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Reaction of Pyridoxal-5′-phosphate-*N*-oxide with Lysine 5,6-Aminomutase: Enzyme Flexibility toward Cofactor Analog

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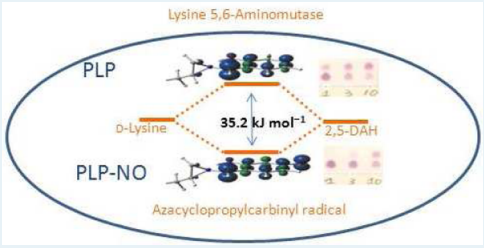
Supporting Information

ABSTRACT: Lysine 5,6-aminomutase (5,6-LAM) is a 5′-deoxyadenosylcobalamin and pyridoxal-5′-phosphate (PLP) codependent radical enzyme that can accept at least three substrates, D-lysine, L-β-lysine and L-lysine. The reaction of 5,6-LAM is believed to follow an intramolecular radical rearrangement mechanism involving formation of a cyclic azacyclopropylcarbinyl radical intermediate ($I^{\bullet}$). Similar $I^{\bullet}$s are also proposed for other radical aminomutases, such as ornithine 4,5-aminomutase (4,5-OAM) and lysine 2,3-aminomutase (2,3-LAM). Nevertheless, experimental proof in support of the participation of $I^{\bullet}$ have been elusive. PLP is proposed to lower the energy of this elusive $I^{\bullet}$ by captodative stabilization and spin delocalization. In this work, we employ PLP-*N*-oxide (PLP-NO) to investigate the flexibility of 5,6-LAM toward cofactor analog and participation of $I^{\bullet}$ in the reaction mechanism. Our calculations show that substitution of PLP-NO for PLP stabilizes $I^{\bullet}$ by 35.2 kJ mol^{-1} as a result of enhanced spin delocalization, which becomes the lowest energy state along the reaction sequence. Kinetic parameters and spectroscopic observations for PLP-NO similar to those of PLP demonstrate that PLP-NO mimics natural cofactor for 5,6-LAM. Interestingly, the flexibility of 5,6-LAM toward cofactor analog PLP-NO makes it an even more promising candidate for biocatalytic applications. Expectedly, the catalytic efficiency (k_{cat}/K_m) is reduced by ∼3 times with PLP-NO as a cofactor. Various factors, including higher stabilization of proposed corresponding $I^{\bullet}$ for PLP-NO than that of PLP, could lead to the decrease in activity.



KEYWORDS: *biocatalysis, aminomutase, D-lysine, pyridoxal-5′-phosphate, 5′-deoxyadenosylcobalamin, DFT, radical mechanism, EPR*

INTRODUCTION

The use of enzymes as biocatalysts has several advantages over chemical synthesis and immense potential for industrial application to produce fine chemicals and pharmaceuticals.[1,2] For example, pyridoxal-5′-phosphate (PLP, the active form of vitamin B_6)-dependent ω-transaminase has been used as an efficient biocatalyst.[3,4] PLP plays important roles in many key enzymatic reactions.[5] PLP is one of the cofactors of radical aminomutases,[6−9] such as lysine 5,6-aminomuatse (5,6-LAM), ornithine 4,5-aminomuatse (4,5-OAM), and lysine 2,3-aminomutase (2,3-LAM). In addition to PLP, these enzymes utilize 5′-deoxyadenosyl ($dAdo^{\bullet}$) radical, generated from either 5′-deoxyadenosylcobalamin (dAdoCbl, coenzyme B_{12}) in the case of 5,6-LAM and 4,5-OAM or (*S*)-adenosyl-L-methionine (SAM) in the case of 2,3-LAM, to perform chemically challenging 1,2-amino shift between adjacent carbon atoms.[6,7] 5,6-LAM catalyzes the reversible interconversion of D-lysine and 2,5-diaminohexanoic acid, and L-β-lysine and 3,5-diaminohexanoic acid. In addition, it can also accept L-lysine as substrate. On the other hand, 2,3-LAM and 4,5-OAM can accept only L-lysine and D-ornithine, respectively, as substrate. 5,6-LAM is most flexible among these enzymes toward substrate and has enormous potential as a biocatalyst.[10] However, to develop 5,6-LAM as a biocatalyst, a thorough knowledge of the mechanism of action of the enzyme is required.

