Self-sacrifice in radical *S*-adenosylmethionine proteins

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The radical SAM superfamily of metalloproteins catalyze the reductive cleavage of *S*-adenosyl-L-methionine to generate a 5′-deoxyadenosyl radical (5′-dA•) intermediate that is obligate for turnover. The 5′-dA• acts as a potent oxidant, initiating turnover by abstracting a hydrogen atom from an appropriate substrate. A special class of these enzymes use this strategy to functionalize unactivated C–H bonds by insertion of sulfur atoms. This review will describe the characterization of three members of this class — biotin synthase, lipoyl synthase, and MiaB protein — each of which has been shown to cannibalize itself during turnover.

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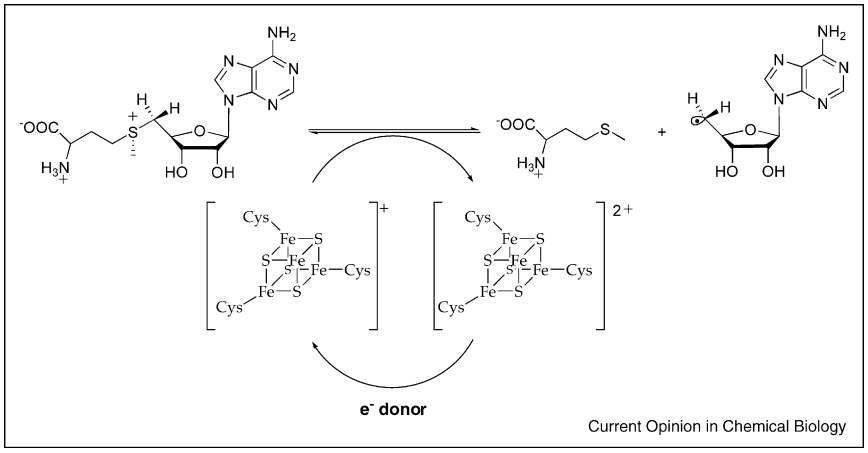
The radical SAM superfamily of enzymes

The radical *S*-adenosylmethionine superfamily of enzymes constitutes a class of metalloproteins that catalyze a reductive cleavage of *S*-adenosyl-L-methionine (SAM) to L-methionine and a 5′-deoxyadenosyl 5′-radical (5′-dA•) (Figure 1) [1,2]. The 5′-dA• is a key and common intermediate, and functions to remove a hydrogen atom from an appropriate substrate. In several instances, the C–H bond that is cleaved is completely unactivated and comparable in energy to C–H bonds cleaved by enzymes that generate high-energy oxidants via the concerted action of a metal-containing cofactor and dioxygen [3]. The closest relative of this radical-generating system is 5′-deoxyadenosyl 5′-cobalamin (coenzyme B_{12}), which reversibly generates a 5′-dA• via a homolytic cleavage of the cofactor's cobalt–5′-carbon bond [2]. Because the sulfur atom in SAM does not contain low-lying d-orbitals that are capable of stabilizing an unpaired electron, as does the cobalt atom in coenzyme B_{12}, a 5′-dA• cannot be generated via simple homolysis of the sulfur–5′-carbon bond of SAM. Radical SAM (RS) proteins require the input of an electron that derives from a reduced iron–sulfur (Fe/S) cluster, $[4Fe–4S]^{+}$, which is an obligate cofactor. In almost all RS proteins the Fe/S cluster is ligated by three cysteine thiolate side chains that are arranged in a CX_3CX_2C motif, which has become a signature sequence for this superfamily. This motif, in combination with glycine-rich segments that signify SAM binding, allows RS proteins to be identified by bioinformatics methods. As of the year 2001, the RS superfamily was predicted to contain over 6000 members spanning all three domains of life [4•]. With the increasing rate at which DNA sequences of genomes from myriad and diverse organisms are determined, membership is growing rapidly. RS proteins have been shown, or are predicted, to catalyze key steps in general metabolism, DNA biosynthesis and repair, and the biosynthesis of a number of cofactors, coenzymes, antibiotics, and herbicides [4•]. Some are also predicted to participate in a variety of other cellular functions such as host defense against viral invasion and cell-cycle regulation [4•,5,6].

