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Letter to the Editor OMICS International

The Birth and Demise of Hypotheses on Evolution of S-Adenosyl-L-methionine and Adenosylcobalamin

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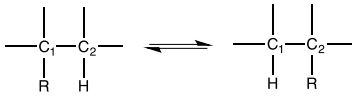
Letter to the Editor

Relationships between adenosylcobalamin and S-adenosyl-L-methionine (SAM)-dependent enzymatic radical reactions are explored with a view toward determining their evolutionary relationships. Adenosylcobalamin is a Vitamin B_{12}-coenzyme, and the vitamin deficiency causes pernicious anemia in humans. Methionine, the precursor of SAM, is a nutritionally essential amino acid. Evidence implicates both SAM and adenosylcobalamin in the generation of the 5'-deoxyadenosyl radical as the initiator of carbon-centered radical chemistry. However, expectations of the evolutionary superiority of the structurally and chemically complex adenosylcobalamin as an initiator of radical biochemistry are contradicted by available information. It is pointed out that adenosylcobalamin functions equally well aerobically and anaerobically, whereas SAM requires strong reducing conditions and electron transfer mediated by a $[4Fe–4S]^{1+}$ cluster to initiate carbon-centered radical chemistry.

This paper focuses on the biochemical relationships between the Vitamin B_{12} coenzyme adenosylcobalamin and S-adenosyl-L-methionine (SAM). Bacteria synthesize Vitamin B_{12} (cobalamin), and bacteria and animals alkylate it to its coenzymatic forms adenosylcobalamin and methylcobalamin. A dietary deficiency of cobalamin leads to pernicious anemia in humans because of the importance of the B_{12}-coenzymatic forms in metabolism. Adenosylcobalamin potentiates the interconversion of succinyl-CoA and methylmalonyl-CoA by methylmalonyl-CoA mutase [1], and methylcobalamin mediates methyltransfer from N^5-methyltetrahydrofolate to homocysteine to form methionine in the action of methionine synthase [2], a methionine salvage reaction. Methionine, the immediate precursor of SAM, is an essential amino acid in humans, and its *de novo* biosynthesis takes place in plants. Defective actions of these enzymes are associated with neurological and red blood cell deficiencies in humans.

Until the late 1980s, SAM was regarded by biochemists as the biological methylating agent, in which SAM donated its methyl group to nucleophilic N and O centers in metabolites, proteins and nucleic acids and was converted into S-adenosylhomocysteine (SAH). In the same time frame, adenosylcobalamin was recognized as the coenzyme for enzymes catalyzing internal isomerizations, following the pattern of Equation 1, in bacteria and animals [3].



(1)

The biochemistry of the adenosyl cofactors is now known to be much more complex. Adenosylcobalamin activates bacterial riboswitches, and SAM activates no fewer than five families of riboswitches, drawing these molecules into the regulation of gene expression [4,5]. Further, adenosylcobalamin serves as a photosensitizer in bacterial gene regulation [6].

In a third dimension of biochemical relationships between adenosyl coenzymes, this laboratory in 1987 undertook to test a hypothesis on the evolution of adenosyl coenzyme-dependent enzymes catalyzing chemically difficult reactions by mechanisms involving carbon-centered radicals. At the time, such radicals were rare in biochemistry. They were formerly regarded as too reactive and difficult to control to be viable as intermediates in enzymatic active sites. However experimental studies had revealed their importance in reactions of adenosylcobalamin [1,3,7].

Adenosylcobalamin was known to be the coenzyme in fewer than a dozen known enzymatic reactions proceeding through carbon-centered radicals initiated by the 5'-deoxyadenosyl radical, which was transiently formed through the homolytic scission of the Co—C bond in the coenzyme (Figure 1A). The resultant 5'-deoxyadenosyl radical mediated hydrogen transfer in these reactions.

Most of the adenosylcobalamin-dependent reactions proceeded with the interchange of a chemical group with a hydrogen atom bonded to an adjacent carbon in a substrate, as illustrated in (Equation 1). The reaction of lysine 2,3-aminomutase [8] apparently followed the same pattern, with the α-amino group in L-lysine migrating from C2 to C3 and the C3-hydrogen cross migrating to C2 to form L–β-lysine. The enzyme was reported to be activated by ferrous iron, pyridoxal phosphate, and SAM, not by adenosylcobalamin [8].