Like other dAdoCbl dependent enzymes,[8,11−16] the proposed reaction mechanism of these radical aminomutases involves several radical intermediates.[6,7] These enzymes are believed to follow similar mechanism; the mechanism for 5,6-LAM[17−22] is shown in Scheme 1. The catalytic cycle begins with formation of external aldimine between the substrate D-lysine and PLP and concurrent hemolytic cleavage of the Co−C bond of dAdoCbl, generating cob(II)alamin and the $dAdo^{\bullet}$ radical, which abstracts a hydrogen from the C5 of lysine to produce a substrate-related radical ($S^{\bullet}$). Intramolecular rearrangement of $S^{\bullet}$ through an azacyclopropylcarbinyl radical ($I^{\bullet}$) results in a product-related radical ($P^{\bullet}$) that accepts a hydrogen from 5′-deoxyadenosine (dAdoH) to generate the product−PLP complex. Finally, release of the product 2,5-diaminohexanoic acid (2,5-DAH) and recombination of $dAdo^{\bullet}$ and cob(II)-alamin to form dAdoCbl completes the cycle. Experimental evidence of the radical mechanism have been obtained by detecting the corresponding $S^{\bullet}$[23] and $P^{\bullet}$[24−26] in the case of 2,3-LAM or inhibitor-based radical intermediate in the case of 4,5-OAM.[27] In the case of 5,6-LAM, the substrate-related radical was detected[28,29] using a substrate analog 4-thialysine and later unambiguously characterized using labeled 4-thialysines.[30−32]

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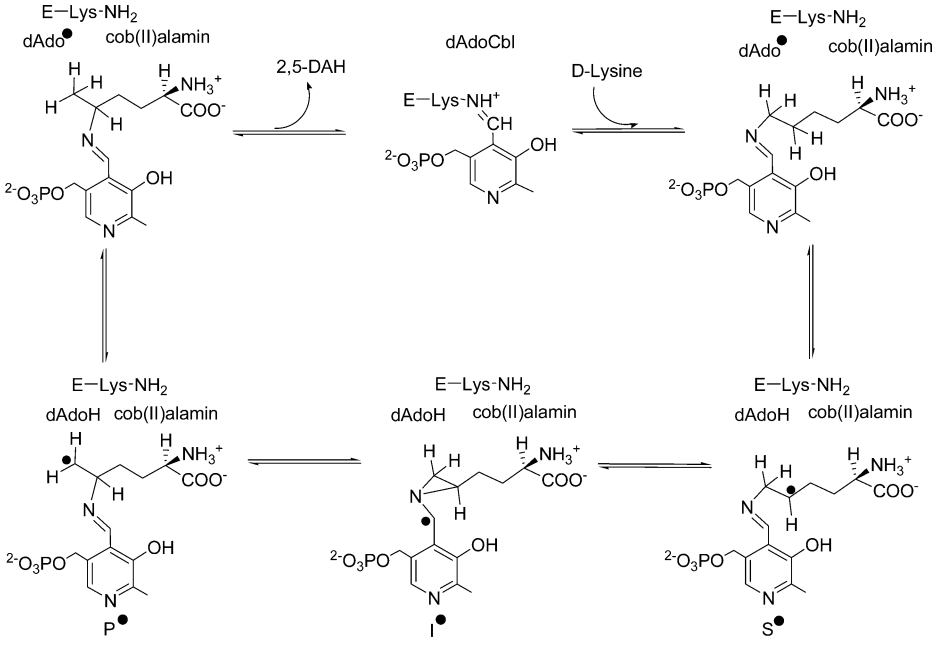
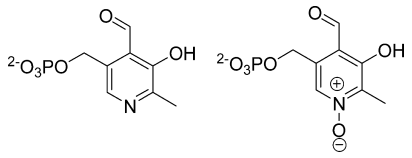
Scheme 1. Reaction Mechanism of 5,6-LAM



High-energy intermediate I• has been eluding experimental detection. DFT computations have been playing complementary roles in the research for radical aminomuatses.[6,7,33−38] Radom and co-workers proposed[35] analogs of PLP having substitution at the 4′-carbon with π acceptors, such as vinyl and acetylenyl are promising candidates for experimental observation of the corresponding I•. Moreover, our recent results predict that another π acceptor cyano substitution offers better prospect for the same purpose.[37] However, the acceptance of these analogs (4′-π-acceptor-PLPs) by 5,6-LAM is a matter of concern.

