

A confluence of radical chemistry: S-Adenosylmethionine and Adenosylcob(III)alamin

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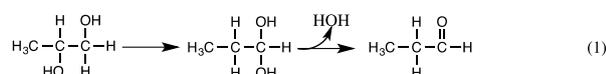
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Abstract

Enzymes requiring adenosylcobalamin (AdoCbl) or complexes of $[4Fe-4S]^{1+}$ -S-adenosylmethionine (AdoMet) function with the intermediate formation of the 5'-deoxyadenosyl radical and catalyze reactions involving carbon-centered radical intermediates. The latter form the radical SAM superfamily and are far more numerous and catalyze much more chemically diverse reactions than the AdoCbl-enzymes. The radical SAM superfamily likely preceded the AdoCbl-enzymes in evolution.

Adenosylcob(III)alamin—a Vitamin B₁₂ coenzyme

As a graduate student under R. H. Abeles in 1964-1968, the author investigated the adenosylcob(III)alamin-dependent reaction of dioldehydrase (DDH), a bacterial enzyme catalyzing the dehydration of propane-1,2-diol to propionaldehyde according to equation 1.



The reaction proceeded with internal transfer of a hydrogen from C1 to C2 and transfer of C2(OH) to C1 [1]. The mechanistic role of adenosylcob(III)alamin (AdoCbl), a Vitamin B₁₂ coenzyme, was of interest. AdoCbl had been found to include a covalent bond between Co(III) of the vitamin and C5' of the adenosyl group [2]. The cobalt-carbon bond was found to be weak and subject to homolytic scission, as illustrated in Figure 1, where 5'-dAdo• is the 5'-deoxyadenosyl radical.

The substrate analogue glycolaldehyde had been shown to inactivate dioldehydrase in the presence of AdoCbl, with irreversible cleavage of

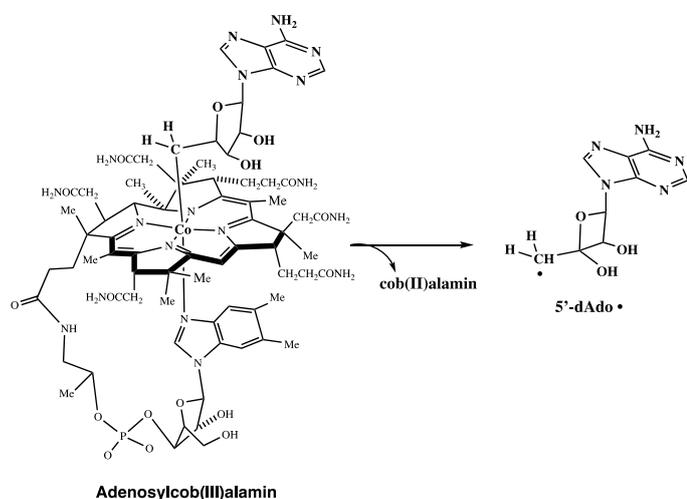
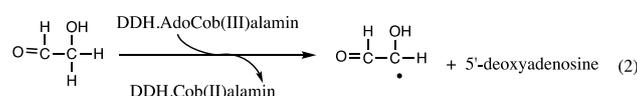


Figure 1. Homolytic cleavage of the Co-C5' bond in adenosylcobalamin to 5'-dAdo• radical

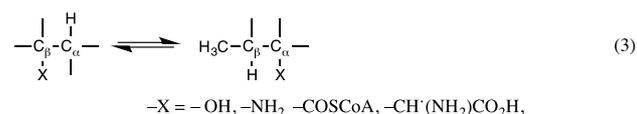
the C—Co(III) bond to form cob(II)alamin [3]. The author undertook to determine the fate of the 5'-adenosyl group and proved it to be 5'-deoxyadenosine [3], so that the inactivation could be described as in equation 2 [4]. Inactivation of DDH by glycolaldehyde proved to be the first mechanism of suicide inactivation of an enzyme by a substrate analogue to be described in chemical terms.



