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Mechanism of Radical Initiation in the Radical S-Adenosyl-L-methionine Superfamily

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CONSPECTUS:

The seeds for recognition of the vast superfamily of radical S-adenosyl-L-methionine (SAM) enzymes were sown in the 1960s, when Joachim Knappe found that the dissimilation of pyruvate was dependent on SAM and Fe(II), and Barker and co-workers made similar observations for lysine 2,3-aminomutase. These intriguing observations, coupled with the evidence that SAM and Fe were cofactors in radical catalysis by these enzyme systems, drew us in the 1990s to explore how Fe(II) and SAM initiate radical reactions. Our early work focused on the same enzyme Knappe had originally characterized: the pyruvate formate-lyase activating enzyme (PFL-AE). Our discovery of an iron-sulfur cluster in this enzyme, together with similar findings for other SAM-dependent enzymes at the time, led to the recognition of an emerging class of enzymes that use iron-sulfur clusters to cleave SAM, liberating the 5'-deoxyadenosyl radical (5'-dAdo●) that initiates radical reactions. A major bioinformatics study by Heidi Sofia and co-workers identified the enzyme superfamily denoted Radical SAM, now known to span all kingdoms of life with more than 100,000 unique sequences encoding enzymes that catalyze remarkably diverse reactions.

Despite the limited sequence similarity and vastly divergent reactions catalyzed, the radical SAM enzymes appear to employ a common mechanism for initiation of radical chemistry, a mechanism we have helped to clarify over the last 25 years. A reduced [4Fe-4S]⁺ cluster provides the electron needed for the reductive cleavage of SAM. The resulting [4Fe-4S]²⁺ cluster can be rereduced either by an external reductant, with SAM acting as a cosubstrate, or by an electron provided during the reformation of SAM in cases where SAM is used as a cofactor. The amino and carboxylate groups of SAM bind to the unique iron of the catalytic [4Fe-4S] cluster, placing the sulfonium of SAM in close proximity to the cluster. Surprising recent results have shown that the initiating enzymatic cleavage of SAM generates an organometallic intermediate prior to liberation of 5'-dAdo●, which initiates radical chemistry on the substrate. This organometallic intermediate, denoted **Q**, has a 5'-deoxyadenosyl moiety directly bound to the unique iron of the [4Fe-4S] cluster via the 5'-C, giving a structure that is directly analogous to the Co-(5'-C) bond of the organometallic cofactor adenosylcobalamin. Our observation that this intermediate **Q** is formed throughout the superfamily suggests that this is a key intermediate in initiating radical SAM

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Notes

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reactions, and that organometallic chemistry is much more broadly relevant in biology than previously thought.

Graphical Abstract

INTRODUCTION

In 1965, Joachim Knappe first described a role for *S*-adenosyl-L-methionine (SAM) in the pyruvate formate-lyase (PFL) reaction, which is central to anaerobic metabolism in *E. coli*.¹ In subsequent years, Knappe and co-workers fractionated the protein components required for this reaction and reported its dependence on Fe(II).^{2,3} Although Knappe could have had no idea at the time, these results were the earliest hints at what would be revealed as one of the largest enzyme superfamilies in nature, the radical SAM superfamily, composed of enzymes that use iron-sulfur clusters and SAM to initiate radical reactions of diverse types throughout all domains of life.^{4,5} In his subsequent studies on this somewhat obscure enzyme system, Knappe would provide additional prescient clues, as he identified PFL and its activating enzyme (PFL-AE),^{6,7} and showed that SAM was involved in the activation of PFL through installation of a radical at G734 of PFL (Figure 1).^{8–11}

Similar observations on lysine 2,3-aminomutase (LAM) were reported by Barker in 1970, where he described the enzyme as oxygen-sensitive and activated by Fe(II) and SAM,¹² and in 1987, Moss and Frey provided experimental evidence implicating SAM as the source of deoxyadenosyl radicals (dAdo•) during LAM catalysis (for a full list of enzymes discussed in this Review and their abbreviations, see Table 1).¹³

