3514 *Biochemistry* **2010,** *49,* 3514–3516
DOI: 10.1021/bi100443a

BIOCHEMISTRY
including biophysical chemistry & molecular biology
Rapid Report

Conformational Changes in Orotidine 5′-Monophosphate Decarboxylase: "Remote" Residues That Stabilize the Active Conformation†

B. McKay Wood,‡ Tina L. Amyes,§ Alexander A. Fedorov,∥ Elena V. Fedorov,∥ Andrew Shabila,‡ Steven C. Almo,∥ John P. Richard,§ and John A. Gerlt*,‡

‡*Departments of Biochemistry and Chemistry, University of Illinois, Urbana, Illinois 61801,* §*Department of Chemistry, University at Buffalo, Buffalo, New York 14260, and* ∥*Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461*

Received March 24, 2010; Revised Manuscript Received April 5, 2010

ABSTRACT: The structural factors responsible for the extraordinary rate enhancement ($\sim 10^{17}$) of the reaction catalyzed by orotidine 5′-monophosphate decarboxylase (OMPDC) have not been defined. Catalysis requires a conformational change that closes an active site loop and "clamps" the orotate base proximal to hydrogen-bonded networks that destabilize the substrate and stabilize the intermediate. In the OMPDC from *Methanobacter thermoautotrophicus*, a "remote" structurally conserved cluster of hydrophobic residues that includes Val 182 in the active site loop is assembled in the closed, catalytically active conformation. Substitution of these residues with Ala decreases k_{cat}/K_m with a minimal effect on k_{cat}, providing evidence that the cluster stabilizes the closed conformation. The intrinsic binding energies of the 5′-phosphate group of orotidine 5′-monophosphate for the mutant enzymes are similar to that for the wild type, supporting this conclusion.

Orotidine 5′-monophosphate decarboxylase (OMPDC)[1] is one of Nature's best catalysts: the reaction occurs with a rate enhancement of $\sim 10^{17}$ and a proficiency of $\sim 10^{23}$ M (*2*). The reaction coordinate includes a vinyl carbanion intermediate (Scheme 1) (*3*, *4*).

Structures of OMPDCs in complexes with UMP or 6-hydroxy-UMP, an intermediate analogue (*5*−*9*), reveal hydrogen-bonded networks proximal to (1) C6 of the pyrimidine [Asp 70-Lys 72-Asp 75 in the OMPDC from *Methanobacter thermoautotrophicus* (MtOMPDC)] and (2) O2, N3, and O4 of the base (Ser 127-Gln 185). These destabilize the substrate (*9*) and stabilize the intermediate, although the structural strategy for the latter is unknown.

The 5′-phosphate group binds in a conserved motif at the ends of the seventh and eighth β-strands of the $(\beta/\alpha)_8$-barrel structure, with interactions to backbone NH groups as well as the guanidinium group of a conserved Arg (Arg 203 in MtOMPDC). These interactions are important for catalysis. (1) The k_{cat}/K_m for OMP exceeds that for orotidine by a factor of $\sim 10^{11}$ for the OMPDC from *Saccharomyces cerevisiae* (ScOMPDC) (*10*). (2) The k_{cat}/K_m for OMP exceeds that for 1-(β-D-erythrofuranosyl)-orotic acid (EO; 5′-truncated OMP analogue) by factors of 5.2×10^8 and 3.6×10^8 for ScOMPDC and MtOMPDC, respectively (*11*, *12*). (3) Phosphite dianion (HP_i) activates decarboxylation of EO by factors of 5.6×10^5 and 2.9×10^5 M^{-1} for ScOMPDC and MtOMPDC, respectively (*11*, *12*). The "intrinsic binding energy" [IBE (*13*)] of the 5′-phosphate/HP_i (1) increases the affinity for the substrate (e.g., OMP vs orotidine/EO) and (2) enables decarboxylation by juxtaposition of the substrate with the active site hydrogen-bonded networks (substrate destabilization and intermediate stabilization). How the IBE promotes catalysis is unknown but required to understand the structural basis for the rate enhancement.

