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An Anchoring Role for FeS Clusters: Chelation of the Amino Acid Moiety of S-Adenosylmethionine to the Unique Iron Site of the [4Fe−4S] Cluster of Pyruvate Formate-Lyase Activating Enzyme

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Iron−sulfur clusters participate in a wide range of critical biochemical reactions.[1] Most Fe−S clusters function in electron transfer, but an essential minority perform catalysis, such as the archetypal [4Fe−4S] cluster of aconitase, which isomerizes citrate to isocitrate. Such 4Fe catalytic clusters are distinct in having a unique Fe ion which is not coordinated to the enzyme by a cysteinyl sulfur. The obvious role of such an Fe is a catalytic one, and indeed, the aconitase cluster isomerizes citrate/isocitrate through a mechanism in which the reactant/product is chelated to the unique cluster Fe.[2] We here demonstrate an alternate role for the unique Fe of a catalytic [4Fe−4S] cluster: the [4Fe−4S] cluster of the pyruvate formate-lyase activating enzyme (PFL-AE) "anchors" its substrate, S-adenosylmethionine (AdoMet), by a classical N/O chelate in which the unique Fe binds the amino and carboxylato groups of the methionine fragment of AdoMet, thereby fixing the substrate geometry for subsequent reaction with the cluster.

Iron−sulfur cluster/AdoMet mediated radical chemistry initially was identified in only a few enzyme systems but now is thought to be ubiquitous in both prokaryotes and eukaryotes.[3,4] Diverse reactions[5−10] are thought to be initiated via a common set of steps which involve the generation of an AdoMet-derived 5′-deoxyadenosyl radical intermediate via interaction of the catalytically active $[4Fe−4S]^+$ cluster with AdoMet,[3] and such is the case with pyruvate formate-lyase activating enzyme (PFL-AE), which ultimately generates the catalytically essential glycyl radical on pyruvate formate-lyase (PFL).[11] How an iron−sulfur cluster might conspire to generate the 5′-deoxyadenosyl radical from AdoMet has been the subject of intense study with several of these enzymes.[3]

Recent ENDOR experiments demonstrated that enzyme-bound AdoMet lies adjacent to the [4Fe−4S] cluster of PFL-AE, weakly interacting with it through an incipient bond/antibond, which was proposed to occur through the sulfonium of AdoMet and one of the μ-3 bridging sulfides of the [4Fe−4S] cluster.[12] This proposal gives the unique iron site no direct role in catalysis, and leaves open the question of the function of this site, which appears to be conserved among the Fe−S/AdoMet enzymes. The Mössbauer parameters of the unique iron site are dramatically perturbed in the presence of AdoMet, suggesting coordination of AdoMet to the cluster,[13] but such perturbation also is seen when the unique Fe is involved in catalysis, as with aconitase. Here we unambiguously establish the function of the unique Fe through Q-band pulsed ENDOR spectroscopic studies of the $[4Fe−4S]^+$/AdoMet complex specifically ^{17}O-, and ^{13}C-labeled in the carboxyl group of the methionine fragment and ^{15}N labeled in the amino group.

Anaerobically isolated PFL-AE was photoreduced to the $[4Fe−4S]^+$ state under anaerobic conditions in the presence of 5-deazariboflavin as previously described,[12] and then a 2-fold excess of the labeled AdoMet was added prior to freezing the sample in liquid nitrogen.[14] EPR and pulsed ENDOR spectra at 35 GHz were then recorded for natural-abundance and isotopically labeled samples. The reduced $[4Fe−4S]^+$-PFL-AE in the presence of AdoMet, denoted [1+/AdoMet], gives a nearly axial EPR signal (g = 2.01, 1.88, 1.87). Figure 1 presents 35-GHz pulsed ENDOR spectra of this state collected with the external field set to g = 1.87 for natural-abundance [1+/AdoMet], for samples in which the carboxyl group of the methionine moiety had been labeled with ^{17}O (top) and ^{13}C (center), and in which the amino group had been labeled with ^{15}N (bottom).