Radical New Roles for FeS Clusters

In this context, we began our work on PFL-AE in 1993. The original objective was to obtain a mechanistic understanding of how PFL-AE could use Fe(II) and SAM to generate the catalytically essential glycyl radical of PFL. John Kozarich kindly provided expression vectors for PFL-AE and PFL as well as helpful expert advice, and Amherst College undergraduates began the challenging work of purifying and characterizing an enzyme, PFL-AE, about which little was known. Early overexpression efforts resulted in protein that was almost entirely in inclusion bodies, making progress slow. Knappe's work indicated that PFL-AE was oxygen sensitive, but we had little in the way of anaerobic equipment in the lab, so we attempted crudely anoxic purifications using nitrogen-purged buffers. In one of these attempts, an undergraduate and technician performing the prep noticed a slightly reddish colored band on the purification column that seemed to be associated with the protein, but the color did not persist in the resulting fractions. By collecting the fractions in a

N_2 -filled glovebag, however, we were able to isolate a protein with a reddish color. The UV-visible spectrum of the purified protein was suggestive of an iron-sulfur cluster, with broad overlapping absorption features in the visible region of the spectrum that decreased in intensity upon addition of dithionite. Ultimately, chemical analysis for iron and sulfide showed that both species were present in the approximately 1:1 ratio expected for an iron-sulfur cluster, and although the total iron and sulfide content seemed low, the enzyme activity correlated with cluster content, indicating that the FeS cluster was essential for activity. Neither the as-isolated nor the dithionite-reduced enzyme was EPR-active, and resonance Raman spectroscopic experiments in collaboration with Mike Johnson revealed that as-isolated protein contained a mixture of $[\text{4Fe-4S}]^{2+}$ and $[\text{2Fe-2S}]^{2+}$ clusters, whereas the reduced enzyme contained only $[\text{4Fe-4S}]^{2+}$ clusters.¹⁴ Reduction in the presence of SAM, however, provided an EPR-active $[\text{4Fe-4S}]^+$ cluster accounting for all of the iron in the protein.¹⁴ The low iron content led us in 1997 to propose that PFL-AE bound one $[\text{4Fe-4S}]$ cluster per homodimer, although subsequent studies would show that one $[\text{4Fe-4S}]$ cluster binds per monomer.

Our recognition of PFL-AE as an iron-sulfur protein clearly placed it alongside LAM, which had been shown in 1991 by Perry Frey, George Reed, and co-workers to use an iron-sulfur cluster and SAM to initiate radical chemistry.¹⁵ Also in 1997, Fontecave and co-workers reported that the activating enzyme for the anaerobic ribonucleotide reductase of *E. coli* (aRNR-AE), which like PFL-AE catalyzes the formation of a glycy radical on its substrate protein,¹⁶ used an iron-sulfur cluster and SAM to initiate catalysis.¹⁷ In the same year, biotin synthase (BioB), which had previously been shown to bind iron-sulfur clusters,¹⁸ was reported to use SAM to generate deoxyadenosyl radicals.¹⁹ A year later, spore photoproduct lyase (SPL), a DNA repair enzyme that catalyzes the repair of a methylene-bridged thymine dimer in bacterial spores, was reported to also have an iron-sulfur cluster and utilize SAM.²⁰ Thus, in the late 1990s there was a convergence of studies showing that numerous enzymes catalyze diverse reactions but apparently with a common set of cofactors: iron-sulfur clusters and SAM. We wrote about these collective observations in a review published in 2001,²¹ a year that also marked a turning point in the field, as a bioinformatics analysis identified these enzymes and hundreds of others as belonging to a new enzyme superfamily dubbed radical SAM.⁴

