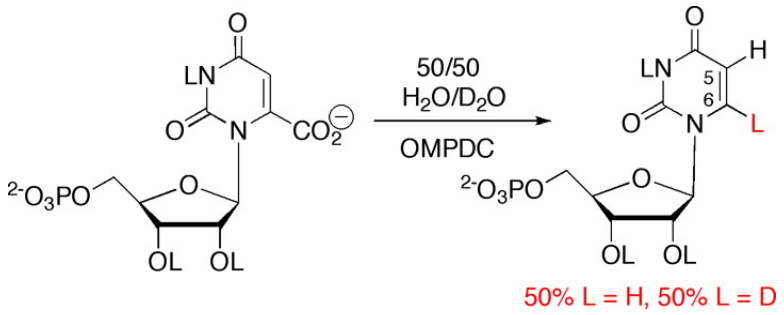
Communication

Product Deuterium Isotope Effect for Orotidine 5'-Monophosphate Decarboxylase: Evidence for the Existence of a Short-Lived Carbanion Intermediate

Krisztina Toth, Tina L. Amyes, Bryant M. Wood, Kui Chan, John A. Gerlt, and John P. Richard

J. Am. Chem. Soc., **2007**, 129 (43), 12946-12947• DOI: 10.1021/ja076222f • Publication Date (Web): 05 October 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/05/2007

Product Deuterium Isotope Effect for Orotidine 5′-Monophosphate Decarboxylase: Evidence for the Existence of a Short-Lived Carbanion Intermediate

Krisztina Toth,[†] Tina L. Amyes,[†] Bryant M. Wood,[#] Kui Chan,[#] John A. Gerlt,*[,#] and John P. Richard*[,†]

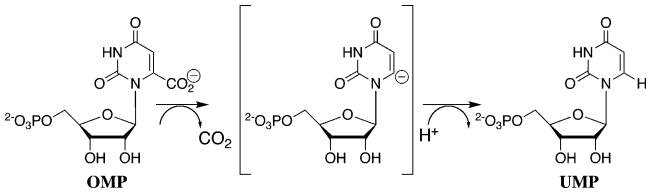
Department of Chemistry, University at Buffalo, Buffalo, New York 14260, and Departments of Biochemistry and Chemistry, University of Illinois, Urbana, Illinois 61801

Received August 17, 2007; E-mail: jrichard@chem.buffalo.edu; j-gerlt@uiuc.edu

We report that equal yields of [6-^{1}H]-uridine 5′-monophosphate (50%) and [6-^{2}H]-uridine 5′-monophosphate (50%) are obtained from the decarboxylation of orotidine 5′-monophosphate (**OMP**) catalyzed by orotidine 5′-monophosphate decarboxylase in a solvent of 50/50 (v/v) H_2O/D_2O. This observation of an unusually small product isotope effect of unity eliminates a proposed mechanism in which proton transfer from Lys-93[1] to C-6 provides electrophilic *push* to the loss of CO_2 from **OMP** in a concerted reaction.[2,3] It provides evidence that proton transfer from the ammonium cation side chain of Lys-93 to a vinyl carbanion intermediate is *faster* than the bond rotation that exchanges the positions of the acidic $N-L^+$ hydrons of this side chain.

Orotidine 5′-monophosphate decarboxylase (OMPDC) is a remarkable enzyme because it employs no metal ions or other cofactors but yet effects an enormous 10^{17}-fold acceleration of the chemically very difficult decarboxylation of **OMP** to give uridine 5′-monophosphate (**UMP**).[4,5] It has been shown that a large fraction of the enzymatic rate acceleration results directly from utilization of the intrinsic binding energy of the remote nonreacting 5′-phosphodianion group of **OMP** in transition state stabilization.[6] The decarboxylation reaction is often proposed to proceed in two steps through a vinyl carbanion intermediate (Scheme 1). However, it has also been suggested that this unstable intermediate might be avoided in a concerted reaction in which decarboxylation and proton transfer to C-6 occur in a single step.[2,3]

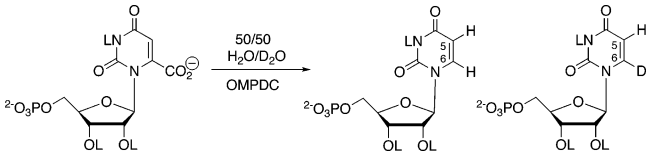
Scheme 1



Experimental and computational studies on OMPDC have focused largely on the partly rate-determining and highly unfavorable loss of CO_2 from **OMP**.[7−9] There are few data pertaining to the proton transfer to C-6 of the pyrimidine ring. Experimental characterization of this proton-transfer step is essential for insight into the existence and lifetime of the putative enzyme-bound vinyl carbanion intermediate.