The ENDOR spectrum from [1+/AdoMet(^{17}O)] shows a broad, asymmetric feature that is not seen for the natural-abundance sample.[15] This can be assigned to the ν_+ branch of an ^{17}O signal from the carboxylate-^{17}O of AdoMet. This signal corresponds to a hyperfine coupling of $A(^{17}O)$ = 12.2 MHz,[15] a value that is essentially the same as the average value for the carboxylate-^{17}O atoms of citrate/isocitrate coordinated to the unique Fe of aconitase,[2] and of amino-cyclopropane carboxylate (ACC) coordinated to the non-heme iron ion of its oxidase (ACCO), after correction for electron spin-coupling.[16] By analogy to the aconitase and ACCO signals, the breadth of the signal can be assigned provisionally to unresolved quadrupole splittings. The spectrum of [1+/AdoMet-(^{13}C)] shows a doublet centered at the ^{13}C Larmor frequency and split by the ^{13}C hyperfine coupling of $A(^{13}C)$ = 0.71 MHz. This too is absent in the natural-abundance sample and thus is to be assigned to the carboxylate carbon of AdoMet. The hyperfine coupling is similar to that observed for the carboxylate ^{13}C of citrate/isocitrate coordinated to the unique Fe of aconitase (∼1 MHz).[2] The results with the two labels establish that a methionine carboxylato oxygen of AdoMet is coordinated to an Fe ion of the 1+ cluster, and in conjunction with the Mossbauer studies[13] this ion must be the unique one.

The spectrum of natural-abundance [1+/AdoMet] shows a broad, poorly resolved feature whose high-frequency edge occurs at ∼8 MHz and which extends to at least 5 MHz. This feature disappears in the spectrum of [1+/AdoMet(^{15}N)] and is replaced by the well-defined ν_+ peak from ^{15}N, whose frequency corresponds to a coupling of $A(^{15}N)$ = 5.8 MHz (as is often true, the ν_- peak was not detected).[17] This coupling is equivalent to those of the ^{15}N of histidines coordinated to the ferrous ion of the Rieske [2Fe−2S] cluster[18] and similar to that of ACC bound to the non-heme iron ACCO,[16] when one takes into account the influence of electron spin-coupling coefficients on the observed hyperfine coupling constants. Thus, it is clear that the value of $A(^{15}N)$ reflects

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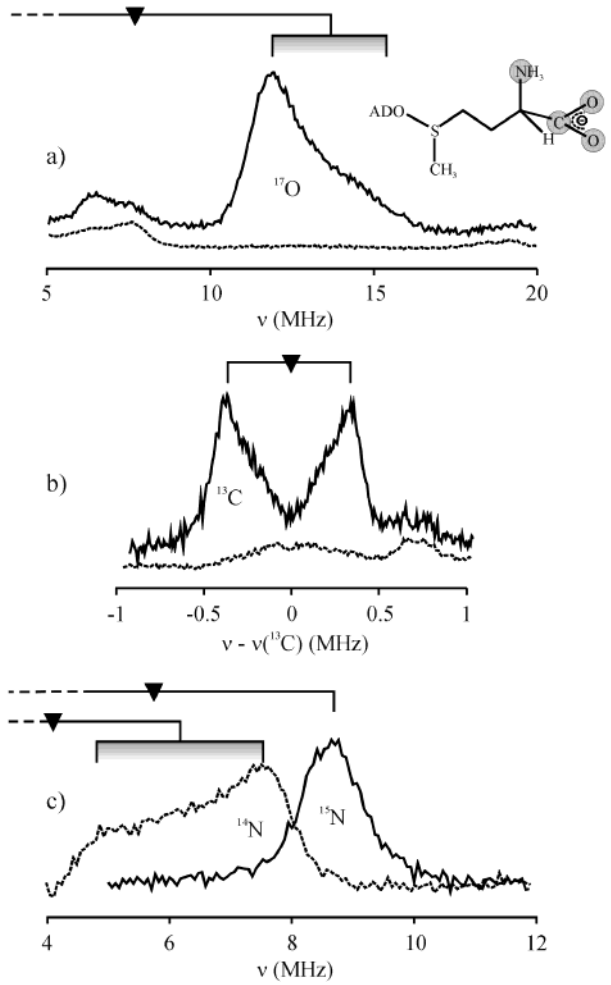

