# Coordination and Mechanism of Reversible Cleavage of S-Adenosylmethionine by the [4Fe-4S] Center in Lysine 2,3-Aminomutase

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Lysine 2,3-aminomutase (LAM) catalyzes the interconversion of L-lysine and L-β-lysine, eq 1, by a radical mechanism initiated by the reversible, reductive homolytic scission of the C5′−S bond in S-adenosylmethionine (SAM) to form methionine and the 5′-deoxyadenosyl radical at the active site.[1−9] LAM is a member of a

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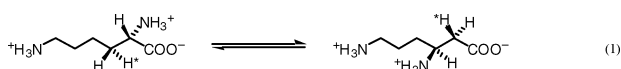
superfamily of enzymes in which a [4Fe-4S]$^+$ cluster with a unique, noncysteinyl coordinated Fe provides the electron required in the cleavage of SAM.[11−21] Little is known of the mechanism by which the electron is inserted into SAM to effect its cleavage, and it is not known whether all enzymes of the family employ the same mechanism.

Selenium X-ray absorption spectroscopy (XAS) of an intermediate in the LAM reaction where *Se*-adenosyl-L-selenomethionine (*Se*SAM) replaces SAM shows that electron transfer from the [4Fe-4S] center occurs by an inner sphere mechanism culminating in direct ligation of selenomethionine to iron upon cleavage of *Se*SAM.[22] In this paper, we use ENDOR spectroscopic methods first employed in studies of the related enzyme, pyruvate formate lyase activating enzyme (PFL-AE),[23,24] to show that SAM binds to LAM by chelating the unique iron of the [4Fe-4S] center through the carboxylato and amine groups of its L-methionine moiety. Through combination of the ENDOR and XAS spectroscopic results, we postulate a mechanism for the cleavage of SAM that involves multiple ligation with the [4Fe-4S] center.

Experiments have been performed on samples with the Fe−S cluster reduced to the 1+ state with dithionite, denoted as [1+/SAM], and reduced samples trapped in the geometry of the 2+ state. The latter were prepared by freezing the enzyme with its cluster in the 2+ state, and then cryoreducing to generate the 1+ state within its frozen matrix; these are denoted as [2+/SAM]$_{red}$. Spectra were obtained using SAM labeled with ^{17}O in the carboxylato group, with ^{15}N in the α-amino group, and with either ^{13}C or ^{2}H$_3$ in the methyl group.[25−27] The EPR spectra of both [1+/SAM] and [2+/SAM]$_{red}$ are characterized by $g = [\sim 2.0, 1.90, 1.85]$ (see Supporting Information Figure S1).[27,28] The ENDOR spectra of [1+/^{17}O-SAM] in Figure 1 show a broad, asymmetric feature that is absent in the unlabeled sample. This is assigned to the ν_+ branch of the ^{17}O ENDOR response from the ^{17}O-carboxylate of SAM. The large coupling ($A(^{17}\text{O}) = 11.4$ MHz at g_2) is comparable to that found for the corresponding complex of PFL-AE[24] and arises from a carboxylato oxygen coordinated directly to the unique Fe of the cluster. The ENDOR spectra of unlabeled [1+/SAM] show the ν_+ branch of a ^{14}N signal; it disappears in the spectrum of [1+/^{15}N-SAM] and is replaced by a well-resolved
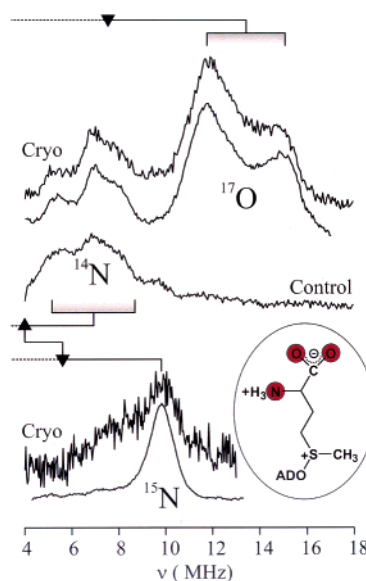
Figure 1. 35 GHz Davies pulsed ^{17}O- and ^{15}N-ENDOR spectra of LAM-[4Fe-4S]$^+$ in complex with ^{17}O- and ^{15}N-SAM.[23b] The upper spectrum is that of [1+/^{17}O-SAM], and the lower is of [1+/^{15}N-SAM]. The offsets displaying greater noise are of [2+/^{17}O-SAM]$_{red}$ and [2+/^{15}N-SAM]$_{red}$. Conditions: MW pulse lengths 80, 40, 80 ns; Rf pulse, 60 μs; $T = 2$ K.