During this time of transformation in the field, we were focused on delineating the detailed mechanistic role for the iron-sulfur cluster in PFL-AE. With better resources available to the project after our move to Michigan State University in 1998, we were able to carry out protein purification under more stringently anoxic conditions. This provided enzyme with higher cluster content and with most of the iron in as-isolated enzyme present in a $[\text{3Fe-4S}]^+$ cluster, as indicated by both EPR and resonance Raman spectroscopies.²² We collaborated with Vincent Huynh to use Mössbauer spectroscopy to characterize all iron species in the protein, confirming the predominance of $[\text{3Fe-4S}]^+$ clusters with lesser amounts of $[\text{2Fe-2S}]^{2+}$ and $[\text{4Fe-4S}]^{2+}$ in the as-isolated enzyme, with all cluster forms converting to $[\text{4Fe-4S}]^{2+/1+}$ upon reduction with dithionite.²³ As our ability to express and purify PFL-AE continued to improve, we ultimately developed methods by which we could isolate PFL-AE with the resting $[\text{4Fe-4S}]^{2+}$ state as the predominant cluster form.²⁴ Some of the methods we

have developed over the years to work with these challenging enzymes are described in a recent *Methods in Enzymology* article.²⁵

A major breakthrough in our mechanistic understanding of PFL-AE came when we demonstrated that the $[4\text{Fe-4S}]^+$ cluster provided the electron required for reductive cleavage of SAM to generate the glycyl radical on PFL.²⁶ This was shown by single turnover experiments in which we utilized 5-deazariboflavin-mediated photoreduction to cleanly and quantitatively reduce the $[4\text{Fe-4S}]^{2+}$ cluster to the $[4\text{Fe-4S}]^+$ state. We then “removed” excess reductant simply by putting the sample in the dark and added PFL. EPR spectroscopy showed a 1:1 correspondence between the amount of $[4\text{Fe-4S}]^+$ originally present in reduced PFL-AE, and the amount of glycyl radical formed on PFL (Figure 2).²⁶ Upon formation of the PFL glycyl radical, the $[4\text{Fe-4S}]^+$ cluster on PFL-AE was oxidized to $[4\text{Fe-4S}]^{2+}$.^{24,26} These were critical results that laid the foundation for further mechanistic studies of radical SAM enzymes, by providing a clear indication of the role of the iron-sulfur cluster in these reactions as the source of the electron required for reductive cleavage of SAM.

A Direct Interaction between SAM and a $[4\text{Fe-4S}]$ Cluster

How does the iron-sulfur cluster provide that electron to SAM? Is it by long-range electron transfer, or direct short-range redox? We were interested in examining whether there was any direct interaction between SAM and the $[4\text{Fe-4S}]$ cluster. EPR spectroscopy had revealed that the presence of SAM perturbs the EPR signal of the $[4\text{Fe-4S}]^+$ cluster,^{22,23} suggesting the potential for direct interaction. By taking advantage of the ability to oxidatively degrade the $[4\text{Fe-4S}]$ cluster to the $[3\text{Fe-4S}]^+$ state and then reconstitute it, we specifically labeled the unique iron site of the $[4\text{Fe-4S}]$ cluster with ^{57}Fe and collaborated with Vincent Huynh to characterize this unique site using Mössbauer spectroscopy. Our results revealed that, in the presence of SAM, this unique iron site underwent a significant change in its isomer shift consistent with direct coordination of SAM to the unique iron of the $[4\text{Fe-4S}]$ cluster.²⁷

