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ADVANCES IN ENZYMOLOGY, v65

Page v, "Contents", line 10

The authors of the article "Pro- and Antioxidant Functions of Quinones and Quinone Reductase in Mammalian Cells" should read: "Enrique Cadenas, Paul Hochstein and Lars Ernster".

Page 393, "Cumulative Author Index", insert after line 5

Ernster, Lars, see Cadenas, E.
LYSINE 2,3-AMINOMUTASE AND THE 
MECHANISM OF THE INTERCONVERSION 
OF LYSINE AND β-LYSINE

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I. Introduction

H. A. Barker and co-workers observed the enzymatic conversion 
of lysine into β-lysine in Clostridia in 1966 (1). They purified and 
described lysine 2,3-aminomutase, the enzyme that catalyzes re-
action 1, in 1970 and showed that the rearrangement proceeds with 
conservation of hydrogen in the substrate; that is, no protons from 
the solvent are incorporated into nonexchangeable positions of ly-
sine or β-lysine (2). Barker and co-workers described the molecular 
properties of lysine 2,3-aminomutase and reported that the enzyme
is activated by S-adenosylmethionine, ferrous ions, and pyridoxal phosphate (PLP) (2).

A. THE MECHANISTIC SIGNIFICANCE OF LYSINE 2,3-AMINOMUTASE

The lysine 2,3-aminomutase reaction is interesting from a mechanistic standpoint because of the cofactor requirements and the mechanistic barrier to 1,2-amino group migrations. In reaction 1 there is cross-migration of an unactivated hydrogen and a neighboring group between adjacent carbon atoms. It is an example of one that follows the pattern of Eq. 2, in which X is the \( \alpha \) amino group and H is one of the \( \beta \)-hydrogen atoms of lysine. These reactions normally require adenosylcobalamin as a cofactor, and, as originally established by Abeles and his associates, adenosylcobalamin mediates hydrogen transfer in these reactions (3). However, lysine 2,3-aminomutase is not activated by adenosylcobalamin and does not contain a \( \text{B}_{12} \) derivative (2). Barker and his associates originally reported that in addition to being activated by ferrous ions, PLP, and S-adenosylmethionine, the purified enzyme contains iron and PLP (2, 4).

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\]

(2)

Recent studies show that the enzyme requires four cofactors: iron-sulfur clusters, PLP, cobalt, and S-adenosylmethionine (5). The adenosyl moiety of S-adenosylmethionine is now known to mediate the transfer of hydrogen between carbon-2 and carbon-3 of the substrate in a manner that appears to be analogous to the action of the adenosyl moiety of adenosylcobalamin (6, 7).
B. THE METABOLIC SIGNIFICANCE OF LYSINE 2,3-AMINOMUTASE

The importance of lysine aminomutases, including lysine 2,3-aminomutase, in *Clostridial* lysine metabolism is reviewed in this series by T. C. Stadtman (8). The amino groups in lysine are not properly placed to allow the molecule to be degraded easily to acetyl CoA. β-Lysine can be oxidized to a β-ketoacid, which can be activated to the CoA ester and cleaved in β-ketothiolase-fashion to acetyl CoA. Lysine 2,3-aminomutase, β-lysine mutase, and D-lysine 5,6-aminomutase play important roles in the isomerization of lysine into structures that are easily degraded to acetyl CoA for use as a cellular fuel and building block. These reactions allow lysine to serve as the carbon and nitrogen source for *Clostridia*. β-Lysine is also significant as a constituent of antibiotics that are produced by *Streptomyces* and *Norcardia* (9–14).

II. Properties of Lysine 2,3-Aminomutase

A. PURIFICATION OF LYSINE 2,3-AMINOMUTASE WITH ITS COFACTORS

In the original purification of lysine 2,3-aminomutase, Barker and co-workers reported that the enzyme contains about 3 atoms of iron and 1.7 molecules of pyridoxal phosphate per hexameric unit, it is activated by S-adenosylmethionine, and its activity is 5 to 7 international units per mg of protein in their standard assay. The original purification has been repeated in this laboratory, and the activity and cofactor composition originally reported has been confirmed (5, 15). Improvements in the growth of *Clostridium SB4*, and in the purification of the enzyme have led to an increase in the specific activity of lysine 2,3-aminomutase to more than 30 units per mg protein (5, 16). The purified enzyme has also been found to contain inorganic sulfide and cobalt, as well as iron and PLP.

