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# Travels with Carbon-Centered Radicals. 5′-Deoxyadenosine and 5′-Deoxyadenosine-5′-yl in Radical Enzymology

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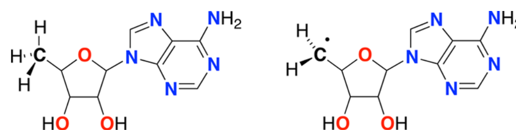
## CONSPECTUS

As a graduate student under Professor R. H. Abeles, I began my journey with 5′-deoxyadenosine, studying the coenzyme $B_{12}$ (adenosylcobalamin)-dependent dioldehydrase (DDH). I proved that suicide inactivation of dioldehydrase by glycolaldehyde proceeded with irreversible cleavage of adenosylcobalamin to 5′-deoxyadenosine. I further showed that suicide inactivation by [2-$^3$H]glycolaldehyde produced 5′-deoxy[$^3$H]adenosine, the first demonstration of hydrogen transfer to adenosyl-C5′ of adenosylcobalamin. The tritium kinetic isotope effect $^Tk$ was 15, which correlated well with the measurement $^Dk = 12$ for transformation of [1-$^2$H]propane-1,2-diol to [2-$^2$H]propionaldehyde by DDH. After establishing my own research program, I returned to the glycolaldehyde inactivation of DDH, showing by EPR that suicide inactivation produced glycolaldehyde-2-yl. In retrospect, suicide inactivation involved scission of adenosylcobalamin to 5′-deoxyadenosine-5′-yl, which abstracted a hydrogen from glycolaldehyde. Captodative-stabilized glycolaldehyde-2-yl could not react further, leading to suicide inactivation.

In 1986, my colleagues and I took up the problem of the mechanism by which lysine 2,3-aminomutase (LAM) catalyzes *S*-adenosylmethionine (SAM) and pyridoxal-5′-phosphate (PLP)-dependent interconversion of L-lysine and L-$\beta$-lysine. Because the reaction followed the pattern of adenosylcobalamin-dependent rearrangements, I postulated that SAM might be an evolutionary predecessor to adenosylcobalamin. Testing this hypothesis, we traced hydrogen transfer from lysine through the adenosyl-C5′ of SAM to $\beta$-lysine. Thus, the 5′-deoxyadenosyl of SAM mediated hydrogen transfer by LAM exactly as in adenosylcobalamin mediated hydrogen transfer in B12-dependent isomerizations. The mechanism postulated that SAM cleaves to form 5′-deoxyadenosine-5′-yl followed by abstraction of C3(H) from PLP-$\alpha$-lysine aldimine to form PLP-$\alpha$-lysine-3-yl. PLP-$\alpha$-lysine-3-yl isomerizes to pyridoxal-$\beta$-lysine-2-yl, and a hydrogen abstraction from 5′-deoxyadenosine regenerates 5′-deoxyadenosine-5′-yl and releases $\beta$-lysine. Of four radicals in the postulated mechanism, three have been characterized by EPR spectroscopy as kinetically competent intermediates.

The analysis of the role of iron allowed researchers to elucidate the mechanism by which SAM is cleaved to 5′-deoxyadenosine-5′-yl. LAM contains one [4Fe−4S] cluster ligated by three cysteine residues. As shown by ENDOR spectroscopy and X-ray crystallography, the fourth ligand to the cluster is SAM, through the methionyl carboxylate and amino groups. Inner sphere electron transfer within the $[4Fe{-}4S]^{1+}{-}$SAM complex leads to $[4Fe{-}4S]^{2+}{-}$Met and 5′-deoxyadenosine-5′-yl.

The iron-binding motif in LAM, CxxxCxxC, found by other groups in four other SAM-dependent enzymes, is the founding motif for the radical SAM superfamily. These enzymes number in the tens of thousands and are responsible for highly diverse and chemically difficult transformations in the biosphere. Available information supports the hypothesis that this superfamily provides the chemical context from which the much more structurally complex adenosylcobalamin evolved.


Carbon-based radicals have come into their own in enzymatic reaction mechanisms during recent decades. Unlike oxygen-based radicals, carbon-based radicals are not known to be toxic in cells but are unexpectedly significant as intermediates in enzymatic reactions. 5′-Deoxyadenosine and 5′-deoxyadenosine-5′-yl, the 5′-deoxyadenosyl radical (Ado-$CH_2^\bullet$) shown in Scheme 1, fuel the most recent expansion of radical enzymology.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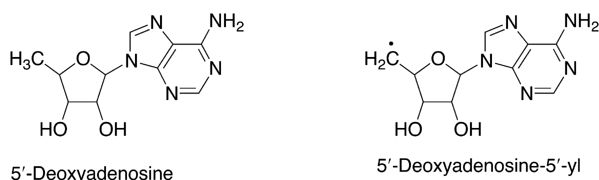
**SCHEME 1**



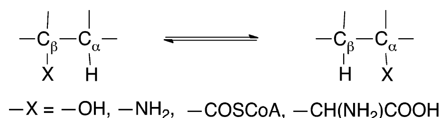

These species arise in the reactions of adenosylcobalamin-dependent enzymes and the *S*-adenosyl-L-methionine (SAM)-dependent radical SAM superfamily of enzymes. In this Account, the author delineates the discovery of 5′-deoxyadenosine in enzymology and its role and that of the 5′-deoxyadenosyl radical in enzymology.

## Propane-1,2-diol Dehydratase (Dioldehydrase)

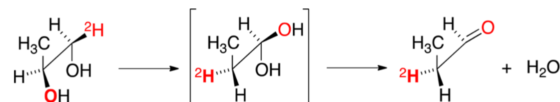
**The Catalytic Reaction.** Dioldehydrase catalyzes the dehydration of propane-1,2-diol to propionaldehyde and water (eq 1).[1] The reaction proceeds with retention of C1(H), that is, without incorporation of solvent hydrogen at C2 of propionaldehyde.[2]

$$CH_3{-}CH(OH){-}CH_2OH \rightarrow CH_3{-}CH_2{-}CHO + H_2O \quad (1)$$

The enzyme activity requires the vitamin $B_{12}$ coenzyme adenosylcobalamin, originally discovered by H. A. Barker as the coenzyme for the reaction of glutamate mutase.[3] The reaction of dioldehydrase follows the pattern of adenosylcobalamin-dependent isomerization reactions shown in eq 2.[4] In the case of dioldehydrase, —X in eq 2 is —OH, so that the immediate product is propionaldehyde hydrate, which undergoes loss of a molecule of water.




As a graduate student under Professor Robert H. Abeles, the author determined the stereochemical specificity for hydrogen transfer at C1 of propane-1,2-diol as his first assignment. He found the 1-*pro-R* hydrogen to be transferred to C2 in the reaction of (*S*)-propane-1,2-diol and the 1-*pro-S* hydrogen to be transferred in the reaction of the R-stereoisomer.[5] Thus, the highlighted deuterium in (1*R*,2*S*)-[1-$^2H_1$]propane-1,2-diol is transferred to C2, as shown in eq 3.[5,6] The rate relative to (2*S*)-propane-1,2-diol indicated a kinetic isotope effect $^Dk = 12$.[5,6] The transfer of the highlighted C2(OH) to C1 in the intermediate was elegantly proven by Rétey et al.[7]



**Suicide Inactivation.** Glycolaldehyde, very likely in its hydrated form as an analogue of propylene glycol, inactivates dioldehydrase in the first suicide inactivation, discovered by R. H. Abeles and his associates.[8] Inactivation proceeds with [*adenosyl*-$^{14}$C]adenosylcobalamin to produce a [$^{14}$C]nucleoside.[8]

The author proceeded to identify the [$^{14}$C]nucleoside produced in glycolaldehyde-dependent inactivation. He chemically synthesized 5′-deoxyadenosine and found it migrated chromatographically with and cocrystallized with the [$^{14}$C]nucleoside.[5] A UV–vis absorbance spectral shift to that of cob(II)alamin suggested homolytic scission of the Co–C bond.[8]

In his Ph.D. dissertation experiments, the author carried out inactivation of dioldehydrase with [2-$^3$H]glycolaldehyde, leading to the formation of 5′-deoxy[$^3$H]adenosine.[5] The tritium in 5′-deoxy[$^3$H]adenosine corresponded to an apparent tritium kinetic isotope effect $^Tk$ of 30. Because the [2-$^3$H]glycolaldehyde was nonstereospecifically labeled, and hydrogen transfer had been shown to be stereospecific,[5,6] the true value of $^Tk$ must have been 15. The value of $^Dk = 12$ for catalysis by dioldehydrase on (2*R*,1*R*-[$^2$H])propane-1,2-diol, and the value of 15 for $^Tk$ in suicide inactivation of glycolaldehyde were in accord with the Swain–Schaad relationship. This correlation supported the idea that hydrogen transfer in the two processes were mechanistically similar.

