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Kinetic and biochemical analysis of the mechanism of action of lysine 5,6-aminomutase

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Abstract

Lysine 5,6-aminomutase (5,6-LAM) catalyzes the reversible and nearly isoenergetic transformations of D-lysine into 2,5-diaminohexanoate (2,5-DAH) and of L-β-lysine into 3,5-diaminohexanoate (3,5-DAH). The activity of 5,6-LAM depends on pyridoxal-5′-phosphate (PLP) and adenosylcobalamin. The currently postulated multistep mechanism involves at least 12 steps, two of which involve hydrogen transfer. The deuterium kinetic isotope effects on k_{cat} and k_{cat}/K_m have been found to be 10.4 ± 0.3 and 8.3 ± 1.9, respectively, in the reaction of DL-lysine-3,3,4,4,5,5,6,6-d_8. The corresponding isotope effects for reaction of DL-lysine-4,4,5,5-d_4 are 8.5 ± 0.7 and 7.1 ± 1.2, respectively. Neither cob(II)alamin nor a free radical can be detected in the steady state by UV–Vis spectrophotometry or electron paramagnetic resonance (EPR) spectroscopy. Therefore, hydrogen abstraction from carbon-5 of the substrate side chain is rate limiting in the mechanism. DL-4-Oxalysine is an alternative substrate for 5,6-LAM. DL-4-Oxalysine reacts irreversibly because the product breaks down into ammonia, acetaldehyde, and DL-serine. The value of K_m for the reaction of DL-4-oxalysine is lower than that for DL-lysine and that of k_{cat} for DL-4-oxalysine is slightly lower than that for DL-lysine. As measured by values of k_{cat}/K_m, 5,6-LAM uses DL-4-oxalysine essentially as efficiently as the best substrates, D-lysine and L-β-lysine, and more efficiently than DL-lysine. DL-4-Oxalysine induces the same suicide inactivation by electron transfer as do the biological substrates. The putative substrate-related radical intermediate is not sufficiently stabilized by the nonbonding 4-oxa electrons to be detectable by EPR spectroscopy.

Keywords: Lysine 5,6-aminomutase; Adenosylcobalamin; Pyridoxal-5′-phosphate; Deuterium isotope effects; 4-Oxalysine; Lysine

Lysine 5,6-aminomutase (5,6-LAM)[1] in *Clostridium sticklandii* participates in the fermentation of DL-lysine by catalyzing the migration of the ε-amino group of either D-lysine or L-β-lysine to the δ-carbon [1]. Concomitant reverse migration of a hydrogen atom from C5 to C6 produces 2,5- or 3,5-diaminohexanoate (2,5/3,5-DAH) [1,2]. Pyridoxal-5′-phosphate (PLP) and adenosylcobalamin are essential coenzymes in the action of 5,6-LAM, which is an $\alpha_2\beta_2$-heterodimeric enzyme [1]. The reaction is reversible with an equilibrium constant for the conversion of D-lysine into 2,5-diaminohexanoate of 1.2 [2]. The overall reaction requires cleavage of unreactive C–H and C–N bonds, both of which are strong and present high-energy barriers to cleavage.

The action of 5,6-LAM is presumed to proceed by a radical mechanism analogous to that of the *S*-adenosyl-L-methionine (SAM) and PLP-dependent L-lysine 2,3-aminomutase (LAM) from *Clostridium subterminale.* The radical mechanism for 1,2-amino group and 1,2-hydrogen migration in the reaction of LAM has been documented by EPR spectroscopic and kinetic characterization of substrate-based radicals [3–6]. The hypothetical mechanism for 5,6-LAM is outlined in Fig. 1. The substrate binds to PLP as the external aldimine through its ε-amino group. Homolytic scission of the Co–C5′ bond in adenosylcobalamin reversibly generates

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[1] *Abbreviations used:* 5,6-LAM, lysine 5,6-aminomutase; SAM, *S*-adenosyl-L-methionine; PLP, pyridoxal-5′-phosphate; 2,5-DAH, 2,5-diaminohexanoate; 3,5-DAH, 3,5-diaminohexanoate; PITC, phenylisothiocyanate; EPPS, *N*-(2-hydroxyethyl)piperazine-*N*′-(3-propanesulfonic acid); EPR, electron paramagnetic resonance.

