Observation of a Second Substrate Radical Intermediate in the Reaction of Lysine 2,3-Aminomutase: A Radical Centered on the β-Carbon of the Alternative Substrate, 4-Thia-L-lysine†

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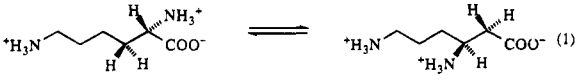
ABSTRACT: Lysine 2,3-aminomutase from *Clostridia* catalyzes the interconversion of lysine and β-lysine by a mechanism in which four organic radicals are postulated as intermediates. One of the intermediates has been identified as the α-radical of β-lysine in imine linkage to pyridoxal phosphate (PLP) [Ballinger, M. D., Frey, P. A., & Reed, G. H. (1992) *Biochemistry 31,* 10782−10788]. We report here the observation of another of the four putative radical intermediates in the reaction of the alternative substrate, 4-thia-L-lysine (*S*-2-aminoethyl-L-cysteine). 4-Thialysine is a substrate for lysine 2,3-aminomutase. The K_m of 4-thialysine is similar to that for lysine, and the V_m is ~3% of that for lysine. Upon mixing 4-thialysine with the activated enzyme in the presence of the required cofactor *S*-adenosylmethionine, followed by freeze-quenching with liquid N_2 in the steady state, a strong EPR signal centered at $g = 2.003$ is observed. This signal exhibits strong hyperfine splitting due to the presence of ^{13}C at carbon-3 of 4-thialysine, and the EPR pattern is narrowed upon the substitution of deuterium at carbon-3. The hyperfine interactions show that the unpaired electron is centered on carbon-3 of 4-thialysine. The hyperfine pattern in the EPR spectrum is also simplified by the use of 4-thia[5,6-$^2H_4$]lysine as the substrate, showing either that the spin is partially delocalized through the sulfur intervening between carbons-3 and -5 or that the conformation is such that protons at carbon-6 are close to carbon-3. The radical appears to be the β-radical of 4-thialysine, the 4-sulfur analog of the β-radical of lysine, a precursor to the previously identified α-radical of β-lysine. The observation of the β-radical derived from 4-thialysine is presumably made possible by the effect of the 4-sulfur in stabilizing the unpaired electron at carbon-3.

Lysine 2,3-aminomutase catalyzes the interconversion of lysine and β-lysine:



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The clostridial enzyme is a hexameric protein with an overall molecular weight of 285 000. It contains iron−sulfur clusters and pyridoxal 5′-phosphate (PLP)[1] and is activated by *S*-adenosylmethionine (Chirpich et al., 1970; Moss & Frey, 1987, 1990; Baraniak et al., 1989; Petrovich et al., 1991; Song & Frey, 1991). The chemical mechanism is novel in that, although the reaction is analogous to that of adenosylcobalamin-dependent aminomutases (Stadtman, 1973), the enzyme does not contain or require any species of vitamin B_{12} coenzyme (Petrovich et al., 1991; Chirpich et al., 1970). The cofactor, *S*-adenosylmethionine, supplies the 5-deoxyadenosyl moiety that acts in mediating hydrogen transfer from carbon-3 to carbon-2 in the manner of the 5′-deoxyadenosyl moiety of adenosylcobalamin in other aminomutases (Moss & Frey, 1987).

Lysine 2,3-aminomutase functions by a radical mechanism that appears to follow the course outlined in Scheme 1. *S*-Adenosylmethionine is postulated to react with a metallic cofactor, most likely the iron−sulfur cluster, by a process that generates the 5′-deoxyadenosyl radical reversibly (Moss & Frey, 1990; Frey & Reed, 1993). This radical is represented as Ado−CH_2• in Scheme 1. The 5′-deoxyadenosyl radical initiates the rearrangement by abstracting a hydrogen atom from carbon-3 of the substrate, which is bound to the enzyme in the form of its aldimine with PLP. This step forms 5′-deoxyadenosine and the β-radical (**1**) of lysine bonded as an aldimine to PLP. The β-radical undergoes rearrangement to an azacyclopropylcarbinyl radical (**2**), a quasi-symmetric species that rearranges further to the α-radical of β-lysine (**3**) bonded as an aldimine to PLP. The α-radical (**3**) abstracts a hydrogen atom from carbon-5′ of 5′-deoxyadenosine to produce the PLP-aldimine of β-lysine and regenerates the 5′-deoxyadenosyl radical.

