Published in final edited form as:

Curr Opin Chem Biol. 2009 February ; 13(1): 74–83. doi:10.1016/j.cbpa.2009.01.022.

Control of radical chemistry in the AdoMet-radical enzymes

Kaitlin S. Duschene, Susan E. Veneziano, Sunshine C. Silver, and Joan B. Broderick^{*}
Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717

Summary

The radical AdoMet superfamily comprises a diverse set of >2800 enzymes that utilize iron-sulfur clusters and S-adenosylmethionine (SAM or AdoMet) to initiate a diverse set of radical-mediated reactions. The intricate control these enzymes exercise over the radical transformations they catalyze is an amazing feat of elegance and sophistication in biochemistry. This review focuses on the accumulating evidence for how these enzymes control this remarkable chemistry, including controlling the reactivity between the iron-sulfur cluster and AdoMet, and controlling the subsequent radical transformations.

Radical AdoMet enzymes: Diverse functions with a common mechanistic thread

The radical SAM superfamily comprises a diverse set of >2800 enzymes that utilize iron-sulfur clusters and S-adenosylmethionine (SAM or AdoMet) to initiate radical transformations [1, 2]. The enzymes are involved in such central pathways as the metabolism of glucose and the reduction of ribonucleotides, as well as in pathways for the biosynthesis of complex cofactors and metal clusters, the modification of tRNA, and the synthesis of antibiotics (Figure 1). Central to each of these diverse reactions are believed to be a common series of mechanistic steps that culminate in the abstraction of a hydrogen atom from substrate (Figure 2). First, AdoMet binds to the unique iron site of the site-differentiated $[4\text{Fe-4S}]^{2+}$ radical AdoMet cluster via the α -carboxyl and α -amino groups of the methionine. This novel interaction between AdoMet and the $[4\text{Fe-4S}]$ cluster was first revealed by detailed electron-nuclear double resonance (ENDOR) spectroscopic studies on one member of the radical AdoMet superfamily, the pyruvate formate lyase activating enzyme [3-5], and was subsequently corroborated for other members of the superfamily by ENDOR [6] and by X-ray crystallography [7-13] of radical AdoMet enzymes complexed to AdoMet. Second, an electron is transferred from an electron donor (in many cases, reduced flavodoxin plays this role *in vivo*) to the radical AdoMet cluster to reduce it to the $[4\text{Fe-4S}]^{1+}$ state, which has been shown to be the catalytically relevant state for these enzymes [14]. Third, inner-sphere electron transfer from the $[4\text{Fe-4S}]^{1+}$ cluster to AdoMet promotes the homolytic cleavage of the S-C5' bond of AdoMet to yield methionine bound to the unique iron site, and a 5'-deoxyadenosyl radical intermediate (dAdo•). The dAdo• abstracts a hydrogen atom (H•) from substrate to generate a substrate radical and 5'-deoxyadenosine (5'-dAdo). For one class of radical AdoMet enzymes, the glycyl radical enzyme activating enzymes (GRE-AEs), this is the final step in catalysis, with the glycyl radical and 5'-dAdo being products of the reaction. For most radical AdoMet enzymes, however, abstraction of H• from substrate

© 2009 Elsevier Ltd. All rights reserved

^{*}To whom correspondence should be addressed at E-mail: jbroderick@chemistry.montana.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

is merely a prelude to sometimes complex and fascinating radical-mediated chemistry that transforms the substrate radical into product. In some cases, most notably lysine 2,3-aminomutase (LAM) and spore photoproduct lyase (SPL), a product radical is generated that re-abstracts H• from 5'-dAdo to re-generate the 5'-dAdo•, which subsequently combines with methionine, with loss of an electron to the [4Fe-4S] cluster, to restore AdoMet (Figure 1). In these two cases and probably others yet to be identified, AdoMet serves as a cofactor in catalysis and is not consumed during turnover. In most of the radical AdoMet enzymes studied to date, however, AdoMet serves as a substrate and is stoichiometrically cleaved to methionine and 5'-deoxyadenosine for each H• that is abstracted from substrate (Figure 1).