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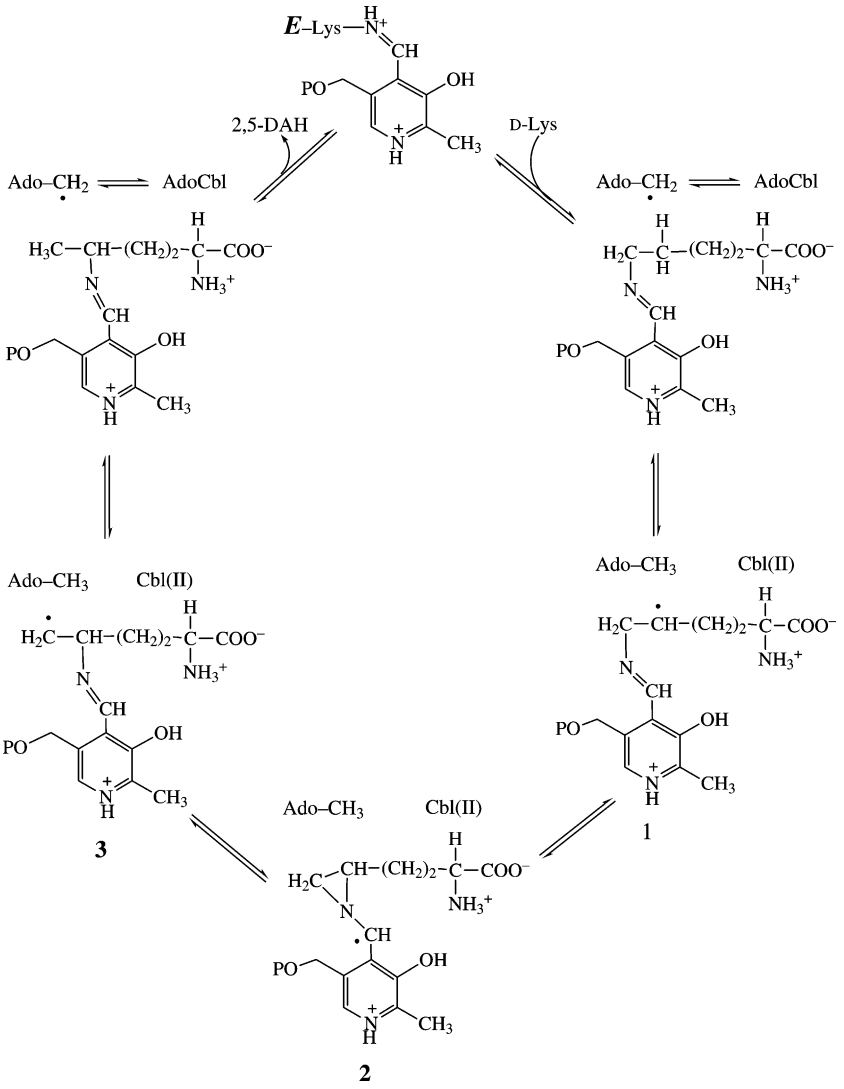

Fig. 1. Hypotical mechanism for the action of 5,6-LAM. The mechanism proposed here for radical rearrangement catalyzed by 5,6-LAM is inspired by the mechanism of action of the *S*-adenosylmethionine-dependent lysine 2,3-aminomutase. In the mechanism for 5,6-LAM, PLP binds lysine through its ε-amino group as the external aldimine. Substrate radical formation is brought about by abstraction of the C5(H) from the side chain of lysine by the 5′-deoxyadenosyl radical derived from reversible homolytic scission of the Co–C5′ bond in adenosylcobalamin. Rearrangement of the substrate-related radical **1** to the product-related radical **3** is facilitated by the imine linkage to PLP and the intermediate formation of the aziridylcarbinyl radical **2**. Hydrogen abstraction by radical **2** from the methyl group of 5′-deoxyadenosine, which remains bound to the active site, leads to the external aldimine of 2,5-DAH and PLP. The release of 2,5-DAH is brought about by transaldimination with the active site lysine to regenerate the internal aldimine of PLP.