The α-radical of β-lysine, **3** in Scheme 1, has been identified and characterized as an intermediate in the reaction of lysine 2,3-aminomutase (Ballinger et al., 1992a,b). Electron spin echo envelope modulation spectroscopy, with samples in which [4′-^{2}H]PLP was substituted for PLP in the reaction, has recently been used to identify PLP as part of the structure of intermediate **3** in Scheme 1 (Ballinger et al.,

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[1] Abbreviations: PLP, pyridoxal 5′-phosphate; DTT, dithiothreitol; 4-thialysine, 4-thia-L-lysine; Epps, *N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid; EPR, electron paramagnetic resonance.

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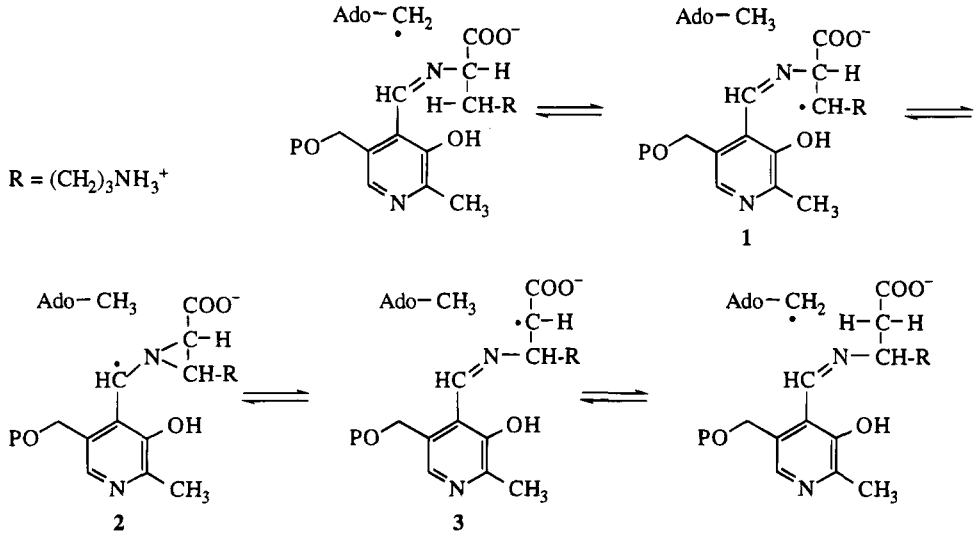
Scheme 1



1995). The α-radical is the only species detectable by EPR at mixing times as short as <10 ms in pre-steady-state kinetic measurements (Ballinger, 1993), and alternative strategies have therefore been explored to trap intermediates corresponding to other steps in Scheme 1. Analogs of lysine that might lead to enhanced stability of the intermediate corresponding to the β-radical, intermediate **1** in Scheme 1, have been investigated as potential substrates of the enzyme. The present paper reports the substrate activity of 4-thialysine (*S*-2-aminoethyl-L-cysteine) in the lysine 2,3-aminomutase reaction and EPR measurements on the β-radical of 4-thialysine that appears in the steady state of the enzymatic conversion of this alternative substrate.

EXPERIMENTAL PROCEDURES

Chemicals and Substrates. 4-Thialysine (*S*-2-aminoethyl-L-cysteine) was purchased from Sigma. L-[3-^{13}C]Cysteine (99% enriched), DL-[3-$^{2}H_2$]cysteine (98% enriched), and 1,2-dibromo[$^{2}H_4$]ethane (99% enriched) were purchased from Cambridge Isotope Laboratories. 2-Bromo[$^{2}H_4$]ethylammonium bromide was prepared according to the method of Gabriel (1888) from 1-bromo-2-phthalimido[$^{2}H_4$]ethane, which was synthesized from 1,2-dibromo[$^{2}H_4$]ethane by the procedure of Sheehan and Bolhofer (1950). DL-4-Thia[3-$^{2}H_2$]lysine, DL-4-thia[5,6-$^{2}H_4$]lysine, DL-4-thia[3,5,6-$^{2}H_6$]-lysine, and L-4-thia[3-^{13}C]lysine were synthesized from the correspondingly labeled cysteines and 2-bromoethylammonium bromides according to the procedure of Cavallini et al. (1955). *S*-Adenosylmethionine was purchased from Sigma as the *p*-toluenesulfonate salt and purified over a CM-cellulose column, with 1 M NaOAc at pH 5.0 as the column preparation buffer, 1 mM NaOAc at pH 5.0 as the column equilibration buffer, and 40 mM HCl as the eluent. Fractions chosen on the basis of A_{260} (ϵ = 15 400 M^{-1} cm^{-1}) were pooled, and aliquots were frozen in liquid N_2 and stored at −135 °C. Typical concentrations of saved fractions ranged from 14 to 22 mM. Buffers and other chemicals were purchased in the highest available purity and used as supplied.