This laboratory considered a hypothesis in three parts: A) in the isomerization of α-lysine to β-lysine, SAM functions in the same manner as adenosylcobalamin; B) as a simple molecule, SAM preceded the evolution of the much more complex adenosylcobalamin as a coenzyme for catalyzing carbon-centered radical reactions; C) the more elegant adenosylcobalamin became dominant because of its chemical reactivity.

In a straight forward test of the role of SAM, [5'-^{3}H]adenosyl-L-methionine activated lysine 2,3-aminomutase and produced β-[^{3}H] lysine and α-[^{3}H]lysine [9]. This proved that SAM functioned exactly as adenosylcobalamin in mediating hydrogen transfer, thereby verifying part A of the hypothesis. This finding supported the intermediate formation of the 5'-deoxyadenosyl radical (Figure 1B). A catalytic mechanism involving carbon-centered radicals was put forward [9], an intermediate lysyl radical was observed and spectroscopically characterized [10], and a fully functional allylic version of the 5'-deoxyadenosyl radical was spectroscopically identified and characterized as an intermediate [11].

In the original discovery of lysine 2,3-aminomutase, the reported activity was low, suggesting a less efficient enzyme than

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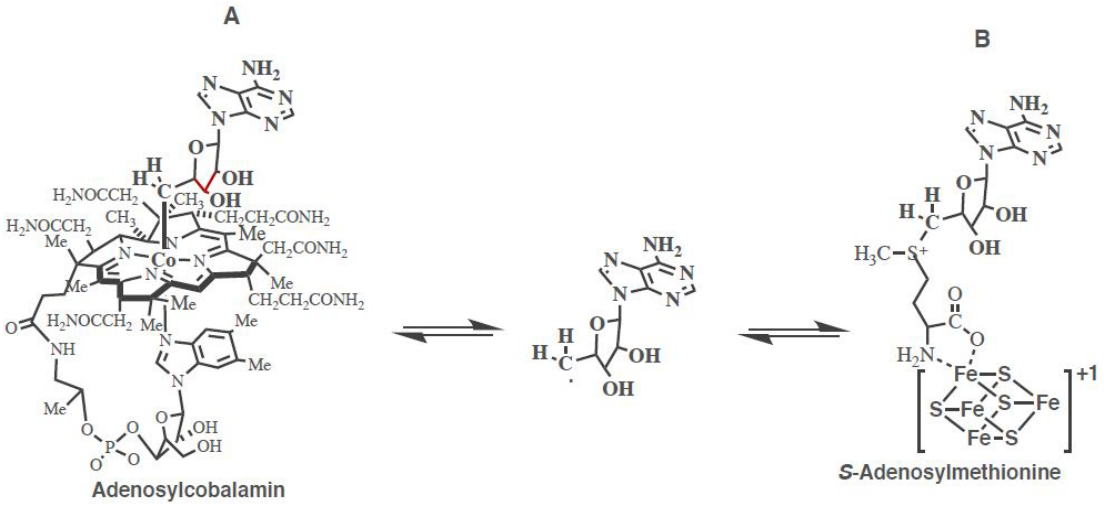

Figure 1: Generation of the 6'-deoxyadenosyl radical from either adenosylcobalamin or SAM.

typical adenosylcobalamin-dependent enzymes [8]. However, with improvements in purification and activation conditions, the enzyme proved to contain a [4Fe–4S] cluster. And optimization of the iron and sulfide content led to high activity, with a turnover number ~50 s^{-s}, similar to the activities of adenosylcobalamin-dependent isomerases [12,13]. Therefore, SAM must have been at least as efficient as the Vitamin B_{12} coenzyme. This contradicted part C of the hypothesis, that adenosylcobalamin would be a superior source of the 5'-deoxyadenosyl radical and superseded SAM in evolution.

