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4′-CyanoPLP presents better prospect for the experimental detection of elusive cyclic intermediate radical in the reaction of lysine 5,6-aminomutase

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The results of our calculations suggest that the reaction of 4′-cyanoPLP with lysine 5,6-aminomutase offers better prospect for the experimental detection of elusive cyclic azacyclopropylcarbinyl radical (I$^\bullet$), which is proposed to be a key intermediate in the reaction of pyridoxal-5′-phosphate dependent radical aminomutases. We have calculated the corresponding hyperfine coupling constants (HFCCs) for ^{14}N and ^{13}C of cyano group using several basis sets to help the characterization of 4′-cyanoI$^\bullet$.

1. Introduction

Pyridoxal-5′-phosphate (PLP) dependent aminomutases [1–4] follow radical mechanisms to carry out chemically challenging 1,2-amino shifts between adjacent carbons. Like in other PLP dependent enzymes, PLP anchors substrate by forming PLP-substrate adduct through external aldimine linkage. In addition to that, PLP plays an important role by stabilizing high energy cyclic azacyclopropylcarbinyl radical (I$^\bullet$, Scheme 1) in the PLP-dependent radical aminomutases. Another common feature of these aminomutases is that 5′-deoxyadenosyl radical (dAdo$^\bullet$) initiates the catalysis. There are three such aminomutases that have been studied extensively. Lysine 2,3-aminomutase (2,3-LAM) uses 5′-deoxyadenosylmethionine (dAdoMet) as the source of dAdo$^\bullet$ while lysine 5,6-aminomutase (5,6-LAM) and ornithine 4,5-aminomutase (4,5-OAM) employ 5′-deoxyadenosylcobalamin (dAdoCbl). 2,3-LAM and 4,5-OAM catalyze the interconversion of L-lysine and L-β-lysine, and D-ornithine and 2,4-diaminopentanoic acid, respectively. 5,6-LAM is more flexible and can accept more than one substrate. It catalyzes the interconversion of D-lysine and 2,5-diaminohexanoic acid, and L-β-lysine and 3,5-diaminohexanoic acid. The reaction of these enzymes is believed to follow similar pathway. dAdo$^\bullet$ abstracts a hydrogen atom from the PLP-substrate adduct to generate a substrate-related radical (S$^\bullet$) and 5′-deoxyadenosine (dAdoH). Then, S$^\bullet$ undergoes internal addition to the imine nitrogen to form cyclic azacyclopropylcarbinyl radical (I$^\bullet$), which rearranges to product-related radical (P$^\bullet$). Finally, a hydrogen atom from dAdoH is transferred to P$^\bullet$ to produce PLP-product complex. The first proof of the radical mechanism was reported [5] in the case of 2,3-LAM as Frey and coworkers characterized corresponding S$^\bullet$. Later, they were also able to characterize [6–8] corresponding P$^\bullet$. In the case of 4,5-OAM, the evidence for radical mechanism was obtained using a substrate homologue [9]. Identification of corresponding S$^\bullet$ in the reaction of 5,6-LAM has been reported recently [10,11] using substrate analogue 4-thialysine. The characterization of that was confirmed by electron paramagnetic resonance (EPR) and electron nuclear double resonance (ENDOR) spectroscopic studies [12] on isotope-edited substrate analogues, 4-thia-[5-^{13}C]lysine [13] and 4-thia-[6-^{13}C]lysine [14]. Although corresponding S$^\bullet$ and P$^\bullet$ have been detected and subsequently characterized, I$^\bullet$, the link between these, could not be detected experimentally in the reaction with any of three aminomutases.

Computational studies, especially density functional theory (DFT), have been employed to complement and explain the proposed mechanism and experimental findings of these enzymes. The observation of corresponding S$^\bullet$ and P$^\bullet$, and probable participation but non-observation of I$^\bullet$ have been explained computationally

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Abbreviations: PLP, pyridoxal-5′-phosphate; HFCCs, hyperfine coupling constants; DFT, density functional theory; EPR, electron paramagnetic resonance; ENDOR, electron nuclear double resonance.