To find an appropriate candidate for experimental detection of I•, we thought that PLP-*N*-oxide (PLP-NO, an analog of PLP, Chart 1), which has been used as a probe to study several

Chart 1. Chemical Structures of PLP and PLP-NO



PLP-dependent enzymes, including aspartate aminotransferase (AAT),[39,40] tryptophanase,[39−43] and glycogen phosphorylase,[44] might be the right choice. PLP-NO has both electronic and structural advantages. Evidently, PLP-NO should stabilize the corresponding I• more than PLP because the spin delocalization would be enhanced in the case of PLP-NO more than PLP. Structurally, PLP-NO would introduce less steric influence with respect to the 4′-π-acceptor-PLPs mentioned above. The steric perturbation is also remote from the radical centers rather than in close proximity as in the cases of 4′-π-acceptor-PLPs. Therefore, it is more promising to test the theoretical prediction with PLP-NO than with 4′-π-acceptor-PLPs. Here, we present the computational and experimental investigations on the reaction of PLP-NO with 5,6-LAM. Our results identify PLP-NO as a functional cofactor and provide experimental evidence for the flexibility of 5,6-LAM toward the cofactor analog.

RESULTS AND DISCUSSION

Following the computational strategies established by Radom and co-workers, we have performed the calculation on PLP-NO models to find out the nature of the relative stabilization of corresponding I•. Figure 1 compares the calculated relative

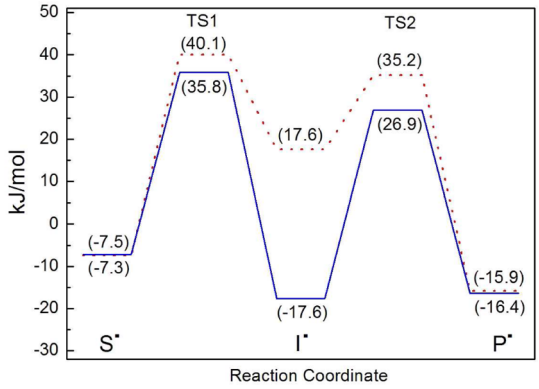


Figure 1. Relative energies (kJ mol^{-1}) of models of S•, I•, and P• derived from PLP-D-lysine (red dash line) and PLP-NO-D-lysine (solid blue line). The sum of the energy for dAdo• and SH is set to zero (Experimental Section and Table S1).

energies at RMP2/G3MP2Large level for the radicals derived from H atom abstraction at the C5 position of PLP-D-lysine and PLP-NO-D-lysine adduct. As expected, relative to the PLP system, PLP-NO substitution has a minimum of effects on the stability of S• and P•. The effect on I• is encouraging because I• becomes the lowest-energy state along the reaction pathway. There is no significant difference in the geometries between PLP and PLP-NO, where captodative stabilization of I• operates. Expectedly, enhanced electron-withdrawing ability of PLP-NO results in a significant change in the spin densities at the primary radical center (exocyclic carbon C4′). The

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calculated RMP2 spin densities at C4′ and the pyridine ring are 0.74 and 0.21 for PLP, but are 0.36 and 0.47 for PLP-NO, respectively. The remaining spin densities (0.15) reside on O1′ of PLP-NO. Thus, enhanced spin delocalization over the PLP-NO framework results in the 35.2 kJ mol^{-1} stabilization of I$^{\bullet}$ in PLP-NO.

The ring closure barrier for formation of I$^{\bullet}$ from S$^{\bullet}$ is reduced by 4.5 kJ mol^{-1} in the PLP-NO system. The ring closure reaction is exothermic by 10.3 kJ mol^{-1} for PLP-NO, but it is endothermic by 25 kJ mol^{-1} for PLP. Consequently, stabilization of I$^{\bullet}$ increases the barrier for subsequent ring opening to P$^{\bullet}$, from 17.6 kJ mol^{-1} for PLP to 44.5 kJ mol^{-1} for PLP-NO. Thus, the depth of the I$^{\bullet}$ energy well offers a great prospect for experimental observation of the hitherto undetected I$^{\bullet}$. The degree of radical stabilization (10.3 kJ mol^{-1}, relative to S$^{\bullet}$) obtained with PLP-NO is comparable with those with 4′-π-acceptor-PLPs using acetylenyl (7.9 kJ mol^{-1}),[35] cyano (9.4 kJ mol^{-1}),[37] or vinyl (18.9 kJ mol^{-1})[35] substitution at the exocyclic 4′-carbon proposed earlier. The question of whether 5,6-LAM can tolerate PLP-NO as a cofactor is of great interest and is subjected to further investigation for its reaction with the enzyme.