With the exception of lysine 2,3-aminomutase, on which an impressive amount of detailed mechanistic information exists, much of the focus of investigations of RS proteins has been on the characterization of their Fe/S clusters. Elegant spectroscopic studies performed on pyruvate formate-lyase activating enzyme (PFL-AE) and lysine 2,3-aminomutase (LAM) indicate that SAM binds in contact with a unique iron atom of the Fe/S cluster — the one that is not coordinated by a cysteine thiolate side chain — forming a bidentate chelate through its α-amino and α-carboxylate groups [7–10]. These findings have been substantiated with the three-dimensional determination of SAM-bound structures of coproporphyrinogen III oxidase (HemN) [11], biotin synthase (BioB) [12••,13], MoaA [13], and LAM [14]. In all instances, the sulfonium atom of SAM is ~3–4 Å away from the nearest atom of the Fe/S cluster, which should allow for facile electron transfer to effect the cleavage reaction. Undoubtedly, the coordination of SAM to the cluster somehow influences cleavage, perhaps by modulating the redox potentials of the Fe/S cluster and SAM; however, the detailed mechanism that underlies this process is still obscure.

During the past 10 years, a new class of RS proteins has emerged, in which the participant members catalyze anaerobic oxidations that involve the insertion of sulfur atoms into unactivated C–H bonds. Three proteins within this class have been isolated and characterized: biotin

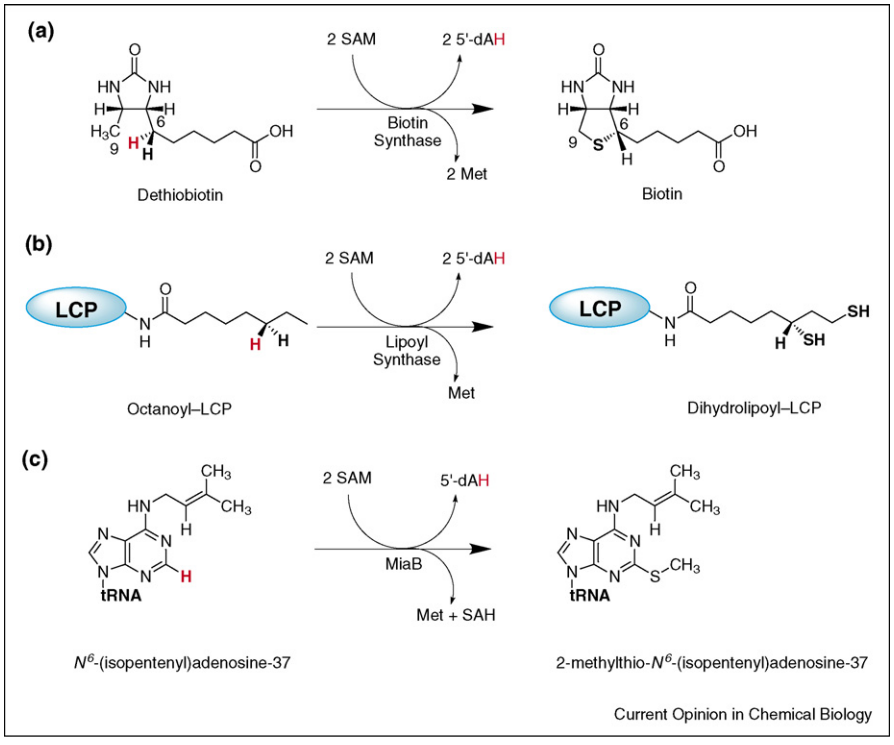
Figure 1



Formation of methionine and a 5′-deoxyadenosyl radical via the reductive cleavage of *S*-adenosylmethionine. The iron–sulfur cluster shown is that which is ligated by protein cysteine thiolates that reside in the CX_3CX_2C motif that is common among radical SAM proteins.

synthase (BS), which catalyzes the insertion of one sulfur atom between two unactivated C–H bonds, resulting in the formation of a thiophane ring; lipoyl synthase (LS), which catalyzes the insertion of two sulfur atoms into two different unactivated C–H bonds; and MiaB protein (MiaB), which catalyzes the insertion of a sulfur atom into an aromatic C–H bond as well as its methylation (Figure 2). The unique aspect of this class of RS proteins is that none of the characterized members have been shown to catalyze more than one turnover. In fact, all are linked by common experimental observations that suggest that each acts as both catalyst — in the sense

Figure 2



Reactions catalyzed by radical SAM enzymes that are known to involve sulfur insertion. SAM, *S*-adenosylmethionine; Met, methionine; 5′-dAH, 5′-deoxyadenosine. Hydrogens shown in red are those that are removed by the 5′-deoxyadenosyl radical **(a)** biotin synthase reaction, **(b)** lipoyl synthase reaction, and **(c)** MiaB reaction.