Control of AdoMet radical chemistry: insights from model studies

Reduced iron-sulfur clusters appear to be well suited to the chemistry described in the previous section. Indeed, Daley and Holm demonstrated that synthetic [4Fe-4S]¹⁺ clusters, whether site-differentiated or not, have the inherent ability to reductively cleave sulfonium cations in overall two electron reductions in which the second electron is donated by a second reduced cluster [15,16]. In addition to reductive cleavage, however, they also observed products formed by electrophilic attack of the sulfonium on the thiolate ligands to the reduced [4Fe-4S]⁺ cluster, a reaction pathway that is not observed in the radical AdoMet enzymes. Also of interest is their observation that the oxidized state of the iron-sulfur cluster (equivalent to a [4Fe-4S]²⁺ in an enzyme) also reacts with sulfonium cations via electrophilic attack of the sulfonium on the terminal thiolate ligands of the cluster. The reactivity observed with these synthetic clusters provides important insights regarding not only the inherent reactivity of iron-sulfur clusters towards sulfonium cations, but also the *control* of AdoMet-derived radical chemistry in the radical AdoMet enzymes. First, these studies reveal that both oxidized and reduced iron-sulfur clusters are susceptible to electrophilic attack by sulfonium cations, a mode of reactivity not observed in the radical AdoMet enzymes; the enzymes, therefore, presumably provide shielding of the cysteinate ligands to the [4Fe-4S] cluster that prevents this type of reaction. Second, these studies demonstrated that if the potentials of the [4Fe-4S] cluster and sulfonium are such that electron transfer to the sulfonium is thermodynamically favorable, reductive cleavage of the sulfonium by the reduced cluster is rapid and complete. In the radical AdoMet enzymes, in contrast, a reduced [4Fe-4S]¹⁺ cluster can bind AdoMet in the absence of substrate in a relatively stable state, with either no or very slow cleavage of AdoMet. The enzymes thus also control the reactivity of the cluster towards reductive cleavage of AdoMet, a mode of control that is biologically important in order to avoid indiscriminate production of the highly reactive 5'-dAdo radical. This Opinion will focus on the evidence accumulating on the means by which the radical AdoMet enzymes control the radical chemistry they catalyze.

Redox potentials and the control of radical AdoMet reactions

As is discussed in a later section of this Opinion, reductive cleavage of AdoMet in the absence of substrate (referred to as uncoupled AdoMet cleavage) has been observed for several of the radical AdoMet enzymes. One way to prevent such uncoupled cleavage would be to control the binding of AdoMet such that it binds only when substrate is available for turnover; Jarrett and coworkers have in fact shown that biotin synthase binds AdoMet and dethiobiotin in an ordered fashion, and that the affinity for AdoMet is increased more than 20-fold in the presence of substrate [17]. Another way to minimize uncoupled AdoMet cleavage physiologically would be to generate the catalytically active [4Fe-4S]⁺ state of the cluster only in the presence of substrate. Indeed, the redox potentials for the [4Fe-4S]^{2+/+} couples of the radical AdoMet enzymes are quite negative (in the ~ -450 mV range), and thus inaccessible for reduction by physiological electron donors such as flavodoxins. For lysine 2,3-aminomutase, however, the [4Fe-4S]^{2+/+} reduction potential has been shown to increase in the presence of AdoMet to potentials within the range of physiological reductants [18,19].

Intriguingly, while the rise in the cluster midpoint potential upon binding of AdoMet renders the cluster in lysine 2,3-aminomutase accessible to reduction by physiological reductants, it simultaneously makes the electron transfer from the reduced cluster to AdoMet even more uphill thermodynamically. In the studies conducted by Daley and Holm, the redox potentials of the clusters and the sulfoniums were such that the electron transfer from the former to the latter was thermodynamically favorable [15,16]. In the radical AdoMet enzymes, however, electron transfer from the $[4\text{Fe-4S}]^{1+}$ cluster to AdoMet is decidedly unfavorable. While the best estimations put the reduction potential for AdoMet at approximately -1.8 V, the $[4\text{Fe-4S}]^{2+/1+}$ couples for the radical AdoMet enzymes are in the -450 mV range. Thus the electron transfer from the $[4\text{Fe-4S}]^{1+}$ cluster of a radical AdoMet enzyme to AdoMet is uphill by ~1.4 V, which corresponds to ~32 kcal mol⁻¹. It is no wonder, then, that no or only slow reductive cleavage of AdoMet is observed in the absence of substrate for these enzymes. The key question then becomes: how is the radical chemistry triggered upon binding of substrate to the E-AdoMet complex? Significant insight into this question has been provided by elegant studies of the radical AdoMet enzyme lysine 2,3-aminomutase [20]. Wang and Frey found that binding of AdoMet to the $[4\text{Fe-4S}]^{2+/1+}$ cluster of LAM contributes 19 kcal mol⁻¹ toward lowering this energy barrier, while binding of lysine to the active site of LAM in the presence of AdoMet contributes an additional 4 kcal mol⁻¹ to the lowering of the energy barrier (Figure 3). Taken together, the contributions of AdoMet and lysine binding to LAM reduce the energy barrier for AdoMet cleavage from 32 kcal mol⁻¹ to 9 kcal mol⁻¹ [20]. An additional consideration to the energy barrier issue is the mechanism of electron transfer itself: transfer of an electron from the $[4\text{Fe-4S}]^{1+}$ cluster to the sulfonium of AdoMet transforms the pentacoordinate iron of the $[4\text{Fe-4S}]^{1+}$ cluster to a more favorable hexacoordinate iron in the $[4\text{Fe-4S}]^{2+}$ cluster in LAM, and this may facilitate inner-sphere electron transfer [20].