In early purification efforts in this laboratory, the major part of lysine 2,3-aminomutase activity was routinely lost from partially purified preparations whenever they were subjected to high resolution chromatographic procedures. This proved to be attributable largely to the separation of tightly bound substrate from the enzyme in the
course of chromatography. The inclusion of 0.1 mM lysine in purification buffers eliminated this problem (15).

Lysine 2,3-aminomutase purified in this laboratory is not more homogeneous than that described by Barker and co-workers, but it is more completely constituted with cofactors. The activity of the enzyme is maximal in crude extracts and throughout the purification procedure, when the bacteria are grown in a medium that is supplemented with 100 μM CoCl₂. Purified enzyme preparations generally contain zinc and copper as well as cobalt and iron; however, the presence of zinc and copper is at the expense of cobalt and of enzymatic activity. The most active preparations contain a minimum of zinc and copper and up to 5 or more gram atoms of cobalt per mole of hexameric enzyme. The zinc and copper content of the enzyme is minimized, and the activity is maximized, by including 5 μM CoCl₂ in the purification buffers (5, 16).

Another purification measure that is required to attain maximal activity in the purified enzyme is the rigorous exclusion of oxygen. Enzyme purified under rigorously anaerobic conditions contains approximately 12 gram atoms of iron and of sulfide per mole of hexameric protein (5). The iron and sulfide content corresponds to six Fe₂S₂ clusters or to three Fe₄S₄ clusters; EPR data are inconsistent with two-iron clusters but are consistent with the presence of four-iron clusters. In this laboratory, all purification steps are now carried out inside an anaerobic chamber. The purification buffers also contain 10 μM PLP and 0.1 mM lysine, which stabilize the activity, in addition to 5 μM CoCl₂. Enzyme purified in the presence of both PLP and lysine contains up to 5.5 gram atoms of PLP per mole of hexameric enzyme (17).

B. MOLECULAR PROPERTIES OF THE ENZYME

Barker and his associates described lysine 2,3-aminomutase from Clostridium SB₄ as a hexameric protein (MW 280-290K) composed of subunits with a molecular weight of 47,000 (2, 4). Recent experiments with lysine 2,3-aminomutase purified in this laboratory further supported this subunit composition (17). (a) Peptide mapping studies indicated that the subunits are chemically identical. Cleavage of the protein by cyanogen bromide gave nine major peptides, in agreement with the presence of eight methionine residues per sub-
unit. If the subunits differed significantly in amino acid sequence, up to twice this number of cyanogen bromide peptides would have been found. (b) Cross-linking experiments were consistent with a hexameric structure of the protein. Partially cross-linked samples of lysine 2,3-aminomutase give bands corresponding to monomers, dimers, tetramers, and hexamers upon polyacrylamide gel electrophoresis in SDS. No trimers or pentamers could be observed, indicating that the hexameric structure is composed of three dimeric units.

The best preparations of lysine 2,3-aminomutase obtained to date contain, per hexameric unit, approximately 12 Fe, 12 S²⁻, 5.5 PLP, and 5 Co, as well as much smaller residues of Zn and Cu (5, 16, 17). The specific enzyme activity increases with increasing amounts of Co. Increases in Co are at the expense of Cu and Zn, and increased specific activity is correlated with decreasing amounts of Zn and of Cu associated with the enzyme. It has so far not been possible to obtain a preparation that is completely free of Zn or Cu. The amount of Zn in the best preparations is near one gram atom per mole hexamer, and that of Cu is about 0.3 gram atom per mole hexamer. It remains possible that Zn is required for activity; however, the simplest interpretation of the available data is that Cu and Zn do not support enzymatic activity but that Co is required.

C. ACTIVATION OF THE ENZYME

The first preparations of purified lysine 2,3-aminomutase were essentially inactive, and all experiments in which the functions of this enzyme were probed had to be preceded by an activation protocol to convert the enzyme into an active form. Barker and co-workers activated the enzyme by incubating it anaerobically with dithionite and glutathione for three hours and then adding S-adenosylmethionine (2). The degree of activation was somewhat increased by substituting dihydrolipoate for glutathione and extending the activation to five hours (15).