ν_+ peak from a ^{15}N with a corresponding coupling $A(^{15}\text{N}) = 9.1$ MHz at g_2 (Figure 1). Such a coupling requires that the amino group of SAM is coordinated to the unique Fe of the 1+ LAM cluster; the coupling indeed is substantially larger than that of ^{15}N-SAM bound to the cluster of PFL-AE.[24] Thus, as with PFL-AE,[24] the ENDOR spectra taken with [1+/SAM (^{14}N, ^{17}O)] disclose that the carboxylato O and amino N of the SAM methionine moiety form a five-membered-ring chelate of the unique iron of the LAM [4Fe-4S] cluster. However, the differences in ^{14,15}N couplings indicate that details of this chelate structure must be different in the two enzymes.

ENDOR spectra taken at g_2 for [1+/SAM] prepared with SAM labeled at the methionine methyl group by ^{13}C and ^{2}H show signals from both labels (not shown). For ^{13}C, the doublet splitting corresponds to $A(^{13}\text{C}) = 0.7$ MHz. The ^{2}H ($I = 1$) spectrum includes an unresolved quadrupole splitting of each branch; the total breadth of the pattern corresponds to $A(^{2}\text{H}) + 3P = 0.7$ MHz, taking as a probable value[23] that $3P \approx 0.1$ MHz yields $A \approx 0.6$ MHz. Preliminary 2-D field-frequency plots for the ^{13}C ENDOR signal from [1+/^{13}C-SAM] show a distinctly dipolar pattern, with the maximum coupling of $A = 0.8$ MHz (not shown), which is larger than the maximum coupling observed from the analogous sample with PFL-AE, $A(^{13}\text{C}) = 0.5$ MHz.[23] Unlike that enzyme, the preliminary 2-D field-frequency ^{13}C ENDOR pattern suggests that there is little or no isotropic component to the coupling. This would

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Scheme 1
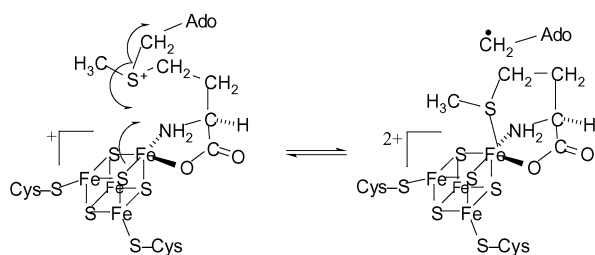
indicate that the methyl group is closer to Fe in LAM, but with little or no covalent interaction between a negatively charged sulfide of the cluster and the positively charged sulfur of the SAM.[23] In contrast, the maximum methyl-deuteron coupling for [1+/^{2}H-SAM], which originates solely in the distance-dependent dipolar interaction, is somewhat smaller than in PFL-AE ($A(^2\mathrm{H})_{max}$ = 0.7 vs 1.0 MHz).[23]

The ENDOR results from four isotopic labels show that both enzymes exhibit the same motif for SAM binding to the [4Fe-4S]$^+$ cluster of the two enzymes: chelation of the unique cluster iron by the amino acid moiety of the SAM methionine; close proximity of the methyl group to the cluster. However, there appear to be significant, and possibly mechanistically important, differences in the details of the binding geometry of SAM.