Enzyme Purification. Lysine 2,3-aminomutase was purified by a modification of the procedure of Moss and Frey (1990) as amended by Petrovich et al. (1991). All procedures were carried out within a Coy anaerobic chamber, except for preparative centrifugations; centrifuge bottles were sealed in the anaerobic chamber, removed for centrifugation, and returned to the chamber before being unsealed. The final purification step was anion exchange chromatography through a 0.1 L column of Q-Sepharose. The purified enzyme was approximately 35 mg mL^{-1} and exhibited a specific activity of approximately 35 IU mg^{-1} protein in the standard assay (Ballinger et al., 1992a).

EPR Measurements. Samples were prepared by the procedure of Ballinger et al. (1992a) with slight modifications. Because 4-thialysine reacts more slowly than lysine, only 10−20 mM 4-thialysine was required to obtain maximum signal intensity. EPR spectra were recorded at X-band on a Varian E3 spectrometer. Sample temperature was maintained in a standard liquid N_2 immersion dewar. The spectrometer was interfaced with an IBM AT computer for data acquisition. Frequency was calibrated with a Hewlett-Packard frequency meter, and the field was calibrated with a Varian flux meter. Spin concentration was determined by double integration and comparison to the standard Varian strong pitch (0.1% pitch in KCl).

Assays. The activation and assay procedure described by Chirpich et al. (1970) and amended by Ballinger et al. (1992a) was modified to accommodate the slower reaction of the alternative substrate, 4-thialysine. Activation and assay procedures were carried out within a Coy anaerobic chamber, and all assay solutions were maintained at 37 °C. Microfuge tubes and pipet tips were autoclaved and brought into the anaerobic chamber while hot at least 24 h before being used.

For reductive incubation preceding an assay, the stock reductive incubation buffer contained 0.20 M Epps at pH 8.0, 4.8 mM ferric ammonium citrate, 2.2 mM PLP, and 87 μM dihydrolipoate. (This solution was prepared by mixing stock solutions of the components in the order indicated.) In one instance, an aliquot (150 μL) of this solution was combined in a 1.5 mL microfuge tube with deaerated water (510 μL) and lysine 2,3-aminomutase (60 μL, 35 mg mL^{-1}) dissolved in the standard purification buffer (30 mM Epps at pH 8.0, 1 mM DTT, 10 μM PLP, and 0.10 mM lysine), and the mixture was incubated at 37 °C for 5 h. In another instance, an aliquot (1.3 mL) of the stock reductive incubation buffer was combined in a 1.5 mL microfuge tube with

lysine 2,3-aminomutase (100 μL, 30 mg mL^{-1}) dissolved in the standard purification buffer, and the mixture was incubated at 37 °C for 5 h. Either of these mixtures could be frozen in liquid N_2 and stored at −135 °C for several weeks before concluding assays, with little loss of activity. In the first instance, after the 5 h reductive incubation (or after thawing), a 100 μL aliquot was removed and diluted into 215 μL of a solution composed of a 1:10 dilution of the stock reductive incubation buffer into 0.20 M Epps at pH 8.0. In the second instance, no further dilution was performed after the reductive incubation.