Isolation of Radical Chemistry: Insights from X-Ray Crystallography

Of the greater than 2800 proteins that have been classified as members of the Radical SAM superfamily in microbial genomes alone, only a handful have been structurally characterized; all of the solved structures contain a partial or complete TIM barrel, with BioB and HydE having a complete TIM barrel ($\beta\alpha$)₈ and the others exhibiting an incomplete ($\beta\alpha$)₆ TIM barrel [7-13]. In addition to the overall fold, these structures reveal common features that likely play a role in sealing off the active site during catalysis, thus preventing side reactions such as the electrophilic attack observed in model studies, as well as uncontrolled reaction of the extremely reactive radical intermediates. First, in all of the structures the site-differentiated $[4\text{Fe-4S}]$ radical AdoMet cluster is bound at one end of the barrel, coordinated by a cluster-binding loop, with the unique iron oriented towards the center of the barrel (Figure 4). In all cases, this unique iron is coordinated by AdoMet through the amino and carboxy groups; this coordination partially seals off the cluster from solvent, perhaps providing the first level of control at the active site in protecting the labile and oxygen-sensitive cluster from degradation. In the structure of the oxygen-independent coproporphyrinogen III oxidase (HemN), two molecules of AdoMet are bound, with one coordinated to the iron-sulfur cluster and the second located deep in the N-terminal domain and adjacent to the first SAM, providing further protection of the cluster and the active site from solvent [7]. In all cases, the AdoMets are not only coordinated to the unique site, but also held in position by a series of conserved residues that are involved in electrostatic, H-bonding, hydrophobic, and π -stacking interactions; this apparently tight control of AdoMet binding speaks to the significance of proper positioning of AdoMet in the active site for optimal control of catalytic function. In the case of biotin synthase (BioB), which catalyzes sulfur insertion to form the thiophane ring of biotin, AdoMet binds in an extended conformation, stretching across the top of the barrel and interacting with several residues following the β -strands; these interactions keep AdoMet buried from solvent and position it for electron transfer from the $[4\text{Fe-4S}]$ cluster and hydrogen atom abstraction from DTB [8].

In addition to bound AdoMet, several of the radical AdoMet crystal structures have substrate bound in the active site, and thus provide further insight into active site architecture and modes for control of radical catalysis. Biotin synthase (Figure 5) was crystallized with both AdoMet and dethiobiotin bound; dethiobiotin lies in core of the TIM barrel between a [2Fe-2S] cluster and AdoMet and exhibits substantial van der Waals interactions with AdoMet including stacking of the carboxylate tail of DTB over the adenine ring of AdoMet [8]. DTB also interacts with conserved residues including a bidentate interaction with Asn222, which may help orient the substrate for hydrogen atom abstraction. The position of DTB relative to AdoMet, with DTB C9 and C6 ~3.9 and 4.1 Å, respectively, from the 5' carbon of AdoMet, is consistent with a mechanism for biotin synthase involving sequential H-atom abstractions from these two carbons, with each followed by sulfur insertion. Interestingly, the DTB is bound not only near to the site of radical initiation on AdoMet, but also to the [2Fe-2S] cluster, which is proposed to be the sulfur donor in biotin biosynthesis [8,19,21,22].

In the X-ray structure of lysine 2,3-aminomutase (Figure 4), L- α -lysine and the required PLP cofactor form an external aldimine which interacts with several residues in the active site; similar to the situation described above for biotin synthase, these interactions uniquely position lysyl-C2 and -C3, where the transfer of an amino group and H-atom occur during the conversion of L- α -lysine to L- β -lysine [11]. The 3-pro-*R* hydrogen on the lysyl side chain of the external aldimine is very near the C5' of AdoMet, putting it in an excellent position for H-atom abstraction upon reductive cleavage of AdoMet [11]. MoaA, like biotin synthase, contains two iron-sulfur clusters; however, in the case of MoaA both are [4Fe-4S] clusters. These clusters bind on opposing sides of the TIM barrel, ~17 Å apart at the closest distance, with a hydrophilic channel in between (Figure 4) [9]. While AdoMet is bound to the radical AdoMet cluster, substrate GTP coordinates to the unique iron of the C-terminal cluster through the purine N1 nitrogen (2.8 Å) and exocyclic amino group (2.4 Å); basic residues lining the hydrophilic channel between the two clusters also form significant interactions with GTP [10]. Several arginine residues are within H-bonding distance of the C6 exocyclic oxo group and the N7 of GTP, and these interactions have been proposed to assist in the rearrangement reaction or in shielding radical intermediates [10].

Pyruvate formate-lyase activating enzyme is the smallest of the radical AdoMet enzymes for which a structure has been determined, and its structure reveals few other secondary structural elements outside the core of the TIM barrel to “close off” the opening to the inner cavity (Figure 4)[13]. The bottom and lateral opening of the barrel both appear to be highly solvent-exposed, whereas the top of the barrel is closed off by loops B and C. However, with the binding of AdoMet, which is bound to the protein via two conserved motifs as well as two histidines that stack against the adenine ring, and the large pyruvate formate lyase substrate (170 kDa dimer), the active site is closed off and protected from the external environment for radical generation (Figure 4). The structure of PFL-AE with a bound 7-mer peptide (corresponding to the conserved sequence around the glycyl radical site of PFL) revealed three residues that appear to facilitate proper orientation of the substrate such as to allow stereospecific H-atom abstraction from the G734 of PFL, with the Gly734 C α positioned 4.1 Å away from C5' of AdoMet.