UV−visible spectral changes observed as a result of the formation of internal aldimine with PLP and PLP-NO is depicted in Figure 2. Free PLP displays an absorption band at

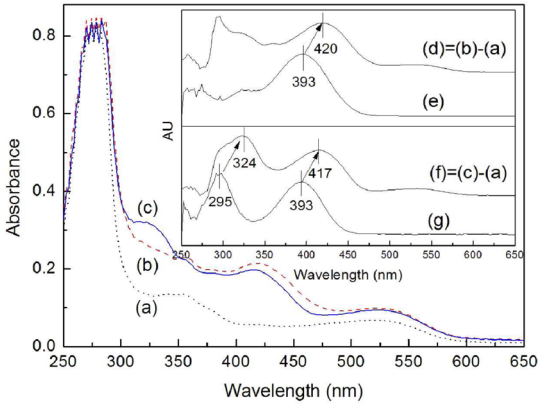

Figure 2. UV−visible spectral changes associated with formation of an internal aldimine. (a) Solution containing 25 μM 5,6-LAM, 25 μM dAdoCbl, and 10 mM β-Me in 100 mM NH_4-EPPS buffer at PH 8.5. (b) Addition of 25 μM PLP and incubated at 37 °C for 30 min, showing absorption at 420 nm. (c) Addition of 25 μM PLP-NO and incubated at 37 °C for 30 min, showing absorption at 324 and 417 nm. Deionized water was used as a blank reference. Spectra were collected at 23 °C. Insert: d = b − a; e = free PLP in 100 mM NH_4-EPPS buffer at pH 8.5; f = c − a; and g = free PLP-NO in 100 mM NH_4-EPPS buffer at pH 8.5. The difference spectra, in which contributions from 5,6-LAM and dAdoCbl are subtracted, allows closer examination of spectral shift due to formation of PLP- and PLP-NO internal aldimine.

393 nm that is shifted to 420 nm upon binding to 5,6-LAM, characteristic of the formation of PLP-Lys144β internal aldimine. Free PLP-NO displays two absorptions at 295 and 393 nm; upon binding to 5,6-LAM, the two bands shift to 324 and 417 nm, respectively. Therefore, the spectral changes are consistent with formation of an aldimine linkage between PLP-NO and Lys144β.

We note that PLP-NO is not converted to PLP during reaction with 5,6-LAM (Figure 3). In free PLP-NO, the ratio of integrated area of the Gaussian peaks (area (A_{393})/area (A_{295})) is 1.2 (Figure 3, left panel), whereas in the case of 5,6-LAM/dAdoCbl-bound PLP-NO, the ratio of the integrated area of the Gaussian peaks (area (A_{417})/area (A_{324})) is 1.1 (Figure 3, right panel). The almost identical area ratios demonstrate that PLP-NO did not decompose into PLP in 5,6-LAM. This is in contrast to the observation of the surprising conversion of PLP-NO to PLP in the reaction with glycogen phosphorylase reported by Pfeuffer and co-workers.[44]

Comparison of UV−visible spectral changes in the reaction of 5,6-LAM/dAdoCbl with D-lysine in the presence of PLP and absence of PLP (PLP-depleted) is shown in Figure S2. The PLP-depleted 5,6-LAM does not induce any obvious spectral changes upon addition of substrate. The addition of D-lysine to the holoenzyme causes decreases in absorbance at 420 nm for PLP and at both 324 and 417 nm for PLP-NO, reflecting transimination to D-lysine (Figure 4). The decreases in absorbance at 380 and 523 nm, characteristic bands for dAdoCbl, reflects the ability of PLP-NO to elicit Co−C bond homolysis rather than binding passively. No spectral component at 470 nm due to formation of cob(II)alamin is observed, suggesting that the degree of I$^{\bullet}$ stabilization afforded by PLP-NO is not enough to allow accumulation in the steady state. A similar increase in absorbance at 357 nm, which is characteristic[20] of production of cob(III)alamin and dAdoH due to electron transfer from cob(II)alamin to substrate or product radicals, is observed in the cases of both PLP (Figure 4 left) and PLP-NO (Figure 4 right). All these observations indicate that PLP-NO is capable of performing the catalysis.