For the assays, a 200 μL aliquot of either enzyme solution was added to 260 μL of a cofactor−substrate solution that had been warmed to 37 °C. The cofactor−substrate solutions were prepared to give the following final concentrations in the assay solutions: 0.26 M Epps at pH 8.0; 2.3 mM sodium dithionite; 1.2 mM *S*-adenosylmethionine; and 2, 4, 6, 12, or 20 mM 4-thialysine, together with activation components carried over with the reduced enzyme. The solution was mixed quickly by swirling gently and incubated at 37 °C. Aliquots of 50 or 100 μL (depending on the substrate concentration) were removed at various times and added to 20 μL (per 50 μL of assay mixture) of 2 M $HClO_4$ to terminate the reaction. Further, 100 μL aliquots of the stock reductive incubation buffer that had been diluted 1:10 with 0.20 M Epps at pH 8.0 were added to 130 μL cofactor−substrate solutions containing the same concentrations of components as before, also at 37 °C. The solutions were mixed quickly by swirling gently and incubated at 37 °C during the course of the assays with the substrate. When the substrate assays were completed, 100 μL aliquots were removed from the reaction mixtures and added to 40 μL of 2 M $HClO_4$. Before derivatization and HPLC analysis, the quenched solutions were spun briefly in a bench-top centrifuge to pellet the precipitated protein.

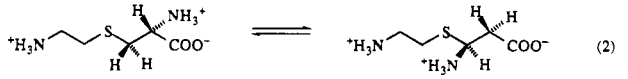
For HPLC analysis, samples were reacted with phenyl isothiocyanate (Heinrikson & Meredith, 1984) and chromatographed through a C8 reversed-phase column using a solvent consisting of a 1:1 mixture of solutions A and B described in the following section for the characterization of formylacetic acid. Elution of the phenylthiocarbamyl derivatives was monitored spectrophotometrically at 254 nm. Areas of chromatographic peaks were used to quantify the unreacted substrate at various reaction times. Peak areas were correlated with concentrations by the use of standard solutions. The initial rates were calculated from the initial slopes of the plots of integrated peak areas versus time.

To determine whether the enzyme lost activity during the course of the 4-thialysine assays, after the 5 h incubation, or after thawing frozen incubated enzyme, samples were assayed by the standard method as follows. A 20 μL aliquot was diluted with 980 μL of the stock reductive incubation buffer that had been diluted 1:10 into 0.20 M Epps at pH 8.0. A 25 μL aliquot of this diluted enzyme was added to 30 μL of a cofactor−[U-^{14}C]lysine solution prepared to give final concentrations of 0.26 M Epps, 44 mM [U-^{14}C]lysine (0.01 μCi μmol^{-1}), 3.2 mM sodium dithionite, and 18 μM *S*-adenosylmethionine, as well as activation components carried over with the reduced, diluted enzyme. The solution was quickly mixed by swirling gently and incubated at 37 °C. The reaction was quenched at 7 min with 30 μL of 0.5 M formic acid. Radiochemically labeled lysine and β-lysine were separated by paper electrophoresis, and their radioactivity was measured by liquid scintillation counting, as described previously (Chirpich et al., 1970). Specific activities in these assays ranged from 28 to 38 IU mg^{-1} protein. The concentration of enzyme was determined spectrophotometrically, assuming an extinction coefficient of 3.6 × 10^5 M^{-1} cm^{-1} at 280 nm (Song & Frey, 1991).

Identification of Formylacetic Acid. An enzymatic reaction mixture (230 μL) consisting of 13 mM 4-thialysine, 1.2 mM *S*-adenosylmethionine, 2.3 mM sodium dithionite, 0.28 M Epps buffer (pH 8.0), and 2.8 mg of reductively incubated lysine 2,3-aminomutase (430 IU mL^{-1}, 43 μM) was prepared in the anaerobic chamber. After 45 min, the reaction was quenched with 20 μL of 2 M $HClO_4$. An aliquot (15 μL) was combined with 30 μL of a solution of ethanol, water, and concentrated H_2SO_4 (2:5:1) saturated with 2,4-dinitrophenylhydrazine. The resulting yellow solution was analyzed by HPLC over a C8 reversed-phase column eluted with a 1:1 mixture of solutions A and B. Solution A was 50 mM NH_4OAc (pH 6.8) and solution B was 0.10 M NH_4OAc (pH 6.8) in 44:10:46 acetonitrile−methanol−water. The elution time of the enzymatically generated formylacetic acid 2,4-dinitrophenylhydrazone was identical with that of the 2,4-dinitrophenylhydrazone derivative of authentic formylacetic acid prepared by base-catalyzed hydrolysis of 1,3-dimethyluracil (Lovett & Lipkin, 1977). In addition, the authentic and enzymatic formylacetic acid samples were treated with dinitrophenylhydrazine at 45 °C. Decarboxylated dinitrophenylhydrazones were obtained from both samples, and the decarboxylated samples comigrated by HPLC (solution B was used as eluent).