Inasmuch as exposure of the enzyme to small amounts of oxygen inactivates it, one might suppose that lysine 2,3-aminomutase that had been rigorously protected from oxygen in the course of purification would be active. This is not true, however, because purification inside an anaerobic chamber does not prevent the enzyme
from losing activity. The enzyme must always be activated with S-adenosylmethionine. Purification under anaerobic conditions protects the metal cofactors from being irreversibly degraded and allows maximal activity to be attained upon activation by S-adenosylmethionine. Although an enzyme that has been purified under strictly anaerobic conditions still requires activation by S-adenosylmethionine, it does not absolutely require the reductive incubation with glutathione or dihydrolipoate. The best preparations exhibit 50 to 75% of their maximum activities upon addition of S-adenosylmethionine with no preliminary reductive incubation (16). However, the reductive incubation leads to maximum activity.

III. The Role of S-Adenosylmethionine in Hydrogen Transfer

Early experiments showed that substrate hydrogen is conserved in the lysine 2,3-aminomutase reaction (2). Stereochemical analysis showed that hydrogen transfer and amino group migration proceed with inversion of configuration (18). The 3-pro-R hydrogen of lysine is transferred to the 2-pro-R position of β-lysine according to reaction 3. Amino group transfer is intramolecular.

\[
\begin{align*}
\text{DH}^+ \text{H}^- \text{N-OH} & \quad \text{NH}_3^+ \text{COO}^- \\
+ \text{H}_3\text{N}^- & \quad + \text{H}_3\text{N}^+ \text{COO}^- \\
+ \text{H}_3\text{N}^- & \quad \text{H}^- \text{N-V}^{\text{P}} \text{OO}^- \quad \text{H}^+ \text{N-V} \text{OO}^-
\end{align*}
\]

(3)

In one experiment, Aberhart and co-workers used lysine 2,3-aminomutase to convert a mixture of lysine and [3,3-2H2]lysine to β-lysine by use of lysine 2,3-aminomutase. They analyzed the product for deuterium and reported that it consisted of monodeutero-β-lysine (18). This experiment showed that deuterium in the 3-pro-R position of [3,3-2H2]lysine is transferred to unlabeled lysine in the production of two molecules of monodeutero-β-lysine, β-[2-2H1]lysine, and β-[3-2H1]lysine. Therefore, although substrate hydrogen is conserved in the product, hydrogen transfer is intermolecular. Baraniak et al varied the ratio [3,3-2H2]lysine/lysine and analyzed the deuterium labeling in the samples of β-lysine produced from the mixtures (7).
Monodeutero-\(\beta\)-lysine and dideutero-\(\beta\)-lysine were detected in the products at all substrate ratios, and a plot of the ratio \(\beta\)-[\(^2\)H\(_2\)]lysine/\(\beta\)-[\(^2\)H\(_1\)]lysine versus [3,3-\(^2\)H\(_2\)]lysine/lysine was a straight line with a positive intercept and a positive slope. The value of 0.11 for the intercept showed that intramolecular deuterium transfer takes place in about 11% of the turnovers as the ratio [3,3-\(^2\)H\(_2\)]lysine/lysine approaches zero. Therefore, the conversion of lysine to \(\beta\)-lysine proceeds with both intramolecular and intermolecular hydrogen transfer.

Adenosylcobalamin-dependent rearrangements also characteristically proceed with conservation of hydrogen in the substrate and with both intermolecular and intramolecular hydrogen transfer (2, 19, 20). This results from the action of the adenosyl moiety of adenosylcobalamin in mediating hydrogen transfer, in which carbon-5' accepts hydrogen from the substrate and transiently becomes the methyl group of 5'-deoxyadenosine (19, 21).

The adenosyl moiety of \(S\)-adenosylmethionine mediates hydrogen transfer in the lysine 2,3-aminomutase reaction in the manner of the adenosyl moiety of adenosylcobalamin in B\(_{12}\)-dependent rearrangements (6, 7). The experimental evidence supporting this role for \(S\)-adenosylmethionine is that hydrogen bonded to the 5'-methylene group of \(S\)-adenosylmethionine becomes part of the pool of substrate hydrogens that appear in the reaction product. Thus, when lysine 2,3-aminomutase is activated with \(S\)-[5'-\(^3\)H\(_2\)]adenosylmethionine and used to catalyze reaction 1, it incorporates tritium into both lysine and \(\beta\)-lysine according to reaction 4 (6, 7).

