Published in final edited form as:

Arch Biochem Biophys. 2014 March 15; 546: 64–71. doi:10.1016/j.abb.2014.01.020.

Glycyl radical activating enzymes: Structure, mechanism, and substrate interactions[☆]

Krista A. Shisler and Joan B. Broderick^{*}

Department of Chemistry & Biochemistry and the Astrobiology Biogeochemistry Research Center, Montana State University, Bozeman, MT 59717, United States

Abstract

The glycyl radical enzyme activating enzymes (GRE-AEs) are a group of enzymes that belong to the radical *S*-adenosylmethionine (SAM) superfamily and utilize a [4Fe–4S] cluster and SAM to catalyze H-atom abstraction from their substrate proteins. GRE-AEs activate homodimeric proteins known as glycyl radical enzymes (GREs) through the production of a glycyl radical. After activation, these GREs catalyze diverse reactions through the production of their own substrate radicals. The GRE-AE pyruvate formate lyase activating enzyme (PFL-AE) is extensively characterized and has provided insights into the active site structure of radical SAM enzymes including GRE-AEs, illustrating the nature of the interactions with their corresponding substrate GREs and external electron donors. This review will highlight research on PFL-AE and will also discuss a few GREs and their respective activating enzymes.

Keywords

Radical *S*-adenosylmethionine (SAM) enzyme; Glycyl radical enzyme activating enzyme (GRE-AE); Glycyl radical enzyme (GRE); Pyruvate formate lyase activating enzyme (PFL-AE)

Introduction

Radical *S*-adenosylmethionine (SAM)¹ enzymes are a large superfamily of enzymes that utilize radical chemistry to catalyze diverse reactions through a similar mechanism for radical initiation. The radical SAM enzymes utilize a [4Fe–4S] cluster that is coordinated to

[☆]This article was originally intended for publication in ABB Special Issue: Cofactor Assisted Enzymatic Catalysis. 544, (15 February 2014) <http://www.sciencedirect.com/science/journal/00039861/544>.

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^{*}Corresponding author. Address: Department of Chemistry & Biochemistry, 103 CBB, Montana State University, Bozeman, MT 59717, United States. Fax: +1 406 994 5407. jbroderick@chemistry.montana.edu (J.B. Broderick).

¹*Abbreviations used:* SAM, *S*-adenosylmethionine; dAdoH, 5'-deoxyadenosine; dAdo[•], 5'-deoxyadenosyl radical; TIM, triosephosphate isomerase; GRE-AE, glycyl radical enzyme activating enzyme; GRE, glycyl radical enzyme; PFL-AE, pyruvate formate lyase activating enzyme; PFL, pyruvate formate lyase; aRNR, anaerobic ribonucleotide reductase; Bss, benzylsuccinate synthase; Gdh, B₁₂-independent glycerol dehydratase; Hpd, 4-hydroxyphenylacetate decarboxylase; choline TMA-lyase, choline trimethylamine-lyase; aRNR-AE, anaerobic ribonucleotide reductase activating enzyme; Bss-AE, benzylsuccinate synthase activating enzyme; Gdh-AE, B₁₂-independent glycerol dehydratase activating enzyme; Hpd-AE, 4-hydroxyphenylacetate decarboxylase activating enzyme; *E. coli*, *Escherichia coli*; CoA, coenzyme A; MTA, 5'-deoxy-5'-methylthioadenosine; ACP, 3-amino-3-carboxypropyl radical; ENDOR, electron nuclear double resonance; EPR, electron paramagnetic resonance; LAM, lysine 2,3-aminomutase; XAS, X-ray absorption spectroscopy; AMP, adenosine 5'-monophosphate; ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate.