Finally, several of the radical AdoMet crystal structures reveal the presence of loops that may undergo conformational changes upon substrate binding to help seal off the active sites for radical chemistry. In biotin synthase, DTB's carboxylate group interacts with the amide of Thr293 and amide and side chain groups of Thr292; these interactions may, upon substrate binding, promote the movement of a loop that would close over the top of the barrel [8]. The crystal structures of PFL-AE in the absence and presence of substrate reveal movement of a loop upon substrate binding; this loop contains a sequence motif (DGXGXR) that is conserved among glycyl radical activating enzymes, and the conserved motif interacts with the peptide

substrate. This loop movement has been proposed to be central to the activation of PFL by inducing a conformational change in PFL and/or by correctly positioning the Gly loop for H-atom abstraction [13]; however, it is also likely that this loop movement helps to seal off the active site and protect radical intermediates from side reactions. In the case of HemN, two loops have been suggested to play roles in sealing off the active site during catalysis [7]. First, a short loop connecting the C- and N-terminal domains has been proposed to play a role in covering and partially filling the binding pocket in order to protect the bound substrate, coproporphyrinogen III, from solution. In addition, residues 4-35 of the N-terminal domain, referred to as the N-terminal 'trip-wire', are relatively lacking in secondary structure though the sequence G₂₀PRYTSYPTA₂₉ is highly conserved among HemN sequences. This 'trip-wire' has been proposed to be important during catalysis in that it may adopt an ordered conformation with the binding of substrate and therefore stimulate the C-terminal domain to "tip and close" over the active site [7]. A slightly different scenario occurs in the case of MoaA, where substrate GTP binding actually causes the active site cavity to widen [10]. However, the electrostatic interactions between the positively charged arginine and lysine residues and the triphosphate moiety may act to hold reactive radical intermediates in the active site for controlled radical chemistry. The crystal structures of both BioB and HydE show the presence of a "lid loop" containing conserved residues that interact with AdoMet; in both cases these interactions could serve to block off the active site by closing the lid loop over the inner cavity when AdoMet is bound (Figure 5) [8,12].

Together, the radical AdoMet crystal structures published to date provide revealing glimpses into the protein contributions to the control of radical chemistry at the active site. The active sites and the radical AdoMet clusters are shielded by the protein structure, thus providing protection from the electrophilic attack on thiolate ligands observed in the model studies. In addition, AdoMet coordinates the unique iron of the cluster, further shielding the cluster, and is positioned for radical chemistry by this coordination and by intricate interactions with protein residues. Substrate binding serves to further shield the site of radical chemistry, and in many cases it appears that movement of protein loops provides one additional means to protect radical intermediates from unwanted side reactions.

Maintaining van der Waals Contacts: Insights from ENDOR Spectroscopy

ENDOR spectroscopy of lysine 2,3-aminomutase has provided additional insights into the control of radical chemistry in the AdoMet-dependent radical enzymes; in this work, Frey, Hoffman, and coworkers probed intermediate states in the catalytic mechanism via the use of analogues of AdoMet or substrate that would produce stable radical intermediates [23]. By coupling radical intermediate stabilization with site-specific incorporation of NMR-active nuclei, they probed distances between the unpaired electron and the NMR active nucleus in a series of intermediate states, thus providing snapshots of atomic movements and a glimpse into how an enzyme controls radical intermediates. The AdoMet analogue S-3',4'-anhydroadenosylmethionine (anSAM), which produces a stable analogue of the 5'deoxyadenosyl radical (dAdo•) upon reductive cleavage, was used to probe the structure of the initial intermediate produced upon reductive cleavage of AdoMet [23-25]. The results demonstrated that the distance between the stable allylic (anhydro-dAdo•) radical and the lysine β -carbon was essentially identical to that observed in the crystal structure between the 5'C of AdoMet and the lysine β -carbon prior to reductive cleavage [23]. Substrate analogues that would result in formation of a stabilized L- α -lysine radical were used to probe the active site geometry in this intermediate state; the ENDOR spectra demonstrated large isotropic hyperfine couplings to 5'-¹³C-dAdo (produced by H-atom transfer from the substrate to the 5'-dAdo•) which could only arise from direct orbital overlap between the substrate radical intermediate and the 5'-methyl of dAdo, indicating direct van der Waals contact between the two moieties (Figure 6) [23]. Comparison of the ENDOR data to the crystal structure of LAM suggests that