\[
\text{Lysine} \quad \xrightarrow{E-[5'-3H_2]AdoMet} \quad [3H]\text{Lysine} + \beta-[^3H]\text{Lysine}
\] (4)

The following properties of lysine 2,3-aminomutase support the conclusion that the adenosyl moiety of \(S\)-adenosylmethionine mediates hydrogen transfer: 1) The enzyme incorporates tritium from \(S\)-[5'-\(^3\)H\(_2\)]adenosylmethionine into both lysine and \(\beta\)-lysine. 2) At chemical equilibrium in the foregoing experiment, the ratio of tritium in lysine and \(\beta\)-lysine is the same as the chemical ratio of lysine and \(\beta\)-lysine at equilibrium. 3) When the enzyme is activated with an
excess of $S\text{-}[5'\text{-}3\text{H}_2]$adenosylmethionine, the amount of tritium incorporated into lysine and $\beta$-lysine depends upon the amount of enzyme used, rather than on the amount of the cofactor in the reaction mixture. 4) When a sample of the enzyme is partially activated with less than a stoichiometric amount of $S\text{-}[5'\text{-}3\text{H}_2]$adenosylmethionine, all of the tritium is transferred from the cofactor to lysine and $\beta$-lysine. These experiments indicate that the activation of lysine 2,3-aminomutase by $S$-adenosylmethionine establishes a pool of hydrogen atoms that participates in the hydrogen transfer process. This pool includes the $5'$-methylene hydrogen atoms of the adenosyl moiety in $S$-adenosylmethionine and either the 3-pro-$R$ hydrogen atom of lysine or the 2-pro-$R$ hydrogen atom of $\beta$-lysine. These experiments also indicated that the activation of lysine 2,3-aminomutase by $S$-adenosylmethionine is stoichiometric and, for practical purposes, irreversible; that is, free $S$-adenosylmethionine does not exchange with enzyme-bound $S$-adenosylmethionine at a rate that compares with that of the enzymatic conversion of lysine into $\beta$-lysine.

If the adenosyl moiety of $S$-adenosylmethionine mediates hydrogen transfer, as required by the foregoing experiments with $S\text{-}[5'\text{-}3\text{H}_2]$adenosylmethionine, the reverse transfer may also take place; that is, the 3-pro-$R$ hydrogen of lysine or the 2-pro-$R$ hydrogen of lysine may be incorporated into $S$-adenosylmethionine, at least into the enzyme bound cofactor. Experiments designed to detect the transfer of deuterium from 3-$[\text{H}]$lysine to $S$-adenosylmethionine by lysine 2,3-aminomutase failed at first to verify such a process (22, 23). These experiments were carried out with $S$-adenosylmethionine in stoichiometric excess of the enzyme, and they implicitly depended upon the activation of lysine 2,3-aminomutase by free $S$-adenosylmethionine being reversible so that enzyme-bound $S$-adenosylmethionine would be in equilibrium with the free cofactor. The experiments also depended on $S$-adenosylmethionine bound to the enzyme being in equilibrium with the adenosyl moiety in its catalytically active chemical form. The former assumption proved to be incorrect, as shown by the observation that the amount of tritium mobilized into substrates from $S\text{-}[5'\text{-}3\text{H}_2]$adenosylmethionine depended upon the amount of enzyme rather than on the amount of cofactor in the reaction mixture (6). In recent experiments, lysine 2,3-aminomutase was activated with a stoichiometrically compa-
rable amount of S-adenosylmethionine and used to convert [3-^3H]lysine into \( \beta \)-lysine. S-Adenosylmethionine was reisolated from the reaction mixture and found to contain tritium (24). These experiments confirmed the earlier conclusion that the adenosyl moiety of S-adenosylmethionine mediates hydrogen transfer in the lysine 2,3-aminomutase reaction.