RESULTS AND DISCUSSION

Substrate Reactivity of 4-Thialysine. 4-Thialysine is an alternative substrate for lysine 2,3-aminomutase as it undergoes normal mutation to 4-thia-L-β-lysine according to eq 2. However, 4-thia-L-β-lysine is an unstable molecule that



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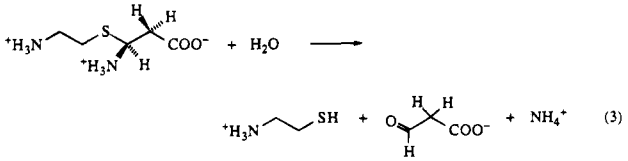
decomposes to β-mercaptoethylammonium ion, formylacetate, and ammonium ion (eq 3). Formylacetate has been



(3)

identified as a product by comparison of the HPLC retention time of its 2,4-dinitrophenylhydrazone with that of an authentic sample, as described in Experimental Procedures. Measurements of the initial rate of reaction of 4-thialysine as a function of its concentration at 37 °C and pH 8.0 gave values of 5.5 ± 0.8 mM and 1.05 ± 0.06 IU mg^{-1} protein for K_m and V_m, respectively. These parameters, when compared with those for lysine (K_m = 7 mM and V_m = 35 IU mg^{-1} protein), show that 4-thialysine is a slow substrate for lysine 2,3-aminomutase.

EPR Spectroscopy. As shown in Figure 1B, reaction mixtures with 4-thialysine as the substrate, frozen in the steady state, exhibit a distinctive EPR signal with a *g* factor and line shape characteristic of an organic radical. (All

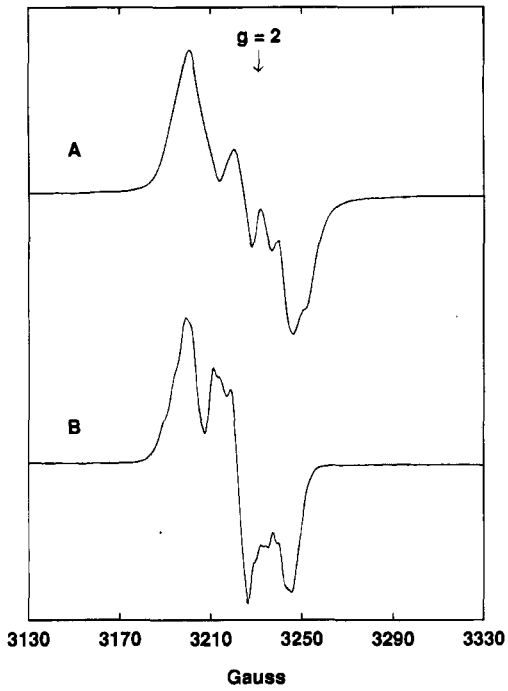


FIGURE 1: EPR spectra of steady-state radical intermediates in the reactions of lysine and 4-thialysine as substrates for lysine 2,3-aminomutase. Activated lysine 2,3-aminomutase was mixed with either lysine or 4-thialysine in EPR tubes and then frozen in liquid N_2 within 30 s. (A) The reaction mixture contained 0.15 M lysine, 1.2 mM *S*-adenosylmethionine, 2.3 mM sodium dithionite, and 440 IU mL^{-1} lysine 2,3-aminomutase (2.8 mg) in 0.23 mL of 80 mM Tris−H_2SO_4 buffer at pH 8.0. (B) The reaction mixture contained 20 mM 4-thialysine in place of lysine in the same reaction mixture. The spectra were obtained at 77 K. Instrument settings: microwave frequency, 9.045 GHz; modulation amplitude, 1.6 G; microwave power, 5 mW. Relative gain: A, 8; B, 1.

