We report that orotidine 5'-monophosphate decarboxylase catalyzes exchange of the C-6 proton of uridine 5'-monophosphate (UMP) for deuterium from solvent in D$_2$O at 25 °C (Scheme 1). Kinetic analysis of deuterium exchange gives pK$_a$ ≤ 22 for carbon deprotonation of enzyme-bound UMP, which is at least 10 units lower than that for deprotonation of an analogue of UMP in water.

Scheme 1.

Orotidine 5'-monophosphate decarboxylase (OMPDC) employs no metal ions or other cofactors but yet effects an enormous 10$^{17}$-fold acceleration of the decarboxylation of orotidine 5'-monophosphate (OMP) to give uridine 5'-monophosphate (UMP). The X-ray structure of the yeast enzyme liganded with 6-hydroxyuridine 5'-monophosphate provides strong evidence that the C-6 proton of the product UMP is derived from the terminal NH$_3^+$ group of Lys-93. The product isotope effect of unity for OMPDC-catalyzed decarboxylation of OMP in 50/50 (v/v) H$_2$O/D$_2$O eliminates a mechanism in which proton transfer from Lys-93 to C-6 provides electrophilic push to the loss of CO$_2$ in a concerted reaction. This result also provides strong evidence for the formation of a short-lived enzyme-bound carbanion intermediate that shows no discrimination between H and D in the proton-transfer step (Scheme 2).

Scheme 2.

The very large kinetic barrier to the nonenzymatic decarboxylation of OMP (t$_{1/2} = 78$ million years) arises mainly from the thermodynamic barrier to formation of the highly unstable C-6 vinyl carbanion. This activation barrier may be reduced either by interactions with OMPDC that destabilize bound OMP relative to the bound carbanion intermediate or by interactions that stabilize the bound carbanion intermediate relative to bound OMP. Computational studies support the proposal that binding of OMP to OMPDC induces either electrostatic stress between the protein and the bound substrate in the ground-state Michaelis complex or conformational stress in the protein at this complex and that this stress is relieved in the transition state for enzyme-catalyzed decarboxylation. However, other calculations suggest that the enzymatic rate acceleration is due mainly to stabilization of the transition state for decarboxylation. These results are difficult to evaluate because there are few experiments that address whether the rate acceleration for OMPDC is due mainly to ground state destabilization, to transition state stabilization, or to both effects.

The exchange for deuterium of the C-6 proton of [6-1$^H$]-uridine 5'-monophosphate (h-UMP) to give [6-2$^H$]-uridine 5'-monophosphate (d-UMP) catalyzed by OMPDC from S. cerevisiae (C1SS mutant) in D$_2$O (Scheme 1) was monitored by $^1$H NMR spectroscopy at 500 MHz. Figure 1 shows partial $^1$H NMR spectra in the region of the anomeric and C-5 protons of recovered UMP ([UMP]$_{total}$ = 2.5 mM) obtained during deuterium exchange at 25 °C catalyzed by OMPDC (0.11 mM, 3.2 mg/mL, monitored for 7 days) in D$_2$O buffered by 100 mM glycyglycine at pH 9.34 (I = 0.1, NaCl). Deuteration exchange results in the disappearance of the doublet (a) at 5.860 ppm due to the C-5 proton of h-UMP and the appearance of an upfield-shifted broad doublet (b) due to the C-5 proton of d-UMP at 5.857 ppm (J $\approx$ 0.5 Hz, coupled to the anomic proton). The broad doublet (c) due to the anomic proton of h-UMP at 5.889 ppm (J = 5.3 Hz, coupled to the C-2' proton) exhibits “shoulders” (d) due to the slightly upfield-shifted doublet for the anomic proton of d-UMP.
\[ f(d\text{-UMP}) = \{A_{\text{D}+\text{H}} - A_{\text{H}}\} / \{A_{\text{D}+\text{H}} + A_{\text{H}}\} \]  

The values of \( k_{\text{obs}} \) (s\(^{-1}\)) determined for enzyme-catalyzed deuterium exchange in D\(_2\)O at pD 9.34 with [UMP]\(_{\text{total}}\) = 2.5–10 mM show a good fit to eq 2 that was derived for Scheme 3 (see Supporting Information), with \( k_{\text{D}} \ll [\text{UMP}]_{\text{obs}}\). The data give the first-order rate constant for deuterium exchange into saturation of enzyme-bound UMP at pD 9.34 as \( k_{\text{D}} = 1.15 \times 10^{-3} \) s\(^{-1}\). Similar experiments using ca. 0.3 mM OMPDC (9 mg/mL) gave values of \( k_{\text{D}} \) (s\(^{-1}\)) for the saturation of UMP (2.5–5 mM) at pD 8.13 (100 mM glycyglycine buffer), and at pD 7.58 and 7.03 (48 mM imidazole buffer), at 25 °C and \( I = 0.1 \) (NaCl).\(^{1,1}\)

\[ k_{\text{obs}} = \frac{k_{\text{ex}}[\text{E}]}{[\text{UMP}]_{\text{total}} + K_d} \]  

