J|A|C|S
COMMUNICATIONS

Published on Web 11/29/2006

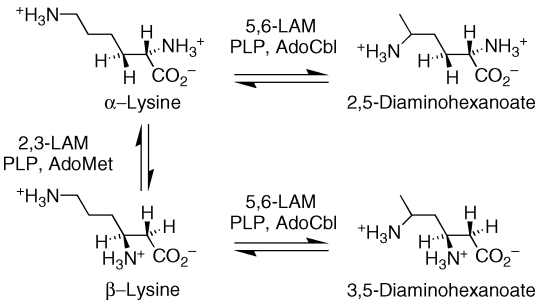
In Search of Radical Intermediates in the Reactions Catalyzed by Lysine 2,3-Aminomutase and Lysine 5,6-Aminomutase

Gregory M. Sandala,*,†,‖ David M. Smith,*,‡ and Leo Radom*,†,‖

School of Chemistry and ARC Centre of Excellence in Free Radical Chemistry and Biotechnology, University of Sydney, Sydney, NSW 2006, Australia, Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia, and Rudjer Boskovic Institute, 10002 Zagreb, Croatia

Received September 22, 2006; E-mail: sandala@chem.usyd.edu.au; david.smith@irb.hr; radom@chem.usyd.edu.au

Radical mechanisms offer the adenosylmethionine (AdoMet)- and adenosylcobalamin (AdoCbl)-dependent aminomutases the opportunity to execute very specific 1,2-amino rearrangements between adjacent carbons.[1] Lysine 2,3-aminomutase (2,3-LAM) and lysine 5,6-aminomutase (5,6-LAM) belong to these intriguing classes of enzymes and their activity is closely related as follows:

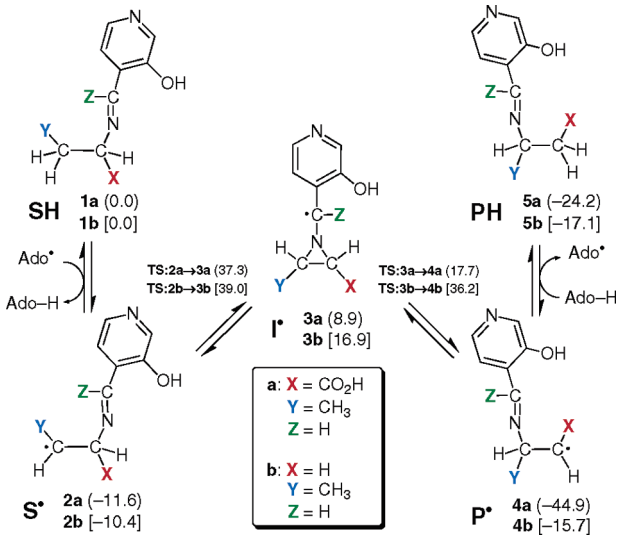


Pyridoxal 5′-phosphate (PLP) is required by both enzymes to facilitate the amino rearrangement via an aldimine linkage to the migrating amino group of the substrate. Adenosylmethionine (AdoMet) and adenosylcobalamin (AdoCbl) contribute the putative high-energy 5′-deoxyadenosyl radical (Ado•) to the process, which, although produced by different means for each cofactor, is responsible for initiating and terminating catalysis via hydrogen transfers. Overall, these reactions appear to be closely related to those catalyzed by other AdoCbl-dependent enzymes.[2]

Initial H-atom abstraction by Ado• from the substrate (**SH**) forms Ado-H plus a substrate-derived radical (**S•**). Rearrangement then generates the product-related radical (**P•**), which may, as is proposed for 2,3-LAM and 5,6-LAM,[3] or may not[2] proceed via a cyclic aziridinylcarbinyl radical (**I•**). The reaction terminates when an H-atom from Ado-H is transferred to **P•** to form the product (**PH**) plus Ado•.