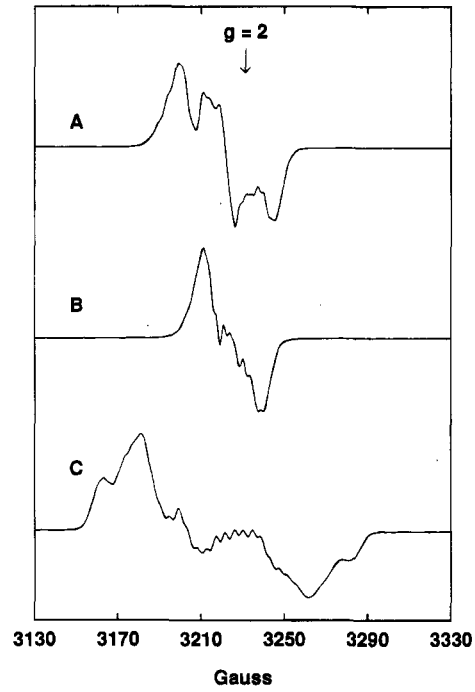


FIGURE 2: Effects of ^2H and ^{13}C substitution at carbon-3 on the EPR spectra of the 4-thialysine radical at the active site of lysine 2,3-aminomutase. The procedure was the same as that employed for Figure 1. The substrates were 4-thialysine in spectrum A, 4-thia-DL-[3-$^2H_2$]lysine in spectrum B, and 4-thia-L-[3-^{13}C]lysine in spectrum C. The enzyme utilizes only L stereoisomers as substrates; the D enantiomers are not inhibitors (Ballinger, 1992b).

signals in the EPR spectra shown in Figures 1−3 require the presence of activated lysine 2,3-aminomutase, that is, the signals depend upon reductive incubation of the enzyme and the presence of *S*-adenosylmethionine and either lysine or 4-thialysine.) The EPR signal of the α-radical of β-lysine (intermediate **3**, Scheme 1) is shown in Figure 1A for reference purposes. The 4-thialysine radical signal is centered at $g = 2.003$ and exhibits partially resolved hyperfine splitting. The concentration of the radical is ~50 μM and that of the enzyme (subunits) is 250 μM. Therefore, the radical concentration corresponds to a minimum of one per hexamer in the steady state.

The unpaired electron in the 4-thialysine radical is centered on carbon-3 of the 4-thialysine skeleton, as shown by the EPR spectra in Figure 2. The spectrum in Figure 2B arises from a sample prepared with 4-thia-DL-[3-$^2H_2$]lysine. The hyperfine splitting pattern in Figure 2B is substantially narrower than that from the protio sample of 4-thialysine in Figure 2A. Such a change in the hyperfine splitting pattern is expected if the unpaired electron resides on carbon-3, where it would be strongly coupled to the remaining proton at this carbon (in the the unlabeled sample). The spectrum generated with 4-thia-L-[3-^{13}C]lysine (Figure 2C) exhibits a large ^{13}C hyperfine splitting expected for the host carbon nucleus of a π-alkyl radical (Morton, 1964). Preliminary analysis suggests principal values for the ^{13}C hyperfine splitting tensor of $A_{xx} \sim 9$; $A_{yy} \sim 9$, and $A_{zz} \sim 80$ (G), which compare favorably with those obtained previously for the α-carbon of the α-radical (Ballinger et al., 1992b). Thus, the *g* value and the strong hyperfine splitting from ^1H and ^{13}C at carbon-3 indicate that the unpaired spin density is localized in a p orbital on carbon-3 of the 4-thialysine skeleton.

Although the unpaired electron is centered on carbon-3 of 4-thialysine, the spectra obtained in reaction mixtures prepared with 4-thia-DL-[5,6-$^2H_4$]lysine and 4-thia-DL-[3,5,6-$^2H_6$]lysine (Figure 3) indicate that part of the hyperfine splitting in the spectrum of the radical comes from protons on carbon-5 (carbon-6) on the "other side" of the 4-sulfur atom of 4-thialysine. Observable hyperfine splitting from these remote protons suggests either that there must be some delocalization of the unpaired spin through the intervening sulfur atom or that the torsion angle defined by carbon-3, sulfur-4, carbon-5, and carbon-6 is close to 0°, thereby bringing the protons at carbon-6 within ~2 Å of carbon-3. It is significant in this regard that the presence of sulfur adjacent to the center of unpaired spin in this radical has little influence on the *g* value of the radical. A more complete accounting of spin density awaits a more exhaustive analysis of the hyperfine splitting in the spectra of the sample with 4-thialysine and the isotopically labeled samples.