PLP-NO was then tested for activity at 37 °C with 5,6-LAM using D-lysine or D-lysine-4,4,5,5-d_4 (CDN Isotopes, Canada) as a substrate and compared with that of PLP and PLP-depleted, on the basis of HPLC analysis of phenylisothiocyanate-derivatized D-lysine and 2,5-DAH.[21] Despite the absence of PLP from the lysis and purification buffers, 5,6-LAM shows measurable activity due to PLP adventitiously bound to the enzyme. The kinetic parameters assigned for the PLP-depleted cofactor in Table 1 refers to this background activity that is typically <1.5% of the activity with PLP. The k_{cat} of 5,6-LAM with PLP-NO is 1 s^{-1}, which is 5 times the background activity and ∼7%, compared with 14.2 s^{-1} with PLP. The K_m value for D-lysine is 4.6 ± 0.7 and 21.0 ± 0.5 for PLP-NO and PLP system, respectively. These kinetic data suggest that PLP-NO is a functional cofactor for 5,6-LAM and demonstrate that 5,6-LAM is flexible toward cofactor. The flexibility of 5,6-LAM toward the cofactor analog PLP-NO in addition to its substrate flexibility enhances its potential for application as biocatalyst.

Another interesting finding is that the catalytic efficiency (k_{cat}/K_m) with PLP-NO as the cofactor is ∼3 times lower than that with PLP. This can be attributed to many reasons: (1) the increased stabilization of I$^{\bullet}$ for PLP-NO with respect to that of PLP (Figure 2) decreases in the rate of rate-limiting step; (2) slowing of Co−C bond cleavage; (3) a change in the conformational equilibrium due to binding of PLP-NO rather than PLP; and others. To elaborate the first point, PLP is proposed to captodatively stabilize I$^{\bullet}$ and lowers the reaction barrier for catalysis. Apparently, 5,6-LAM optimizes the catalytic efficiency by controlling the stability of I$^{\bullet}$; over stabilization of this radical retards the formation of the desired product. Enhanced electron-withdrawing ability of PLP-NO results in the delocalization of spin throughout the PLP-NO framework and puts the cyclic intermediate in a potential well 44.5 kJ mol^{-1} deep in the forward direction. Therefore, the participation of I$^{\bullet}$ in the reaction sequence should result in the

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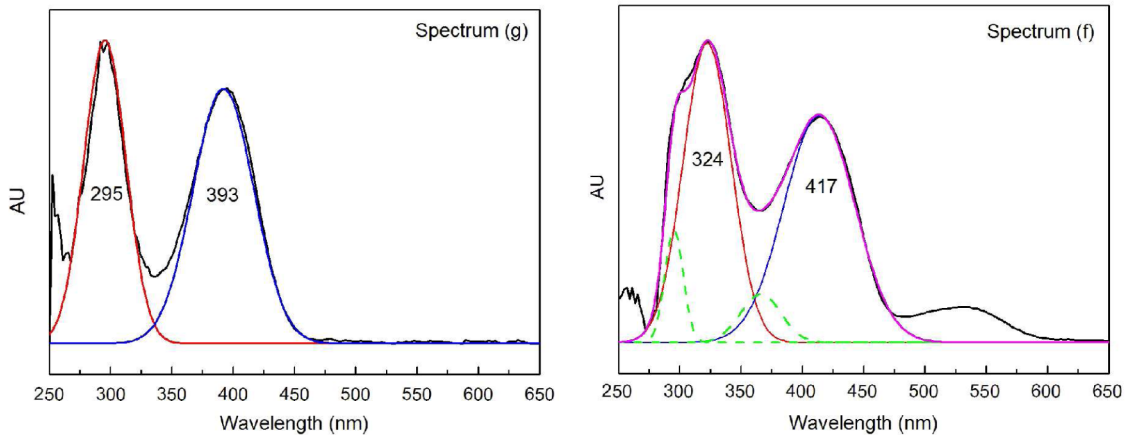

Figure 3. Decomposition of free PLP-NO absorption band (left, spectrum g in Figure 2) at 295 and 393 nm and 5,6-LAM/dAdoCbl-bound PLP-NO absorption band (right, spectrum f in Figure 2) at 324 and 417 nm. In the right panel, two additional peaks (green dashed lines), due to the presence of enzyme and dAdoCbl, were included to fit the overall experimental spectrum. The sum of the four peaks (pink line) superimposed well with the experimental data (black line). The computer program FitYK, version 0.9.8 was used for spectra fitting and data analysis.