A loop located at the end of the seventh β-strand closes over the active site when OMP binds (Figure 1). Although the active site loops differ in both length and sequence in divergent OMPDCs (*12*), each includes a spatially conserved Gln (Gln 185 in MtOMPDC) hydrogen-bonded to both the 5′-phosphate and O2 of the pyrimidine as well as a conserved Ser at the end of the fifth β-strand that also is hydrogen-bonded to N3 of the

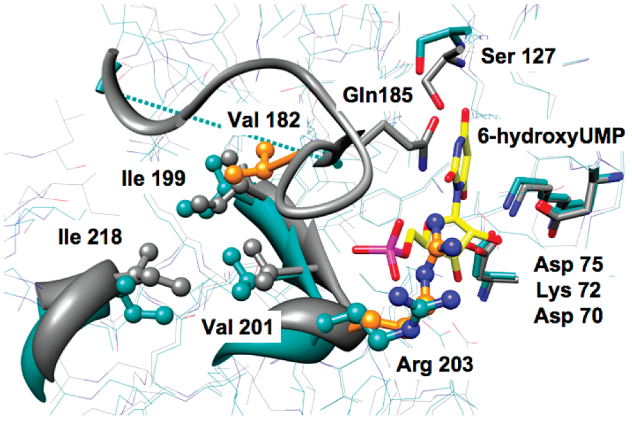
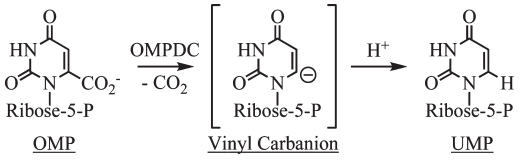


FIGURE 1: Superposition of the active site of wild-type MtOMPDC in the absence (cyan; disordered loop depicted with the dotted line) and presence (gray) of 6-hydroxyUMP. The carbons of 6-hydroxy-UMP are colored yellow; the carbons of Val 182 and Arg 203 in the liganded structure are colored orange.

Scheme 1



†This work was supported by National Institutes of Health Grants GM039754 to J.P.R. and GM065155 to J.A.G.

*To whom correspondence should be addressed. Telephone: (217) 244-7414. Fax: (217) 244-6538. E-mail: j−gerlt@uiuc.edu.

[1]Abbreviations: OMP, orotidine 5′-monophosphate; OMPDC, OMP decarboxylase; MtOMPDC, OMPDC from *Methanobacter thermoautotrophicus*; ScOMPDC, OMPDC from *Saccharomyces cerevisiae*; EO, 1-(β-D-erythrofuranosyl)orotic acid; HP_i, phosphate dianion; IBE, intrinsic binding energy.

 Published on Web 04/06/2010

Table 1: Kinetic Constants for OMP, EO, and EO·HP_i and Intrinsic Binding Energies of the 5′-Phosphate Group of OMP at pH 7.1 and 25 °C

MtOMPDC	$(k_{cat})_{OMP}$ (s^{-1})	$(k_{cat}/K_m)_{OMP}$ (M^{-1} s^{-1})	$\Delta\Delta G^{\ddagger}$ (kcal/mol)[a]	$(k_{cat}/K_m)_{EO}$ (M^{-1} s^{-1})	$\Delta\Delta G^{\ddagger}$ (kcal/mol)[a]	$(k_{cat}/K_m)/(K_D)_{EO\cdot HP_i}$[b] ($M^{-2}$ s^{-1})	$\Delta\Delta G^{\ddagger}$ (kcal/mol)[a]	5′-phosphate IBE[c] (kcal/mol)
wild type	4.6	2.9×10^6		8.7×10^{-3}		2500		11.6[d]
V182A	3.4	1.4×10^5	1.8	1.3×10^{-3}	1.1	190	1.5	10.9
I199A	3.9	9.1×10^5	0.7	1.9×10^{-3}	0.9	980	0.6	11.8
V201A	4.0	9.5×10^5	0.7	3.1×10^{-3}	0.6	690	0.8	11.5
I218A	3.3	2.8×10^5	1.4	2.3×10^{-3}	0.8	340	1.2	11.0
V182A/I199A	3.1	4.9×10^4	2.4	3.9×10^{-4}	1.8	81	2.0	11.0
V182A/V201A	2.5	4.9×10^4	2.4	5.0×10^{-4}	1.7	30	2.6	10.9