**IV. The Formation of Substrate Radicals as Intermediates**

Inasmuch as the lysine 2,3-aminomutase reaction is formally similar to adenosylcobalamin-dependent rearrangements, and the hydrogen transfer mechanisms also appear to be similar, the question of whether organic radicals participate as intermediates naturally arises because organic radicals are intermediates in some or all of the adenosylcobalamin-dependent rearrangements. Organic radicals are generated in the course of the lysine 2,3-aminomutase reaction, as shown by the electron paramagnetic resonance (EPR) spectra in Fig. 1 (25). The EPR spectra in Fig. 1 are those of four samples of the enzyme at 77 K that differ only in the presence or absence of lysine and S-adenosylmethionine, singly or together. Spectrum A is that of lysine 2,3-aminomutase that had been incubated with dihydrolipoate and PLP in preparation for activation by S-adenosylmethionine; no organic radical appears to be present. Spectrum B is an identical sample to which lysine had been added before freezing the solution for EPR analysis, and spectrum C is another identical sample to which S-adenosylmethionine had been added before freezing. Neither spectrum shows evidence of the presence of an organic radical. Spectrum D is of a sample to which both lysine and S-adenosylmethionine had been added before freezing, and this spectrum clearly shows an EPR signal centered at \( g = 2.003 \). The radical spectrum shows extensive structure corresponding to hyperfine couplings of the unpaired electron spin with neighboring nuclear spins.

The EPR signal of the organic radical in Fig. 1 is always observed in repetitions of the experiment, and it corresponds in signal intensity to between 9 and 10% of the active sites when the reaction solution is frozen in the steady-state after addition of lysine and before very much \( \beta \)-lysine is produced. In the course of the reaction, the intensity of the radical signal declines to an equilibrium value at a rate that corresponds closely to the rate of approach to chemical
Figure 1. Electron paramagnetic resonance spectrum of organic radical intermediates in the lysine 2,3-aminomutase reaction.

The samples contained 31 μM lysine 2,3-aminomutase (hexamer), which were frozen at 77 K within 90 s after the following treatments. (A) The EPR spectrum of the enzyme after reductive incubation in preparation for activation. (B) The enzyme reduced as in A plus 200 mM L-lysine. (C) The enzyme reduced as in A plus 1.2 mM S-adenosylmethionine. (D) The enzyme reduced as in A plus 200 mM L-lysine and 1.2 mM S-adenosylmethionine.
equilibrium between lysine and β-lysine. Moreover, the intensity of the signal at equilibrium is directly proportional to the amount of enzyme activity in the reaction mixture (25). Finally, the signal changes slightly in the course of the approach to equilibrium, showing that the signal is inhomogeneous at equilibrium. The signal at equilibrium includes the signal for a second radical that does not appear to be an intermediate.

The properties of the EPR signal at $g = 2$ indicate that organic radicals may be intermediates in the lysine 2,3-aminomutase reaction. Radical intermediates would appear only in the presence of the active enzyme and a substrate, which is the case in Fig. 1. The concentration of an intermediate will be determined by the concentration of the enzyme, as is the concentration of the radical in Fig. 1. The concentration of an intermediate will also be determined by the ratio of the concentrations of lysine and β-lysine. Under conditions in which the concentrations of lysine and β-lysine change, as they do in the course of approaching equilibrium, the concentration of an intermediate is likely to change owing to differences in kinetic parameters of steps connecting the intermediate to the substrate or product. Thus, in a simple isomerization of a substrate to a product through one intermediate $X$ according to Scheme 1, the substrate and product are connected to $X$

$$
\text{Scheme I} \\
E + S \xrightleftharpoons[k_2]{k_1} E\cdot S \xrightarrow[k_4]{k_3} X \xrightarrow[k_6]{k_5} E\cdot P \xrightarrow[k_8]{k_7} E + P
$$

through steps governed by different rate constants. The fraction of enzyme complexes that are in the form of $X$ will depend on the relative values of the rate constants and the ratio of substrate to product. Within the first few seconds of mixing the substrate with the activated enzyme, little product is present and the concentration ratio of $X$ to total enzyme, $[X]/[E_T]$, is determined largely by the concentration of the substrate and the values of $k_1$, $k_2$, $k_3$, and $k_4$. At equilibrium, the concentration of the substrate is decreased and that of the product is increased because of the equilibrium constant ($K_{eq} = 6$), and the ratio $[X]/[E_T]$ is governed by the values of all the rate constants and the ratio of substrate to product. Inasmuch as the product concentration is larger than the substrate concentra-
tion at equilibrium in the lysine 2,3-aminomutase reaction, the values of \( k_5, k_6, k_7, \) and \( k_8 \) must be very important in determining the ratio \([X]/[E_T]\). The change in signal intensity for the organic radical in lysine 2,3-aminomutase in the course of the approach to equilibrium is in accord with these expectations.