The [$^{14}$C]glycolaldehyde product of suicide inactivation proved to be [$^{14}$C]glyoxal after work-up in air.[8] Because glyoxal is two-electron oxidized relative to glycolaldehyde, a balanced chemical equation for the inactivation could not be written that included cob(II)alamin, a one-electron lower oxidation state than adenosylcobalamin. The author left the field of adenosylcobalamin to pursue other interests after receiving his Ph.D. degree, and Professor Abeles did not pursue this issue.

After 33 years, the author had occasion to revisit glycolaldehyde and dioldehydrase. In a collaboration with Drs. A. Abend, G. H. Reed, and V. Bandarian, EPR analysis of the inactivation solution revealed the formation of an organic paramagnetic species.[9] This was initially proposed to be the

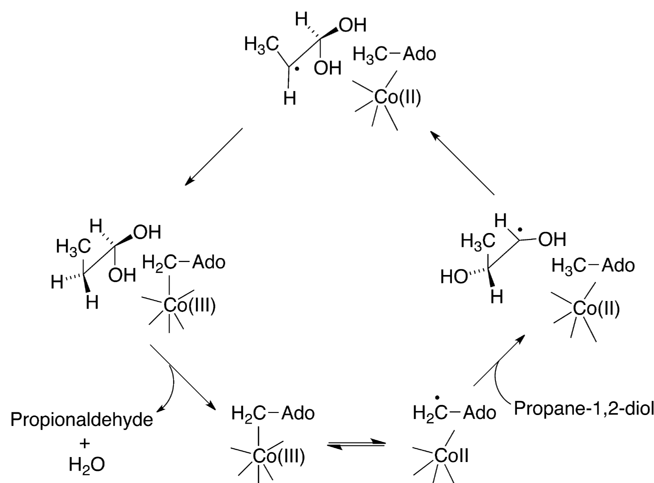
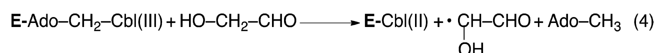



**FIGURE 1.** Radical mechanism for the action of dioldehydrase. The 5′-deoxyadenosyl radical from adenosylcobalamin abstracts C1(H) from propane-1,2-diol. The resultant radical rearranges to the hydrated radical propionaldehyde-2-yl, which abstracts a C5′(H) from 5′-deoxyadenosine to form the product and regenerate adenosylcobalamin.

*cis*-ethanesemidione radical[9] and later modified to be glycolaldehyde-2-yl, the glycolaldehyde radical.[10] Thus, the balanced chemical equation for suicide inactivation could be written as eq 4.




The glycolaldehyde radical in eq 4 is so stable, owing to the captodative effect of the $\alpha$-carbonyl (capto) and hydroxyl (dative) substituents, that the reaction cannot proceed at signficant rates either forward or reverse. This radical appears to be the first example of suicide inactivation through the formation of an ultrastable carbon-centered radical.

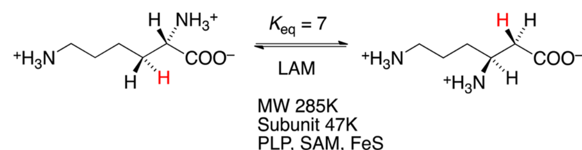
The foregoing experiments on suicide inactivation of dioldehydrase proved for the first time that a close analogue of the substrate transferred a hydrogen to 5′-deoxyadenosine derived from adenosylcobalamin, this process being accompanied by the transformation of the inactivator to a free radical.

**The Radical Mechanism of Dioldehydrase.** The author's further research as a graduate student demonstrated the same hydrogen transfer from the substrate [1-$^3$H]propane-1,2-diol to adenosyl-C5′ of adenosylcobalamin as an intermediate step in the catalytic mechanism.[5,11,12] Thus, adenosylcobalamin mediated hydrogen transfer by way of adenosyl-C5′. Upon abstracting a tritium from a substrate, the methylene group in the 5′-deoxyadenosyl moiety of adenosylcobalamin became a tritiated methyl group in 5′-deoxy[5′-$^3$H]adenosine. Any one of the hydrogen isotopes at C5′ could be transferred to the product in a later step. Subsequently, research in other laboratories confirmed hydrogen transfer mediated by adenosyl-C′-5 in reactions of the other adenosylcobalamin-dependent enzymes.[4]

Abeles and his associates discovered substrate-derived carbon-based radicals in the reaction of dioldehydrase.[13] This proved to be in accord with the weakness of the cobalt−carbon bond in adenosylcobalamin.[14,15] The propane-1,2-diol-derived radical was identified as propane-1,2-diol-1-yl.[16] These findings, when integrated with the hydrogen transfer results, allowed an overall reaction mechanism to be written involving the 5′-deoxyadenosyl radical as an intermediate, shown in Figure 1. Analogous mechanisms could be written for the other adenosylcobalamin-dependent isomerization reactions.[4]

## Lysine 2,3-Aminomutase: A SAM-Dependent Isomerase

**LAM, the Enzyme.** Lysine 2,3-aminomutase (LAM) from *Clostridium subterminale SB4*, described by H. A. Barker and his associates in 1970, catalyzes the interconversion of L-lysine and L-$\beta$-lysine according to eq 5.[17] The reaction follows the course typical of




adenosylcobalamin-dependent enzymatic isomerizations,[4] in that the C2($NH_2$) group migrates to C3, concomitant with the cross-migration of a C3(H) to C2 The internal $NH_2$/H-switch is analogous to the reaction of dioldehydrase, in which the C2(OH) group of propane-1,2-diol migrates to C1, with cross-migration of a hydrogen from C1 to C2. However, LAM does not require adenosylcobalamin. As originally described, LAM contains pyridoxal-5′-phosphate (PLP) and iron and is activated by a reducing agent and SAM.[17]

The initial report on LAM lay unexplored in the literature for 17 years until the author, in collaboration with graduate student Marcia Moss, took up a detailed analysis.[18] The author's experience in adenosylcobalamin biochemistry inspired the study of LAM. Because the biosynthesis of adenosylcobalamin required at least 24 enzymatic steps, it did not seem likely that adenosylcobalamin would have appeared in a primitive organism by incremental evolution as

a coenzyme that would convey biological survival advantage. It seemed more likely for carbon-centered radical chemistry to be available to primitive organisms and for adenosylcobalamin to evolve in the context of that chemistry. In this scenario, an evolutionary predecessor to adenosylcobalamin might have existed. The author thought that SAM, which includes the 5′-deoxyadenosyl moiety, might be such a predecessor, as implied by the structures in Figure 2.

**The Reaction Mechanism.** In the first successful test of whether SAM could function similarly to adenosylcobalamin, LAM together with [*adenosyl*-5′-$^3$H]SAM catalyzed the reaction of L-lysine to the equilibrium mixture of L-[$^3$H]lysine and L-$\beta$-[$^3$H]lysine.[18,19] These experiments proved that SAM functioned in the reaction of LAM in the very same capacity as adenosylcobalamin in coenzyme $B_{12}$-dependent reactions. All of the tritium in [*adenosyl*-5′-$^3$H]SAM could be transferred to products when LAM was in excess of [*adenosyl*-5′-$^3$H]SAM.[20] Moreover, further research with postdoctoral associate J. Baraniak using deuterated substrates proved that deuterium transfer between substrate and product proceeded both intramolecularly and intermolecularly.[20] Still further research implicated 5′-deoxyadenosine as an intermediate.[21] The hydrogen transfer properties of SAM in the reaction of LAM exactly matched those of adenosylcobalamin.

