. (2000) Direct FeS cluster involvement in generation of a radical in lysine2,3-aminomutase. *Biochemistry 39*, 15668–15673.
5. Hinckley, G. T., and Frey, P. A. (2006) Cofactor-dependence in reduction potentials for $[4Fe\text{-}4S]^{2+/1+}$ in lysine 2,3-aminomutase. *Biochemistry 45*, 3219–3225.
6. Wang, S. C., and Frey, P. A. (2007) Binding energy in the one-electron reductive cleavage of S-adenosylmethionine by lysine 2,3-aminomutase, a radical SAM enzyme. *Biochemistry 46*, 12889–12895.
7. Nicolet, Y., Amara, P., Mouesca, J.-M., and Fontecilla-Camps, J. C. (2009) Unexpected electron transfer mechanism upon AdoMet cleavage in radical SAM proteins. *Proc. Natl. Acad. Sci. 106*, 14867–14871.
8. Jarrett, J. T. (2003) The generation of 5′-deoxyadenosyl radicals by adenosylmethionine-dependent radical enzymes. *Curr. Opin. Chem. Biol. 7*, 174–182.
9. Broderick, J. B. (2010) A radically different enzyme. *Nature 465*, 877–878.
10. Kampmeier, J. A., Jordan, R. B., Jordan, M. S., Liu, H. Y., and Bishop, D. J. (1978) The geometry of free radical displacements at di- and trivalent sulfur, in Organic Free Radicals; Pryor, W. A., Ed.; ACS Symposium Series No. 69, pp 275−289, American Chemical Society, Washington, DC.
11. Beckwith, A. L. J., and Boate, D. R. (1986) Stereochemistry of intramolecular homolytic substitution at the sulphur atom of a chiral sulphoxide. *J Chem. Soc. Chem. Commun*. 189–190.
12. Musher, J. I. (1969) The chemistry of hypervalent molecules. *Angew. Chem., Int. Ed. 8*, 54–68. Perkins, C. W., Martin, J. C., Arduengo, A. J., Lau, W., Alegria, A., and Kochi, J. K. (1980) An electrically neutral σ-sulfuranyl radical from the homolysis of a perester with neighboring sulfenyl sulfur: 9-S-3 species. *J. Am. Chem. Soc. 102*, 7753–7759. Kampmeier, J. A., Hoque, M., Saeva, F. D., Wedegaertner, D. K., Thomsen, P., Ullah, S., Krake, J., and Lund, T. (2009) Regioselectivity in the reductive bond cleavage of diarylalkylsulfonium salts: Variation with driving force and structure of sulfuranyl radical intermediates. *J. Am. Chem. Soc. 131*, 10015–10022.
13. Layer, G., Moser, J., Heinz, D. W., Jahn, D., and Schubert, W. D. (2003) Crystal structure of coproporphyrinogen III oxidase reveals cofactor geometry of Radical SAM enzymes. *EMBO J. 22*, 6214–6224.
14. Berkovitch, F., Nicolet, Y., Wan, J. T., Jarrett, J. T., and Drennan, C. L. (2004) Crystal structure of biotin synthase, an 5-adenosylmethionine-dependent radical enzyme. *Science 303*, 76–79.
15. Hanzelmann, P., and Schindelin, H. (2004) Crystal structure of the S-adenosylmethionine-dependent enzyme MoaA and its implications for molybdenum cofactor deficiency inhumans. *Proc. Natl. Acad. Sci. U.S.A. 101*, 12870–12875.
16. Lepore, B. W., Ruzicka, F. J., Frey, P. A., and Ringe, D. (2005) The X-ray crystal sructure of lysine 2,3-aminomutase from *Clostridium subterminale*. *Proc. Natl. Acad. Sci. U.S.A. 102*, 13819–13824.
17. Vey, J. L., Yang, J., Li, M., Broderick, W. E., Broderick, J. B., and Drennan, C. L. (2008) Structural basis for glycyl radical formation by pyruvate formate-lyase activating enzyme. *Proc. Natl. Acad. Sci. U.S.A. 105*, 16137–16141.
18. Zhang, Y., Zhu, X., Torelli, a. T., Lee, M., Dzikovski, B., Koralewski, R. M., Wang, E., Freed, J., Krebs, C., Ealick, S. E., and Lin, H. (2010) Diphthamide biosynthesis requires an organic radical generated by an iron-sulphur enzyme. *Nature 465*, 891–896.
19. Yan, F., LaMarre, J. M., Rohrich, R., Wiesner, J., Jomaa, H. J., Mankin, A. S., and Fujimori, D. G. (2010) RlmN and Cfr are radical SAM enzymes involved in methylation of ribosomal RNA. *J. Am. Chem. Soc. 132*, 3953–3964.