the 5'-carbon of dAdo and lysine move 0.5 to 1.0 Å closer upon H-atom transfer from the substrate to dAdo•, a movement that results in the van der Waals contacts between dAdo and the substrate radical [23]. Further, the sharp ENDOR spectra point to a very well-defined geometry for the 5'-methyl of dAdo and the substrate radical, suggesting that the reacting partners are constrained to a single, optimized geometry. Significant movement of PLP during the reaction is suggested by the ³¹PENDOR spectra that reveal an isotropic coupling to the stabilized substrate radical; these results point to the considerable shifting of PLP in going from the resting enzyme (as observed in the crystal structure of LAM, 7 Å from the phosphorus to the spin-bearing carbons) to the substrate radical intermediate (<~4.5 Å as observed by ENDOR). Based on these ENDOR spectra, Lees et al. proposed a model wherein the reactive C5' carbon of dAdo• contacts the 3-pro-R hydrogen of lysine and this close contact persists after H-atom transfer produces the substrate radical intermediate (Figure 6) [23]. In the final stage of the catalytic cycle, the active site of LAM contains the [4Fe-4S]²⁺ cluster bound to methionine, in addition to adenosine, PLP and the β-lysine α-carbon radical [23]. The large isotropic coupling observed in the ENDOR spectra of the product radical (β-lysine•) demonstrates that the methyl of 5'-deoxyadenosine remains in van der Waals contact with the product lysine radical (Figure 6). Overall, the van der Waals contacts that are enforced throughout the isomerization reaction catalyzed by LAM facilitate hydrogen transfer and serve to eliminate or minimize side reactions from the highly reactive intermediates produced through the catalytic cycle [23].

The Problem of Uncoupled Reductive Cleavage of AdoMet

Uncoupled AdoMet cleavage, which refers to reductive cleavage of AdoMet in the absence of substrate, or reductive cleavage of AdoMet that exceeds the stoichiometry required for catalysis, has been observed for a number of the radical AdoMet enzymes. Such uncoupling is a reflection of a *lack* of control of the radical AdoMet chemistry and is difficult to envision as anything but wasteful and potentially damaging to the organism; the observation of uncoupling, however, is quite common among members of the superfamily. Several members of the superfamily, including the activating enzyme of anaerobic ribonucleotide reductase [26,27], spore photoproduct lyase [28-30], HydE and HydG [31], biotin synthase [32-34], lipoyl synthase [35,36], and HemN [37] catalyze the uncoupled cleavage of AdoMet under certain conditions. In several cases, the uncoupled cleavage has been observed to be diminished or even absent when physiological reducing systems, rather than dithionite or photoreduced deazariboflavin, were used [32,33,36,37], suggesting again that reduction of the [4Fe-4S] cluster is an important control point in radical AdoMet chemistry. It was suggested in the case of LipA that uncoupling was a result of damaged enzyme, either the cluster or the protein itself, that was able to cleave AdoMet but unable to catalyze the remaining steps in the reaction; such a correlation between enzyme damage and uncoupling of AdoMet cleavage may well hold for the other radical AdoMet enzymes as well. The physiological relevance, if any, of uncoupled AdoMet cleavage remains to be established.

Concluding Remarks

The radical AdoMet enzymes utilize multiple modes to control the inherent reactivity of the reduced iron-sulfur cluster and the AdoMet sulfonium, and to sequester the resulting radical chemistry. First, there is control at the level of redox potentials, with a large negative reduction potential for the cluster preventing generation of the catalytically active [4Fe-4S]⁺ state in the absence of AdoMet. There is also a dramatic mismatch in redox potential between the cluster and AdoMet, thus preventing the rapid and complete reaction observed in the case of the model systems. Studies on lysine 2,3-aminomutase have provided insights into how this energetic barrier is overcome: by perturbation of redox potentials upon binding of AdoMet and substrate to the enzyme. It appears therefore that the catalytically active state of the cluster is generated

under physiological conditions only when AdoMet and substrate are available for reaction, and further that AdoMet and substrate binding to the enzyme perturb potentials sufficiently to allow reductive cleavage of AdoMet coupled to H atom abstraction from substrate. The protein architecture plays an essential role not only in preventing the electrophilic attack of cluster ligands that is so prominent in the reactivity of the model complexes, but also in controlling and sequestering radical chemistry in these enzymes. AdoMet is bound in place both by its coordination to the unique iron of the cluster, and by significant interactions with the protein; its presence shields the iron-sulfur cluster from solvent and thus from unwanted side reactions. Substrate binding further seals off the active site, providing a protected location for controlled generation of radical intermediates; these substrates are also held in place by numerous interactions with protein, and these interactions position the site of H atom abstraction within striking distance of the 5'-C of AdoMet, the site of the initial radical formed upon reductive cleavage of AdoMet. Intriguingly, several of these enzymes also appear to have a loop that moves into position upon substrate binding, further sealing off the active site. Finally, the detailed interactions in the active site appear to facilitate maintenance of van der Waals contacts between reacting intermediates, providing intricate control of the catalytic mechanism.

The remarkable chemistry catalyzed by these enzymes is at one level primitive, utilizing a primary carbon-centered radical, one of the most reactive species known in biology, to perform difficult chemical transformations. And yet the chemistry by which this radical is generated, and the intricate control that these enzymes apparently exercise over the radical transformations they catalyze, represents the height of elegance and sophistication in biochemistry.