Direct evidence for SAM coordination to the unique iron of the $[4\text{Fe-4S}]$ cluster, however, came from a series of experiments initially conceived by two of us (W.E.B. and B.M.H.) while sitting at an outdoor cafe in Minneapolis during the ninth International Conference on Bioinorganic Chemistry (ICBIC-9). The plan was to introduce specific isotopic labels into SAM and then look for hyperfine coupling of the introduced magnetic nuclei to the electron spin of the $[4\text{Fe-4S}]^+$ cluster. The labeled SAMs were synthesized from commercially available labeled methionine or ATP by use of SAM synthetase. Initial experiments utilized SAM labeled with ^{13}C or ^2H at the methyl, and the results provided the first unequivocal evidence that SAM binds near to the $[4\text{Fe-4S}]$ cluster.²⁸ In fact, analysis of the coupling revealed that the sulfonium of SAM must be in orbital overlap with the $[4\text{Fe-4S}]^+$ cluster, providing a plausible pathway for electron transfer to reductively cleave SAM.²⁸ We also synthesized SAM labeled at the amino with ^{15}N , at the carboxyl with ^{17}O , and at the carboxyl C1 with ^{13}C . Subsequent ENDOR measurements provided irrefutable evidence that the amino acid end of SAM directly coordinates the unique iron of the $[4\text{Fe-4S}]$ cluster of PFL-AE (Figure 3).²⁹ This work paralleled earlier studies of substrate/product coordination

to the unique Fe of the aconitase [4Fe-4S] cluster^{30,31} and was in fact the first direct demonstration that the methionyl moiety of SAM coordinates the unique iron of the [4Fe-4S] cluster in radical SAM enzymes; the following year saw the first³² of a series³³⁻³⁵ of X-ray crystal structures that corroborated this mode of interaction' showing it in other radical SAM enzymes.

Active Site Structure and Substrate Binding

In collaboration with Catherine Drennan at MIT, we used X-ray crystallography to structurally characterize PFL-AE in complex with SAM and a heptamer peptide comprising the sequence surrounding G734 in PFL.³⁶ The interaction of SAM with the [4Fe-4S] cluster was consistent with that we had previously demonstrated using ENDOR spectroscopy.^{24,28,29} The peptide substrate bound such that the central glycyl residue was poised in close proximity to SAM with the *Ca* pro-*S* hydrogen close (4.1 Å) to the 5' carbon of SAM (Figure 4). The system appeared to be perfectly set up for direct and immediate pro-*S* H atom abstraction from the glycyl residue upon reductive cleavage of SAM to generate a 5'-deoxyadenosyl radical. A structure in the absence of heptamer peptide was also obtained, but this structure had partial occupancy of SAM, and the details of SAM binding were not well-resolved. Comparison of these two structures revealed that a peptide loop undergoes a significant structural change upon peptide substrate binding, perhaps playing a role in reactions with the full PFL substrate of recognizing and extruding the G734 loop, which is buried in the interior of PFL.³⁷ Through subsequent fluorescence and EPR studies coupled with activity assay measurements, we provided direct evidence for large conformational changes in PFL during its interaction with, and activation by, PFL-AE.³⁸

During the mid-2000s, we observed variations in the EPR signals for reduced PFL-AE and its complex with SAM that were ultimately traced back to differences in buffer composition, specifically the presence of NaCl or KCl in the buffer. This led to a detailed study of the effects of monovalent cations on both the spectroscopic properties and enzymatic activity. The use of alkali metal cations from lithium to cesium, as well as ammonium, revealed a clear dependence of enzymatic activity on cation size, with K⁺ providing the highest activity and activity falling off sharply with increasing or decreasing cation size.³⁹ The EPR signals of the [4Fe-4S]⁺ cluster were significantly affected by the presence and identity of the monovalent cations in a way that reflected the effects on activity. ENDOR spectroscopy revealed hyperfine interactions between several of the monovalent cations and the [4Fe-4S]⁺ cluster and indicated that the monovalent cation was bound close to the active-site cluster in all cases.³⁹ Interestingly, reexamination of the X-ray structural data revealed evidence for a bound monovalent cation that had not been previously recognized. The monovalent cation is coordinated by two conserved aspartic acid residues as well as a backbone carbonyl and an oxygen of the carboxylate of SAM, providing a direct link between the monovalent cation, SAM, and the catalytic [4Fe-4S] cluster (Figure 5). The precise role for the monovalent cation in catalysis remains under investigation.