The author further showed that inactivation of DDH by $[2\text{-}^3\text{H}]$ glycolaldehyde led to $[^3\text{H}]5'$ -deoxyadenosine, suggesting that the 5'-deoxyadenosyl group in AdoCbl might participate in hydrogen transfer; and he proved this in the catalytic reaction by showing that the reaction of $[1\text{-}^3\text{H}]$ propane-1,2-diol as a substrate produced $[5'\text{-}^3\text{H}]$ AdoCbl at the active site of DDH [5,6]. Reaction of $[5'\text{-}^3\text{H}]$ AdoCbl with DDH and propane-1,2-diol produced $[^3\text{H}]$ propionaldehyde.

The foregoing observations led to a reaction mechanism initiated by the 5'-deoxyadenosyl radical and proceeding at the active site of DDH through a sequence of carbon-centered radicals, as depicted in Figure 2.

In addition to DDH, reactions of other AdoCbl-dependent isomerases, including glutamate mutase, methylmalonyl-CoA mutase, glycerol dehydratase, lysine 5,6-aminomutase, and ornithine 4,5-aminomutase, follow the pattern of equation 3, β -interchange of hydrogen and another group between adjacent carbon atoms (equation 3). As subsequently shown, adenosyl-C5' of AdoCbl mediates hydrogen transfer in each reaction [7].



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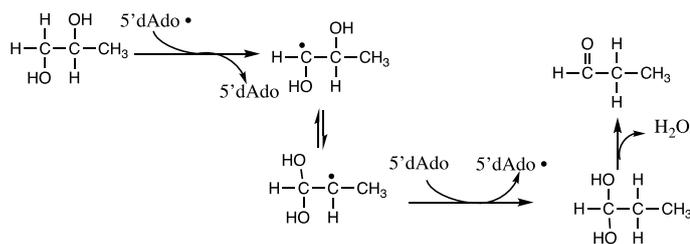


Figure 2. The chemical roles of the 5'-deoxyadenosyl radical and other carbon-centered radicals at the active site of dioldehydrase (DDH)

An exception is the AdoCbl -dependent ribonucleotide reductase [8,9], in which C5' of AdoCbl initiates chemistry by hydrogen abstraction from Cys408 [10]. Thus, hydrogen transfer is proven for chemical reactions of all AdoCbl -dependent enzymes. Substrate-radical species, as detected in the reaction of DDH [11], are intermediates in all of these reactions [7].

S-Adenosylmethionine and radical enzymology

Prior to the 1980s, AdoMet was known as the biological methylating agent, for its role in donating the methyl group to numerous substrates engaged in essential biological functions. These reactions proceeded with methyl transfer by AdoMet in a polar displacement mechanism to nucleophilic nitrogen or oxygen atoms in the substrates to form the methylated products and S -adenosylhomocysteine.

An exception to methyl transfer in the activation of pyruvate formate lyase (PFL) proved to be essential [12], in which AdoMet was cleaved by the PFL activase to 5'-deoxyadenosine [13], concomitant with the production of a radical form of PFL, identified as a glycol radical [14].

The author and his associates discovered the mechanistic role of AdoMet in the reaction of lysine 2,3-aminomutase (LAM), which had been discovered by H. A. Barker and his associates in 1970 and remained obscure in the literature until 1987 [15]. The enzyme catalyzed the interconversion of L-a-lysine and L-b-lysine according to the pattern of AdoCbl-dependent reactions (equation 3) but did not require a Vitamin B₁₂ coenzyme. LAM required AdoMet and pyridoxal-5'-phosphate, and the activity was increased by the presence of added iron and a reducing agent. To determine whether the 5'-adenosyl group of AdoMet mediated hydrogen transfer, as in AdoCbl, the action of LAM on L-lysine was examined upon activation with S-[5'-³H]adenosylmethionine [16]. This reaction produced the equilibrium mixture of L-a-[3H]lysine and L-b-[3H]-lysine, proving hydrogen transfer by the 5'-deoxyadenosyl group of AdoMet. Deuterium transfer experiments proved that hydrogen transfer took place both inter- and intramolecularly [17], as required if 5'-deoxyadenosine is an intermediate in hydrogen transfer.

The author and his associates proposed that LAM somehow produced the 5'-deoxyadenosyl radical from AdoMet, and that this radical initiated the chemical mechanism in Figure 3. Of the four carbon-centered radicals in Figure 3, three have been observed and characterized spectroscopically and shown to be kinetically competent intermediates [18-20]. Radical 3 was the dominant radical in the steady state in the reaction of L-lysine [18]. 4-Thia-L-lysine proved to be a substrate, and the 4-thia-analog of radical 1 proved to be the dominant radical in the steady state [19]. An analogue of AdoMet, S-[3',4'-anhydro]adenosylmethionine, proved to be fully functional, and the allylic analogue of 5'-dAdo•, 3',4'-anhydro-5'-Ado•, was the dominant radical in the steady state [20].