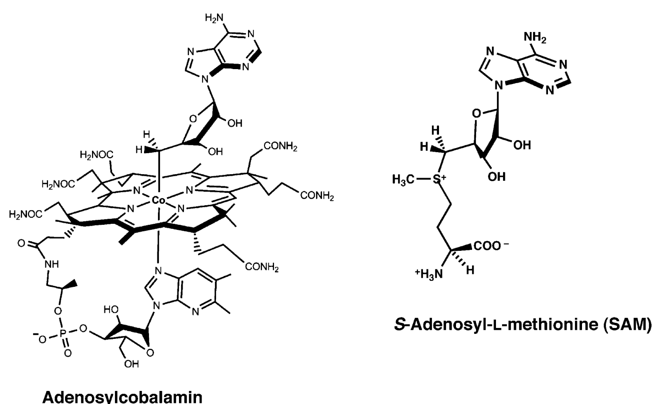



**FIGURE 2.** Structures of adenosylcobalamin and SAM.

The function of SAM, when correlated with the known chemistry of adenosylcobalamin, suggests a chemical mechanism in the reaction of LAM.[19] As shown in Figure 3, PLP exists as an internal aldimine with Lys346 of LAM.[22] L-Lysine initially undergoes transaldimination to form the conventional external aldimine with PLP. As first proposed in the mechanism of Figure 3,[19] SAM somehow undergoes homolytic cleavage between adenosyl-C5′ and sulfur, and the resultant 5′-deoxyadenosyl radical abstracts C3-hydrogen from the lysyl side chain to form 5′-deoxyadenosine and the substrate side chain C3-radical **1**. The unpaired electron at C3 of **1** undergoes addition to the imine nitrogen of the external PLP-aldimine to form the azacyclopropylcarbinyl radical **2**, with the unpaired electron residing on PLP-C4′.

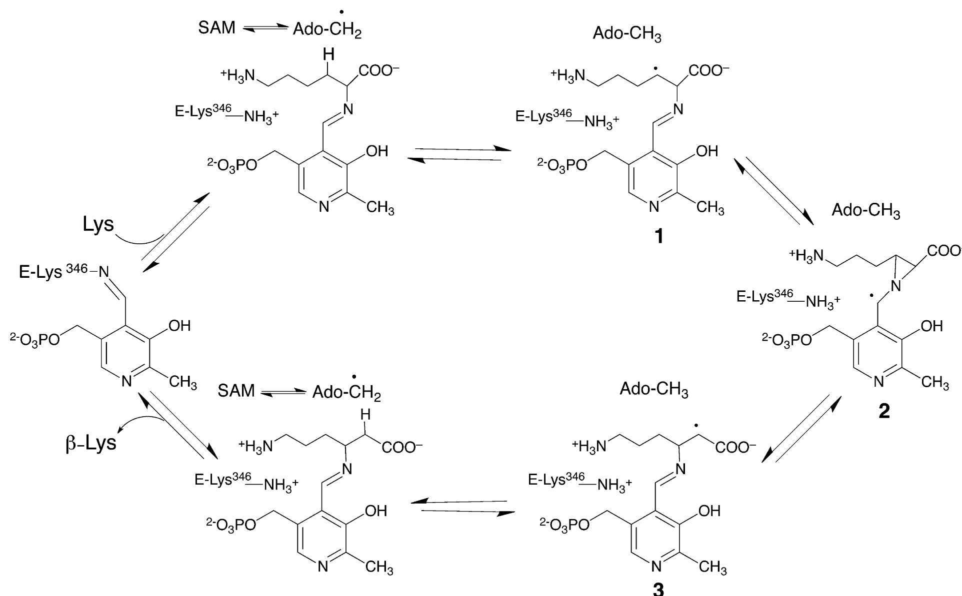



**FIGURE 3.** The chemical mechanism of the action of LAM. Upon L-lysine and SAM binding to the internal PLP-aldimine of LAM, transaldimination by L-lysine leads to the external PLP-lysyl aldimine at the active site of LAM. SAM undergoes homolytic $S^+$—C5′ cleavage to the 5′-deoxyadenosyl radical (Ado-$CH_2^•$) by a mechanism to be elucidated, initiating the radical chemistry by abstraction of C3(H). Internal cyclization of the resultant radical **1** produces radical **2**, the azacyclopropylcarbinyl-PLP radical. Ring-opening in the forward direction produces the PLP-L-$\beta$-lysine-2-yl radical **3**. Abstraction of a C5′(H) from Ado-$CH_3$ leads in two steps to L-$\beta$-lysine.

The cyclic intermediate **2** can open in either the forward or reverse direction, and in the forward direction, it forms the $\beta$-lysyl-related radical **3**. Abstraction of a hydrogen by radical **3** from the methyl group of 5′-deoxyadenosine to C2 regenerates the 5′-deoxyadenosyl radical and forms the external aldimine of PLP with L-$\beta$-lysine. The stereochemistry of H-transfer is shown in eq 5.[23] Recombination of 5′-deoxyadenosyl radical with methionine regenerates SAM, and transaldimination releases L-$\beta$-lysine to regenerate the internal PLP aldimine. The mechanism explained for the first time how SAM and PLP could catalyze a radical reaction.

In the same time frame, J. Knappe and his associates were studying pyruvate formate lyase (PFL) from *Escherichia coli*. In 1976, they found PFL to be activated by a SAM-dependent activating enzyme (PFL-AE) that cleaved SAM to adenine, hypoxanthine, 5-deoxyribose, and an unidentified product.[24,25] In 1984, they observed a PFL-radical as a product of PFL-AE, and they also identified 5′-deoxyadenosine as a product of SAM cleavage.[26] At the time, the favored interpretations of these findings were the intermediate formation of an adenosyl–Fe complex[25,26] and "formal hydride transfer" in the reductive cleavage of SAM.[24,25] The properties of LAM and PFL-A soon converged (see below).

**Characterization of the Iron in LAM.** The question of how the strong $S^+$—C5′ bond in SAM could be homolytically cleaved to form 5′-deoxyadenosine could not be answered by the known chemistry of SAM or PLP. This left iron as the prime suspect. The original report on LAM included little information about iron associated with LAM.[17] The iron content was reported to be very low; however, added ferrous iron slightly increased the activity. Activity also increased upon extended preliminary incubation with glutathione and PLP.[17]

The author and graduate student Robert Petrovich together with Dr. Frank Ruzicka and Prof. George H. Reed characterized the iron in LAM.[27,28] All steps in the purification of LAM were carried out in an anaerobic chamber. Anaerobic purification of LAM increased the iron and sulfide content 10-fold, accompanied by a corresponding increase in catalytic activity. The highly pure LAM displayed a UV–vis absorption spectrum typical of an iron–sulfur protein. Iron and sulfide analyses indicated one [4Fe–4S]-cluster per subunit in the purified enzyme. EPR analysis indicated the presence of an oxidized iron–sulfur cluster that appeared inactive but was easily reduced by glutathione and iron to an EPR-silent species, presumably a $[4Fe{-}4S]^{2+}$ cluster. Reduction with dithionite in the presence of SAM led to the EPR-positive $[4Fe{-}4S]^{1+}$ cluster and maximum catalytic activity.[29] Upon mild oxidation, the cluster lost iron, becoming a [3Fe–4S] cluster, and the enzyme became inactive. Upon addition of $Fe^{2+}$ and dithiothreitol, the $[4Fe{-}4S]^{2+}$ cluster was reconstituted.[28] The results implicated just three cysteines as ligands to iron, with one unique iron site being labile.[28]

**Identification of Radical Intermediates.** The availability of highly purified LAM fully activated by [4Fe–4S] clusters allowed mechanistic analysis to proceed. The definitive establishment of a multistep chemical mechanism requires the identification of the intermediates. Carbon-centered radical intermediates in Figure 3 are paramagnetic and potentially detectable by electron paramagnetic resonance (EPR) spectroscopy.