Figure 1. 35-GHz pulsed ENDOR spectra of PFL-AE with ^{17}O **(a)** and ^{13}C **(b)** carboxylato-labeled and ^{15}N-amino-labeled **(c)** AdoMet compared with data from an unlabeled sample, at $g_\perp$. *Conditions:* $T = 2$ K, $\nu_{MW} = 34.9$ GHz, rf pulse length = 60 μS; (a) ^{17}O-labeled, Davies ENDOR, MW pulse lengths = 80, 40, 80 ns, number of averaged transients at each point: ^{17}O = 288, unlabeled = 200. (b) ^{13}C-labeled, Mims ENDOR, MW pulse lengths = 80 ns, $\tau = 552$ ns, number of averaged transients: ^{13}C-labeled = 144, unlabeled = 600. (c) ^{15}N-labeled, Davies ENDOR: MW pulse lengths = 80, 40, 80 ns, number of averaged transients: ^{15}N-labeled = 80, unlabeled = 624.

coordination of the amino group, as well as the carboxylato group, to the unique Fe of [1+/AdoMet(^{15}N)].

The results reported here indicate that the methionine end of AdoMet forms a five-membered-ring chelate to the unique Fe of the [4Fe−4S] cluster of PFL-AE, with the amino nitrogen and one carboxylate oxygen as ligands to this Fe. (Note, the amino acid ACC likewise chelates the non-heme Fe of ACCO.[16]) This interaction "anchors" the nonreacting end of AdoMet, thereby positioning the substrate in the proper configuration for the subsequent radical chemistry with the cluster at the reactive end of AdoMet (Figure 2). As suggested by our previous results, this active configuration likely involves a close interaction of the AdoMet sulfonium and a μ-3 bridging sulfide of the cluster, an interaction that may provide a pathway for inner-sphere electron transfer from the cluster to the AdoMet.

The site-differentiated [4Fe−4S] cluster of PFL-AE appears to be conserved throughout the Fe−S/AdoMet family, as all members have the same three cysteine (CX_3CX_2C) cluster binding motif.

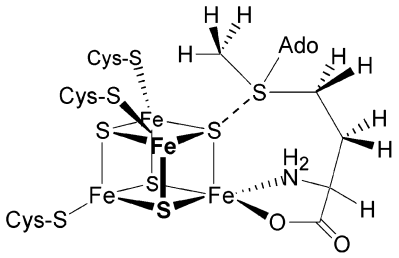

Figure 2. AdoMet forms a classical N/O chelate with the unique iron site of the [4Fe−4S] cluster of PFL-AE.

On the basis of the results presented here, we propose that the unique site is conserved for its essential role in anchoring AdoMet during catalysis.

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(14) Detailed procedures for the preparation of isotopically labeled AdoMets will be provided in detail elsewhere. Carboxy-^{17}O-enriched methionine was prepared by incubating L-methionine·HCl with $H_2{}^{17}O$ (Isotec) prior to removing excess water. Amino-^{15}N-methionine (Isotec), carboxy-^{13}C-methionine (CDN Isotopes), and carboxy-^{17}O-methionine were used to synthesize the isotopically labeled AdoMets using AdoMet synthetase, as previously described.[12]
(15) For ^{17}O($I = {}^5/_2$) a single molecular orientation gives a signal with two branches, denoted $\nu_\pm$, with features centered at the ^{17}O Larmor frequency ($\nu(^{17}O) = 7.7$ MHz in Figure 1) and split by the ^{17}O hyperfine coupling: $\nu_\pm = \nu(^{17}O) \pm A(^{17}O)/2$. The individual branches are further split, in principle, into a quintet by the quadrupole interaction, but this usually is not resolved for ^{17}O.
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