REFERECES

1. Sofia HJ, Chen G, Hetzler BG, Reyes-Spindola JF, Miller NE. 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* 2001;29:1097–1106. [PubMed: 11222759]
2. Frey PA, Hegeman AD, Ruzicka FJ. The radical SAM superfamily. *Crit. Rev. Biochem. Mol. Biol* 2008;43:63–88. [PubMed: 18307109]** This is the most current general review on the radical SAM superfamily, which discusses the biochemical activities and crystal structures for the radical SAM enzymes that have been characterized to date. This review presents the enzymes according to their reactivity with SAM, either as a cofactor or substrate.
3. Walsby C, Ortillo D, Yang J, Nnyepi M, Broderick WE, Hoffman BM, Broderick JB. Spectroscopic approaches to elucidating novel iron-sulfur chemistry in the “Radical SAM” protein superfamily. *Inorg. Chem* 2005;44:727–741. [PubMed: 15859242]
4. Walsby CJ, Hong W, Broderick WE, Cheek J, Ortillo D, Broderick JB, Hoffman BM. Electron-nuclear double resonance spectroscopic evidence that S-adenosylmethionine binds in contact with the catalytically active $[4\text{Fe-4S}]^+$ cluster of pyruvate formate-lyase activating enzyme. *J. Am. Chem. Soc* 2002;124:3143–3151. [PubMed: 11902903]
5. Walsby CJ, Ortillo D, Broderick WE, Broderick JB, Hoffman BM. An anchoring role for FeS Clusters: Chelation of the amino acid moiety of S-adenosylmethionine to the unique iron site of the $[4\text{Fe-4S}]$ cluster of pyruvate formate-lyase activating enzyme. *J. Am. Chem. Soc* 2002;124:11270–11271. [PubMed: 12236732]
6. Chen D, Walsby C, Hoffman BM, Frey PA. Coordination and mechanism of reversible cleavage of S-adenosylmethionine by the $[4\text{Fe-4S}]$ center in lysine 2,3-aminomutase. *J. Am. Chem. Soc* 2003;125:11788–11789. [PubMed: 14505379]
7. Layer G, Moser J, Heinz DW, Jahn D, Schubert W-D. Crystal structure of coproporphyrinogen III oxidase reveals cofactor geometry of Radical SAM enzymes. *EMBO J* 2003;22:6214–6224. [PubMed: 14633981]
8. Berkovitch F, Nicolet Y, Wan JT, Jarrett JT, Drennan CL. Crystal structure of biotin synthase, an S-adenosylmethionine-dependent radical enzyme. *Science* 2004;303:76–79. [PubMed: 14704425]