In the case of lysine 2,3-aminomutase, both the signal intensity and the signal shape change in the course of approaching equilibrium, which suggests that the radical signal is inhomogeneous at equilibrium and represents the presence of more than one organic radical. An inhomogeneous signal could arise from two radicals corresponding to two intermediates that are chemically connected by a reversible step. If two radical intermediates, \( X_1 \) and \( X_2 \), are on the reaction pathway, Scheme 2 can account for the change in the radical signal intensity in the course of

\[
\text{Scheme 2} \\
E + S \xrightarrow{k_1/k_2} E \cdot S \xrightarrow{k_3} X_1 \xrightarrow{k_5} X_2 \xrightarrow{k_7} E \cdot P \xrightarrow{k_9} E + P
\]

approaching equilibrium for the same reasons as in Scheme 1, except that the ratio of total radical to total enzyme is \([X_1 + X_2]/[E_T]\). If radical intermediates \( X_1 \) and \( X_2 \) are at equilibrium with each other throughout the approach to equilibrium, the EPR signal will not change in shape with time. However, if two intermediates are not at equilibrium but are connected by one or more steps in a steady-state, the ratio of \( X_1 \) to \( X_2 \) will change with the approach to equilibrium between the substrate and product. This appears to be the case because the EPR signal changes significantly in the course of the reaction, indicating the presence of two or more radicals at equilibrium (25). As explained in the following section, the radical signal that is observed in the steady-state immediately after mixing L-lysine with the activated enzyme is that of a single radical species; the second species appears later but does not seem to be an intermediate.

Initial experiments with isotopically-substituted samples of lysine show that the carbon skeleton of lysine hosts the steady-state radical. Resolution in the spectrum obtained with \([3,3,4,4,5,5,6,6-^{2}H_6]\)lysine as the substrate is substantially improved relative to that with lysine shown in Fig. 1 (25). The major splittings are, however, preserved in the spectrum with \([3,3,4,4,5,5,6,6-^{2}H_6]\)lysine, and the
enhanced resolution arises from the abolition of longer range couplings to the radical center by protons at carbons 3 through 6 upon substitution of deuterium at those positions. The fact that the unpaired spin is detectably coupled to these protons indicates that the organic radical is located on the carbon skeleton of lysine. There is only a slight increase in spectral resolution of samples prepared in D₂O, and this observation shows that the unpaired electron spin is very weakly coupled to exchangeable protons (25). Recent EPR measurements with [2-¹³C]lysine, [2-²H]lysine, and [2-¹⁵N]lysine identify the steady-state radical—the major species in Fig. 1—as a β-lysine imine radical with the unpaired electron on the α carbon. The EPR data show that the bond linking carbon-3 to nitrogen is approximately coplanar with the p-orbital on carbon-2 containing the unpaired electron, as shown in the following structure (26).

This conformation is fully compatible with the stereochemistry of the reaction.

V. The Role of Pyridoxal Phosphate in Amino Group Migration

The mechanistic importance of PLP in the lysine 2,3-aminomutase reaction and in other aminomutase reactions has been unclear for many years and remains uncertain to date. The catalytic function of PLP in nearly all known enzymatic and model nonenzymatic reactions is to stabilize carbanionic intermediates through its ability to delocalize electron pairs (27). (An exception to this rule is glycogen phosphorylase, in which PLP is required in a different capacity that does not entail imine formation with the substrate.) In the case of lysine 2,3-aminomutase, EPR measurements implicate substrate based organic radicals as intermediates. The transient formation of organic radicals does not rule out carbanionic interme-
diates if an electron transfer step is required in the mechanism; however, the amino acid carbanions that would be required for 1,2-amino group migrations include species that would not be stabilized by PLP.

A potential nonenzymatic model reaction for 1,2-amino group migration through the intermediate formation of organic radicals is based on the well-known cyclopropyl carbinyl radical rearrangement shown in reaction 5 (28). The reverse reaction proceeds with a rate constant of about $10^8 \text{ s}^{-1}$. If imine radicals undergo the analogous process according to reaction 6, a route by which PLP could facilitate a 1,2-amino group migration would be available. Reaction of an amine with PLP to form an imine, followed by radical formation at the carbon atom adjacent to the imine nitrogen would allow the rearrangement in reaction 6 to take place.