the 5′-deoxyadenosyl radical and cob(II)alamin. Abstraction of a C5(H) from the side chain of the lysine-PLP aldimine by the 5′-deoxyadenosyl radical produces 5′-deoxyadenosine and substrate-related radical **1**, which undergoes internal addition to the imine–N to form the aziridylcarbinyl-PLP radical **2**. Analogous retroaddition by cleavage of the C6–N bond in a fashion similar to that of cyclopropyl carbinyl radical clocks [7] moves the chemistry forward to the product-related radical **3**. Abstraction of a hydrogen atom from the methyl group of 5′-deoxyadenosine by radical **3** completes the rearrangement of the amino group and regenerates the 5′-deoxyadenosyl radical, which in turn recombines with cob(II)alamin to regenerate adenosylcobalamin. Finally, 2,5-DAH is released from the active site after transaldimination with the active site lysine to regenerate the internal aldimine of PLP. The active site lysine is Lys^{144} in the β-subunit [8].

Deuterium kinetic isotope effects have been reported for several adenosylcobalamin-dependent isomerases. Product release is likely to be the rate-limiting step in the overall reaction of methylmalonyl-CoA mutase [9]; however, a large deuterium kinetic isotope effect has been reported in the cleavage of adenosylcobalamin in the reaction of [2-CD_3]methylmalonyl-CoA [10]. A very large deuterium isotope effect (k_H/k_D) has been

measured in the reaction of glutamate mutase (range from 28 to 35) [11], and a large deuterium kinetic isotope effect (>10) is estimated for the reactions of ethanolamine ammonia lyase (EAL) [12] and diol dehydratase [13]. In this paper, we report measurements of deuterium kinetic isotope effects in steady-state kinetic experiments with 5,6-LAM. We observe relatively large deuterium kinetic isotope effects on k_{cat} and k_{cat}/K_m in the formation of 2,5-DAH. Furthermore, DL-4-oxalysine, a substrate analog, is found to be an alternative substrate. The steady-state kinetic parameters for D-lysine, DL-lysine, L-β-lysine, and DL-4-oxalysine are reported and compared.

Experimental procedure

Materials

DL-Lysine was obtained from Sigma, DL-lysine-4,4,5,5-d_4 and DL-lysine-3,3,4,4,5,5,6,6-d_8 were from Isotec. DL-4-Oxalysine was prepared by a described procedure [14]. L-β-Lysine was synthesized enzymatically from L-lysine with L-lysine 2,3-aminomutase [15]. Phenyl isothiocyanate (PITC), triethylamine, and pyridine were purchased from Pierce. HPLC-grade methanol and acetonitrile were from Fisher Scientific. The recombinant 5,6-LAM from *Porphyromonas gingivalis* was produced by expression in *Escherichia coli* as described previously [8]. The concentration of enzyme was determined spectrophotometrically using an extinction coefficient calculated from the deduced amino acid sequence of 5,6-LAM ($\varepsilon_{280} = 9.9 \times 10^4\,M^{-1}\,cm^{-1}$).

Spectrophotometric studies

The kinetic UV–Vis spectra were measured on a Hewlett–Packard model 8452A diode array spectrophotometer with the cuvette holder temperature maintained by a circulating water bath. The inactivation rate was measured using an OLIS Inc. RSM-1000 stopped-flow spectrophotometer, monitoring the appearance of cob(III)alamin at 358 nm.