OMPDC catalyzes incorporation of a hydron from solvent into the **UMP** product and it has been reported that the decarboxylation of saturating **OMP** is 30% faster in H_2O than in D_2O.[7] While the origin of this solvent isotope effect on k_{cat} is unclear, it may represent a secondary solvent kinetic isotope effect (SKIE). By contrast, a product isotope effect (PIE) determined in experiments in which H and D in a mixed solvent of H_2O/D_2O compete for reaction with enzyme-bound **OMP** to form **UMP** labeled at C-6 (Scheme 2) would provide insight into the changes in bonding at the transferred hydron that occur on proceeding to the transition state for the product-determining step.[10] PIEs are more precise and easier to interpret than SKIEs determined as the ratio of rate constants for reactions in H_2O and D_2O because (1) there are no complications from any secondary SKIE when the H- and D-labeled products are formed in the same mixed H_2O/D_2O solvent and (2) there are no errors due to differences in the conditions for separate reactions in H_2O and D_2O, such as enzyme concentration, temperature, and pL.

Scheme 2



The product distribution for the decarboxylation of **OMP** catalyzed by OMPDC in 50/50 (v/v) H_2O/D_2O was determined by ^{1}H NMR spectroscopy at 500 MHz. Figure 1 shows the partial ^{1}H NMR spectrum of **UMP** obtained from the decarboxylation of **OMP** (2 mM) catalyzed by OMPDC from *S. cerevisiae* (C155S mutant, 24 nM, 1 h, >90% reaction) in 50/50 (v/v) H_2O/D_2O at pL 7.3 and 25 °C (I = 0.10, NaCl).[11,12] The value of PIE = 1.0 was calculated using eq 1, where A_H is the integrated area of the doublet due to the C-6 proton of [6-^{1}H]-**UMP** (7.990 ppm), and A_D is the integrated area of the singlet due to the C-5 proton of [6-^{2}H]-**UMP** (5.865 ppm).[13] By comparison, PIEs of 7.3−8.1 for proton transfer to ring-substituted aryl vinyl ethers from lyonium ion in 50/50 (v/v) H_2O/D_2O have been reported recently.[10]

$$\mathrm{PIE} = A_H/A_D \qquad (1)$$

We used similar procedures to determine values of PIE = 1.0 for decarboxylation of **OMP** (2 mM) catalyzed by OMPDC from both *E. coli* (40 nM) and *M. thermoautotrophicum* (40 nM) in 50/50 (v/v) H_2O/D_2O at pL 7.3 and 25 °C (I = 0.10, NaCl). The essentially identical PIEs determined for OMPDC from different sources is significant, because these enzymes exhibit somewhat different architectures at their active sites.[2,9a,14,15]

The value of PIE = 1.0 for the OMPDC-catalyzed decarboxylation of **OMP** in 50/50 (v/v) H_2O/D_2O shows that the deuterium enrichment of the hydron used to protonate **OMP** or an intermediate carbanion at the reaction transition state (50%) is the same as that

[†] University at Buffalo.
[#] University of Illinois.

10.1021/ja076222f CCC: $37.00

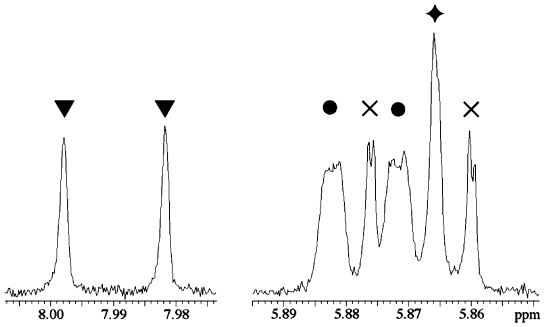
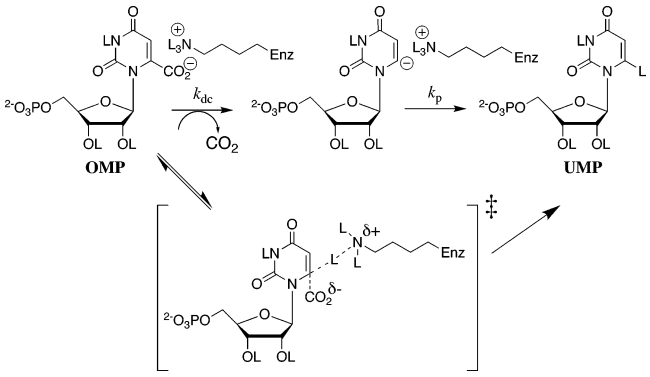