ENDOR spectra from [2+/SAM]$_{red}$ prepared with each of the four labels, ^{17}O, ^{15}N (Figure 1), and ^{13}C, ^{2}H (not shown), are indistinguishable from the corresponding spectra from [1+/SAM], indicating that SAM binds with essentially the same geometry in [2+/SAM] and [1+/SAM]. Thus, the interactions of SAM with the cluster and the active-site pocket appear to be independent of the oxidation state of the cluster.

Correlating the ENDOR results with the earlier selenium XAS data obtained with *Se*SAM,[22] we propose the mechanism in Scheme 1 for the reversible cleavage of SAM at the active site of LAM. Cleavage begins with SAM bound to the unique Fe of the [4Fe-4S] cluster by the carboxylato and amino ligands, with the sulfonium group held close to the cluster. Electron transfer then homolytically cleaves the C5′−S bond of SAM to form the 5′-deoxyadenosyl radical, while the sulfur of methionine becomes the sixth ligand to the unique iron. Such a six-coordinated octahedral geometry for the unique Fe of a [4Fe-4S] cluster is well documented for aconitase.[29,30] This mechanism allows for reversible cleavage of SAM, as required in the LAM mechanism. In an alternative cleavage mechanism put forward for PFL-AE, the sulfur becomes bonded to a sulfide in the cluster.[24] In contrast to LAM, the cleavage of SAM by PFL-AE is irreversible.

Supporting Information Available: EPR spectra of [2+/Ado-Met]$_{red}$ and [1+/AdoMet] (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(25) (a) L-[*methyl*-^{2}H$_3$]Methionine, L-[*methyl*-^{13}C]methionine, and L-[2-^{15}N]-methionine were obtained from commercial suppliers. L-[1-^{17}O]Methionine was prepared by acid-catalyzed exchange of L-methionine with H$_2$^{17}O. Acid-catalyzed exchange of carboxyl oxygens with H$_2$^{18}O has been described.[26] Mass spectrometric analysis of the [1-^{17}O]methionine indicated an ^{17}O-enrichment of ~48.5%. Taking into account the equivalence of carboxylate oxygens and the ^{16}O, ^{17}O, and ^{18}O content of the H$_2$^{17}O used, the isotopic profile of the exchanged methionine at C1 was as follows: ^{16}O$_2$, 10.4%; ^{17}O$_2$, 40%; ^{18}O$_2$, 20%; ^{16}O$_1$, 4.5%; ^{17}O$_1$, 17%; ^{18}O$_1$, 8.6%. The labeled samples of L-methionine were used with ATP and SAM synthetase to produce labeled SAM as previously described, with minor modifications.[23,24] 35 GHz CW/pulsed EPR and pulsed ENDOR experiments were carried out at 2 K as previously described.
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(27) ENDOR samples were prepared as follows inside an anaerobic chamber. The purified LAM was prepared as described.[13,14] Samples (~90 μM) were reductively incubated with 10 mM dihydrolipoate in the presence of 0.42 mM PLP, 1.0 mM ammonium iron (II) sulfate, and 15% glycerol in 42 mM Epps buffer at pH 8.0 and 37 °C for 4 h. The reductively incubated enzyme was concentrated by ultrafiltration through Microcon 30 (Millipore) to ~500 μM. The concentrated enzyme was mixed with SAM or labeled SAM in the presence or absence of 6 mM sodium dithionite, transferred to ENDOR sample tubes, and quickly frozen in liquid nitrogen. Iron−sulfur clusters of samples prepared in the absence of dithionite were cryoreduced by γ-irradiation as desribed.[23,24] In all samples, the concentrations of the enzyme and SAM were ~400 μM and 3.7 mM, respectively.
(28) g_1 is obscured by signals from a Mn^{2+} impurity, but examination of the field dependence of the ENDOR indicates that $g_1 = 2.00-2.05$.
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