The intriguing and facile iron-sulfur cluster interconversions we observed with PFL-AE in vitro led us to question whether such cluster transformations might have functional significance in vivo. We grew *E. coli* cells overexpressing PFL-AE in ⁵⁷Fe-enriched

medium, subjected the culture to either aerobic or anaerobic conditions, and then harvested the cells by centrifugation.⁴⁰ Mössbauer spectroscopy showed that the iron-sulfur cluster content in PFL-AE in these whole cells was dependent on the growth conditions: cells harvested after anaerobic incubation contained primarily the [4Fe-4S] cluster, whereas cells harvested after aerobic incubation had both [2Fe-2S] and [4Fe-4S] clusters.⁴⁰ Further, the clusters could interconvert by incubating the cultures under alternating aerobic and anaerobic conditions. This suggests the possibility that cluster interconversions play a role in regulating PFL-AE activity, which is beneficial only under anaerobic conditions. Perhaps the most intriguing outcome of these studies was the observation that fully 100% of the [4Fe-4S]²⁺ cluster in PFL-AE in whole cells was valence-localized,⁴⁰ whereas in purified PFL-AE the [4Fe-4S]²⁺ cluster is valence delocalized.²³ Valence-localized [4Fe-4S] clusters are unusual in biology with the best-studied example being the ferredoxin-thioredoxin reductase. The origin and significance of valence localization in PFL-AE in vivo remains under investigation in our lab. We have been able to show that adding certain adenosyl-based molecules (including AMP, ADP, and adenosine) to purified PFL-AE causes valence localization.⁴⁰ Structural studies are underway to probe how these molecules modify the cluster environment to induce valence localization.

Other Radical SAM Enzymes

Over the years, we examined mechanistic aspects of other radical SAM enzymes as well. In 2002, we demonstrated that spore photoproduct lyase (SPL; see Table 1 for abbreviations and other information on enzymes discussed) catalyzed the repair of the UV photoproduct spore photoproduct (SP) via *direct* H atom abstraction from the C6 position of SP.⁴¹ We subsequently synthesized both the *5R*- and *5S*-spore photo-products and identified the substrate of SPL as the *5R* diastereomer;^{42,43} the fact that SPL can repair only a single diastereomer demonstrates that it, like PFL-AE, catalyzes a stereospecific H atom abstraction. We also examined the human antiviral protein viperin, showing that it binds a [4Fe-4S] cluster and catalyzes the reductive cleavage of SAM to generate methionine and 5'-deoxyadenosine, thereby identifying it as a radical SAM enzyme.⁴⁴ In our studies of the maturation of the [FeFe]-hydrogenase, we have characterized the iron-sulfur clusters and enzymatic activity of the radical SAM enzyme HydG, showing that it synthesizes CO and CN⁻ using tyrosine as a substrate,^{45,46} binds two distinct iron-sulfur clusters,⁴⁷ and catalyzes a reversible reductive cleavage of SAM even though it uses SAM as a substrate and not a cofactor.⁴⁸ In subsequent work, Britt and co-workers showed that the C-terminal iron-sulfur cluster on HydG was an unusual [5Fe-4S] cluster in which the fifth iron is coordinated by free cysteine, which bridges this iron to the [4Fe-4S] cluster.⁴⁹⁻⁵² Their evidence supports a mechanism in which the fifth iron of the cluster serves as a site for CO and CN⁻ binding to form a [Fe(CO)₂(CN)] "synthon" that is transferred to the scaffold protein HydF. We have also pursued characterization of the elusive HydE, a radical SAM enzyme thought to synthesize the dithiomethylamine ligand of the H-cluster of hydrogenase but for which no enzymatic activity has been demonstrated.⁵³

Probing Radical Intermediates

More recently we have pursued an understanding of the features that *control* radical SAM catalysis using the SAM analogue 3',4'-anhydro-*S*-adenosyl-L-methionine (anSAM).⁵⁴