To address the question of how the 5'-deoxyadenosyl radical (5'-Ado• in Figure 3) could arise from AdoMet, the author and associates investigated the role of iron in the action of LAM. They found that LAM, purified under strictly anaerobic conditions, contained a [4Fe-4S]¹⁺ cluster that was required for activity [21,22]. Oxidation of the cluster to [3Fe-4S]²⁺ eliminated activity. Apparent cleavage of the strong S-C5' bond in AdoMet (>60 kcal mol⁻¹) required electron transfer, presumably from the [4Fe-4S]¹⁺ cluster. Like the pyruvate formate lyase (PFL) activating enzyme [23], AdoMet was found to be ligated directly to Fe in the [4Fe4S] cluster, as illustrated in Figure 4 [24]. Intimate binding between the cluster and AdoMet facilitated electron transfer from {Fe-4S}¹⁺ to AdoMet and cleavage to the 5'-deoxyadenosyl radical, as shown in Figure 4. This cleavage of AdoMet to the 5'-deoxyadenosyl radical consolidated the parallel between the functions of AdoCbl and AdoMet.

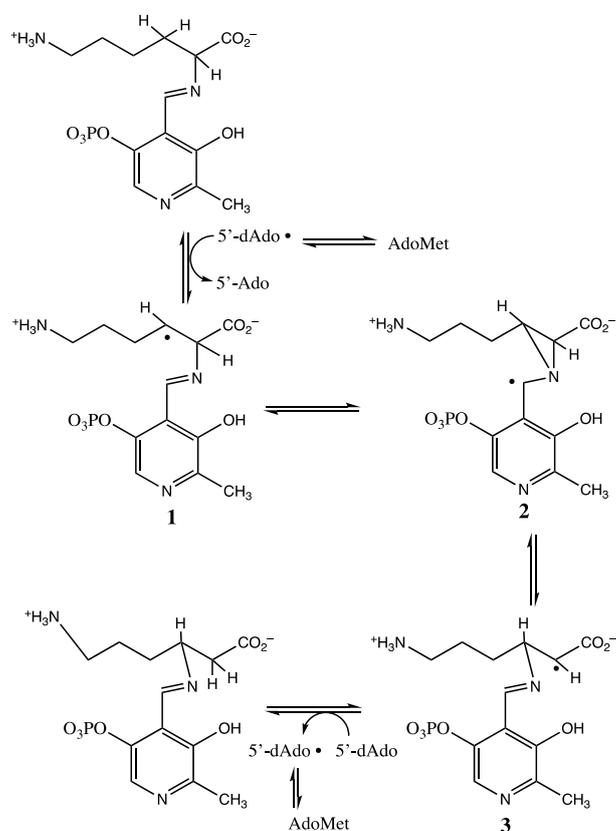


Figure 3. The carbon-centered radical mechanism in the interconversion of lysine and β-lysine by lysine 2,3-aminomutase

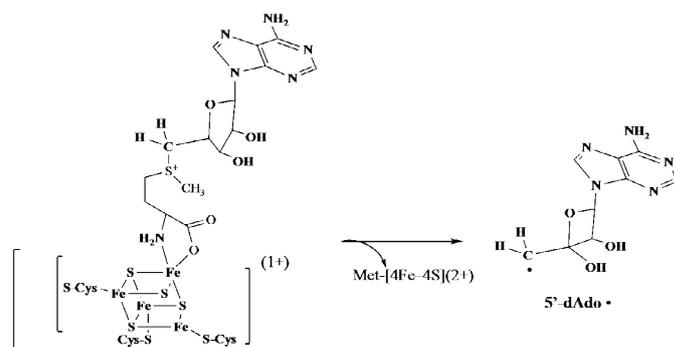


Figure 4. Homolytic cleavage of the S-C5' bond in adenosylmethionine to 5'-dAdo• radical

In the 1990s several other enzymes not engaged in methyl transfer became known to require AdoMet and iron-sulfur clusters. These included anaerobic ribonucleotide reductase, biotin synthase and lipoyl synthase [25-33]. The latter two catalyzed the insertion of sulfur into unreactive C—H bonds of precursors. The ribonucleotide reductase in *E. coli* grown anaerobically and activated by AdoMet proved to contain a [4Fe–4S] cluster. And the PFL activating enzyme was found to contain iron-sulfur clusters, and when purified anaerobically to contain a [3Fe–4S] cluster [34,35]. All of the reactions of these enzymes were found to proceed by radical mechanisms.