A longstanding goal in AdoMet- and AdoCbl-dependent biochemistry is the characterization of the radical intermediates in these reactions so as to verify the postulated reaction pathways and thus better understand the mechanism of action. Using native substrates and their analogues, Frey, Reed and co-workers have provided solid evidence in support of radical-mediated rearrangements in 2,3-LAM. For instance, electron paramagnetic resonance (EPR) spectroscopy has been able to identify the **P•** of β-lysine[4] and the **S•** of the analogue 4-thialysine.[5] An allylic analogue of Ado• has provided additional evidence for the role of Ado• in mediating the H-atom-transfer steps for this enzyme.[6] However, observation of the cyclic intermediate (**I•**) linking **S•** and **P•** in these reactions remains elusive, and direct evidence of any type of radical intermediate (i.e., **S•** , **I•**, or **P•**) has yet to be obtained in the reactions of 5,6-LAM. In the present contribution, we seek to address these outstanding issues by examining energy profiles of model substrates and their analogues.

Scheme 1. Energies along the Proposed Pathways for the 2,3-LAM- (**a**) and 5,6-LAM- (**b**) Catalyzed Reactions (kJ mol^{-1})



Geometries and scaled vibrational frequencies were obtained with the B3-LYP/6-31G(d,p) procedure, and improved relative energies at 0 K were calculated at RMP2/G3MP2Large.[7] This level of theory has previously been demonstrated to be adequate for aminomutase-catalyzed reactions.[8] Lysine and PLP have been truncated as indicated to allow for computational tractability. We have chosen the neutral form of the pyridine ring within our model of PLP, since the crystal structures of 2,3-LAM[9a] and 5,6-LAM[9b] suggest full protonation to be unlikely. 5′-Deoxyadenosine (Ado-H) was modeled with 2-methyltetrahydrofuran-3,4-diol.

Scheme 1 displays results for models aimed to reflect the reactions of 2,3-LAM (**a**) and 5,6-LAM (**b**) with lysine. In both cases, a mildly exothermic initial H-atom abstraction by Ado• from **1** is followed by modest barriers for ring closure of **2** to form **3** in reactions that are endothermic by 20−25 kJ mol^{-1}. Ring-opening is moderately exothermic for both **a** and **b**, although more so for **a** because of the stabilizing effect of CO_2H adjacent to the radical center in **4a**.[10]

Frey, Reed and co-workers have examined the O (Y = OCH_3),[3] S (Y = SCH_3),[5] and allylic (Y = $CH{=}CH_2$)[11] C4-derivatives of lysine to further characterize the pathway outlined in Scheme 1. Table 1 displays our calculated energies for the steps along the reaction pathway for model analogues for 2,3-LAM (X = CO_2H) and 5,6-LAM (X = H). The first two rows of Table 1 correspond

† University of Sydney.
‖ Australian National University.
‡ Rudjer Boskovic Institute.

10.1021/ja0668421 CCC: $33.50

Table 1. Energies (kJ mol^{-1}) for Steps along the Reaction Pathway for Model Species Pertinent to the 2,3-LAM- (X = CO_2H) and 5,6-LAM- (X = H) Catalyzed Reactions (see Scheme 1)

Y	X	S$^\bullet$	TS1	I$^\bullet$	TS2	P$^\bullet$
CH_3	CO_2H	−11.6	37.3	8.9	17.7	−44.9
	H	−10.4	39.0	16.9	36.2	−15.7
OCH_3	CO_2H	−24.8	22.7	7.8	10.7	−42.9
	H	−29.2	18.9	4.7	25.8	−26.6
SCH_3	CO_2H	−36.8	16.1	10.5	15.1	−40.1
	H	−37.7	19.0	12.3	38.6	−22.9
CH=CH_2	CO_2H	−70.1	26.0	17.0	25.0	−29.3
	H	−77.4	20.3	15.0	39.1	−11.8

to **2** (**S**$^\bullet$), **3** (**I**$^\bullet$), and **4** (**P**$^\bullet$) of Scheme 1, and provide a baseline against which results for the other systems can be compared.