AnSAM was previously synthesized in Perry Frey's lab and shown to support catalytic activity but also allowed buildup of spectroscopically observable quantities of anAdo•, the allylically stabilized analogue of the primary carbon radical 5'-dAdo•.⁵⁵ By mixing anSAM and isotopically labeled lysine substrates with reduced LAM followed by freezing in liquid nitrogen, we generated intermediate samples in which we examined the hyperfine coupling between the unpaired electron on anAdo• and the NMR-active nuclei on the substrate lysine. Analysis of ENDOR spectra of the intermediate samples demonstrated that the 5'-C of the anAdo• radical was very close to the target H atom of the lysine substrate with very little movement required for subsequent H atom abstraction. Further, the model of the intermediate state based on ENDOR spectroscopy showed that the 5'-C had moved only ~0.6 Å toward the target H atom, a distance that could be accounted for simply by the S-C bond cleavage. Altogether, our data pointed to a tightly constrained active site in which the intermediate anAdo•, and by analogy the 5'-dAdo•, were controlled by van der Waals interactions that effectively target the reactive primary radical to the C(3)-H, preventing unwanted side reactions. In contrast, the 5'-dAdo• generated by adenosylcobalamin (AdoCbl or B₁₂) radical enzymes, which catalyze reactions analogous to those of radical SAM enzymes, are *less* geometrically constrained as more movement is required to enable the C5' radical to reach the target H atom (Figure 6).

We postulate that this difference in radical constraint in the radical SAM vs AdoCbl enzymes may have contributed to the preponderance of radical SAM enzymes in biology with only a few AdoCbl radical enzymes found in a limited number of organisms.

A Novel Organometallic Intermediate

Our early interest in the mechanism of radical initiation and intermediates in radical SAM enzymes led us to carry out rapid freeze-quench (RFQ) experiments on PFL-AE/PFL in collaboration with Vincent Huynh. We trapped a species at 500 ms with an unusual EPR signal that did not appear to be the putative 5'-dAdo• intermediate. Recently, we followed up on these early RFQ experiments, providing exciting new insights into the early steps of radical initiation. ENDOR studies of the 500 ms radical using samples specifically isotopically labeled both in SAM and the [4Fe-4S] cluster revealed this novel species, which we have called **Ω**, to be an organometallic complex in which the 5'-C of a SAM-derived deoxyadenosyl moiety is directly bound to the unique iron of the [4Fe-4S] cluster in the PFL-AE active site (Figure 7, left).^{56,57} Further, we showed that **Ω** could convert directly to the PFL glycyl radical in a frozen solid upon annealing at temperatures of 170-220 K, indicating that **Ω** is a reaction intermediate (Figure 8).⁵⁶ The progression from **Ω** to PFL glycyl radical must occur via homolytic cleavage of the Fe-C(5') of **Ω** to generate a 5'-dAdo• intermediate that abstracts a H atom from G734 of PFL; formation of **Ω**, therefore, precedes the liberation of the 5'-dAdo• radical that was explored using anSAM as described above.

The similarities between **Ω** and AdoCbl, the only other cofactor in nature known to liberate 5'-deoxyadenosyl radicals, is striking (Figure 7). Both are organometallic complexes in which the 5'-C of an adenosyl moiety is directly bound to a metal ion: the unique iron of a [4Fe-4S]³⁺ cluster in the case of **Ω**, and the Co(III) of cobalamin in the case of

adenosylcobalamin (Figure 7). In both cases, homolytic M-C(5') bond cleavage yields the one-electron reduced metal center ([4Fe-4S]²⁺ and Co(II), respectively) and a 5'-deoxyadenosyl radical (5'-dAdo•). Once generated, the 5'-dAdo• in both cases abstracts a hydrogen atom from the substrate. The stabilities of the metal-carbon bond in these two species are quite different with AdoCbl being a stable cofactor at ambient temperatures and **Q** being a transient intermediate that reacts at less than 200 K, but the parallels in structure and reactivity suggest how useful nature has found the organometallic metal-adenosyl species as a precursor for initiating difficult radical reactions.