Further research in many laboratories led to the discovery of other SAM-dependent enzymes catalyzing chemically difficult reactions. An activating enzyme (PFL-activase) for pyruvate formate-lyase (PFL) was discovered by Knappe and coworkers in 1976 [14]. By 1984 it had been shown to cleave SAM into 5'-deoxyadenosine and methionine and to generate a radical in PFL [15]. PFL-activase was subsequently shown to contain an Fe-S cluster [16]. Three other SAM-dependent enzymes were discovered in the 1990s and shown to contain iron-sulfur clusters and to cleave SAM. Biotin synthase and lipoyl synthase catalyzed the SAM-dependent insertion of sulfur into chemically unreactive C—H bonds [17-19]. Anaerobic ribonucleotide reductase was discovered, shown to catalyze the SAM-dependent reduction of ribonucleotides to deoxyribonucleotides, and to contain an iron-sulfide cluster [20].

In due course, the amino acid sequences of the foregoing enzymes became known. Being aware of this, Dr. Heidi Sofia[1] and her associates found the sequence motif CxxxCxxC in common among these enzymes. She and her associates searched the genomic database in 2001 for proteins incorporating this motif and found nearly 600 such proteins—in both animal and plant kingdoms—in the database [21]. These proteins were associated with many diverse and complex chemical transformations, including the chemical methylations of nucleic acids and proteins and the biosyntheses of vitamins, coenzymes and antibiotics. This family was named the radical SAM superfamily [21].

The explosion of genomic information has led to the identification of more than 100,000 sequences in the radical SAM superfamily, and more than 2800 potential enzymes [22,23]. Only a small number of these enzymes have been studied mechanistically. To date, those investigated have been found to incorporate [4Fe–4S] complexes, with three Fe ligated to cysteine residues in the CxxxCxxC motif and the fourth ligated to the carboxylate and amino group of SAM. In the most detailed studies, the iron-sulfur center has been found to function in reduced $[4Fe–4S]^{1+}$-form and to cleave SAM by inner sphere electron transfer (Figure 1B).

All experimental evidence verifies part A of the hypothesis, that radical SAM and adenosylcobalamin enzymes share the same capacity to generate the 5'-deoxyadenosyl radical and initiate mechanisms involving carbon-centered radicals by abstraction of hydrogen from C—H groups. Part C, that the more complex and elegant adenosylcobalamin became dominant because of its chemical reactivity, is contradicted by the sheer size of the radical SAM superfamily. The question whether SAM preceded adenosylcobalamin in evolution, part B of the hypothesis, might be correct. A point in favor includes the fact that radical SAM enzymes are found in plants as well as animals and microorganisms. Plants do not contain adenosylcobalamin or any Vitamin B_{12}-related cobalamin or cobamide. Further, *de novo* biosynthesis of methionine takes place mainly in plants and would be available for the appearance of radical SAM enzymes.

The possibility of co-evolution in microorganisms could be considered. The author has been asked at lectures about the evolutionary forces leading to the appearance of Vitamin B_{12}, given the existence of the radical SAM superfamily. The response has been the importance of methylcobalamin in mediating methyl transfer in one-carbon metabolism. This too has been brought into focus by the radical SAM superfamily. Certain radical SAM enzymes are methyltransferases that methylate non-nucleophilic centers, such as phosphorus in phosphonates and trigonal carbons in amino acids and nucleic acid bases [24]. Moreover, certain methyltransferases require both SAM and a cobalamin, generally methylcobalamin [24,25]. The biochemical and mechanistic relations between SAM and cobalamins might be indicative of special relationships that eventually led to adenosylcobalamin-dependent enzymes.

The foregoing nevertheless begs the question of the evolutionary factor(s) leading to the appearance of adenosylcobalamin in biology. In this connection, there is reason to consider whether adenosylcobalamin displays any chemical property that could give it an evolutionary

[1]Dr. Sofia received her Ph. D. degree from the University of Wisconsin-Madison. Although she was not a member of the author's research group, the author served as her adviser of record in the writing and defense of her Ph. D. dissertation in the absence of her research supervisor.

advantage. A reasonable response would be that it serves as a source of the 5'-deoxyadenosyl radical without the need for a reducing agent. As illustrated in Figure 1, SAM requires the fully reduced iron sulfur cluster $[4Fe–4S]^{1+}$ in order to generate the 5'-deoxyadenosyl radical. For this reason, all radical SAM enzymes require a reducing system to display activity, e.g., dithionite and anaerobicity *in vitro* or an enzymatic reducing system *in vivo*. Adenosylcobalamin works without such systems either aerobically or anaerobically.

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