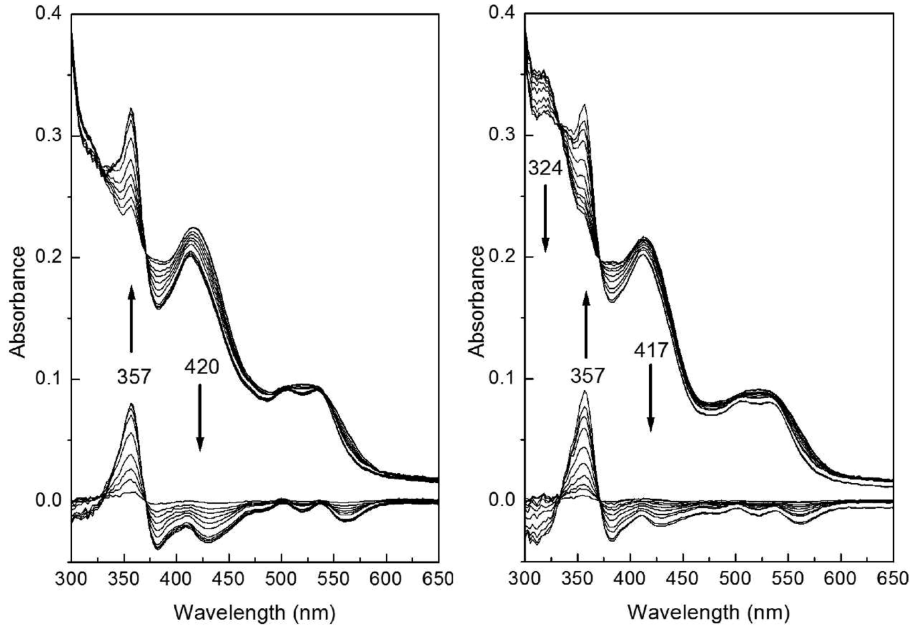

Figure 4. UV−visible spectral changes upon the addition of D-lysine. (left) PLP and (right) PLP-NO systems. 5,6-LAM (25 μM) was preincubated with an equimolar amount of dAdoCbl and PLP or PLP-NO for 30 min in 100 mM NH_4-EPPS buffer at PH 8.5 with 10 mM β-Me. The reaction was started by the addition of D-lysine aerobically. Spectra were recorded at 0.5, 1, 2, 3, 5, 10, 20, 40, 60, 90, and 136 min at 23 °C. Difference spectra were obtained by subtracting the spectra recorded prior to mixing with D-lysine.

Table 1. Kinetic Parameters of D-Lysine and D-Lysine-4,4,5,5-d_4 with 5,6-LAM

	D-lysine		D-lysine-4,4,5,5-d_4	
cofactor	K_m (mM)	k_{cat} (s^{-1})	$^Dk_{cat}$	$^D(k_{cat}/K_m)$
PLP	21.0 ± 0.5	14.2 ± 0.3	5.5 ± 0.5	4.4 ± 1.0
PLP-NO	4.6 ± 0.7	1.0 ± 0.04	2.5 ± 0.3	2.5 ± 0.8
PLP-depleted	3.2 ± 0.2	0.2 ± 0.03		

reduction in catalytic efficiency. However, it is noted that other mechanisms mentioned above may contribute to the same cause. To provide further evidence, we performed experiments to determine deuterium kinetic isotope effect. The reaction of 5,6-LAM with D-lysine-4,4,5,5-d_4 in the presence of PLP displays deuterium isotope effects. The value of $^Dk_{cat}$ is 5.5 ± 0.5 and that of $^D(k_{cat}/K_m)$ is 4.4 ± 1.0 (Table 1). The results suggest that abstraction of the hydrogen atom from the substrate-PLP adduct by dAdo$^\bullet$ is the rate-limiting step. It is less likely that abstraction of a hydrogen atom from dAdoH by P$^\bullet$ was the rate-limiting step because no sign of either absorbance build-up at 470 nm corresponding to cob(II)alamin formation or a paramagnetic EPR signal by EPR could be detected. Now, the reduced KIEs for PLP-NO ($^Dk_{cat}$ = 2.5 ± 0.3 and $^D(k_{cat}/K_m)$ = 2.5 ± 0.8) suggest that formation or breakdown of I$^\bullet$ may contribute to the rate limitation. However, a similar observation is also expected in the case of rate-limiting Co−C homolysis, or altered conformational equilibrium.