To determine whether **Q** is widespread in the radical SAM superfamily or a feature unique to only PFL-AE, we carried out similar RFQ-EPR experiments on six additional radical SAM enzymes that spanned the known structural and functional diversity of the superfamily. Remarkably, we found that *all* of the enzymes tested formed **Q**, suggesting that this novel intermediate is ubiquitous throughout the superfamily and indeed central to the radical initiation mechanism (Figure 9).

The indication that **Q** plays a central mechanistic role in radical initiation in the vast radical SAM superfamily is a stunning finding that dramatically expands the scope of organometallic chemistry in biology to encompass this large superfamily spanning all kingdoms of life. It is particularly surprising given the mechanistic paradigm that had developed over the previous 20 years, wherein the reductive cleavage of SAM leads to 5'-dAdo• formation, which promptly abstracts a H atom from the substrate. Instead, our results require a paradigm shift to a mechanism involving **Q** as a cofactor *and* intermediate, analogous to but more reactive than AdoCbl. Key questions remain as to how and why **Q** forms in radical SAM reactions. Regardless, we view **Q** formation as a *direct mechanistic consequence* of the reductive cleavage process.

CONCLUDING REMARKS

Radical SAM enzymes have emerged in the last 20 years as one of the largest superfamilies in all of biology with the number of unique sequences at over 100,000 and continuing to grow through ongoing bioinformatics efforts.^{58,59} They are found in all domains of life and catalyze diverse reactions in key processes such as glucose metabolism, ribonucleotide reduction, DNA repair, the antiviral response, and cofactor and antibiotic synthesis with new functions and new chemistry continuing to be found.⁵ The mechanism by which iron and SAM could initiate radical reactions that are similar to those catalyzed by AdoCbl enzymes was and continues to be an intriguing question. We have shown that radical initiation in radical SAM enzymes involves novel iron-sulfur cluster chemistry. First, a site-differentiated [4Fe-4S] cluster serves as a site for coordination of SAM as well as a redox cofactor to provide an electron to promote SAM cleavage. Most recently, we have demonstrated that radical initiation proceeds through a novel organometallic species (**Q**) in which the adenosyl moiety derived from SAM is covalently bound to the unique iron of the [4Fe-4S] cluster. The intermediate **Q** is remarkably similar to AdoCbl, and like AdoCbl generates a 5'-dAdo• by homolytic metal—carbon bond cleavage; these similarities suggest that nature has selected for metal-adenosyl species as an effective and versatile means to initiate radical catalysis in biology. Given that radical SAM enzymes are thought to be evolutionarily

ancient, we suggest that Ω was the original metal-adenosyl species in biocatalysis with the more biosynthetically complex AdoCbl evolved later for certain specialized situations. The observation of Ω broadly throughout the radical SAM superfamily further indicates that organometallic chemistry, once viewed as a niche aspect of biocatalysis, is in fact a major component of bioinorganic chemistry.

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Biographies

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Brian M. Hoffman received a Ph.D. from Caltech (Harden McConnell) and did postdoctoral research at MIT (Alex Rich). He is a long time member of the Northwestern faculty, where his early career was shaped by interactions with Fred Basolo, Jim Ibers, and Robert Burwell. He shares his time between Chemistry and, with his wife Janet, watching their children and grandchildren blossom.

Joan B. Broderick received a B.S. in Chemistry from Washington State University in 1987 and a Ph.D. from Northwestern University in 1992 with Tom O'Halloran. After postdoctoral research with JoAnne Stubbe at MIT, she became Assistant Professor at Amherst College in 1993, moved to Michigan State University in 1998, and to Montana State University in 2005, where she is currently Professor and Head of the Department of Chemistry and Biochemistry.

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Figure 1.

Activation of PFL by PFL-AE utilizes the reductive cleavage of SAM to carry out a stereospecific H atom abstraction from G734 of PFL.

Figure 2.