The α-radical **3** in Scheme 1 has recently been characterized as the dominant organic radical in the steady state of the reaction of lysine as the substrate for lysine 2,3-aminomutase (Ballinger et al., 1992a,b). This radical is a catalytic intermediate and the only one seen in the EPR spectrum in the steady state, presumably because it is the most stabilized radical in the mechanism of Scheme 1. Stabilization arises from the delocalization of 15−20% of the electron spin into the carboxyl group of **3**. Less stabilization is available to the other radicals in Scheme 1. Although delocalization of the unpaired electron into the

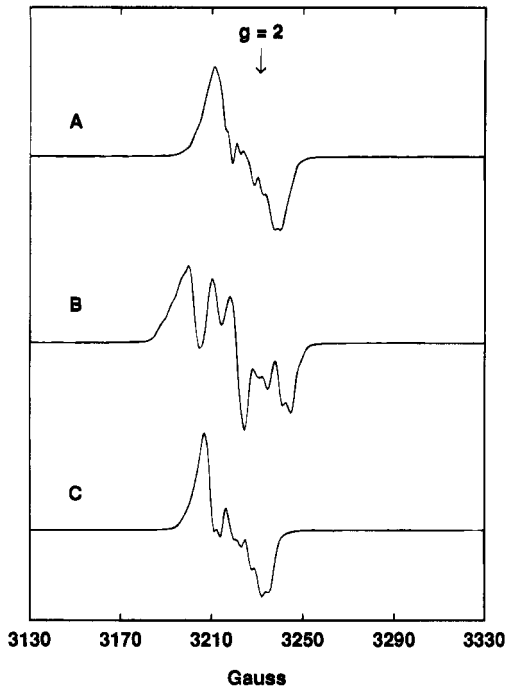


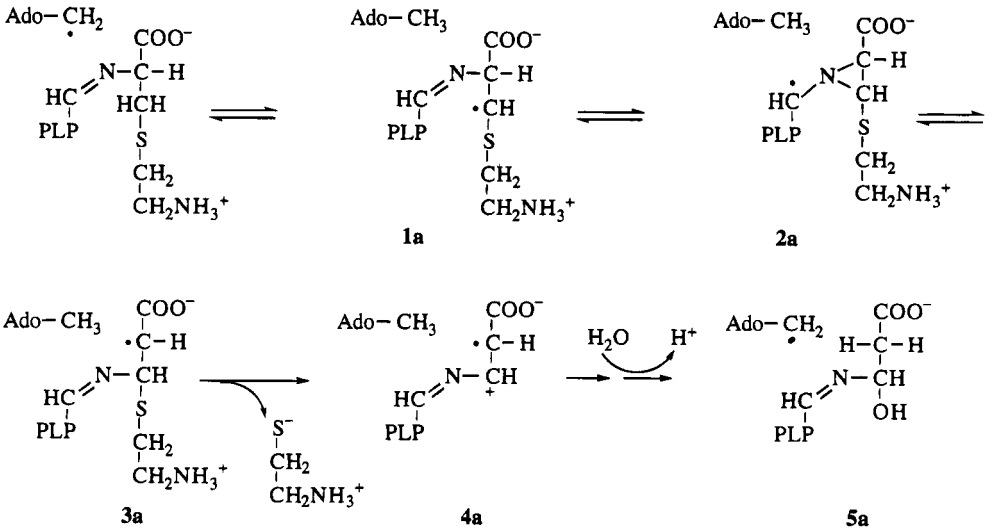
FIGURE 3: Effects of deuterium substitution at carbons-3, -5, and -6 on the EPR spectra of 4-thialysine radicals at the active site of lysine 2,3-aminomutase. The procedure was the same as that employed for Figure 1. The substrates were 4-thia-DL-[3-$^2H_2$]lysine in spectrum A, and 4-thia-DL-[5,6-$^2H_4$]lysine in spectrum B, and 4-thia-DL-[3,5,6-$^2H_6$]lysine in spectrum C.

pyridine ring of **2** should stabilize the radical, the strain in the aziridine ring counteracts the stabilization due to delocalization. Apparently, the summation of these effects leaves **2** too unstable to be observed in the presence of the dominant radical **3**. Earlier studies clearly demonstrate that no radicals other than **3** in Scheme 1 can be observed by EPR spectroscopy with lysine or β-lysine as the substrate and *S*-adenosylmethionine as the cofactor.