the enzyme via a conserved cysteine motif, most commonly CX₃CX₂C, that coordinates three of the four irons of the cluster. The fourth iron of the cluster is then free to bind SAM through its amino and carboxylate moieties (Fig. 1). In the reduced state, the [4Fe–4S]⁺ cluster transfers an electron to SAM, resulting in homolytic cleavage of SAM to produce methionine and the highly reactive 5'-deoxyadenosyl radical (dAdo[•]) intermediate. The dAdo[•] abstracts a hydrogen atom from substrate to produce 5'-deoxyadenosine (dAdoH) and a substrate radical (Fig. 2, blue arrow) which can be the product of the reaction or can undergo further transformation [1–3]. In addition to a common mechanism, the radical SAM enzymes exhibit a conserved fold, with the [4Fe–4S] cluster bound within a partial (α/β)₆ or full (α/β)₈ triosephosphate isomerase (TIM) barrel (Fig. 3) [1]. Other variations of the cluster binding motif [4,5] and enzyme fold [6,7] have been identified in radical SAM enzymes or radical SAM-like enzymes. SAM has also been reported to undergo alternative cleavage reactions: in a radical SAM-like enzyme [6,7] as well as one GRE–AE [8], cleavage of the S—C(γ) bond has been reported (Fig. 2, green arrow), while the radical SAM enzyme TsrM cleaves the S—C(methyl) bond of SAM but in a non-radical mechanism [9]. This review will focus on the radical SAM enzyme pyruvate formate lyase activating enzyme (PFL–AE) as well as other radical SAM enzymes that utilize SAM to abstract a hydrogen atom from a protein glycine residue, placing them in a group known as glycy radical enzyme activating enzymes (GRE–AEs).

The glycy radical enzymes: substrates for the GRE–AEs

The GRE–AEs are a subclass of radical SAM enzymes which, after the production of the dAdo[•], abstract a hydrogen atom from the alpha carbon of a highly conserved glycine residue in the enzymes known as glycy radical enzymes (GREs). The resulting glycy radical is catalytically essential for the GRE, and during GRE catalysis, it abstracts an H-atom from a conserved cysteine residue to produce a thiyl radical followed by generation of a substrate radical (Fig. 4). The GREs include pyruvate formate lyase (PFL) [10–15], anaerobic ribonucleotide reductase (aRNR) [16–21], benzylsuccinate synthase (Bss) [22–26], B₁₂-independent glycerol dehydratase (Gdh) [27–29], 4-hydroxyphenylacetate decarboxylase (Hpd) [30–32], and CutC (more recently named choline trimethylamine-lyase or choline TMA-lyase) [33,34]. Each of these enzymes have a specific activating enzyme, and current results indicate no cross-reactivity between the GRE–AE and any non-partner GRE.

Although the GREs catalyze a diverse set of reactions, they share considerable sequence and structural homology. They are most commonly homodimeric proteins with a subunit size of 80–100 kDa, although Bss and Hpd contain additional subunits [25,35,36]. The core structure of a GRE monomer consists of a 10-stranded β-barrel surrounded by α-helices (Fig. 5) [10,11,20,28,30,37]. The GREs have half-site reactivity where only one monomer is activated by its activating enzyme with the catalytic glycine residing on a finger loop with a highly conserved motif, RVXG[FWY]X_{6–8}[FL]X₄QX₂[IV]X₂R [36] and is in close proximity to the active site cysteine in the crystallized inactive state (Fig. 6). The glycy radical produced during activation is highly stable under anaerobic conditions, with a half life of more than 24 h in the case of PFL [38,39]. The radical is then transferred to a conserved cysteine, or two cysteines sequentially in the case of PFL [13,40], whereupon this

thiyl radical abstracts an H-atom from substrate to produce a substrate radical. Product is then formed through re-abstraction of an H-atom to reproduce the thiyl radical (Fig. 4). Further details on individual GREs and their cognate activating enzymes are provided in the following sections.

Pyruvate formate lyase

PFL catalyzes the reaction of pyruvate and CoA to formate and acetyl-CoA (Fig. 7), providing the sole source of acetyl-CoA for cells under anaerobic conditions [12–15]. The glycyl radical abstracts an H-atom from a catalytic cysteine, and the resulting thiyl radical is then transferred to an adjacent cysteine residue. The second thiyl radical attacks the pyruvate carbonyl carbon, cleaving the carbon–carbon bond [10,11] to form a formate anion radical and the acetyl-S-Cys-enzyme. The formate anion radical abstracts a hydrogen from the catalytic glycine and the acetyl moiety reacts with CoA to form acetyl-CoA [13–15]. Each monomer in PFL is 85 kDa and composed of a 10-stranded β -barrel surrounded by α -helices, forming an α/β barrel with the active site located in the center (Fig. 5a) [10,11]. Upon oxygen exposure, PFL is irreversibly cleaved at the glycyl radical, forming two fragments of 82 kDa and 3 kDa in size. The 14 kDa *Escherichia coli* (*E. coli*) protein YfiD can restore full activity in O₂-damaged PFL, possibly by replacement of the C-terminal 3 kDa portion through complexation with PFL [41].