In adenosylmethionine or dAdoCbl-dependent enzymes, EPR experiments[11−14] are often employed to detect a radical intermediate and to reflect the relative positions of the substrate-derived radical and low-spin Co^{2+} in cob(II)alamin

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by analysis of spin−spin interaction parameters.[45−49] No paramagnetic intermediate can be detected by EPR in the reaction of 5,6-LAM/dAdoCbl/PLP with natural substrate D-lysine. Presumably, the radicals are too short-lived to accumulate to detectable amounts. Our calculations predict that observation of I• may become possible because PLP-NO substitution stabilizes I• by 35.2 kJ mol^{-1} with respect to PLP (Figure 1). In contrast, no radical intermediate was detected in the reaction of 5,6-LAM/dAdoCbl/PLP-NO with D-lysine during room temperature steady state turnover or under freeze-quenching between 20 and 900 ms (data not shown). This suggests that the relative stabilization of I• is not enough to accumulate it to an EPR detection level. To investigate whether the active site is still capable of accommodating identical radical chemistry observed with a natural cofactor, we performed the experiment with 4-thia-L-lysine as a suicide inhibitor. The reaction of PLP-depleted 5,6-LAM/dAdoCbl with 4-thia-L-lysine did not produce any detectable signal. EPR spectrum generated from reaction of 5,6-LAM/dAdoCbl/PLP-NO with 4-thia-L-lysine at 5 min is exactly identical to that generated from 5,6-LAM/dAdoCbl/PLP (Figure 5) in both line shape and spin-counting, indicating that the native active site structure is maintained with PLP-NO substitution.

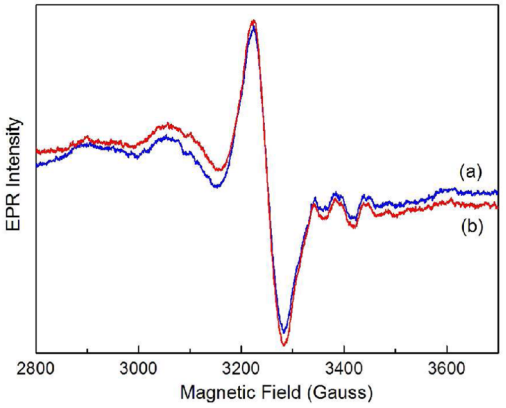

Figure 5. EPR spectra (77 K) generated in the reaction of 5,6-LAM with 4-thia-L-lysine. 5,6-LAM (165 μM) was preincubated with 300 μM dAdoCbl and (a) 300 μM PLP or (b) 300 μM PLP-NO for 30 min at 37 °C in 100 mM NH_4-EPPS buffer at PH 8.5. The reaction was initiated by addition of 30 mM 4-thia-L-lysine and allowed to proceed for 5 min aerobically.

The observed steady state radical is actually a tautomer of a transient radical derived from H-abstraction at the C5 position of 4-thialysine. It was proposed that the tautomerization process requires at least one active site acid−base group to catalyze the proton transfer from C6 of the lysyl side chain to C4′ of PLP. This suggests that the cofactor substitution is also inconsequential in the tautomerization process. This further establishes that the active site of 5,6-LAM is flexible toward not only substrates but also the cofactor analog.

CONCLUSION

Our results demonstrate that PLP-NO, an analog of natural cofactor PLP, can act as a functional cofactor for 5,6-LAM. The native structural and functional properties of the enzyme are maintained with PLP-NO. Moreover, the kinetic data complemented by computational results provides evidence in support of participation of I• that has been proposed to be an important intermediate in the reaction of radical aminomutases or involvement of other mechanisms, such as rate-limiting Co−C homolysis, or altered conformational equilibrium. Taken together, the knowledge of flexibility of 5,6-LAM toward cofactor in addition to substrates is a significant finding. Studies are in progress to trap I• by performing the reaction with PLP-NO at low temperature. Engineering of low temperature (<−20 °C) ms freeze-quenching is also necessary to study the fast decay kinetics of the transient species.