Figure 1. Partial ^{1}H NMR spectrum (500 MHz) of **UMP** from decarboxylation of **OMP** (2 mM) catalyzed by OMPDC from *S. cerevisiae* (24 nM) in 50/50 (v/v) H_2O/D_2O at pL 7.3 and 25 °C: (▼) doublet due to the C-6 proton of [6-^{1}H]-**UMP**; (●) doublets (not resolved) due to the anomeric protons of [6-^{1}H]-**UMP** and [6-^{2}H]-**UMP**; (×) doublet due to the C-5 proton of [6-^{1}H]-**UMP**; (◆) singlet due to the C-5 proton of [6-^{2}H]-**UMP**.

of the 50/50 (v/v) H_2O/D_2O solvent. The product-determining step is thought to be proton transfer from the NL_3^+ group of the side chain of Lys-93 to **OMP** or to a reaction intermediate (Scheme 3).[15] Values of $\phi_{NL3+} \approx 1.0$ have been reported for the H/D fractionation between L_2O and R-NL_3^+, so that the deuterium enrichment of the NL_3^+ group of Lys-93 should be similar to that of the solvent L_2O.[16] Therefore the PIE of 1.0 is essentially equal to the primary kinetic isotope effect for reaction of the H- and D-labeled NL_3^+ group of Lys-93 to form [6-^{1}H]-**UMP** and [6-^{2}H]-**UMP**.

Scheme 3



A significant primary product isotope effect is expected for a reaction in which there is *movement* of the proton in the transition state for the product-determining step,[17a] and there is no precedent for PIEs as small as 1.0 when carbanion protonation is the product-determining step.[17a,18] The observed PIE of 1.0 requires that all of the zero-point energy present in the N-L^+ bonds of Lys-93 be maintained at the transition state for the step that determines whether the **UMP** product is labeled at C-6 with H or D. This PIE is not consistent with a mechanism in which proton transfer from Lys-93 to C-6 of **OMP** provides electrophilic *push* to the loss of CO_2 in a concerted reaction that avoids formation of an unstable vinyl carbanion intermediate (bottom pathway, Scheme 3).[2,3,19]

We suggest that the essentially statistical yields of [6-^{1}H]-**UMP** and [6-^{2}H]-**UMP** from the OMPDC-catalyzed decarboxylation of **OMP** are established at a step that occurs prior to hydron transfer to a vinyl carbanion intermediate. This could be the decarboxylation step, if an N−L^+ bond of Lys-93 is already correctly positioned to deliver a hydron to a vinyl carbanion (k_{dc}, Scheme 3). Alternatively it may be a step that orients an N-L^+ bond of Lys-93 into a "reactive position" where hydron transfer to a vinyl carbanion intermediate can occur. In both cases the PIE of 1.0 *requires* that the chemical step of hydron transfer to the carbanion be *faster* than any molecular motion that allows its discrimination between reaction with H and D at the NL_3^+ group of Lys-93.[17] We therefore propose that hydron transfer from the side chain of Lys-93 to a vinyl carbanion intermediate (k_p) is faster than any movement that exchanges the positions of the N−L^+ hydrons and which would allow the carbanion to *select* for reaction with H or D.[17] In water, the rate constant for such a step is ca. 10^{11} s^{-1}.[20]

The X-ray crystal structure of yeast OMPDC complexed with 6-hydroxyuridine 5′-monophosphate shows that the $CH_2-NH_3^+$ group of Lys-93 is anchored by two hydrogen bonds to the carboxylate groups of Asp-91 and Asp-96 that are proposed to direct the third ammonium hydron of Lys-93 toward the putative vinyl carbanion intermediate.[15] These hydrogen bonds should also restrict rotation about the carbon-nitrogen bond of the terminal CH_2-NL_3^+ group of Lys-93 ($k_{rot} \ll 10^{11}$ s^{-1}). This would favor the observed unselective proton transfer from the remaining free (non-hydrogen-bonded) hydron to a vinyl carbanion intermediate.