The radical SAM superfamily

In due course the nucleotide sequences of genes encoding the foregoing AdoMet/[4Fe–4S]-dependent enzymes became known. Heidi L. Sofia and her associates examined the translated amino acid sequences and discovered the motif CxxxCxxC in common among these enzymes. They searched the available genomic databases in 2001 and found nearly 600 homologs, which they named the Radical SAM superfamily [36]. They found superfamily members throughout biology in both the animal and plant kingdoms. With the passage of time, the genomic database grew to include more than 100,000 homologous sequences in the Radical SAM superfamily encoding enzymes engaged in more than 70 amazingly diverse functions [37-39].

Members of the superfamily include enzymes that catalyze nucleic acid and protein modifications, enzymes that catalyze key steps in biosynthesis of vitamins, coenzymes, analogues of nucleotide bases, antibiotics and many other biological species. A number of Radical SAM enzymes require both AdoCbl and AdoMet and catalyze methylations of non-nucleophilic carbon and phosphorus atoms. Enzymes of the radical SAM superfamily might catalyze the most chemically diverse reactions of any superfamily in biochemistry.

Fewer than a third of all Radical SAM enzymes have been experimentally investigated in detail. Most of them appear to function by mechanisms involving carbon-centered radicals. However, in some of them, AdoMet displays bimodal functions, polar methylation in one step and as a source of the 5'-deoxyadenosyl radical in another step [40,41]. It remains possible that one or another Radical SAM enzyme might engage only in polar methyl group transfer. Whether all Radical SAM enzymes catalyze carbon-centered radical chemistry remains to be determined in future research.

Origins

Cob(III)alamins are structurally complex, requiring many enzymatic steps to assemble. Adenosylcob(III)alamin synthetase produces AdoCbl in a single step from ATP and cob(I)alamin. Cobalamins are produced in bacteria as vitamin B₁₂, and both bacteria and animals contain the synthetase. In contrast, *de novo* synthesis of the simpler methionine occurs only in plants, and it is a nutritionally essential amino acid for animals. Iron-sulfur clusters are among the most primitive cofactors. All things considered, the Radical SAM enzymes likely preceded AdoCbl enzymes in evolution.

The diverse radical SAM enzymes catalyze amino acid isomerizations; methylation of non-nucleophilic phosphorus [39] and carbon atoms [40,41]; modifications of proteins and nucleic acids, including deazaguanine biosynthesis [40]; glycol radical formation; thyl radical formation; chemically difficult steps in vitamin, cofactor, and antibiotic biosyntheses; and many other processes [37,38]. The less diverse AdoCbl proteins catalyze isomerizations and ribonucleotide reduction, and also serve as photosensors [43].

While a significant number of radical SAM enzymes have been found to function by way of the transient 5'-deoxyadenosyl radical, this is not a universal property of radical SAM enzymes. At least one member of the superfamily, the TsrM methylase, might not catalyze a radical reaction [44]. Another member of the superfamily catalyzes both radical and polar methylation [43]. Other members of the superfamily remain to be studied in detail.

LAM and its relative glutamate 2,3-aminomutase are as active or more active than AdoCbl dependent enzymes catalyzing analogous reactions. Therefore, the two coenzymatic systems should be equally efficient sources of the 5'-deoxyadenosyl radical. The sheer size of the radical SAM superfamily means that it is an evolutionary survivor, and AdoCbl enzymes are supplemental and mechanistically related sources of the 5'-deoxyadenosyl radical. In a possibly significant mechanistic advantage, AdoCbl produces the 5'-deoxyadenosyl radical without a requirement for a reducing agent. Radical SAM enzymes require a reducing system to generate the [4Fe–4S]¹⁺ cluster in the cleavage of AdoMet (Figure 4).

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