Anaerobic ribonucleotide reductase

The anaerobic ribonucleotide reductase (aRNR) catalyzes the conversion of ribonucleotides to their corresponding 2'-deoxyribonucleotides through the reduction of the C2'—OH bond in the ribonucleotide (Fig. 7) [16–19,42]. The reaction is initiated by H-atom abstraction of the ribonucleotide 3'-hydrogen by the catalytic cysteine (Cys290), generating a 3'-nucleotide radical. The 2'OH is protonated by another cysteine (Cys79) and then leaves as water, generating a 3'-keto-2'-deoxynucleotide radical. This radical is reduced by formate, producing CO₂. Two electron-coupled proton transfer steps generate the 3'-deoxynucleotide radical. This radical can abstract a hydrogen from the catalytic Cys290, generating product and a thiyl radical which regenerates the glycyl radical. [16,17,19,42,43]. The complete enzyme contains a large homodimer, α_2 , with the catalytic glycine and a small homodimer, β_2 , which contains the radical SAM [4Fe–4S] cluster [21]. The β_2 homodimer is tightly bound to the α_2 homodimer under normal conditions, but has been isolated and shown to function catalytically as an activating enzyme for α_2 . Only the α_2 subunit has been crystallographically characterized, and it shows a strikingly similar topology to PFL with an α/β barrel and two opposing finger loops that meet to form the active site (Fig. 5b) [20,37]. The aRNR α_2 subunit was found to contain four conserved cysteines not needed for complexation with aRNR-AE (β_2) but are essential for glycyl radical activation of aRNR [44]. These cysteines coordinate one Zn ion per monomer which is thought to play a structural role [44,45].

Benzylsuccinate synthase

Benzylsuccinate synthase (Bss) catalyzes the formation of (*R*)-benzylsuccinate through the transfer of the methyl group of toluene to the double bond of fumarate through carbon–

carbon bond formation (Fig. 7) [22,23,25,46]. The reaction proceeds through H-atom abstraction from toluene, generating a benzyl radical. This resulting benzyl radical then adds to fumarate, generating a benzylsuccinyl radical intermediate which regenerates the cysteine thiyl radical to produce (*R*)-benzylsuccinate [23,46]. Bss contains three subunits as an $\alpha_2\beta_2\gamma_2$ heterohexamer: the 98 kDa α_2 subunit has high similarity to other GREs and the 8.5 kDa β_2 and 6.5 kDa γ_2 subunits are homologous to those in other enzymes involved in activation of hydrocarbons such as xylenes, cresols, and alkanes to form succinate adducts [24,47]. The additional β_2 and γ_2 subunits contain two [4Fe–4S] clusters possibly coordinated by conserved cysteine residues and are necessary for the structural integrity of the complete complex [24,26].

In addition to Bss containing additional clusters, its activating enzyme, Bss-AE, contains two ferredoxin-like cysteine motifs in addition to the radical SAM cluster binding site [25]. These auxiliary Fe–S clusters have become a rising theme in radical SAM enzymes [2]. These additional cluster(s) have been shown to facilitate sulfur insertion into product [48–50] or to assist in methylthiotransferase reactions as the sulfur source [51–53]. Other possible purposes for auxiliary Fe–S clusters include reducing the radical SAM [4Fe–4S] cluster [54], acting as an electron acceptor during catalysis [55], or substrate coordination [56].