Scheme 3.

\[ \text{E} + \text{h-UMP} \rightleftharpoons \text{End-UMP} \rightleftharpoons \text{Enz-UMP} \rightleftharpoons \text{E} + d-\text{UMP} \]

Figure 2 shows the pH-rate profile of the values of \( k_{\text{ex}} \) (s\(^{-1}\)) for turnover of enzyme-bound h-UMP to give d-UMP by yeast OMPDC in D\(_2\)O at 25 °C and \( I = 0.1 \) (NaCl). The solid line shows the calculated profile for a catalytic base of \( pK_a = 8.0 \).

We have shown that proton transfer from protonated Lys-93 to the vinyl carbanion must be faster than any molecular motion that exchanges the positions of the N–L\(^+\) hydrons of Lys-93, so that \( k_p \gg k_{\text{rot}} \) (Scheme 4).\(^4\) Therefore, the observed deuterium exchange reaction consists of the pre-equilibrium reversible chemical step of proton transfer from UMP to Lys-93, followed by the occasional rotation of the terminal CH\(_2\)–NH\(^+\) group of Lys-93 into a position to deliver a deuterium to the vinyl carbon (Scheme 4). The CH\(_2\)–NH\(^+\) group of Lys-93 is hydrogen-bonded to the carboxylate groups of Asp-91 and Asp-96,\(^2\) and the barrier to CH\(_2\)–NH\(^+\) bond rotation and hydron exchange is expected to be at least 5 kcal/mol, so that \( k_{\text{rot}} \leq 10^8 \) s\(^{-1}\). The limit of \( k_{\text{rot}} \leq 10^8 \) s\(^{-1}\) can be substituted into eq 3, derived for the mechanism shown in Scheme 4, with \( (k_{\text{ex}})_{\text{max}} = 1.24 \times 10^{-5} \) s\(^{-1}\) and \( (k_{2})_{\text{Lys}} = 10^{-8} \) M to give \( p(K_{a}^{\text{UMP}}) \geq 22 \) for the C-6 proton of enzyme-bound UMP.

\[ (k_{\text{ex}})_{\text{max}} = \frac{k_p}{k_{\text{p}}} k_{\text{rot}} = \frac{(K_a^{\text{UMP}})_{\text{Lys}}}{K_{d}} k_{\text{rot}} \]  

Scheme 4.

The observation of enzyme-catalyzed deuterium exchange via formation of a stabilized carbanion provides convincing evidence for decarboxylation of OMP by yeast OMPDC to give the same carbanion.\(^{1,13}\) The value of \( pK_a \leq 22 \) for the C-6 proton of enzyme-bound UMP determined here is at least 10 units lower than the estimated values of \( pK_a = 30–34 \) for the C-6 proton of 1,3-dimethyluracil in water.\(^{16–18}\) Therefore, yeast OMPDC stabilizes the bound vinyl carbanion by at least 14 kcal/mol. We conclude that OMPDC also provides substantial stabilization of the late carbanion-like transition state for the decarboxylation of OMP, and that this transition state stabilization constitutes a large fraction of, but probably not the entire, enzymatic rate acceleration. Further experimental studies directed at elucidating the origin of the transition state stabilization for OMPDC will provide insight into its so far unexplained extraordinary catalytic power.

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Supporting Information Available: Experimental procedures, kinetic analysis, semilogarithmic plots, table of values of \( k_{\text{ex}} \) and \( k_{\text{D}} \). This material is available free of charge via the Internet at http://pubs.acs.org.

References


(11) The reported failure to detect deuterium exchange in an earlier study is likely due to the use of only a small amount of enzyme and a relatively short reaction time: Smiley, J. A.; DelFraino, B. J.; Simpson, B. A. J. Am. Chem. Soc. 2003, 125, 267–271.

(12) The exchange reaction does not occur in the presence of a saturating amount of the competitive inhibitor 6-aza-uridine 5\(^{\prime}\)-monophosphate.

(13) The pH-dependence of \( k_{\text{ex}} \) for turnover of OMP indicates the involvement of the acidic form of a catalytic residue of \( pK_a = 8.8 \). Porter, D. J.; Short, S. A. Biochemistry 2000, 39, 11788–11800.

(14) \( k_{\text{ex}} = 10^{11} \) s\(^{-1}\) \(^{15}\) For unhindered rotation within an ion pair in water: Richard, I. P.; Tsujii, Y. J. Am. Chem. Soc. 2001, 122, 3963–3964.

(15) We have observed that deuterium exchange into UMP is also catalyzed by the OMPDCs from Methanobacterium thermoautotrophicum and Esherichia coli.


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