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by Radom and coworkers [15,16]. Moreover, authors proposed the possible experimental observation of I$^{\cdot}$ if the reaction of 5,6-LAM is performed with either 4′-vinylPLP or 4′-acetylenylPLP (Scheme 2) [16]. 4′-VinylPLP is relatively more stabilized, and hence more promising, than 4′-acetylenylPLP. DFT has been used to study the transitions between the conformational states of 5,6-LAM [17] and 4,5-OAM [18]. We have recently investigated the case of 5-fluorolysine as an alternative substrate in the reaction of 5,6-LAM and found that the substrate related radical (5-FS$^{\cdot}$) could be detected [19]. In this work, following the protocol used by Radom [15,16] and co-workers we have performed calculations on models of radical intermediates expected from the reaction of 4′-cyanoPLP and 5,6-LAM.

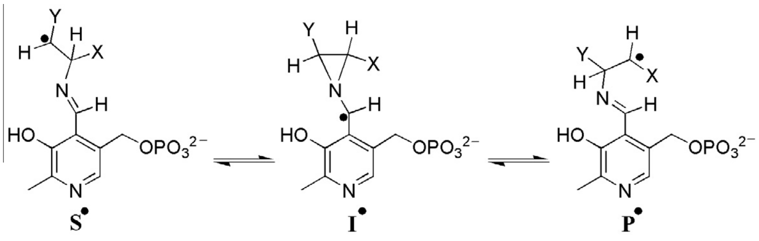
2. Materials and methods

All geometry optimizations were performed at the hybrid B3LYP functional level with 6-31G(d,p) basis in gas phase, as implemented in the Gaussian 03 program package [20]. Energies were calculated at RMP2/G3MP2Large and corrected for zero point vibrational energies [16]. Hyperfine coupling constants (HFCCs) were calculated of the B3LYP/6-31G(d,p) optimized structures at B3LYP level using relevant basis sets. 4′-cyanoPLP is modeled similarly as 4′-vinylPLP or 4′-acetylenylPLP following the procedure of Radom (Scheme 3).

3. Results and discussion

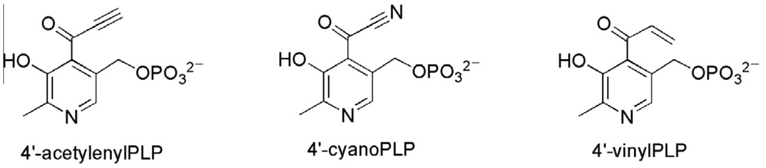
The results show that the cyano group stabilizes the corresponding I$^{\cdot}$ (4′-cyanoI$^{\cdot}$) to make it the lowest energy radical intermediate among three important radical intermediates (Fig. 1). The exothermicity of formation of 4′-cyanoI$^{\cdot}$ from corresponding S$^{\cdot}$ is 9.4 kJ mol^{-1} and formation of corresponding P$^{\cdot}$ is endothermic by 4.7 kJ mol^{-1}. Both values lie in between those for corresponding 4′-acetylenylI$^{\cdot}$ and 4′-vinylI$^{\cdot}$ (Table 1). Therefore, 4′-cyanoPLP, like 4′-vinylPLP and 4′-acetylenylPLP, also offers good prospect for the experimental observation of corresponding I$^{\cdot}$. If energy values are only considered, 4′-vinylPLP is the best candidate among these. However, active site of enzyme is compact and has very little space to accommodate extra atoms or groups. Therefore, the less perturbation is introduced the more likelihood to be accepted by the enzyme. Interestingly, cyano group introduces less perturbation than both vinyl and acetylenyl. The group contributions to the van der Waals volume of vinyl, acetylenyl, and cyano group are 20.41, 19.6, and 14.70 cm^3 mol^{-1}, respectively [21]. Evidently, it would be much easier for the enzyme to accommodate 4′-cyanoPLP than 4′-vinylPLP or 4′-acetylenylPLP. We suspect that 5,6-LAM might not accommodate 4′-vinylPLP in the active site. The question whether 5,6-LAM can accommodate 4′-cyanoPLP still remains open. But this is the most promising candidate among the modified PLPs, which are predicted to give rise stabilized I$^{\cdot}$. Furthermore, the presence of nitrogen atom in 4′-cyanoPLP is beneficial as it can act as probe for characterization by EPR and ENDOR spectroscopy. Nitrogen has two magnetic nuclei ^{14}N ($I = 1$; natural abundance = 99.64%; magnetogyric ratio = 2.0378×10^{27} J T^{-1}) and ^{15}N ($I = ½$; natural abundance = 0.36%; magnetogyric ratio = -2.8585×10^{27} J T^{-1}) [22]. An unequivocal characterization can be performed by using natural abundant PLP analogue and ^{15}N enriched analogue. However, to achieve this, a significant amount of spin density should reside on nitrogen atom. A sizeable amount of spin density is expected on nitrogen atom due to the delocalization of spin density from C4′ through C≡N bond. Expectedly, the calculations reveal that there is significant amount of spin density resides on nitrogen atom of cyano group (Fig. 2). Moreover, the carbon atom of cyano group has also significant amount of spin density. Hence, ^{13}C enriched PLP analogue would also help unequivocal characterization of the radical intermediate.