The third and fourth rows of Table 1 highlight the reaction energies of 4-oxalysine (4-OL), which is an alternative substrate for 5,6-LAM.[3] We can see a large stabilizing effect in **S**$^\bullet$ of the OCH_3 substituent on the adjacent radical center. Relative stabilization of **S**$^\bullet$ has the effect of increasing the endothermicity for the formation of **I**$^\bullet$ from **S**$^\bullet$.

Rows five and six of Table 1 represent results for models of 4-thialysine (4-TL), an analogue that has allowed characterization of the **S**$^\bullet$ in 2,3-LAM.[5] Overall, the reaction exothermicity for the initial H-atom abstraction increases across the series CH_3 < OCH_3 < SCH_3 from ca. 10 to ca. 40 kJ mol^{-1}. The relatively large exothermicities accompanying H-abstraction from **SH** when Y = SCH_3 are consistent with an accumulation of the **S**$^\bullet$ of 4-TL with 2,3-LAM[5] and suggest a similar outcome might be expected with 5,6-LAM. Indeed, the formation of **I**$^\bullet$ is predicted to be endothermic by ca. 50 kJ mol^{-1}, with barriers for ring closure of ca. 55 kJ mol^{-1} for both X = CO_2H and H.

The last two rows in Table 1 display the reaction enthalpies obtained with models for allylic analogues of lysine. The reaction of 2,3-LAM with *trans*-4,5-dehydrolysine (*t*-4,5-DL) has been found to result in mechanism-based inactivation, and the C3-derived radical of *t*-4,5-DL was assigned as the causative agent for inactivation.[11] We find that the initial H-atom abstraction reaction at C3 when X = CO_2H is exceptionally exothermic at 70.1 kJ mol^{-1}. The barrier for formation of **I**$^\bullet$ is calculated to be 96.1 kJ mol^{-1}, and the reaction is endothermic by 87.1 kJ mol^{-1}. The depth of this energy well provides an explanation for the inability of *t*-4,5-DL to proceed to products as well as for the absence of H-atom exchange in the 2,3-LAM-catalyzed reaction with *t*-4,5-DL.[11] Such behavior has been previously observed in calculations associated with the suicide inactivation in some AdoCbl-dependent enzymes.[12] Our results in Table 1 with X = H suggest a similar outcome might be observed in the reaction of 5,6-LAM with *trans*-3,4-dehydrolysine (*t*-3,4-DL).[13]

The results in Table 1 and Scheme 1 are thus consistent with the experimental observations of **P**$^\bullet$ for the 2,3-LAM-catalyzed reaction of β-lysine and of **S**$^\bullet$ in the corresponding reaction of 4-TL. Can we now design a system for which there is a reasonable prospect for observation of the hitherto unobserved **I**$^\bullet$? The results in Figure 1 for PLP derivatives with substituents (Z) at the radical center in **I**$^\bullet$ are striking in this respect.

Relative to the unperturbed system (Z = H), methyl substitution (Z = CH_3) is predicted to add little to the stability of **I**$^\bullet$; hence, it remains the highest energy intermediate for models relevant to both 2,3- and 5,6-LAM. In contrast, the π-acceptors C≡CH and CH=CH_2 are shown to stabilize **I**$^\bullet$ by 30−40 kJ mol^{-1}. For the 5,6-LAM-catalyzed reactions (X = H), this is particularly significant because **I**$^\bullet$ becomes the lowest energy radical intermediate (at −7.9 and −18.9 kJ mol^{-1} for Z = C≡CH and CH=CH_2, respectively),

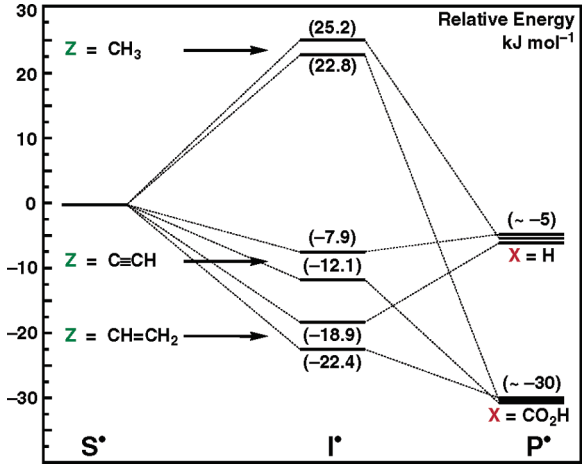


Figure 1. Relative energies of **S**$^\bullet$, **I**$^\bullet$, and **P**$^\bullet$ for various Z-modified PLP derivatives pertinent to the 2,3-LAM- (X = CO_2H) and 5,6-LAM- (X = H) catalyzed reactions (see Scheme 1).[7]