EXPERIMENTAL SECTION

Purification of 5,6-LAM. The recombinant 5,6-LAM from *Clostridium sticklandii* was produced by expression in *Escherichia coli* and purified following the procedures described by Chang and Frey[19] with the exception that PLP was omitted from the lysis and purification buffers.

Synthesis of PLP-NO. PLP-NO was synthesized following the literature procedure.[40,50] All the operations involving PLP-NO were performed in the dark to avoid its decomposition.

Measurement of Kinetic Parameters. Using D-lysine or D-lysine-4,4,5,5-d_4 (CDN Isotopes, Canada) as a substrate, the kinetic parameters of 5,6-LAM were measured based on HPLC analysis of phenylisothiocyanate (PITC)-derivatized D-lysine and 2,5-DAH. 5,6-LAM (5 μM) was preincubated with 10 μM dAdoCbl and 10 μM PLP or 10 μM PLP-NO or without addition of PLP (PLP-freed) for 30 min at 37 °C in 100 mM NH_4-EPPS buffer at pH 8.5 in a total volume of 50 μL. The reaction was initiated by addition of various concentrations of D-lysine (10, 30, 50, 70, 90, 110, 130, and 150 mM for PLP; 1, 3, 6, 10, 15, 20, 25, and 30 mM for PLP-NO and PLP-depleted), and the reaction mixture was incubated at 37 °C. At different time points (20, 60, 90s), 30 μL aliquots were taken, quenched with 20 μL of 2 N $HClO_4$, and centrifuged. A C_{18} Sep-Pak cartridge (Waters) was used to remove 5′-deoxyadenosine and adenosylcobalamin. The D-lysine and 2,5-DAH were eluted with deionized water, and the volume of eluted solution was concentrated to 20 μL. The PITC-derivatized D-lysine and 2,5-DAH were applied to a C_{18} column (XBridge, Waters) and subjected to HPLC analysis.

UV−Visible Measurements. An anaerobic holoenzyme mixture (5,6-LAM/dAdoCbl/PLP or/PLP-NO) and bulk solution containing substrates were prepared by repeated evacuation and backfilling with argon. The argon-saturated holoenzyme and substrate solutions were mixed in an Applied Photophysics SX20 stopped-flow spectrophotometer mixer under aerobic condition. We note that it has been shown that suicide inactivation of 5,6-LAM is an O_2-independent process. Spectra were collected using the photodiode array detector. The light shutter is on only during data collection, which ensures that the sample is dark 99% of the time. Data processing was accomplished using the software package supplied with the instrument.

EPR Measurements. EPR data were collected using a Bruker EMX spectrometer equipped with a Bruker TE102 cavity. The microwave frequency was measured with a Hewlett-Packard 5246L electronic counter. Temperatures were maintained between 77 and 80 K by an Advanced Research System Helitran continuous flow cryostat. Typical EPR instrument settings: microwave frequency, 9.5 GHz; microwave power, 20 mW; modulation amplitude, 4 G at 100 kHz.

DFT Computation. The methyl group and the phosphate handle of PLP and PLP-NO were replaced with hydrogen atoms.[35] The N1 of the pyridine ring of PLP is not protonated.

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The structure of substrate D-lysine was not truncated.[22,36] We used tetrahydro-5-methylfuran-3,4-diol as a model for 5′-deoxyadenosine (dAdOH). Geometry optimizations were performed in gas phase at the B3-LYP level with 6-31G(d,p) basis. Fully optimized structures were characterized by the absence of imaginary vibrational frequencies. Improved relative energies at 0 K were evaluated at the RMP2/G3MP2Large level. A factor of 0.9806 was used to scale the zero-point energies and added to the RMP2 energy. Relative energies (kJ mol^{-1}), with respect to the sum of SH (substrate-PLP) and dAdO$^{\bullet}$, for steps along the reaction pathway are given in Table S1. The Gaussian03 suite of programs[51] was used for all calculations.

■ ASSOCIATED CONTENT

Supporting Information

The following file is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.5b00671.

Supporting table (relative energies for steps along the reaction pathway) and figure (^{1}H NMR of PLP-NO in D_2O) and Gaussian archive entries (PDF)

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Notes

The authors declare no competing financial interests.

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