To identify and characterize the radical structure observed experimentally, the EPR spectra need to be simulated using various parameters. HFCCs are the most important parameter among those. HFCCs can be calculated for potential radical intermediates. Comparison of these values with experimental values would lead to characterization of the radical observed. The HFCCs can be separated into an isotropic (A_{iso}) and three anisotropic (T_{XX}, T_{YY}, and T_{ZZ}) components. Unlike anisotropic components, A_{iso} is difficult to calculate accurately. Therefore, the choice of basis set is very important. A_{iso} corresponds to the spin density at the nucleus. Isotropic HFCCs calculated for different nuclei belonging to first three rows of periodic table of a large number of both organic and inorganic radicals using the combination of B3LYP functional and EPR-III basis set showed excellent agreement with experimental values [23,24]. Surprisingly, for ^{14}N nuclei a smaller and less computationally demanding 6-31G(d) basis set yielded HFCC values closer to the experimental values, with a notable exception in the case of cyanomethyl radical for which calculated value using EPR-III basis set is in better agreement with the experimental value [25]. The reason behind this apparent anomaly is that cyanomethyl radical is C-centered radical unlike others, which are either N-centered or NO-centered radicals. 4′-cyanoI$^{\cdot}$ is essentially a substituted cyanomethyl radical. Therefore, we believe that values predicted by EPR-III would be closer to experimental values. However, we employed both the basis sets to provide wider comparison with the experimental values and find out reliability of various basis sets. We also included 6-311G(2d,p) basis set which has been successfully used to explain radical mechanism of dAdoCbl dependent enzyme [26]. ^{14}N HFCCs calculated for cyano N along with ^{13}C HFCCs of cyano C of 4′-cyanoI$^{\cdot}$ using various basis sets is tabulated in Table 2 and those of 4′-cyanoS$^{\cdot}$, and 4′-cyanoP$^{\cdot}$ in Supporting information. The values reveal that ^{14}N A_{iso} of 4′-cyanoI$^{\cdot}$ are in the range of 4-8 MHz while those of 4′-cyanoS$^{\cdot}$ and 4′-cyanoP$^{\cdot}$ are negligible. A value of 6 MHz is predicted by EPR-III basis set, which is best suited for these radicals as discussed above. As expected, anisotropic values varies little with different basis sets. A successful simulation of EPR spectrum

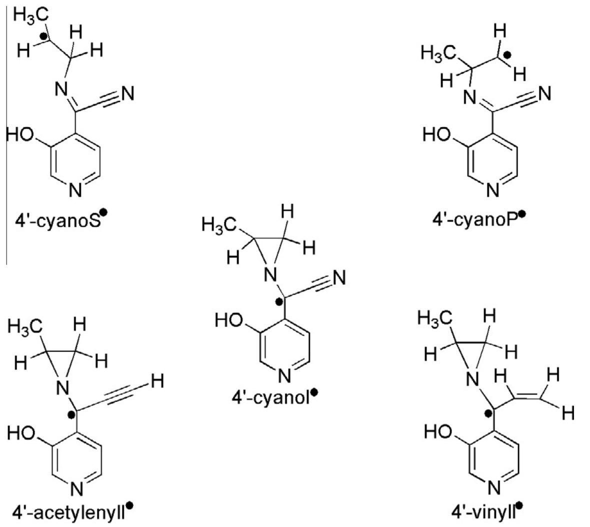


5,6-LAM: X = H; Y = $CH_2CH_2CH(NH_3^+)COO^-$
4,5-OAM: X = H; Y = $CH_2CH(NH_3^+)COO^-$
2,3-LAM: X= COO^-; Y = $CH_2CH_2CH_2NH_3^+$

Scheme 1. Chemical structures of important radical intermediates.



Scheme 2. Chemical structures of PLP analogues.



Scheme 3. Chemical structures of models of radical intermediate analogues.