B₁₂-independent glycerol dehydratase

B₁₂-independent glycerol dehydratase (Gdh) is the GRE involved in the microbial dehydration of glycerol to 3-hydroxypropionaldehyde, which is ultimately converted to 1,3-propanediol (Fig. 7) [27–29]. The reaction was originally proposed to involve the transfer of the hydroxyl group from the central carbon to a terminal carbon where the diol intermediate cleaves to produce water and 3-hydroxypropionaldehyde [28]. A recent study suggests that the active site of Gdh does not facilitate this migration of the hydroxyl group [27]. Instead, surrounding amino acids act as proton donors and acceptors where a histidine residue donates a proton to the central hydroxyl group, releasing water. Glutamate accepts a proton, forming a carbonyl group at C-1 on glycerol followed by H-atom abstraction from cysteine by the substrate to reproduce the thiyl radical [27]. This reaction takes place in a 10-stranded β -barrel surrounded by α -helices similar to PFL and aRNR with no additional subunits (Fig. 5c) [28].

The activating enzyme for Gdh, Gdh-AE, has been reported to cleave SAM not through the C5'-S bond to produce dAdo[•] and methionine but rather through an alternative bond to form 5'-deoxy-5'-methylthioadenosine (MTA) and a proposed 3-amino-3-carboxylpropyl radical (ACP) (Fig. 2) [8]. Dph2, termed a radical SAM-like enzyme because it lacks the sequence and structural characteristics of the superfamily (including the CX₃CX₂C the TIM barrel fold) but still utilizes a [4Fe–4S] cluster to carry out SAM radical chemistry, also cleaves the S—C(γ) bond of SAM to produce MTA and the proposed ACP radical [6,7]. How radical SAM enzymes control the regioselectivity of SAM cleavage is unclear but the orientation of SAM with respect to the [4Fe–4S] cluster likely plays a role [7,57]. In addition to the radical SAM cysteine binding motif, Gdh-AE contains two additional cysteine-rich domains, CX₂CX₂-CX₃C, which are ferredoxin-like [4Fe–4S] cluster binding domains [8,29]. It was

proposed that these auxiliary clusters may contribute to the altered site of SAM cleavage but this possibility is still under investigation.

4-Hydroxyphenylacetate decarboxylase

4-Hydroxyphenylacetate decarboxylase (Hpd) catalyzes the last step in the fermentation pathway of tyrosine through the decarboxylation of 4-hydroxyphenylacetate (HPA) to *p*-cresol (Fig. 7) [30–32,58,59]. From the crystal structure and substrate binding, a Kolbe-type decarboxylation reaction was proposed for Hpd [30]. The reaction is initiated through a radical transfer between the catalytic cysteine and the substrate carboxylic group and a proton transfer from the hydroxyl group of the substrate to a glutamate residue. Decarboxylation is then coupled with proton transfer from a glutamate residue (Glu637) to the phenolic hydroxyl group, producing a *p*-hydroxybenzyl radical. Glu505 transfers a proton to the catalytic cysteine which is abstracted to produce the product *p*-cresol [59]. Hpd is a heterotetramer ($\beta_4\gamma_4$) with a 100 kDa β_4 subunit containing the catalytic glycine and cysteine and a 9.5 kDa γ_4 subunit which binds two [4Fe–4S] clusters (Fig. 5d). This smaller subunit has been proposed to be involved in the regulation of the oligomeric state and the activity of the enzyme [30,32]. The N-terminal cluster is coordinated by three cysteines and a histidine while the C-terminal cluster is coordinated by four cysteines buried in the $\beta_4\gamma_4$ heterodimer interface.

Like Bss-AE and Gdh-AE, the activating enzyme for Hpd, Hpd-AE, contains two cysteine rich motifs in addition to the radical SAM [4Fe–4S] cluster which are thought to bind auxiliary Fe–S clusters. Chemically reconstituted Hpd-AE contained about 8 Fe per protein [32] and more recently 12 Fe per protein after as-isolated protein was treated with iron–sulfur cluster (ISC) assembly proteins followed by chemical reconstitution [58]. The functional roles for these additional clusters in Hpd-AE are still under investigation.