In collaboration with Professor George H. Reed, graduate students M. Ballinger and C. Chang in the author's laboratory succeeded in identifying radical **3** in Figure 3 as a kinetically competent intermediate,[30–32] W. Wu, S. Booker, and K. Lieder employing structural analogues of L-lysine identified steady-state radicals that are structural analogues of radical **1** in Figure 3,[33,34] and O. Magnusson using a fully functional structural analogue of SAM identified the corresponding kinetically competent allylic analogue of the 5′-deoxyadenosyl radical.[35] In the design of the structural analogues, functional groups were introduced into the structures of L-lysine or the adenosyl moiety of SAM that would not increase molecular volume but would delocalize and thereby stabilize the unpaired electron.

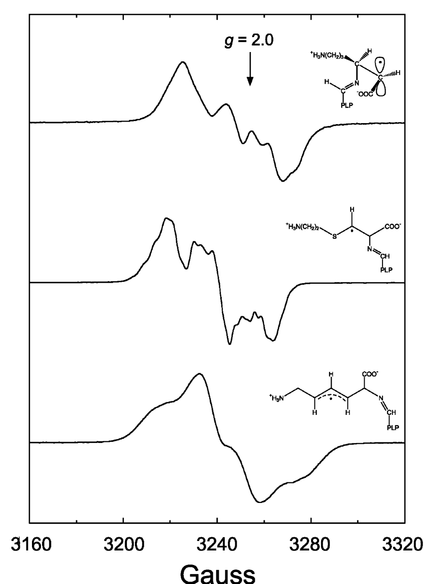
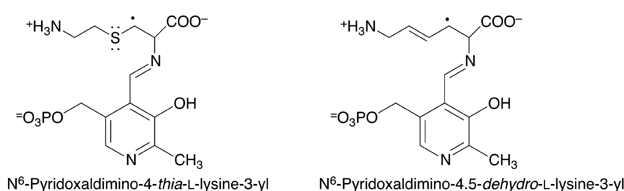



**FIGURE 4.** EPR spectra of radical **3** and analogues of radical **1** in Figure 3.

**SCHEME 2**




Radical **3** in Figure 3 appears in freeze-quenched samples of activated LAM, SAM, and L-lysine in the steady state. The upper EPR spectrum in Figure 4 is that of radical **3** in Figure 3, the product-related radical. The unpaired electron in **3** is delocalized into the carboxylate group, making it the most stable radical in the mechanism. Deuterium labeling at C3 of L-lysine narrows the radical **3** signal, and $^{13}$C3-labeling broadens the signal, in accord with the known effects of these nuclei on the coupling between the magnetic nuclei and unpaired radical electron.

The radical analogues of **1** appear in freeze-quenched samples of LAM, SAM, and either 4-thia-L-lysine[33] or *trans*-4,5-dehydro-L-lysine.[34] The EPR spectra of these radicals are shown in the central and lower spectra of Figure 4. The structures of these radicals are shown in Scheme 2. Substitution of C4(H$_2$) in L-lysine with S introduces two nonbonding electron pairs adjacent to the unpaired electron in the 4-thia analogue of radical **1**. Electron exchange between the unpaired electron on C3 and the nonbonding electron pairs on S stabilizes the radical. Thus, with 4-thia-L-lysine as the substrate, the 4-thia analogue of radical **1** in Figure 3 is the most stable radical in the mechanism and the only one observed by EPR spectroscopy in the steady state. The structure of this radical is proven by deuterium and $^{13}$C-labeling as described above for the identification of radical **3**. 4-Thia-L-lysine is a true substrate of LAM that reacts at about 3% of the rate of L-lysine; however, it reacts irreversibly because the product, 4-thia-L-$\beta$-lysine, is chemically unstable and decomposes to $\beta$-mercaptoethylamine and malonaldehyde.

Reaction of *trans*-4,5-dehydro-L-lysine with LAM and SAM leads to the 4,5-dehydro-radical and the EPR spectrum at the bottom of Figure 4. The radical at the right in Scheme 2 can be written with the unpaired electron at C4, the alternative resonance form of the allylic radical. Deuterium and $^{13}$C-labeling proves the location of the unpaired electron. This allylic radical is so stable that it does not react further. Therefore, *trans*-4,5-dehydro-L-lysine is a suicide inactivator of LAM leading to a stable radical, just as glycolaldehyde, the suicide inactivator of dioldehydrase, reacts to form a stable radical.

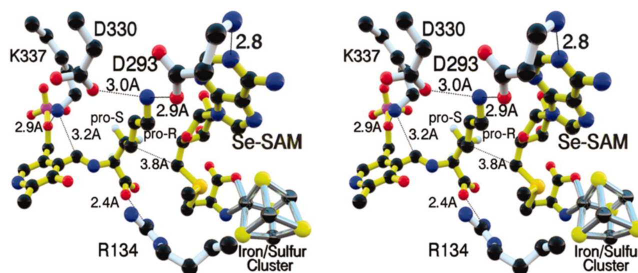
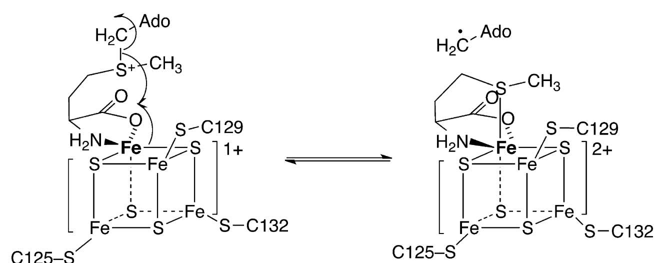



**FIGURE 5.** Active site structure of the complex of LAM, SAM, and L-lysine.[41] Reprinted from Figure 3 of ref 41, with permission from the National Academy of Sciences of the U.S.A.

**SCHEME 3**




**Reductive Cleavage of SAM to the 5′-Deoxyadenosyl Radical.** SAM dependence in the reduction of [4Fe$-$4S]$^{2+}$ to [4Fe$-$4S]$^{1+}$ implicated SAM in a direct interaction with the iron$-$sulfur cluster.[29] Postdoctoral researcher Dr. S. Booker extended research on the interaction of SAM with [4Fe$-$4S] in LAM in a collaboration with Professor Robert Scott and Dr. N. Cosper at the University of Georgia.[36] Dr. Booker synthesized the selenium analogue of SAM, SeSAM, and found it to be nearly as effective as SAM in activating LAM. He then prepared samples for selenium X-ray absorption spectroscopic experiments carried out by Drs. Cosper and Scott at the Stanford synchrotron radiation laboratory. The results implicated a direct ligand interaction between Se in SeSAM and Fe in [4Fe$-$4S]$^{2+}$ in LAM after addition of the suicide inactivator *trans*-4,5-dehydro-L-lysine.[36] This complex was an excellent analogue of intermediate radical **1** in Figure 3. The same ligation occurred upon addition of seleno-L-methionine to the complex of LAM with [4Fe$-$4S]$^{2+}$. By slight extension, these findings implicated S$-$Fe ligation in the reductive cleavage of SAM to generate the 5′-deoxyadenosyl radical.