Recording UV–Vis spectra under anaerobic conditions

In anaerobic experiments, the substrate or substrate analog, in the side arm of anaerobic cuvette, and enzyme in the main chamber (the cuvette, 1.00 mL) were made anaerobic by repeated evacuation followed by purging with oxygen-free argon. The enzyme mixtures for spectrophotometry were made by mixing 5,6-LAM (40 μM) in 100 mM NH_4EPPS (pH 8.5), 45 μM PLP, and 20 mM substrate or substrate analog. After the reference spectra, containing all components except adenosylcobalamin, were obtained, inactivation was initiated by addition of adenosylcobalamin (45 μM final concentration). Spectra from 190 to 800 nm were obtained every 11 s for the first minute, and the time interval was then doubled between each succeeding spectrum.

Determination of the inactivation rate constant (k_{inact})

Inactivation reactions were monitored by observing the generation of cob(III)alamin, the decomposition product of adenosylcobalamin, by UV–Vis spectrophotometry (27 °C) or by rapid scanning stopped-flow spectrophotometry (16 °C). The dramatic rise at 358 nm is characteristic of cob(III)alamin production, while the absorbance decreases at 466 nm. The distinctive bands at 505 and 534 nm that develop with increasing incubation time also signal cob(III)alamin formation. The rate of inactivation was determined by plotting A_{358} versus time. The data were fitted to the single-exponential growth function, $A_{358} = a(1 - e^{-kt}) + b$, in which k (k_{inact}) is the rate constant of inactivation.

Activity assay

5,6-LAM was pre-incubated at 37 °C for 2 min with 100 mM K^+EPPS buffer at pH 8.5, 5 mM β-ME, 200 μM adenosylcobalamin, and PLP in a total volume of 300 μl, and the reaction was initiated by the addition of various concentrations of unlabeled or deuterium-labeled DL-lysine, L-β-lysine, or DL-4-oxalysine. Aliquots (15 μl) were taken at various time intervals and the reaction was quenched immediately by the addition of 2 μl of 2 N $HClO_4$. After the removal of protein precipitates, 5′-deoxyadenosine and adenosylcobalamin were removed by loading onto a C_{18} Sep-Pak cartridge (Waters), which had been washed with methanol. The lysine or its analog and their turnover products were eluted with de-ionized water, and the volume of eluted solution was reduced to 50 μl using a SpeedVac apparatus.

Derivatization and HPLC analysis of amino acids

DL-Lysine and 2,5-DAH, L-β-lysine and 3,5-DAH, DL-4-oxalysine, and DL-serine were derivatized by reaction with phenylisothiocyanate (PITC) as described [16]. The PITC-derivatized DL-lysine and 2,5-DAH; L-β-lysine and 3,5-DAH; or DL-4-oxalysine and DL-serine were applied to an analytical C_8 column (Vydac). DL-Lysine and 2,5-DAH, L-β-lysine, and 3,5-DAH were eluted with a gradient of 65% buffer A (0.05 M ammonium acetate) to 100% buffer B (0.1 M ammonium acetate in 44% H_2O, 46% acetonitrile, and 10% methanol) at pH 6.8 over a period of 30 min at a flow rate of 1 ml/min. DL-4-Oxalysine and DL-serine were eluted with a gradient of 50% buffer A to 100% buffer B over a period of 25 min at a flow rate of 1 ml/min. The relative areas of the peaks corresponding to DL-lysine and 2,5-DAH

were calculated to determine the fraction of DL-lysine (retention time 28 min) converted to 2,5-DAH (retention time 29.3 min). The initial velocities of 2,5-DAH formation were calculated from assays of 2,5-DAH formation at various times during the incubation. To determine the amount of 2,5-DAH formed at each point in time, the ratio of the area of the peak of 2,5-DAH-PITC to the area of the sum of the peaks for DL-lysine and 2,5-DAH-PITCs was multiplied by the total DL-lysine initially present.