9. Hänzelmann P, Schindelin H. Crystal structure of the S-adenosylmethionine-dependent enzyme MoaA and its implications for molybdenum cofactor deficiency in humans. *Proc. Natl. Acad. Sci. U.S.A* 2004;101:12870–12875. [PubMed: 15317939]
10. Hänzelmann P, Schindelin H. Binding of 5'-GTP to the C-terminal FeS cluster of the radical S-adenosylmethionine enzyme MoaA provides insights into its mechanism. *Proc. Natl. Acad. Sci. U.S.A* 2006;103:6829–6834. [PubMed: 16632608]* The crystal structure of MoaA with bound substrate, 5'-GTP, confirms residues involved with coordinating the substrate as well as offers insights into the ensuing radical reaction.
11. Lepore BW, Ruzicka FJ, Frey PA, Ringe D. The x-ray crystal structure of lysine-2,3-aminomutase from *Clostridium subterminale*. *Proc. Natl. Acad. Sci. U.S.A* 2005;102:13819–13824. [PubMed: 16166264]
12. Nicolet Y, Rubach J, Posewitz M, Amara P, Mathevon C, Atta M, Fontecave M, Fontecilla-Camps J: X-ray structure of the [FeFe]-hydrogenase maturase HydE from *Thermotoga maritima*. *J.Biol.Chem* 2008;283:18861–18872. [PubMed: 18400755]
13. Vey JL, Yang J, Li M, Broderick WE, Broderick JB, Drennan CL. Structural basis for glycyl radical formation by pyruvate formate-lyase activating enzyme. *Proc. Natl. Acad. Sci. U.S.A* 2008;105:16137–16141. [PubMed: 18852451]* providing the first structure of a radical SAM activase, this paper effectively demonstrates a structural basis for the direct and stereospecific H-atom abstraction from PFL-AE's substrate, Gly734 of pyruvate formate-lyase.
14. Henshaw TF, Cheek J, Broderick JB. The $[4\text{Fe-4S}]^+$ cluster of pyruvate formate-lyase activating enzyme generates the glycyl radical on pyruvate formate-lyase: EPR-detected single turnover. *J. Am. Chem. Soc* 2000;122:8331–8332.
15. Daley CJA, Holm RH. Reactivity of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-,3-}$ clusters with sulfonium cations: analogue reaction systems for the initial step in biotin synthase catalysis. *Inorg. Chem* 2001;40:2785–2793. [PubMed: 11375696]
16. Daley CJA, Holm RH. Reactions of site-differentiated $[\text{Fe}_4\text{S}_4]^{2+,+}$ clusters with sulfonium cations: reactivity analogues of biotin synthase and other members of the S-adenosylmethionine enzyme family. *J. Inorg. Biochem* 2003;97:287–298. [PubMed: 14511891]
17. Ugulava N, Frederick KK, Jarrett JT. Control of adenosylmethionine-dependent radical generation in biotin synthase: A kinetic and thermodynamic analysis of substrate binding to active and inactive forms of BioB. *Biochemistry* 2003;42:2708–2719. [PubMed: 12614166]
18. Hinckley GT, Frey PA. Cofactor dependence of reduction potentials for $[4\text{Fe-4S}]^{2+/1+}$ in lysine 2,3-aminomutase. *Biochemistry* 2006;45:3219–3225. [PubMed: 16519516]** The midpoint reduction potentials of the $[4\text{Fe-4S}]^{2+/1+}$ couple in the presence of AdoMet and other AdoMet analogues were measured by spectroelectrochemistry. This work shows that binding of AdoMet raises the reduction potential of the cluster giving reduction potentials in the midrange for ferredoxin-like $[4\text{Fe-4S}]$ clusters.
19. Ugulava NB, Gibney BR, Jarrett JT. Biotin synthase contains two distinct iron-sulfur cluster binding sites: Chemical and spectroelectrochemical analysis of iron-sulfur cluster interconversions. *Biochemistry* 2001;40:8343–8351. [PubMed: 11444981]
20. Wang SC, Frey PA. Binding energy in the one-electron reductive cleavage of S-adenosylmethionine in lysine 2,3-aminomutase, a radical SAM enzyme. *Biochemistry* 2007;46:12889–12895. [PubMed: 17944492]** This work focuses on the importance of shifting of the binding energies in the active site of LAM in the presence of AdoMet and substrate in order to lower the energy barrier necessary for reductive cleavage of AdoMet.
21. Ugulava NB, Sacanell CJ, Jarrett JT. Spectroscopic changes during a single turnover of biotin synthase: Destruction of a $[2\text{Fe-2S}]$ cluster accompanies sulfur insertion. *Biochemistry* 2001;40:8352–8358. [PubMed: 11444982]
22. Ugulava NB, Surerus KK, Jarrett JT. Evidence from Mössbauer spectroscopy for distinct $[2\text{Fe-2S}]^{2+}$ and $[4\text{Fe-4S}]^{2+}$ cluster binding sites in biotin synthase from *Escherichia coli*. *J. Am. Chem. Soc* 2002;124:9050–9051.
23. Lees NS, Chen D, Walsby C, Behshad E, Frey PA, Hoffman BM. How an enzyme tames reactive intermediates: Positioning of the active-site components of lysine 2,3-aminomutase during enzymatic turnover as determined by ENDOR spectroscopy. *J. Am. Chem. Soc* 2006;128:10145–10154. [PubMed: 16881644]** Using ENDOR to probe the active site of LAM, this group was able to

monitor the interactions of the components involved in catalysis to further discern how an enzyme controls radical intermediates.