and therefore systems of this type offer good prospects for experimental observation.[14]

In summary, our calculations provide a rationalization for the previous experimental observations of substrate (**S**$^\bullet$) and product (**P**$^\bullet$) radicals in specific 2,3-LAM-catalyzed reactions, and suggest that 4-TL and *t*-3,4-DL may permit observation of **S**$^\bullet$ in 5,6-LAM as well.[13] Our calculations also suggest strategies for modifying PLP that might lead to the first observation of the aziridinylcarbinyl radical intermediate (**I**$^\bullet$) in the lysine−aminomutase-catalyzed reactions, which would provide further support for the mechanism of Scheme 1.

Acknowledgment. The award of an ANU IPRS Fellowship (to G.M.S.), an ARC Discovery Grant (to L.R.), and supercomputer time from APAC, ANUSF, and AC3 are gratefully acknowledged.

Supporting Information Available: Details of the calculations, archive entries, and total energies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Frey, P. A.; Chang, C. H. In *Chemistry and Biochemistry of B_{12}*; Banerjee, R. Ed.; Wiley-Interscience: New York, 1999; pp 835−857.
(2) See, for example: (a) Banerjee, R. *Chem. Rev.* **2003**, *103*, 2083−2094. (b) Toraya, T. *Chem. Rev.* **2003**, *103*, 2095−2127.
(3) Tang, K.-H.; Casarez, A. D.; Wu, W.; Frey, P. A. *Arch. Biochem. Biophys.* **2003**, *418*, 49−54.
(4) Ballinger, M. D.; Frey, P. A.; Reed, G. H. *Biochemistry* **1992**, *31*, 10782−10789.
(5) Wu, W.; Lieder, K. W.; Reed, G. H.; Frey, P. A. *Biochemistry* **1995**, *34*, 10532−10537.
(6) Magnusson, O. T.; Reed, G. H.; Frey, P. A. *Biochemistry* **2001**, *40*, 7773−7782.
(7) For full details, see the Supporting Information.
(8) Wetmore, S. D.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2001**, *123*, 8678−8689.
(9) (a) Lepore, B. W.; Ruzicka, F. J.; Frey, P. A.; Ringe, D. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 13819−13824. (b) Berkovitch, F.; Behshad, E.; Tang, K.-H.; Enns, E. A.; Frey, P. A.; Drennan, C. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15870−15875.
(10) Henry, D. J.; Parkinson, C. J.; Mayer, P. M.; Radom, L. *J. Phys. Chem. A* **2001**, *105*, 6750−6756.
(11) Wu, W.; Booker, S.; Lieder, K. W.; Bandarian, V.; Reed, G. H.; Frey, P. A. *Biochemistry* **2000**, *39*, 9561−9570.
(12) See, for example: (a) Sandala, G. M.; Smith, D. M.; Radom, L. *J. Am. Chem. Soc.* **2004**, *126*, 8856−8864. (b) Sandala, G. M.; Smith, D. M.; Coote, M. L.; Golding, B. T.; Radom, L. *J. Am. Chem. Soc.* **2006**, *128*, 3433−3444 and references therein.
(13) We note that the **P**$^\bullet$ states in the 5,6-LAM- and 2,3-LAM-catalyzed reactions are likely to be accessible from the **PH** state of the substrate analogues *t*-4,5-DL and *t*-3,4-DL, respectively. See the Supporting Information for further details.
(14) Of course, the question of whether LAM enzymes can tolerate such systems remains open.

JA0668421