Research on PFL-AE, alluded to above, influenced the elucidation of the mechanism by which the reductive cleavage of the S$^+$—C5′ bond in SAM takes place. J. Kozarich and his associates cloned and expressed the gene encoding the *Escherichia coli* PFL-AE.[37] J. Broderick and her associates

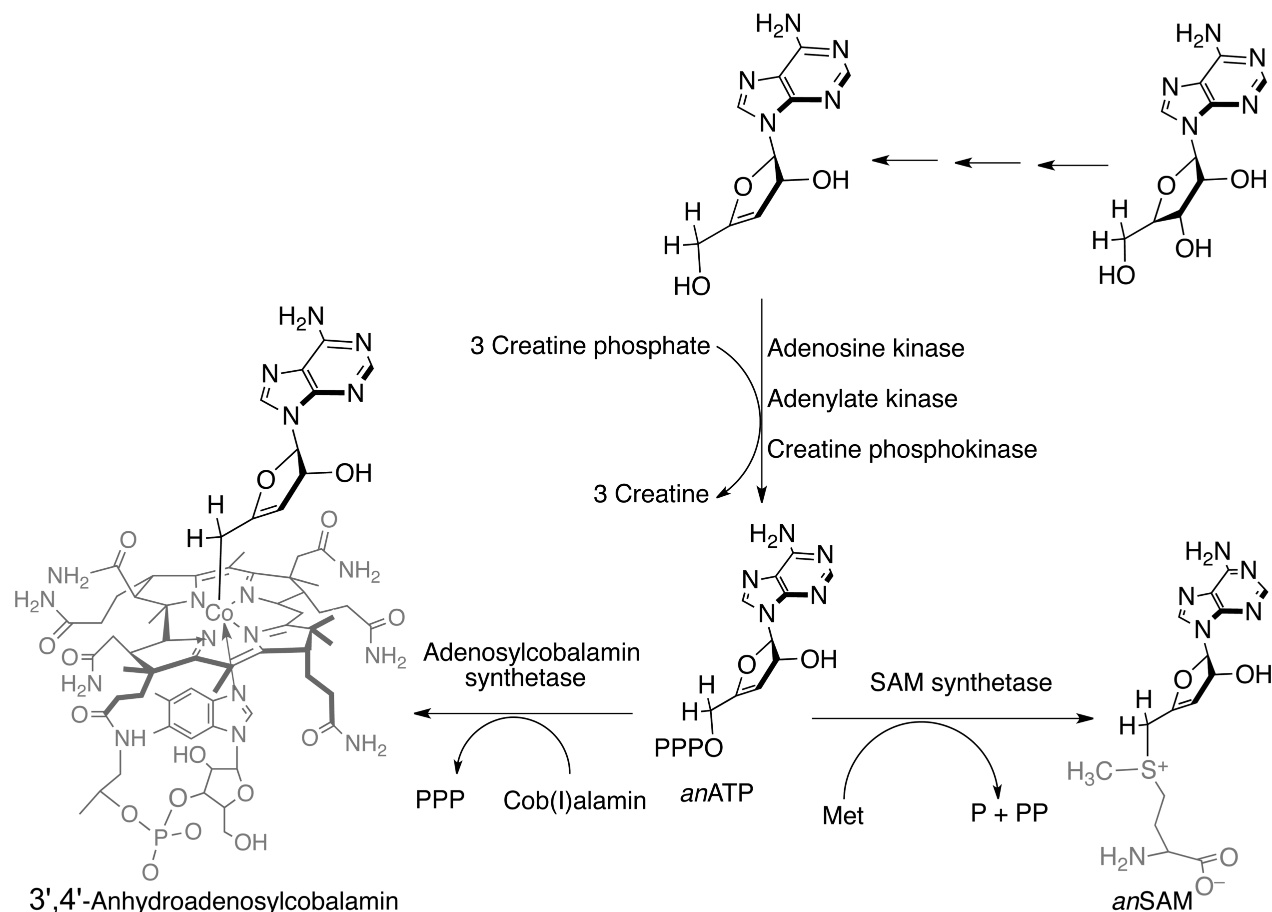



**FIGURE 6.** Synthesis of *an*SAM and 3′,4′-anhydroadenosylcobalamin. 3′,4′-Anhydroadenosine (*an*Ado) was synthesized as described[43] and enzymatically phosphorylated to *an*ATP using adenosine kinase, adenylate kinase, and creatine phosphokinase in the presence of catalytic ATP. Purified *an*ATP reacted with methionine catalyzed by SAM synthetase to *an*SAM. Similar reaction of *an*ATP with the nucleophilic cob(I)alamin was catalyzed by adenosylcobalamin synthetase.

discovered iron and sulfide in anaerobically purified PFL-AE and showed it to be a [4Fe−4S] cluster.[38] In a major mechanistic advance, Professors J. Broderick and B. Hoffman and their associates showed in $^{15}N$- and $^{13}C$-ENDOR experiments the carboxyl and amino groups of SAM ligated to the unique iron in the [4Fe−4S] cluster of PFL-AE.[39] Professor Hoffman, Dr. C. Walsby, and the author and associate Dr. D. Chen confirmed this mode of binding between SAM and [4Fe−4S] in LAM.[40] The proven ligation of SAM to the unique iron in LAM, coupled with the proof of ligation between Se of SeSAM upon reductive cleavage of SeSAM, led directly to the SAM cleavage mechanism in Scheme 3.[40]

The identities of the cysteine ligands in Scheme 3 are known from the X-ray crystal structure of LAM with SAM and L-lysine bound at the active site (Figure 5).[41] The author's senior associate, Dr. F. Ruzicka, developed crystals of this complex in an anaerobic chamber. In a collaboration with crystallographers Professor D. Ringe and B. Lepore of Brandeis University, the structure was solved by MAD phasing with the selenomethionyl derivative of LAM. In the structure, the atom closest to the selenium in SeSAM is the unique iron in the cluster, perfectly consistent with the $S^+$—C5′ cleavage mechanism in Scheme 3. The structure also shows all of the binding contacts to the L-lysine-PLP aldimine, as well as showing C3 of the lysyl side chain in very close proximity to *adenosyl*-C5′ of SAM, perfectly positioned for abstraction of C3(H) by the 5′-deoxyadenosyl radical. This structure is consistent with a rationale for regiospecific cleavage of the C5′—$S^+$-bond advanced by Professor J. Kampmeier.[42]

**The 5′-Deoxyadenosyl Radical: Physicochemical Evidence.** Much indirect information implicates the 5′-deoxyadenosyl radical in reaction mechanisms of adenosylcobalamin-dependent enzymes and LAM. Direct evidence is sparse but available. Synthesis in this laboratory of 3′,4′-*anhydro*-SAM (*an*SAM) and 3′,4′-*anhydro*-adenosylcobalamin by graduate student O. Magnusson (Figure 6) presents the opportunity to observe the allylic analogue of the 5′-deoxyadenosyl radical by EPR spectroscopy in the steady

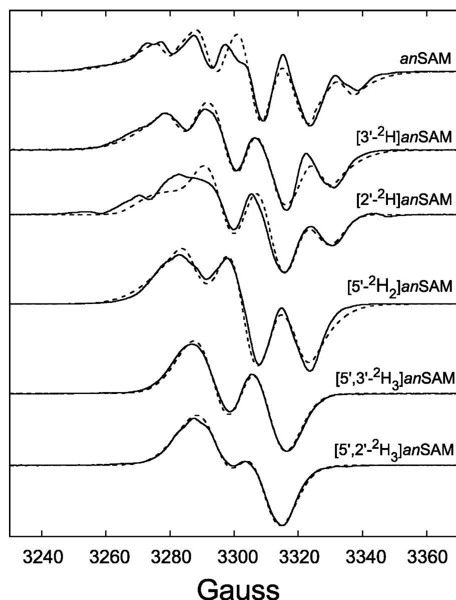


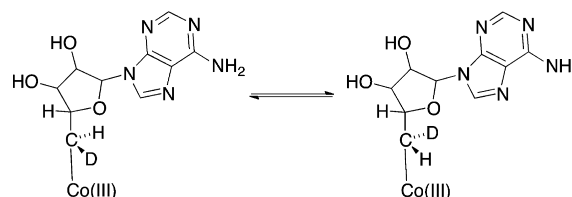

**FIGURE 7.** EPR spectroscopic identification of 3′,4′-anhydro-5′-deoxyadenosyl radical. Activation of LAM by *an*SAM followed by addition of L-lysine and freeze quenching produced the top EPR spectrum in the frozen sample. The spectrum showed at least four nuclear hyperfine couplings with hydrogen nuclei.[35] Substitution of hydrogens with deuterium narrowed the spectra in accordance with the hydrogen coupling pattern. Adapted with permission from ref 35. Copyright 2001 American Chemical Society.

state of reactions. The 3′,4′-double bond stabilizes the unpaired electron at C5′ as an allylic radical and allows the steady state concentration of the nucleoside radical to appear at levels observable by EPR. Thus, the EPR spectrum of 3′,4′-anhydro-5′-deoxyadenosyl radical appears in the steady state of the action of LAM on L-lysine.[35] *an*SAM functions perfectly well in place of SAM, but at a slower rate. The EPR spectra of this radical with deuterium labeling in the ribose ring of *an*SAM proves the structure of this radical (Figure 7), which is kinetically competent.