Similarly, the initial velocities of 3,5-DAH formation (produced from L-β-lysine) and DL-serine production (produced from DL-4-oxalysine) were obtained by the same method as that employed for DL-lysine. When eluted by the same gradient as in the separation of DL-lysine and its products, the retention time for L-β-lysine is around 23.7 min, 25.7 min for 3,5-DAH, 18 min for DL-4-oxalysine, and 7 min for DL-serine.

Measurement of kinetic parameters

To determine the values of V_m and K_m for each substrate, 5,6-LAM was pre-incubated at 37 °C for 2 min with 100 mM K^+EPPS buffer at pH 8.5, 5 mM β-mercaptoethanol, 200 μM adenosylcobalamin, and PLP in a total volume of 20 μl, and the reaction was initiated by the addition of DL-lysine, D-lysine, L-lysine, L-β-lysine, or DL-4-oxalysine at appropriate concentrations. After incubation at 37 °C for 2 min, the reactions were quenched by the addition of 2 μl of 2 N $HClO_4$. The precipitated protein was removed by centrifugation and the supernatant fluid was loaded onto a C_{18} Sep-Pak cartridge (Waters) to remove 5′-deoxyadenosine and adenosylcobalamin. The lysine or its analog and their turnover products were eluted with de-ionized water and assayed as described in the preceding section.

In measurements of deuterium isotope effects in the steady state, the initial velocity of product formation was measured within the first 0.50 min rather than 2 min, to minimize the effects of substrate-induced suicide inactivation [2]. Data points were obtained in triplicate to attain a 5% standard deviation from the mean. The kinetic or inhibition data were fitted to Eqs. (1) or (2) (Lineweaver–Burk double-reciprocal plot) as appropriate:

$$1/v = 1/V + K_m/V_m[S], \quad (1)$$

$$1/v = 1/V + (K_m/V_m[S])(1 + [I]/K_i), \quad (2)$$

where V_m is the maximal velocity; [S] and [I] are the substrate and inhibitor concentrations; K_m is the Michaelis–Menten constant; and K_i is the inhibition constant for the inhibitor I. Fits of kinetic data were made using the KaleidaGraph (Abelbeck Software). Values of k_{cat} were calculated from the experimental values of V_m and the enzyme concentrations used in the experiments.

Results and discussion

Deuterium kinetic isotope effects

The mechanism in Fig. 1 shows six steps, and the complete mechanism including binding steps to form substrate and product Michaelis complexes, two steps for each transimination process, and two steps for the homolytic scission and recombination of the Co–C5′ bond would come to at least 12 steps overall. Of these, only two involve C–H bond cleavage, the abstraction of substrate C5(H) by the 5′-deoxyadenosyl radical to form radical **1** and abstraction of hydrogen from the methyl group of 5′-deoxyadenosine by radical **3**. To clarify the mechanism of action of 5,6-LAM, we undertook to determine whether one of these two steps might be rate limiting. The experimental test for rate limitation by hydrogen transfer is the observation of primary deuterium kinetic isotope effects on the steady-state kinetic parameters in the reaction of deuterium-labeled substrates.

The kinetic parameters for the reactions of DL-lysine with and without deuterium substitution are listed in Table 1. The results suggest that the abstraction of C5(H) limits the overall rate of the reaction of 5,6-LAM. The isotope effect for the reaction with deuterium labeling at C3, C4, C5, and C6 is slightly larger than that for the reaction with deuterium at C4 and C5 alone. The C4 hydrogens do not participate in the reaction, so that the deuterium substitution for C5(H) must lead to the kinetic isotope effect. The value of k_{cat} for lysine labeled with deuterium at C5 is about one-tenth of the value for unlabeled lysine. In the forward reaction of DL-lysine-3,3,4,4,5,5,6,6-d_8, the deuterium isotope effect on k_{cat} ($^Dk_{cat}$) is 10.4 ± 0.3 and the effect on k_{cat}/K_m [$^D(k_{cat}/K_m)$] is 8.3 ± 1.9. In the reaction of DL-lysine-4,4,5,5-d_4 the value of $^Dk_{cat}$ is 8.5 ± 0.7 and that of $^D(k_{cat}/K_m)$ is 7.1 ± 1.2. We believe it is unlikely that hydrogen transfer at C6 significantly limits the rate, for reasons presented in the further discussion, although the experimental isotope effects alone cannot exclude this possibility. The kinetic parameters for the reaction of L-lysine-3,3,4,4,5,5,6,6-d_8 are also listed in Table 1. Comparison with the values for unlabeled L-lysine in Table 2 indicates that the value of $^D(k_{cat})$ is 10 and that of $^D(k_{cat}/K_m)$ is 14 for the L-enantiomer. The similarities in