CutC

A newly characterized GRE, CutC or choline TMA-lyase, catalyzes the conversion of choline to trimethylamine (TMA) (Fig. 7) [33,34]. The reaction involves breaking a C—N bond, which is unusual for glycy radical enzymes. CutC shows active site similarity to Gdh [34]. Little characterization has been conducted on CutC or its activating enzyme, CutD, but two mechanisms for CutC catalysis have been proposed. Both involve abstraction of an H-atom from C1 of choline by a thiyl radical generated by the glycy radical, followed by migration of the trimethylamino group at C2 to C1 to produce a carbinolamine radical; the mechanistic proposals differ in the details of this migration and the release of TMA [33,34]. Further biochemical and mechanistic studies need to be conducted to establish a mechanism and structure of this novel GRE.

PFL-AE as a model for the GRE–AEs

Most of the GRE–AEs have proven difficult to study due to instability, difficulty in overexpression, lability of the iron-sulfur cluster, or other reasons. PFL-AE is the exception, and after the initial discovery of the iron–sulfur cluster in this enzyme [60], considerable

understanding of radical SAM enzymes in general, and GRE-AEs in particular, has come about via detailed studies of this enzyme.

Interaction of PFL-AE and PFL

Initial work on PFL suggested that an activase, later identified as PFL-AE, was required for catalysis [61,62]. It was later shown that the pro-S hydrogen of the PFL Gly734 was abstracted by a SAM-derived dAdo[•] during PFL activation [12]. The catalytic glycine residue in PFL, as in the other GREs, is located on a finger loop which is buried in the interior of PFL [10]. In the unactivated state of PFL characterized by crystallography, the catalytic glycine on the finger loop is positioned near an opposing loop housing the two catalytic cysteines involved in the conversion of pyruvate and CoA to formate and acetyl-CoA; we have referred to this state of PFL as the “closed state” (Fig. 6) [10,11,63]. In the “open state” of PFL, the finger loop is flipped out to interact with PFL-AE [63]. The structure of PFL-AE crystallized with a peptide portion of this finger loop of PFL showed that the finger loop is located next to SAM and the [4Fe-4S] cluster inside the partial TIM barrel of PFL-AE (Fig. 3) [64]. The catalytic glycine is positioned near SAM in preparation for H-atom abstraction. The dynamics of the open and closed state are regulated by the presence or absence of PFL-AE, with more PFL in the open state at higher concentrations of PFL-AE [63]. This conformational change is proposed to be similar in other GREs due both to their sequence and structural similarities and to their need to undergo direct H-atom abstraction in the GRE-AE active site.

Nature of the iron-sulfur cluster in PFL-AE and its interaction with SAM

The initiation of catalysis in all radical SAM enzymes is thought to occur through a common mechanism where a [4Fe-4S]²⁺ cluster is reduced by an external electron donor and the electron is then transferred from the cluster to SAM through inner-sphere electron transfer, whereupon SAM is cleaved and abstracts a hydrogen atom from substrate to produce a substrate radical. The unique coordination mode of SAM, in which the amino and carboxylate moieties of SAM chelate the unique iron of the [4Fe-4S] cluster, was first demonstrated via spectroscopic studies of PFL-AE [65–67] and was further substantiated by X-ray crystal structures of PFL-AE and other radical SAM enzymes [4,64,68–76].

Using UV-vis absorption, resonance Raman, electron paramagnetic resonance (EPR), and Mössbauer spectroscopy, PFL-AE was initially found to contain a [4Fe-4S] cluster in its active site [60,77,78]. Mössbauer studies demonstrated that the unique Fe coordination changes upon addition of SAM, and an increase in coordination number with a ligand other than sulfur was proposed [65]. EPR experiments further emphasized SAM binding to the cluster: the cluster signal converted from a rhombic to a nearly axial signal in the presence of SAM [66]. Electron nuclear double resonance (ENDOR) spectroscopic studies of PFL-AE bound to a series of SAMs containing NMR-active nuclei introduced at specific positions (e.g. the methyl carbon and hydrogens, the carboxylate oxygen, or the amino nitrogen) revealed coupling between the paramagnetic [4Fe-4S]⁺ cluster and these nuclei, demonstrating that SAM coordinates to the cluster through the amino and a carboxyl oxygen of SAM (Fig. 1) [67] and is in direct orbital overlap with the cluster [66]. This coordination

allows for the sulfonium ion to orient itself close to the unique iron in preparation for electron transfer.