3′,4′-Anhydroadenosylcobalamin also functions in place of adenosylcobalamin with dioldehydrase, and the 3′,4′-*anhydro*-5′-deoxyadenosyl radical, spin-coupled with $Co^{2+}$ in cob(II)alamin, gives a prominent spin-coupled EPR pattern in the steady state.[44]

The adenosylcobalamin-dependent Class II ribonucleoside triphosphate reductase (NTPR) catalyzes the reduction of ribonucleoside triphosphates (NTPs) to 2-deoxyribonucleoside triphosphates (dNTPs) by dithiol reagent such as reduced thioredoxin or dithiothreitol. The first step is the adenosylcobalamin-dependent generation of a thiyl radical at Cys408 of NTPR, presumably through abstraction of a hydrogen from the thiol group of Cys408 by the 5′-deoxyadenosyl radical.

To test a proposal that hydrogen abstraction from Cys408 takes place by a concerted mechanism, postdoctoral associate A. Abend synthesized (5′-*S*)-[5′-$^{2}H_{1}$]adenosylcobalamin in the author's laboratory and studied its interaction with RTPR in collaboration with Professor J. Stubbe. Abend observed epimerization at C5′ according to eq 6 catalyzed by RTPR and by the specifically mutated C408A-RTPR and C408S-RTPR.




The epimers are distinguished by the NMR chemical shifts for C5′(H). Scientist D. Chen in the author's laboratory carried out detailed kinetic analyses, finding epimerization at adenosyl-C5′(H,D) to be much faster than the overall reduction of NTPs by RTPR, as did the specifically mutated C408A- and C408S-RTPR.[45] Thus, Cys408 is not required for cleavage of the Co−C5′ bond by RTPR, ruling out a concerted hydrogen transfer mechanism.

The simplest and most reasonable explanation for fast epimerization at C5′ is rapid and reversible cleavage of the Co−C5′-bond reversibly generating the 5′-deoxyadenosyl radical. The adenosylcobalamin-dependent methylmalonyl-CoA mutase also catalyzes this epimerization.[46]

**Energetics in the Cleavage of the $S^{+}$—C5′ Bond in SAM.** The mechanism of SAM cleavage in Scheme 3 described but did not explain the reductive cleavage of SAM. Unexplained was the how this process could proceed given the reduction potentials of the [4Fe−4S] cluster and the sulfonium center in SAM. This issue was solved in this laboratory by graduate student G. T. Hinckley and postdoctoral associate S. C. Wang. By direct electrochemical measurement, the reduction potential of LAM-[4Fe−4S] was found to depend on the nature of the ligand to the unique iron. With SAM as the ligand, the measured potential was −430 mV.[47] Given the reduction potential of −1800 mV for the reductive cleavage of a generic sulfonium ion, the difference signaled an intrinsic barrier of 1.4 V, corresponding to 32 kcal/mol.

The intrinsic barrier had to be decreased for the reaction to take place at an observable rate. Upon simulation of the binding of L-lysine to the LAM-[4Fe−4S] complex by substitution of L-alanine plus ethylamine, the reduction potential for [4Fe−4S] decreased to −600 mV.[48] By measurement of the $K_{eq}$ for the formation of the 3′,4′-anhydro-5′-deoxyadenosyl

radical ($an$Ado-$CH_2\cdot$) at the active site of LAM, the reduction potential for *an*SAM could be calculated. Knowing from independent experiments that $an$Ado-$CH_2^{\bullet}$ is 7 kcal/mol more stable than Ado-$CH_2^{\bullet}$, it became possible to calculate the reduction potential of SAM ligated to [4Fe−4S] at the active site of LAM. The value proved to be −990 mV, just 390 mV more negative than 600 mV. This meant that reductive SAM cleavage could occur with a $K_{eq} \approx 10^{-5}$ M, that is, over a barrier of ~9 kcal/mol.[48]

**The Radical SAM Superfamily.** In the 1990s, biotin synthase and lipoyl synthase, which catalyze the insertion of sulfur atoms into alkanyl groups, were found to require SAM and to cleave it to 5′-deoxyadenosine.[49,50] The activase for Class III anaerobic ribonucleotide reductase also required SAM and cleaved it in a process similar to PFL-AE.[51] The amino acid sequences of these enzymes and of LAM were available by 2000.[52] H. J. Sofia and her associates examined the sequences and found all to include the sequence motif CxxxCxxC, the three cysteine ligands of the [4Fe−4S] clusters. She searched the available genomic databases and found 600 proteins incorporating this motif.[53] She named them the radical SAM superfamily. The proteins were associated with antibiotic and vitamin biosyntheses, metabolism, DNA repair, methylation of non-nucleophilic centers, assembly of enzyme cofactors, and many unknown functions. By 2007, the genomic information allowed the identification of nearly 3000 such proteins, and by 2012, the superfamily had grown to more than more than 48000 proteins in more than 60 families. Newer functions included carbide insertion into the FeMo cluster of nitrogenase and methane formation in bacteria.[54,55] The superfamily stimulated a continuing series of reviews of enzymes catalyzing very difficult reactions by radical mechanisms initiated by SAM.[56−62]

## Conclusion

Economist-sociologist Thorsten Veblen said "The outcome of any serious research can only be to make two questions grow where only one grew before." By this standard, the search for an evolutionary predecessor of adenosylcobalamin is serious. Discovery of SAM as a predecessor opens the question of how it is cleaved to the 5′-deoxyadenosyl radical and the question of the nature of the chemical context in which it functions. Cleavage is by inner sphere electron transfer within the complex $[4Fe{-}4S]^{1+}$−SAM. The chemical context in which SAM functions survives in the form of the chemical reactions catalyzed by the radical SAM superfamily. Available information does not prove SAM as a predecessor of adenosylcobalamin. However, the reverse could not be the case. SAM participates in seven steps of adenosylcobalamin biosynthesis, and iron−sulfur clusters were among the earliest cofactors. Moreover, both the Animal and Plant Kingdoms contain radical SAM enzymes, whereas only the Animal Kingdom has vitamin $B_{12}$. Radical SAM chemistry was almost certainly the chemical context for development of adenosylcobalamin chemistry. The survival of the much more highly diverse radical SAM superfamily attests to its chemical vitality.

### BIOGRAPHICAL INFORMATION

**Perry Allen Frey** was educated in Chemistry at The Ohio State University, B.S. 1959. He was a chemist with the USPHS from 1960 to 1964. He received his Ph.D. in Biochemistry from Brandeis University in 1968 and served as Postdoctoral Fellow in Chemistry at Harvard in 1968. He was Assistant Professor, Ashland Oil Foundation Associate Professor, and Professor of Chemistry at Ohio State from 1969 to 1981. He was Professor of Biochemistry and the Robert H. Abeles Professor of Biochemistry at the University of Wisconsin—Madison in 1981−2008. He has been Professor of Biochemistry Emeritus at Wisconsin since 2008.

### FOOTNOTES

The authors declare no competing financial interest.