Table 1
Kinetic parameters for the 5,6-LAM-catalyzed reactions of deuterium-labeled lysine

Substrate	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}\,mM^{-1}$)
DL-Lysine	560 ± 16	11.2 ± 1.0
DL-Lysine-3,3,4,4,5,5,6,6-d_8	54 ± 3	1.35 ± 0.29
DL-Lysine-4,4,5,5-d_4	66 ± 5	1.57 ± 0.22
L-Lysine-3,3,4,4,5,5,6,6,-d_8	5.0 ± 0.7	0.18 ± 0.02

Table 2
Kinetic parameters for the reactions of DL-4-oxalysine and other substrates of 5,6-LAM[a]

Substrate	K_m (mM)	k_{cat} (min^{-1})	k_{cat}/K_m ($mM^{-1}\,min^{-1}$)	k_{inact}[b] (min^{-1})
D-Lysine[c]	20 ± 1	750 ± 44	37.5 ± 0.9	0.70 ± 0.04
L-Lysine[c]	20 ± 6	51 ± 7	2.55 ± 0.84	0.57 ± 0.04
DL-Lysine	50.2 ± 4.3	560 ± 16	11.2 ± 1.0	0.62 ± 0.04
L-β-Lysine	8.7 ± 0.7	255 ± 6	29.3 ± 2.5	>18
DL-4-Oxalysine	16 ± 1	454 ± 45	28.4 ± 3.3	0.68 ± 0.05

[a] The values of K_m, k_{cat}, and k_{cat}/K_m were measured as described in the Experimental procedure at 37 °C.
[b] Values of k_{inact} were determined by stopped-flow spectrophotometry, observing the chromophoric changes corresponding to the formation of cob(III)alamin shown in Fig. 2.
[c] These values were reported in [2] and were redetermined in this work.

the isotope effects for the reactions of DL- and L-lysine indicate that the isotope effects are similar for D- and L-lysine.

The deuterated substrates in this study are labeled at both diastereotopic positions of either C5 or of C5 and C6, and both hydrogens of the C5′-methylene group in adenosylcobalamin become deuterium labeled within a few turnovers in the pre-steady-state phase. Therefore, it is likely that secondary deuterium kinetic isotope effects contribute to the measured effects in the steady state. Secondary isotope effects are much smaller than the effects observed in this work, of the order of 5% of primary isotope effects. Therefore, the kinetic isotope effects in Table 1 represent mainly the primary kinetic isotope effects, while including the much smaller secondary kinetic isotope effects.

The provisional assignment of rate limitation to the abstraction of C5(H) from the side chain of lysine cannot be sustained by the kinetic isotope effects alone. This is because the stepwise mechanism leads to the formation of 5′-[5′-$^2H_3$]deoxyadenosine as an intermediate in the reaction of DL-lysine-4,4,5,5-d_4. Deuterium transfer from this intermediate to C6 of radical **3** in Fig. 1 could also limit the rate. If this were rate limiting, the preceding complex would accumulate in the steady state and this complex would contain cob(II)alamin and radical **3**. The UV–Vis absorption spectrum of cob(II)alamin is very different from that of adenosylcobalamin. The absorption spectrum of the reaction mixture containing 5,6-LAM, adenosylcobalamin, PLP, and D-lysine in the steady state is typical of adenosylcobalamin and PLP, with no sign of an increase at 477 nm, which would indicate the formation of cob(II)alamin [2]. Furthermore, no accumulation of the product-related radical **3,** or of cob(II)alamin, in the steady state could be detected by EPR spectroscopy. Therefore, hydrogen abstraction from C5 most likely limits the rate.