In order to control uncoupled SAM cleavage, radical SAM enzymes utilize the large redox barrier between [4Fe–4S] clusters (–450 mV) and SAM (–1.8 V). This barrier of about 1.4 V or 32 kcal mol^{–1} is lowered to more favorable SAM cleavage conditions through binding of SAM to the cluster, contributing to a 19 kcal mol^{–1} decrease, and the binding of substrate, contributing to a 4 kcal mol^{–1} decrease in the case of lysine 2,3-aminomutase (LAM) [79,80]. The reduction is largely attributed to the coordination of SAM to the [4Fe–4S] cluster. Upon electron transfer, the unique iron transforms from a pentacoordinate state in the [4Fe–4S]⁺ cluster to the more favorable hexacoordinate state in the [4Fe–4S]²⁺ cluster. The sulfur of SAM is also within van der Waals contact of the Fe, facilitating inner-sphere electron transfer [79]. To investigate the interaction between SAM and the [4Fe–4S] cluster, sulfur K-edge X-ray absorption spectroscopy (XAS) and DFT calculations were utilized [81]. An increase in the intensity of the pre-edge feature with the presence of SAM was detected, which is indicative of decreased covalency of the Fe–S bonds of the cluster. The orientation of the C–S σ^* orbital in relation to the unique iron may contribute, possibly in conjunction with the polarity of the medium, to decreasing the large reduction potential barrier for the cleavage of SAM through the backbonding interactions. Control of the dielectric conditions, which are lowered upon substrate binding, could also aid in the decrease of uncoupled SAM cleavage in the absence of substrate [81]. Another contributing factor may be proximity of the sulfonium ion of SAM and the unique Fe as enzymes with a shorter Fe–S_{SAM} distance show an increase in the rate of uncouple SAM cleavage.

The state of the iron–sulfur cluster in vivo

Previously, PFL-AE had been shown to purify with a mixture of [2Fe–2S], [3Fe–4S], and [4Fe–4S] clusters [77,78] with the conversion to [4Fe–4S] clusters under reducing conditions [60,78]. In order to probe PFL-AE *in vivo*, Yang et al. investigated the clusters in *E. coli* whole cells overexpressing PFL-AE under aerobic and anaerobic conditions [82]. Under aerobic conditions, the cells contained mixed iron states in the form of [4Fe–4S] clusters, [2Fe–2S] clusters, high spin Fe^{III}, and high spin Fe^{II}, but after anaerobic incubation, the only clusters observed were [4Fe–4S] with some free Fe^{II} species. When converting back to aerobic conditions, [2Fe–2S] clusters reappeared with a decrease in [4Fe–4S] clusters [82].

While investigating the cluster conversion in whole cells, Yang et al. surprisingly found that the [4Fe–4S] clusters in the cells under anaerobic conditions were in a valence localized state [82]. Typically [4Fe–4S] clusters contain two delocalized Fe^{II}–Fe^{III} pairs; no other radical SAM enzymes have shown this valence localization event and only one other case of protein-bound [4Fe–4S] clusters in this state has been found [83]. For the [4Fe–4S] cluster of PFL-AE in whole cells, three quadrupole doublets were observed in the Mössbauer spectrum that correspond to a delocalized Fe^{II}Fe^{III} pair, a high spin Fe^{II}, and a Fe^{III} site in a 2:1:1 ratio, indicating that 100% of the [4Fe–4S] clusters were in this valence-localized state [82]. The valence localized state observed in PFL-AE in whole cells is likely to arise from altered coordination at the unique iron site of the cluster; that 100% of the cluster is in this

state indicates that whatever binds to the cluster to induce valence localization is likely an abundant small molecule. In order to explore possible inducers of valence localization, a series of small molecules including SAM degradation products (MTA, dAdoH, methionine, adenine, and ribose), molecules associated with PFL (pyruvate, CoA, and acetyl-CoA), and cellular metabolites (ATP, ADP, and AMP) were added to purified PFL-AE and the Mössbauer spectra were recorded [82]. Upon addition of MTA, dAdoH, AMP, and ADP, partial valence localization in the purified protein was observed; AMP was proposed as the most likely candidate to induce valence localization in whole cells due to its highest abundance in *E. coli* cells [82]. When SAM was added to PFL-AE containing the small molecules, complete conversion to SAM-bound clusters was observed, demonstrating that SAM is able to displace these small molecules in preparation for catalysis [82].