### REFERENCES


1 Abeles, R. H.; Lee, H. A. An intramolecular oxidation reduction requiring a vitamin B12 coenzyme. *J. Biol. Chem.* **1961**, *236*, 2347–2350.
2 Brownstein, A. M.; Abeles, R. H. *J. Biol. Chem.* **1961**, *236*, 1199–1200.
3 Barker, H. A.; Weissbach, H.; Smyth, R. D. A coenzyme containing pseudovitamin B12. *Proc. Natl. Acad. Sci. U. S. A.* **1958**, *44*, 1093–1097.
4 Frey, P. A. Cobalamin coenzymes in enzymology. In *Comprehensive Natural Products II Chemistry and Biology*; Mander, L., Liu, H.-W., Eds.; Begley, T., Vol. Ed.; Elsevier: Oxford, 2010; Vol. 7, pp 501−546.
5 Frey, P. A. Studies on the mechanism of the dioldehydrase reaction and the role of vitamin $B_{12}$ coenzyme. PhD Dissertation, Brandeis University, University Microfilms, Ann Arbor, MI, 1967, pp 16−160.
6 Zagalak, B.; Frey, P. A.; Karabatsos, G. L.; Abeles, R. H. The stereochemistry of the conversion of D and L 1,2-propanediols to propionaldehyde. *J. Biol. Chem.* **1966**, *241*, 3028–3035.
7 Rétey, J.; Umani-Ronchi, A.; Seibl, J.; Arigoni, D. Zum mechanismus der propandioldehydrase-reaction. *Experientia* **1966**, *22*, 502–503.
8 Wagner, O. W.; Lee, H. A., Jr.; Frey, P. A.; Abeles, R. H. Studies on the mechanism of action of cobamide coenzymes. Chemical properties of the enzyme-coenzyme complex. *J. Biol. Chem.* **1966**, *241*, 1751–1762.
9 Abend, A.; Bandarian, V.; Reed, G. H.; Frey, P. A. Identification of cis-ethanesemidione as the organic radical derived from glycolaldehyde in the suicide inactivation of dioldehydrase and of ethanolamine ammonia-lyase. *Biochemistry* **2000**, *39*, 6250–6257.
10 Sandala, G. M.; Smith, D. M.; Coote, M. L.; Radom, L. Suicide inactivation of dioldehydrase by glycolaldehyde and chloroacetaldehyde: an examination of the reaction mechanism. *J. Am. Chem. Soc.* **2004**, *126*, 12206–12207.
11 Frey, P. A.; Abeles, R. H. The role of the $B_{12}$ coenzyme in the conversion of 1,2-propanediol to propionaldehyde. *J. Biol. Chem.* **1966**, *241*, 2732–2733.
12 Frey, P. A.; Essenberg, M. K.; Abeles, R. H. Studies on the mechanism of hydrogen transfer in the cobamide coenzyme-dependent dioldehydrase reaction. *J. Biol. Chem.* **1967**, *242*, 5369–5377.
13 Finlay, T. H.; Valinsky, J.; Mildvan, A. S.; Abeles, R. H. Electron spin resonance studies with dioldehydrase. Evidence for radical intermediates in reactions catalyzed by coenzyme B12. *J. Biol. Chem.* **1973**, *248*, 1285–1290.
14 Halpern, J. Mechanisms of coenzyme $B_{12}$-dependent rearrangements. *Science* **1985**, *227*, 869–875.

15 Hay, B. P.; Finke, R. G. Thermolysis of the cobalt-carbon bond of adenosylcobalamin. 2. Products, kinetics, and cobalt-carbon bond dissociatin energy in aqueous solution. *J. Am. Chem. Soc.* **1986**, *108*, 4820–4829.

16 Yamanishi, M.; Ide, H.; Murakami, Y.; Toraya, T. Identification of the 1,2-propanediol-1-yl as an intermediate in adenosylcobalamin-dependent diol dehydratase reaction. *Biochemistry* **2005**, *44*, 2113–2118.

17 Chirpich, T. P.; Zappia, V.; Costilow, R. N.; Barker, H. A. Lysine 2,3-aminomutase. Purification and properties of a pyridoxal phosphate and S-adenosylmethionine-activated enzyme. *J. Biol. Chem.* **1970**, *245*, 1778–1789.

18 Frey, P. A.; Moss, M. L. S-Adenosylmethionine and the mechanism of hydrogen transfer in the lysine 2,3-aminomutase reaction. *Cold Spring Harbor Symp. Quant. Biol., Evol. Catal. Funct.* **1987**, *LII*, 571–577.

19 Moss, M. L.; Frey, P. A. The role of S-adenosylmethionine in the lysine 2,3,-aminomutase reaction. *J. Biol. Chem.* **1987**, *262*, 14859–14862.

20 Baraniak, J.; Moss, M. L.; Frey, P. A. Lysine 2,3-aminomutase. Support for a mechanism of hydrogen transfer involving S-adenosylmethionine. *J. Biol. Chem.* **1989**, *264*, 1357–1360.

21 Moss, M. L.; Frey, P. A. Activation of lysine 2,3-aminomutase by S-adenosylmethionine. *J. Biol. Chem.* **1990**, *265*, 18112–18115.

22 Chen, D.; Frey, P. A. Identification of lysine 346 as a functionally important residue for pyridoxal 5′-phosphate binding and catalysis in lysine 2,3-aminomutase from Bacillus subtilis. *Biochemistry* **2001**, *40*, 596–602.

23 Aberhart, D. J.; Gould, S. J.; Lin, H. J.; Thiruvengadam, T. K.; Weiller, B. Stereochemistry of lysine 2,3-aminomutase isolated from Clostridium subterminale strain SB4. *J. Am. Chem. Soc.* **1983**, *105*, 5461–5470.

24 Knappe, J.; Schmit, T. A Novel reaction of S-adenosyl-L-methionine correlated with the activation of pyruvate-formate lyase. *Biochem. Biophys. Res. Commun.* **1976**, *71*, 1110–1117.

25 Conradt, H.; Hohmann-Berger, M.; Hohmann, H. P.; Blaschkowski, H. P.; Knappe, J. Pyruvate formate-lyase (inactive form) and pyruvate formate-lyase activating enzyme of Escherichia coli: isolation and structural properties. *Arch. Biochem. Biophys.* **1984**, *228*, 133–142.

26 Knappe, J.; Neugebauer, F. A.; Blaschkowski, H. P.; Gänzler, M. Post-translational activation introduces a free radical into pyruvate formate-lyase. *Proc. Natl. Acad. Sci. U. S. A.* **1984**, *81*, 1332–1335.

27 Petrovich, R.; Ruzicka, F.; Reed, G. H.; Frey, P. A. Metal cofactors of lysine-2,3-aminomutase. *J. Biol. Chem.* **1991**, *266*, 7656–7660.

28 Petrovich, R. M.; Ruzicka, F. J.; Reed, G. H.; Frey, P. A. Characterization of iron-sulfur clusters in lysine 2,3-aminomutase by electron paramagnetic resonance spectroscopy. *Biochemistry* **1992**, *31*, 10774–10781.

29 Lieder, K. W.; Booker, S.; Ruzicka, F. J.; Beinert, H.; Reed, G. H.; Frey, P. A. S-Adenosylmethionine-dependent reduction of lysine 2,3-aminomutase and observation of the catalytically functional iron-sulfur centers by electron paramagnetic resonance. *Biochemistry* **1998**, *37*, 2578–2585.

30 Ballinger, M. D.; Reed, G. H.; Frey, P. A. An organic radical in the lysine 2,3-aminomutase reaction. *Biochemistry* **1992**, *31*, 949–953.

31 Ballinger, M. D.; Frey, P. A.; Reed, G. H. Structure of a substrate radical intermediate in the reaction of lysine 2,3-aminomutase. *Biochemistry* **1992**, *31*, 10782–10789.

32 Chang, C. H.; Ballinger, M. D.; Reed, G. H.; Frey, P. A. Lysine 2,3-aminomutase: Rapid mix-freeze-quench electron paramagnetic resonance studies establishing the kinetic competence of a substrate-based radical intermediate. *Biochemistry* **1996**, *35*, 11081–11084.

33 Wu, W.; Lieder, K. W.; Reed, G. H.; Frey, P. A. Observation of a second substrate radical intermediate in the reaction of lysine 2,3-aminomutase: A radical centered on the $\beta$-carbon of the alternative substrate, 4-Thia-L-lysine. *Biochemistry* **1995**, *34*, 10532–10537.