Acknowledgment. We acknowledge the National Institutes of Health (Grant GM39754 to J.P.R. and Grant GM65155 to J.A.G.) for generous support of this work.

References

(1) Residues are numbered according to the sequence for the enzyme from yeast.
(2) Begley, T. P.; Appleby, T. C.; Ealick, S. E. *Curr. Opin. Struct. Biol.* **2000**, *10*, 711−718.
(3) Begley, T. P.; Ealick, S. E. *Curr. Opin. Chem. Biol.* **2004**, *8*, 508−515.
(4) Miller, B. G.; Wolfenden, R. *Annu. Rev. Biochem.* **2002**, *71*, 847−885.
(5) Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90−93.
(6) Amyes, T. L.; Richard, J. P.; Tait, J. J. *J. Am. Chem. Soc.* **2005**, *127*, 15708−15709.
(7) Ehrlich, J. I.; Hwang, C.-C.; Cook, P. F.; Blanchard, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 6966−6967.
(8) Rishavy, M. A.; Cleland, W. W. *Biochemistry* **2000**, *39*, 4569−4574.
(9) (a) Wu, N.; Mo, Y.; Gao, J.; Pai, E. F. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2017−2022. (b) Gao, J.; Byun, K. L.; Kluger, R. *Top. Curr. Chem.* **2004**, *238*, 113−136. (c) Warshel, A.; Strajbl, M.; Villa, J.; Florian, J. *Biochemistry* **2000**, *39*, 14728−14738.
(10) Tsang, W.-Y.; Richard, J. P. *J. Am. Chem. Soc.* **2007**, *129*, 10330−10331.
(11) ^{1}H NMR spectra were recorded on a Varian Unity Inova-500 spectrometer using a sweep width of 6000 Hz, a 90° pulse angle, an acquisition time of 6 s, a relaxation delay between pulses of 80 s ($>7T_1$) and with suppression of the water peak. Baselines were subjected to first-order drift correction before integration of the signals.
(12) Reaction mixtures were buffered with 50 mM 3-(*N*-morpholino)propanesulfonic acid (50% free base). Values of pL were obtained by adding 0.18 to the reading of the pH meter [Pentz, L.; Thornton, E. R. *J. Am. Chem. Soc.* **1967**, *89*, 6931−6938].
(13) Control experiments showed that A_H/A_D (eq 1) remains constant over a period of *ca.* 20 hours. The variation in this ratio determined from integration of different NMR spectra is less than 3%.
(14) Harris, P.; Poulsen, J.-C. N.; Jensen, K. F.; Larsen, S. *J. Mol. Biol.* **2002**, *318*, 1019−1029.
(15) Miller, B. G.; Hassell, A. M.; Wolfenden, R.; Milburn, M. V.; Short, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 2011−2016.
(16) Schowen, K. B.; Schowen, R. L. *Methods Enzymol.* **1982**, *87*, 551−606.
(17) (a) Thibblin, A.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 4963−4973. (b) Jencks, W. P. *Acc. Chem. Res.* **1980**, *13*, 161−169.
(18) Fishbein, J. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1988**, *110*, 5075−5086.
(19) The primary ^{13}C KIE on OMPDC-catalyzed decarboxylation of **OMP** labeled at the carboxylate carbon decreases from 1.043 for reaction in H_2O to 1.034 for the 25% slower reaction in D_2O (ref 7), which shows that decarboxylation is *less* rate-determining in D_2O than in H_2O. This result is not easily rationalized by a mechanism in which proton transfer from Lys-93 to C-6 of **OMP** is concerted with the loss of CO_2 because the change from H_2O to D_2O should raise the barrier to a reaction in which proton transfer is concerted with loss of CO_2, as a result of a normal primary KIE. This would cause the loss of CO_2 to become more rate-determining in a multistep enzymatic reaction in D_2O and would result in an increase, rather than the observed decrease, in the ^{13}C isotope effect.
(20) Richard, J. P.; Tsuji, Y. *J. Am. Chem. Soc.* **2000**, *122*, 3963−3964.

JA076222F