The reason for valence localization is still under investigation but protecting the cluster from oxidative damage and/or reactivity control are possibilities. The Fe–S clusters in radical SAM enzymes are generally highly oxygen sensitive, degrading to [3Fe–4S] and [2Fe–2S] clusters upon oxygen exposure, as discussed previously. Under aerobic conditions, these small molecules could protect the unique iron site, maintaining the [4Fe–4S] cluster which was observed after purified protein was exposed to air for 30 min [82].

Interaction with *in vivo* electron donors

In vitro studies have shown that flavodoxins can be used as external electron donors to reduce the [4Fe–4S] clusters in radical SAM enzymes and is thought to be one of the electron donors *in vivo* [84–86]. In order for flavodoxin to donate an electron to PFL-AE, flavodoxin must contain its flavin mononucleotide cofactor (FMN); even the interaction between flavodoxin and PFL-AE requires FMN, which was shown using surface plasmon resonance (SPR) experiments [87]. Using the crystal structure of PFL-AE, docking studies of PFL-AE with flavodoxin showed possible sites of interaction and essential amino acids for electron transfer. A tryptophan residue, W57, in PFL-AE is thought to facilitate the electron transfer from flavodoxin to the [4Fe–4S] cluster [87]. Sequence alignments show that other radical SAM enzymes have similar conserved regions as PFL-AE and are thought to be electron donor binding sites.

Conclusions

The GRE–AE enzymes are a unique class of radical SAM enzymes, activating a much larger protein. One of the most prominent GRE–AEs, PFL-AE, was one of the first radical SAM enzymes characterized and has provided considerable insights into the SAM-cluster interactions for this superfamily. PFL-AE has also shown unusual active site electronic structure *in vivo* that can be replicated in the presence of small molecules *in vitro*, and may provide insights into control of reactivity in the GRE–AEs. Recent studies have provided insight into how PFL-AE catalyzes direct H-atom abstraction on a buried glycine residue of PFL, and similar complex protein–protein interactions are also likely for the structurally related GRE–AE/GRE pairs described herein. It is likely that additional GREs and their cognate activating enzymes will continue to be discovered, and the understanding of PFL

and PFL-AE will provide an important foundation for elucidating their structures, mechanisms, and interactions.

Acknowledgments

Research on radical SAM activating enzymes in the Broderick laboratory is supported by the National Institutes of Health (GM54608).

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Fig. 1.
SAM coordinated to the [4Fe-4S] cluster in radical SAM enzymes.

Fig. 2.

SAM cleavage reactions. Blue: Traditional radical SAM cleavage to produce the 5'-deoxyadenosyl radical. Green: SAM cleavage to produce the proposed 3-amino-3-carboxylpropyl radical. Red: SAM cleavage to produce a methyl radical.

Fig. 3.
Crystal structure of PFL-AE with the finger loop peptide of PFL (purple sticks) and SAM (teal sticks) (PDB ID: 3CB8).

Fig. 4.
General activation reaction for GREs involving the catalytic glycine and cysteine.

Fig. 5.
Crystal structures of the GREs and their substrates (orange sticks). (A) PFL with pyruvate (PDB ID: 1H18); (B) aRNR with dGTP and Zn (purple sphere) (PDB ID: 1HK8); (C) Gdh with glycerol (PDB ID: 1R9D); (D) Hpd with HPA (PDB ID: 2YAJ).

Fig. 6. Active site of PFL. The finger loop, shown in tan, contains the catalytic glycine (purple sticks) which resides next to the two catalytic cysteines (blue sticks) in the unactivated state. Also shown is the substrate pyruvate (brown sticks) which is located next to the catalytic cysteines. The distances between the abstracted hydrogens (green) and the substrate pyruvate oxygen (red) are shown in angstroms. The radical moves along the roman numerals from glycine (I) to pyruvate (IV) (PDB ID: 1H18).

Fig. 7.
Reactions catalyzed by GREs.