34 Wu, W.; Booker, S.; Lieder, K. W.; Bandarian, V.; Reed, G. H.; Frey, P. A. Lysine 2,3-aminomutase and trans-4,5-dehydrolysine: Characterization of an allylic analogue of a substrate-based radical in the catalytic mechanism. *Biochemistry* **2000**, *39*, 9561–9570.

35 Magnusson, O. T.; Reed, G. H.; Frey, P. A. 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.

36 Cosper, N. J.; Booker, S. J.; Ruzicka, F.; Frey, P. A.; Scott, R. A. Direct FeS cluster involvement in generation of a radical in lysine 2,3-aminomutase. *Biochemistry* **2000**, *39*, 15668–15673.

37 Wong, K. K.; Murray, B. W.; Lewisch, S. A.; Baxter, M. K.; Ridky, T. W.; Ulissi-DeMario, L.; Kozarich, J. W. Molecular properties of pyruvate formate-activating enzyme. *Biochemistry* **1993**, *32*, 14102–14110.

38 Broderick, J. B.; Henshaw, T. F.; Cheek, J.; Wojtuszewski, K.; Smith, S. R.; Trojan, M. R.; McGhan, R. M.; Kopf, A.; Kibbey, M.; Broderick, W. E. Pyruvate formate-lyase activating enzyme: Strictly anaerobic isolation yields active enzyme containing a $[3Fe{-}4S]^{+}$ cluster. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 451–456.

39 Walsby, C. J.; Ortillo, D.; Broderick, W. E.; Cheek, J.; Ortillo, D.; Broderick, J. B.; Hoffman, B. M. Electron-nuclear double resonance spectroscopic evidence that S-adenosylmethionine binds in contact with the catalically active $[4Fe{-}4S]^{+}$ cluster of pyruvate formate-lyase activating enzyme. *J. Am. Chem. Soc.* **2002**, *124*, 3143–3151.

40 Chen, D.; Walsby, C.; Hoffman, B. M.; Frey, P. A. Coordination and mechanism of reversible cleavage of S-adenosylmethionine by the [4Fe−4S] center in lysine 2,3-aminomutase. *J. Am. Chem. Soc.* **2003**, *125*, 11788–11789.

41 Lepore, B. W.; Ruzicka, F. J.; Frey, P. A.; 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.

42 Kampmeier, J. A. Regioselectivity in the homolytic cleavage of S-adenosylmethionine. *Biochemistry* **2010**, *49*, 10770–10772.

43 Robins, M. J.; Jones, R. A.; Mengel, A. Nucleic acid related compounds. 23. Transformation of ribonucleoside 2′,3′-O-ortho esters into unsaturated and deoxy sugar nucleosides via enol ester-substituted iodo intermediates. *J. Am. Chem. Soc.* **1976**, *98*, 8213–8217.

44 Mansoorabadi, S. O.; Magnusson, O.Th.; Poyner, R.; Frey, P. A.; Reed, G. H. Analysis of the cob(II)alamin-5′-deoxy-3′,4′-anhydroadenosyl radical triplet spin system in the active site of diol dehydrase. *Biochemistry* **2006**, *45*, 4362–4370.

45 Chen, D.; Abend, A.; Stubbe, J.; Frey, P. A. Epimerization at carbon-5′ of (5′R)-[5′-$^{2}$H] adenosylcobalamin by ribonucleoside triphosphate reductase: Cysteine 408-independent cleavage of the Co-C5′ bond. *Biochemistry* **2003**, *42*, 4578–4584.

46 Gaudemer, A.; Zylber, J.; Zylber, N.; Baran-Marszac, M.; Hull, W. E.; Fountulakis, M.; König, A.; Wölfle, K.; Rétey, J. Reversible cleavage of the cobalt-carbon bond to coenzyme B12 catalyzed by methylmalonyl-CoA mutase from Propionibacterium shermanii. The use of coenzyme $B_{12}$ stereospecifically deuterated in position 5′. *Eur. J. Biochem.* **1981**, *119*, 279–285.

47 Hinckley, G. T.; Frey, P. A. Cofactor-dependence in reduction potentials for $[4Fe\text{-}4S]^{2+/1+}$ in lysine 2,3-aminomutase. *Biochemistry* **2006**, *45*, 3219–3225.

48 Wang, S. C.; Frey, P. A. 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.

49 Miller, J. R.; Busby, R. W.; Jordan, S. W.; Cheek, J.; Henshaw, T. F.; Ashley, G. W.; Broderick, J. B.; Cronan, J. E., Jr.; Marletta, M. A. Escherichia coli LipA is a lipoyl synthase: In vitro biosynthesis of lipoylated pyruvate dehydrogenas complex from octanoyl-acyl carrier protein. *Biochemistry* **2000**, *39*, 15166–15178.

50 Florentin, D.; Bui, B. T.; Marquet, A.; Oshino, T.; Izumi, Y. On the mechanism of biotin synthase of Bacillus sphaericus. *C. R. Acad. Sci. III* **1994**, *317*, 485–488.

51 Eliasson, R.; Fontecave, M.; Jörnvall, H.; Krook, M.; Pontis, E.; Reichard, P. The anaerobic ribonucleotide reductase from Escherichia coli requires S-adenosylmethionine as a cofactor. *Proc. Natl. Acad. Sci.U. S. A.* **1990**, *87*, 3314–3318.

52 Ruzicka, F. J.; Lieder, K. W.; Frey, P. A. Lysine 2,3-aminomutase from Clostridium subterminale SB4: Mass spectral characterization of cyanogen bromide-treated peptides and cloning, sequencing, and expression of the gene kamA in Escherichia coli. *J. Bacteriol.* **2000**, *182*, 469–476.

53 Sofia, H. J.; Chen, G.; Hetzler, B. G.; Reyes-Spindola, J. F.; Miller, N. E. Radical SAM. A novel rotein superfamily linking unresolved steps in familiar biosynthetic pathways with radical mechanisms: functional characterization using new analysis and information visualization methods. *Nuleic Acids Res.* **2001**, *29*, 1097–1106.

54 Wiig, J. A.; Hu, Y.; Lee, C. C.; Ribbe, M. W. Radical SAM-dependent carbon insertion into the nitrogenase M-cluster. *Science* **2012**, *337*, 1672–1675.

55 Kamat, S. S.; Williams, H. J.; Dangott, L. J.; Chakrabarti, M.; Raushel, F. M. The catalytic mechanism for anaerobic formation of methane by bacteria. *Nature* **2013**, *497*, 132–136.

56 Booker, S. J.; Cicchillo, R. M.; Grove, T. L. Self-sacrifice in radical S-adenosylmethionine proteins. *Curr. Opin. Chem. Biol.* **2007**, *11*, 543–552.

57 Frey, P. A.; Hegeman, A. D.; Ruzicka, F. J. The radical SAM superfamily. *Crit. Rev. Biochem. Mol. Biol.* **2008**, *43*, 63–88.

58 Booker, S. J. Anaerobic functionalization of unactivated C-H bonds. *Curr. Opin. Chem. Biol.* **2009**, *13*, 58–73.

59 Roach, P. L. Radicals from S-adenosylmethionine and their application to biosynthesis. Booker, S. J. Anaerobic functionalization of unactivated C-H bonds. *Curr. Opin. Chem. Biol.* **2011**, *15*, 267–275.

60 Zhang, Q.; van der Donk, W. A.; Liu, W. Radical-mediated enzymatic methylation: a tale of two SAMS. *Acc. Chem. Res.* **2012**, *45*, 555–564.

61 Shisler, K. A.; Broderick, J. B. Emerging themes in radical SAM chemistry. *Curr. Opin. Struct. Biol.* **2012**, *22*, 701–710.

62 Fujimori, D. G. Radical SAM-mediated methylation reactions. *Curr. Opin. Chem. Biol.* **2013**, *17*, 597–604.