DL-4-Oxalysine as a substrate of 5,6-LAM

The best substrates are D-lysine and L-β-lysine, although L-lysine also serves as a poor substrate [2]. We now find that DL-4-oxalysine is a good substrate for 5,6-LAM. The presumed product, 4-oxa-5-methylornithine, in the reaction of DL-4-oxalysine is very unstable and decomposes into DL-serine, acetaldehyde, and ammonium ion, so that the reaction of 4-oxalysine is irreversible. The conversion of DL-oxalysine into serine is proven by the observation of the production of serine, detected as its phenylisothiocyanate derivative in the HPLC assay for activity. That both enantiomers of DL-4-oxalysine reacted and not just one of them is deduced from the fact that the DL-4-oxalysine is quantitatively transformed into serine when large amounts of enzyme are employed (data not shown).

The kinetic parameters in Table 2 show that DL-4-oxalysine is approximately as good a substrate as D-lysine and L-β-lysine and a better substrate than DL-lysine. The value of k_{cat}/K_m for DL-4-oxalysine is similar to that for L-β-lysine, slightly lower than that for, D-lysine, and about four times that for DL-lysine. Evidently the slight steric difference introduced by the 4-oxa group is essentially immaterial in the overall reaction of DL-oxalysine.

The reaction of DL-4-oxalysine is initially zero order but falls off with the passage of time. Other substrates also display this property and it has been traced to substrate-induced suicide inactivation. In the mechanism of Fig. 1, the radical intermediates co-exist with cob(II)alamin, and in suicide inactivation electron transfer from cob(II)alamin to substrate- or product-related radicals ***1*** and ***3*** leads to cob(III)alamin and 5′-deoxyadenosine, thereby permanently cleaving the adenosylcobalamin and inactivating the enzyme [2]. The simplest signal of this process is the transformation of adenosylcobalamin into cob(III)alamin, which has a prominent absorption band at 358 nm. The appearance of this band under both aerobic and anaerobic conditions concomitant with irreversible inactivation indicates suicide inactivation by electron transfer [2]. As shown in Fig. 2, DL-4-oxalysine shares this property with the other substrates of 5,6-LAM. The spectral changes facilitate the measurement of the inactivation rate constant (k_{inact}) for suicide inactivation. The value of the inactivation rate constant for DL-4-oxalysine is

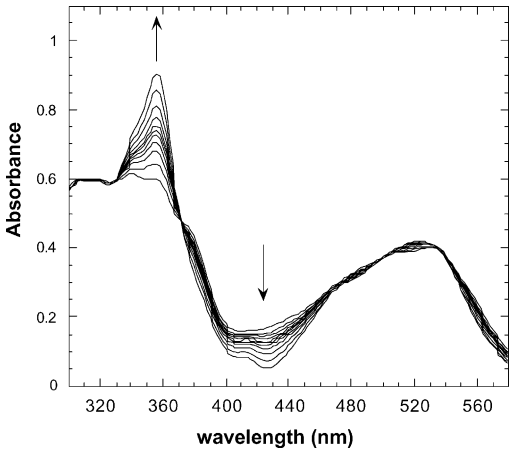

Fig. 2. Suicide inactivation of 5,6-LAM by the substrate DL-4-oxalysine. The reaction conditions are described in the Experimental procedure. The increase in the cob(III)alamin chromophore at 358 nm directly signals the formation of suicide inactivation products.

very similar to those observed with D-lysine, L-lysine, and DL-lysine, as shown in Table 2. Suicide inactivation induced by L-β-lysine is much faster than inactivation induced by the other substrates.