A precedent for reaction 6 appears to be the conversion of the benzaldimine of α-methyl-β-bromoalanine ethyl ester into a mixture of the benzaldimine derivatives of α-methylalanine ethyl ester 1 and α-methyl-β-alanine ethyl ester 2 in the presence of a free radical chain initiation system, as illustrated in Fig. 2 (29). Under kinetic conditions allowing rearrangement, the formation of the rearranged product 2 in Fig. 2 is favored over the formation of product 1. This can be rationalized by the fact that the α radical in Fig. 3 is more stable than the β radical. Because of the reversible connection between the two radicals through an isomerization mechanism such as that of reaction 6, the more stable radical will be the predominant form at equilibrium and will be quenched to the corresponding prod-
Figure 2. A nonenzymatic model for an α,β-imino radical rearrangement.

Abstraction of the bromine atom from the benzaldimine of 2-methyl-3-bromoalanine ethyl ester produces the 3-radical, which is either quenched or undergoes rearrangement to the 2-radical. Quenching of the 2-radical produces the rearrangement product (29). This reaction is thought to be a model for the action of PLP in facilitating α,β-imino rearrangements of radical intermediates in aminomutase reactions.
Figure 3. A hypothetical mechanism for the α,β-imino rearrangement of β-imino radicals.

This mechanism is analogous to the well documented cyclopropyl carbinyl radical rearrangement in which one of the ring atoms is nitrogen instead of carbon. It may be regarded as an azocyclopropyl carbinyl radical rearrangement.

uct. The greater stability of the α radical in Fig. 3 is due to the delocalization of the unpaired electron into the ester group. Under the conditions of the experiment, the ratio of $1:2$ was found to be $1:13$ (29).

The reaction in Fig. 2 can be a model for the isomerization of lysine and β-lysine if PLP forms an imine with the α-amino group of lysine at the active site of the enzyme, if the rearrangement is not impeded by the substitution of the pyridoxylidene group for the benzylidene group, and if the organic radical intermediates in the enzymatic reaction are structurally analogous to those in the model reaction. The intermediates in the putative model reaction are thought to be those in Fig. 3; however, the mechanism has not yet been definitively proven, although it appears to be reasonable.

There is little evidence for the direct participation of PLP in the mechanism of the lysine 2,3-aminomutase reaction. The enzyme is activated by PLP, and it is stabilized by the presence of PLP in purification buffers. However, all efforts to reduce PLP onto the enzyme or the substrate by treatment with NaBH$_4$ or NaB$_3$H$_4$ have failed to afford convincing evidence of imine formation, either between PLP and an amino group at the active site of the enzyme or between PLP and lysine (30). If PLP forms an imine at the active site, it is either not reactive with NaBH$_4$ or not accessible to the reagent. In the mechanism of Fig. 2 and 3, the imine is not protonated, and there appears to be no mechanistic advantage for a protonated imine relative to an unprotonated imine in this isomerization.
Unprotonated imines are much less reactive with NaBH₄ than protonated imines. If a lysine-PLP imine is an intermediate in the lysine 2,3-aminomutase reaction, it may be unprotonated and therefore less reactive with NaBH₄ than the protonated imines of other PLP-dependent enzymes. Moreover, such a species might be shielded from reaction with external reagents by other interactions with the enzyme or/and a metallic cofactor.

The EPR measurements in D₂O described above indicate that the migrating nitrogen atom in the radical intermediate is not bonded to more than one hydrogen atom. The EPR data are, therefore, most compatible with an imine nitrogen in the radical, and this constitutes the only direct evidence for PLP being linked as an imine with the migrating nitrogen atom.

VI. The Importance of Iron-Sulfur Clusters and Cobalt

Very little is known about the roles of the iron-sulfur clusters and cobalt in the lysine 2,3-aminomutase reaction. The presence of iron in the enzyme and the activating effect of added iron have been known since 1970, but inorganic sulfide and cobalt have only recently been discovered as constituents of the active enzyme (5).

The evidence for the presence of iron-sulfur complexes as constituents of this enzyme consists of a) the presence of both iron and sulfide in approximate stoichiometric equivalence in the purified enzyme, b) the long wavelength visible absorption spectrum of the enzyme, and c) the fact that the purified enzyme exhibits an EPR signal at 10 K that corresponds to an iron-sulfur cluster (5, 31). The EPR signal is complex and not yet characterized fully (31). The EPR signal is abolished by the reductive incubation that is required to attain maximum activation by S-adenosylmethionine. When interpreted in the context of the vast literature on the properties of iron-sulfur clusters, the experimental facts leave little doubt that iron-sulfur clusters are associated with the enzyme.