Role of the [4Fe-4S] cluster of PFL-AE was demonstrated via single turnover experiments. PFL-AE was reduced to the [4Fe-4S]⁺ state by photoreduction, and SAM was added. The EPR spectra on the left show the increasing intensity of the [4Fe-4S]⁺ signal with increasing time of photoreduction up to 30 min. In the dark, to eliminate the source of reductant, PFL was added, resulting in the spectra shown on the right, which are due to the glycyl radical on PFL. There was a 1:1 correspondence between the amount of [4Fe-4S]⁺ on PFL-AE to begin, and the amount of glycyl radical generated on PFL, demonstrating that the [4Fe-4S]⁺ provides the electron necessary for the reductive cleavage of SAM to generate the glycyl radical. Adapted with permission ref 26. Copyright 2000, American Chemical Society.

Figure 3.
Coordination of SAM via the amino and carboxylate groups to the unique iron of the [4Fe-4S] cluster as first demonstrated using ENDOR studies of PFL-AE.

Figure 4.

View of the active site of PFL-AE as determined using X-ray crystallography. SAM (green) coordinates the unique iron of the [4Fe-4S] cluster, as previously demonstrated using ENDOR spectroscopy. The peptide substrate (teal) binds such that the G734 is poised for H atom abstraction. Adapted with permission from ref 36. Copyright 2008, National Academy of Sciences.

Figure 5. Sodium ion (teal) bound in the monovalent cation binding site of PFL-AE. The ion is coordinated by two conserved aspartate residues (orange) and an oxygen of the carboxylate of SAM (purple).

Figure 6.

Geometric arrangement in a radical SAM enzyme (LAM) compared to that in a B₁₂ radical enzyme (ethanolamine ammonia lyase, EAL). Whereas little movement is required for H atom abstraction in LAM, a significant movement of the 5'C is required in EAL. Adapted with permission from ref 54. Copyright 2015, American Chemical Society.

Figure 7.

Intermediate Ω (left) is similar to the adenosylcobalamin cofactor (right) employed by B_{12} radical enzymes. Adapted with permission from ref 57. Copyright 2018, American Chemical Society.

Figure 8.

Conversion of Ω to the glycy radical of PFL ($G\bullet$) by annealing at the indicated temperatures for the time shown. Quantification of the signals demonstrates quantitative conversion from Ω to $G\bullet$. Reproduced with permission from ref 56. Copyright 2016, American Association for the Advancement of Science.

Figure 9.

Normalized EPR spectra of Ω formed in RS reactions freeze-quenched at 500 ms after mixing RS enzyme with (substrate + SAM). RS enzymes are PFL-AE, the hydrogenase maturase HydG, the epimerases PoyD and OspD, the anaerobic ribonucleotide reductase activating enzyme (RNR-AE), LAM, and the DNA repair enzyme spore photoproduct lyase (SPL). Conditions: frequency, 9.375 GHz; modulation amplitude, 10 G; $T = 40$ K.

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Table 1

Enzymes Discussed in This Review

enzyme name	abbreviation	type of reaction or pathway	substrate	product
pyruvate formate-lyase	PFL	conversion of pyruvate to formate	pyruvate + CoA	formate + acetyl-CoA
pyruvate formate-lyase activating enzyme	PFL-AE	enzyme activation by glycylyl radical formation	inactive PFL	active PFL containing glycylyl radical
lysine 2,3-aminomutase	LAM	lysine rearrangement	α -lysine	β -lysine
anaerobic ribonucleotide reductase activating enzyme	aRNR-AE	enzyme activation by glycylyl radical formation	inactive ribonucleotide reductase	active ribonucleotide reductase containing glycylyl radical
biotin synthase	BioB	sulfur insertion into dethiobiotin	dethiobiotin	biotin
spore photoproduct lyase	SPL	DNA repair	spore photoproduct (methylene-bridged thymine dimer)	repaired TT sequence in DNA
viperin		unknown reaction during antiviral response	unknown	unknown
	HydG	[FeFe]-hydrogenase maturation	tyrosine	<i>p</i> -cresol, CO, CN ⁻
	HydE	[FeFe]-hydrogenase maturation	unknown	dithiomethylamine ligand of hydrogenase