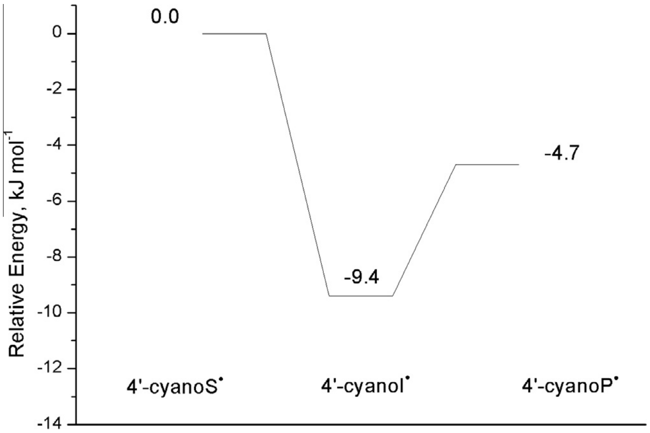


Fig. 1. Relative energies of radical intermediates.

Table 1
Reaction enthalpies (kJ mol^{-1}) of radical intermediates.

Models	Exothermicity of formation of I·	Endothermicity of formation of P·	
4′-AcetylenylI·	7.9	2.9	[16]
4′-CyanoI·	9.4	4.7	This work
4′-VinylI·	18.9	13.9	[16]

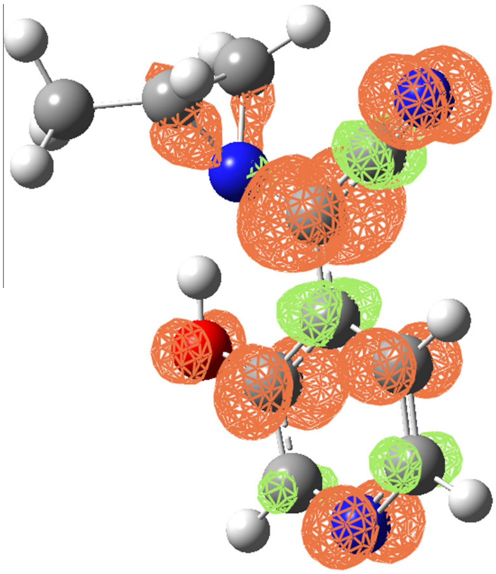


Fig. 2. B3LYP/EPR-III calculated spin density of 4′-cyanoI·.

Table 2
^{14}N and ^{13}C hyperfine coupling constants (HFCCs) in MHz.

Radical	Atom		6-31G(d)	6-311G(2d,p)	EPR-III
4′-cyanoI·	Cyano N	A_{iso}	8.0	4.1	6.0
		T_{XX}	−10.4	−11.5	−11.5
		T_{YY}	−7.9	−8.6	−8.8
		T_{ZZ}	18.3	20.0	20.3
	Cyano C	A_{iso}	−37.7	−40.4	−40.6
		T_{XX}	−11.3	−12.0	−12.0
		T_{YY}	−1.8	−1.6	−1.4
		T_{ZZ}	13.1	13.6	13.4

using these sets of values, more likely with those of EPR-III basis set, would indicate that the radical is 4′-cyanoI·. Furthermore, significantly high values of ^{13}C A_{iso} (~40 MHz) of cyano C for 4′-cyanoI· in contrast to negligible amount of those for 4′-cyanoS· and 4′-cyanoP· suggest that EPR and ENDOR experiment with ^{13}C enriched 4′-cyanoPLP would provide unequivocal characterization of the radical. Additionally, ^{15}N enriched 4′-cyanoPLP would provide further support for the characterization of the radical. It is noteworthy that our results also suggest that the reaction of 4,5-OAM with 4′-cyanoPLP should also result in experimental observation of corresponding I·. However, 4,5-OAM is less flexible with the substrate and less probable to accept modified PLP.

In summary, our results suggest that 4′-cyanoPLP promises better prospect for the experimental detection and characterization of elusive I· in the reaction of 5,6-LAM. These results would encourage the synthesis of 4′-cyanoPLP followed by reaction with 5,6-LAM to observe and characterize I·.

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Appendix A. Supplementary data

^{14}N and ^{13}C hyperfine coupling constants (HFCCs), Gaussian Archive Entries for the B3LYP/6-31G(d,p) Optimized Geometries. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.12.076.

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