The evidence for the presence of cobalt consists of a) all samples of the enzyme contain cobalt, and b) the purified enzyme exhibits an EPR spectrum at 4 K that is characteristic of high spin Co(II) (5). The spectrum of Co(II) in unactivated lysine 2,3-aminomutase is shown in Fig. 4.

The mechanistic importance of iron-sulfur clusters and cobalt in
Figure 4. EPR spectrum of high-spin cobalt(II) bound to lysine 2,3-aminomutase at 4.3 K. The inset is an expansion of the $g = 7$ region, showing the $\text{Co}^{2+}$ hyperfine structure.

The action of lysine 2,3-aminomutase is a subject of speculation and experimental exploration. The only currently available evidence for their importance as cofactors is that the activity of the enzyme depends on the presence of both iron-sulfur clusters and cobalt (5). Although no experiment directly implicates either of these cofactors in catalysis, it seems likely that one or both of them participate in the reaction; one may be required for activation of the enzyme by $S$-adenosylmethionine and the other for an essential catalytic function of the activated enzyme.
A key process in the activation of lysine 2,3-aminomutase appears to be the chemical reaction of a reduced form of the enzyme with S-adenosylmethionine. Reaction of the enzyme with a thiol such as glutathione or dihydrolipoate places it in a chemical state in which it can react with the adenosyl moiety of S-adenosylmethionine reversibly. The chemical nature of this process allows the 5'-methylene group to participate in the transfer of substrate hydrogen between the α and β carbon atoms of the substrate in a manner that is analogous to the function of 5'-deoxyadenosine-5'yl—hereafter referred to as the 5'-deoxyadenosyl radical—in adenosylcobalamin-dependent rearrangements. The 5'-deoxadenosyl moiety in the catalytically active enzyme is most likely cleaved from methionine and bonded to one of the metallic cofactors. The properties of the adenosyl cofactor at the active site may include the capacity to generate the 5'-deoxyadenosyl radical reversibly, according to the scheme in Fig. 5. This radical could initiate the rearrangement by abstracting the 3-pro-R hydrogen atom from lysine, or the 2-pro-R hydrogen from β-lysine. The resulting substrate radical could undergo the reversible isomerization shown in Fig. 6 if lysine is bound as an α-imine to PLP. The structure of the substrate radical intermediate that can be observed in EPR experiments, the spectrum of which is shown in Fig. 1, corresponds to intermediate 5 in Fig. 6.

Cleavage of the 5'-deoxyadenosyl moiety from S-adenosylmethionine appears to be mandated by the fact that both hydrogen atoms bonded to carbon-5' are subject to be transferred to lysine and β-lysine in reaction 4. The most reasonable rationale for the participation of both hydrogen atoms of the 5' methylene group in the hydrogen transfer pool is that the 5'-deoxyadenosyl moiety becomes detached from methionine by a process that allows free rotation of the methylene group about the bond linking it to carbon-4' in its catalytically functional form in Fig 5. Thus, tritium in both stereotopic positions of carbon-5' in [5'-3H2]adenosylmethionine is subject to transfer to lysine and β-lysine through positional isotope exchange in the intermediate 5'-deoxyadenosine, as shown in Fig. 6.

Experimental evidence for the cleavage of S-adenosylmethionine is provided by the fact that it is cleaved to 5'-deoxyadenosine and methionine in the course of the reaction (15). Upon mixing S-aden-
Figure 5. Hypothetical steps in the activation of lysine 2,3-aminomutase.

The activation process begins with the incubation of lysine 2,3-aminomutase in the presence of a reducing system. This prepares the enzyme for activation by S-adenosylmethionine. This incubation is required to attain maximum activity; however, enzyme that has been purified under rigorously anaerobic conditions exhibits 50 to 70 percent of maximum activity without preliminary incubation. Addition of S-adenosylmethionine then leads to the active enzyme. The 5'-deoxyadenosyl moiety is probably transferred to a metal cofactor, which allows it to form the 5'-deoxyadenosyl radical reversibly.