In the mechanism of Fig. 1, radical intermediate ***1*** has an unpaired electron on C5, which is presumably a π-radical center. It is a high-energy secondary radical that could not exist at a concentration that could be detected by EPR. In the reaction of DL-4-oxalysine, the corresponding C5-π-radical would be bonded to the 4-oxa group, which bears nonbonding electron pairs. The 4-oxa group should stabilize a π-radical at C5 through delocalization of the unpaired electron by overlap with the π-orbitals occupied by the nonbonding electrons. In the event of sufficient stabilization, the C5-π-radical (radical ***1*** in Fig. 1) might accumulate to a detectable concentration. This phenomenon has been observed in the reaction of lysine 2,3-aminomutase when L-4-thialysine was used as a substrate analog [5,6]. In the case of DL-4-oxalysine reacting as a substrate for 5,6-LAM, EPR experiments failed to detect a radical species in the steady state of the reaction (data not shown). The radical stabilization energy of the oxa group in the methoxy substituted radical has been estimated to be about 4 kcal mol^{-1} [17]. Evidently the radical stabilization afforded by the 4-oxa group in DL-4-oxalysine is not sufficient to enhance the concentration of a radical intermediate to a detectable level in the reaction of 5,6-LAM.

The mechanism in Fig. 1 is inspired by the fact that adenosylcobalamin-dependent rearrangements proceed by radical mechanisms, and the rearrangement is analogous to that established in the reaction of the SAM-dependent lysine 2,3-aminomutase [3–6,18]. The mechanism is also supported by a theoretical analysis showing that other radical mechanisms would involve higher activation energies [19].

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References

[1] J.J. Baker, T.C. Stadtman, in: D. Dolphin (Ed.), B_{12}, vol. 2, Wiley, New York, 1982, pp. 203–232.
[2] K.-H. Tang, C.H. Chang, P.A. Frey, Biochemistry 40 (2001) 5190–5199.
[3] M.D. Ballinger, P.A. Frey, G.H. Reed, Biochemistry 31 (1992) 10782–10789.
[4] M.D. Ballinger, P.A. Frey, G.H. Reed, R. LoBrutto, Biochemistry 34 (1995) 10086–10093.
[5] W. Wu, K.W. Lieder, G.H. Reed, P.A. Frey, Biochemistry 34 (1995) 10532–10537.
[6] J. Miller, V. Bandarian, G.H. Reed, P.A. Frey, Arch. Biochem. Biophys. 387 (2001) 281–288.
[7] V.W. Bowry, J. Lusztyk, K.U. Ingold, J. Am. Chem. Soc. 113 (1991) 5687–5698.
[8] K.-H. Tang, A. Harms, P.A. Frey, Biochemistry 41 (2002) 8767–8776.
[9] T.W. Meier, N.H. Thoma, P.F. Leadlay, Biochemistry 35 (1996) 11791–11796.
[10] R. Padmakumar, R. Banerjee, Biochemistry 36 (1997) 3713–3718.
[11] E.N.G. Marsh, D.P. Ballou, Biochemistry 37 (1998) 11864–11872.
[12] V. Bandarian, G.H. Reed, Biochemistry 39 (2000) 12069–12075.
[13] B. Zagalak, P.A. Frey, G.L. Karabatsos, R.H. Abeles, J. Biol. Chem. 241 (1996) 3028–3035.
[14] G.I. Tesser, G.H.L. Nefkens, Recueil 78 (1959) 404–405.
[15] T.P. Chirpich, M.M. Herbst, H.N. Edmunds, B.G. Baltimore, R.N. Costilow, H.A. Barker, Prep. Biochem. 3 (1973) 47–52.
[16] R.L. Heinrikson, S.C. Meredith, Anal. Biochem. 136 (1984) 65–74.
[17] C. Leory, D. Peeters, C. Wilante, Theochem 5 (1982) 217–233.
[18] P.A. Frey, O.Th. Magnusson, Chem. Rev. (2003) in press.
[19] S.D. Wetmore, D.M. Smith, L. Radom, J. Am. Chem. Soc. 123 (2001) 8678–8689.