Carbon-centered radicals adjacent to sulfur have been reported previously (Gilbert et al., 1987). Results of the present experiments (Figures 1−3) indicate that the sulfur atom in 4-thialysine does indeed stabilize the corresponding β-radical relative to the other radicals in the rearrangement pathway. The EPR properties of this β-radical, however, indicate that it is carbon-centered. Preliminary analysis by simulation indicates that the major hyperfine splittings arise from the C3 proton and the nitrogen and proton attached to C2. These hyperfine splittings appear quantitatively similar to those from the α-proton, β-nitrogen, and β-proton of the α-radical intermediate **3** in the reaction of lysine. Major differences in the spectra (Figure 1A,B) result from additional hyperfine splitting from protons on carbons-5 and -6 of the 4-thialysine radical. Preliminary analysis indicates that the EPR spectra are homogeneous, i.e., a single radical contributes nearly all of the observed intensity.

4-Thialysine Is a Substrate for Lysine 2,3-Aminomutase. In the simplest rationale for its reactivity, 4-thialysine may be postulated to react in place of lysine according to Scheme 1 to produce 4-thia-L-β-lysine as the product released from the enzymatic site. This product would decompose to formylacetate, β-mercaptoethylammonium ion, and ammonium ion in aqueous solution, according to eqs 2 and 3. Alternatively, the enzymatic mechanism may follow Scheme 1 to a point and then diverge into an alternative pathway leading to the same products. Considering the potential reactivity of the 4-thia analog of radical **3** in Scheme 1, its decomposition to products might conceivably follow a different course. Scheme 2 illustrates how the 4-thia analog of the α-radical **3a**, which is analogous to **3** in Scheme 1, might undergo elimination of 2-ammonioethylthiolate ion to form an azo radical cation, **4a**. This species could accept a hydrogen atom from 5′-deoxyadenosine and a hydroxyl group from water to form **5a** at the active site. Hydrolysis of **5a** would produce the aminal of formylacetate, which would decompose to formylacetate and ammonium ion in aqueous solution. The formation of the azo radical cation **4a** in Scheme 2 is analogous to the formation of oxy radical cations in the reactions of glycols with radical chain initiators (Walling & Johnson, 1975). No specific evidence supporting the mechanism corresponding to steps 4 and 5 in Scheme 2 is available. The alternative scheme is provided here to stress that the most obvious mechanism for the reaction of 4-thialysine is not necessarily the only conceivable reaction course. In any case, the dominant radical in the reaction of 4-thialysine as a substrate is **1a** in Scheme 2, the 4-thia analog of the β-radical **1** in Scheme 1.

Scheme 2



The EPR spectra in Figures 1−3 do not specifically implicate PLP in the structure of the 4-thialysine radical, which is likely to be **1a** in Scheme 2. The relatively high steady-state concentration of the 4-thialysine radical should facilitate further characterization of this species and its linkage to PLP by electron spin echo and electron nuclear double-resonance techniques. These studies, together with the detailed kinetic and conformational characterization of the 4-thialysine radical, are currently in progress.

The identification in Figures 1−3 of the 4-thialysine radical at the active site of lysine 2,3-aminomutase as the 4-thia analog of the β-radical **1** constitutes the observation of the second of the four radical species in the mechanism outlined in Scheme 1. The α-radical **3** is the first radical to be observed in this reaction (Ballinger et al., 1992a,b). The azacyclopropylcarbinyl radical **2** and the 5′-deoxyadenosyl radical remain to be detected as components of the mechanism. The strategy of thioether substitution adjacent to the site of carbon radical formation might be useful in other enzymatic systems, wherein the concentrations of radical intermediates are too low to permit detection by EPR methods.

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