24. Magnusson OT, Reed GH, Frey PA. Spectroscopic Evidence for the participation of an allylic analogue of the 5'-deoxyadenosyl radical in the reaction of lysine 2,3-aminomutase. *J. Am. Chem. Soc* 1999;121:9764–9765.
25. Magnusson OT, Reed GH, Frey PA. Characterization of an Allylic Analogue of the 5'-Deoxyadenosyl Radical: An Intermediate in the Reaction of Lysine 2,3-Aminomutase. *Biochemistry* 2001;40:7773–7782. [PubMed: 11425303]
26. Ollagnier S, Mulliez E, Schmidt PP, Eliasson R, Gaillard J, Deronzier C, Bergman T, Gräslund A, Reichard P, Fontecave M. Activation of the Anaerobic Ribonucleotide Reductase from *Escherichia coli*. The essential role of the iron-sulfur center for S-adenosylmethionine reduction. *J. Biol. Chem* 1997;272:24216–24223. [PubMed: 9305874]
27. Mulliez E, Padovani D, Atta M, Alcouffe C, Fontecave M. Activation of class III ribonucleotide reductase by flavodoxin: A protein radical-driven electron transfer to the iron-sulfur center. *Biochemistry* 2001;40:3730–3736. [PubMed: 11297442]
28. Chandor-Proust A, Berteau O, Douki T, Gasparutto D, Ollagnier-de-Choudens S, Fontecave M, Atta M. DNA repair and free radicals, new insights into the mechanism of spore photoproduct lyase revealed by single amino acid substitution. *J. Biol. Chem* 2008;283:36361–36368. [PubMed: 18957420]
29. Rebeil R, Nicholson WL. The subunit structure and catalytic mechanism of the *Bacillus subtilis* DNA repair enzyme spore photoproduct lyase. *Proc. Natl. Acad. Sci. U.S.A* 2001;98:9038–9043. [PubMed: 11470912]
30. Pieck JC, Hennecke U, Pierik AJ, Friedel MG, Carell T. Characterization of a new thermophilic spore photoproduct lyase from *Geobacillus stearothermophilus* (SplG) with defined lesion containing DNA substrate. *J. Biol. Chem* 2006;281:36317–36326. [PubMed: 16968710]
31. Rubach J, Brazzolotto X, Gaillard J, Fontecave M. Biochemical characterization of the HydE and HydG iron-only hydrogenase maturation enzymes from *Thermatoga maritima*. *FEBS Lett* 2005;579:5055–5060. [PubMed: 16137685]
32. Guianvarc'h D, Florentin D, Bui BTS, Nunzi F, Marquet A. Biotin synthase, a new member of the family of enzymes which uses S-adenosylmethionine as a source of deoxyadenosyl radical. *Biochem. Biophys. Res. Commun* 1997;236:402–406. [PubMed: 9240449]
33. Ollagnier-de-Choudens S, Sanakis Y, Hewitson KS, Roach P, Münck E, Fontecave M. Reductive cleavage of S-adenosylmethionine by biotin synthase from *Escherichia coli*. *J. Biol. Chem* 2002;277:13449–13454. [PubMed: 11834738]
34. Shaw N, Birch O, Tinschert A, Venetz V, Dietrich R, Savoy L. Biotin synthase from *Escherichia coli*: Isolation of an enzyme-generated intermediate and stoichiometry of S-adenosylmethionine. *Biochem. J* 1998;330:1079–1085. [PubMed: 9494071]
35. Blight S, Larue R, Mahapatra A, Longstaff D, Chang E, Zhao G, Kang P, Green-Church K, Chan M, Krzycki J. Direct charging of tRNA sub(CUA) with pyrrolysine in vitro and in vivo. *Nature* 2004;431
36. Cicchillo RM, Iwig DF, Jones AD, Nesbitt NM, Baleanu-Gogonea C, Souder MG, Tu L, Booker SJ. Lipoyl synthase requires two equivalents of S-adenosyl-L-methionine to synthesize one equivalent of lipoic acid. *Biochemistry* 2004;43:6378–6386. [PubMed: 15157071]
37. Layer G, Grage K, Teschner T, Schunemann V, Breckau D, Masoumi A, Jahn M, Heathcote P, Trautwein A, Jahn D. Radical S-adenosylmethionine enzyme coproporphyrinogen III oxidase HemN: functional features of the [4Fe-4S] cluster and the two bound S-adenosyl-L-methionines. *J. Biol. Chem* 2005;280:29038–29046. [PubMed: 15967800]

Figure 1.

Representative reactions catalyzed by the radical-AdoMet enzymes. Abbreviations used: GRE-AE, glycyl radical activating enzymes such as pyruvate formate-lyase activating enzyme; LAM, lysine 2,3-aminomutase; BioB, biotin synthase; LipA, lipoyl synthase; MoaA, molybdopterin cofactor biosynthesis enzyme; HemN, oxygen-independent coproporphyrinogen oxidase; SPL, spore photoprotect lyase; MiaB, tRNA methylthiolation enzyme; ThiC and ThiH, enzymes involved in thiamine biosynthesis, TYW1, tRNA modification enzyme; AtsB, formylglycine-generating enzyme.

Figure 2.
Common mechanistic steps proposed to be involved in the radical AdoMet enzymes.

Figure 3. Binding energetics and redox potentials in the radical AdoMet enzyme lysine 2,3-aminomutase. The blue scale indicates the changes in redox potential for the $[4\text{Fe-4S}]^{2+/+}$ couple for the cluster in the free enzyme, for the cluster with the AdoMet bound, and for the cluster with AdoMet and lysine bound. The red scale indicates the redox potential for trialkylsulfoniums such as AdoMet (at -1800 mV) and for AdoMet bound to LAM and substrate (at -990 mV). The binding of AdoMet and substrate to the active site reduces the redox potential gap to ~300 mV.

Figure 4.

Views of the X-ray crystal structures of lysine 2,3-aminomutase (LAM, left, minus its C-terminus), MoaA (center), and pyruvate formate-lyase activating enzyme (PFL-AE, right), each with both AdoMet and substrate bound. Note the location of the radical-AdoMet cluster at one end of the TIM barrel, with AdoMet and substrate effectively sealing off the active site. Note also that as the substrate size increases from left to right, the size of the lateral opening in the TIM barrel also increases.

Figure 5.
Views of the X-ray crystal structures of biotin synthase (BioB, left) and HydE (right), with the lid loop highlighted in orange.

Figure 6.

Illustration of the results of ENDOR spectroscopic studies utilizing stabilized substrate and product radical analog intermediates. In all cases, van der Waals contacts are maintained between the 5'-methyl of 5'-deoxyadenosine (dAdo) and the substrate/product radicals. Illustrations are for the substrate radicals generated upon reaction with *trans*-4,5-dehydro-L-lysine (DHLys, left), 4-thia-L-lysine (SLys, center), and the product radical generated upon equilibration of the reduced state of the enzyme with AdoMet and L- α -lysine (right).