[a]Calculated from the ratio of the second-order or third-order rate constants for the wild-type and mutant enzyme. [b]Third-order rate constant for reaction of EO·HP_i. [c]Transition state stabilization by the 5′-phosphate group of OMP, calculated from the ratio of the values of k_{cat}/K_m for OMP and EO. IBEs for phosphite dianion can be calculated from the ratio of $(k_{cat}/K_m)/K_D$ for EO·HP_i and k_{cat}/K_m for EO. [d]This value is a lower estimate because the value of k_{cat}/K_m for OMP is partially diffusion-controlled (*1*).

pyrimidine (Ser 127). We characterized the importance of these "clamp" residues in ScOMPDC (Gln 215-Ser 154) using EO·HP_i and confirmed that the 5′-phosphate·HP_i "switch" is required to activate the enzyme (*14*).

The structures of divergent OMPDCs reveal that OMP binding is always accompanied by a conformational change (Figure 1). The most obvious component is closure of the active site loop. However, the $(\beta/\alpha)_8$-barrel structure can be divided into two domains, one formed from the second, third, fourth, and fifth β-strands (where the hydrogen-bonded Asp 70-Lys 72-Asp 75 motif and Ser 127 are located) and the second from the sixth, seventh, eighth, and first β-strands (where the phosphate binding motif and the active site loop, including Gln 185, are located) (*15*). OMP binding reorients the domains, with the latter domain moving toward the former, forcing the orotate carboxylate group to be juxtaposed vis-à-vis the Asp 70-Lys 72-Asp 75 motif and, also, allowing formation of the Ser 127-Gln 185 clamp. Thus, the transition between the open and closed conformations is more complicated than "simple" hinge motion of the loop on the rigid framework of the $(\beta/\alpha)_8$-barrel structure. In this work, we identify "remote" residues involved in this conformational change and quantitate their importance in promoting and stabilizing the catalytically competent form of the enzyme.

The active site loop of MtOMPDC, P180-G181-V182-G183-A184-Q185-G186-G187-D188, is disordered in the absence of substrate but ordered and closed in its presence (Figure 1). In the liganded structure, two residues in the loop make contacts with the $(\beta/\alpha)_8$-barrel scaffold. (1) Gln 185 is hydrogen-bonded to Ser 127 (vide infra), and (2) Val 182 is embedded in a hydrophobic cluster also formed by Ile 199, Val 201, and Ile 218. The structural conservation of this hydrophobic cluster in all OMPDCs suggests its importance in a common catalytic strategy. We probed this strategy by mutagenesis of these hydrophobic residues.

Ala substitutions for residues in the hydrophobic cluster cause substantial decreases in k_{cat}/K_m for decarboxylation of OMP but have little effect on k_{cat} (Table 1). We determined high-resolution X-ray structures (≤1.4 Å) for each mutant in the presence of 6-hydroxyUMP (Figure 2). The liganded structures superimpose well with that of the wild type, with only small differences observed at the sites of the substitutions (panel A). The active sites are identical to that of the wild type (panel B), explaining the minimal impact on k_{cat}.

The mutated residues are remote from the active site (Figures 1 and 2). Thus, the effects of the substitutions on k_{cat}/K_m cannot be explained by altered direct interactions with the substrate.

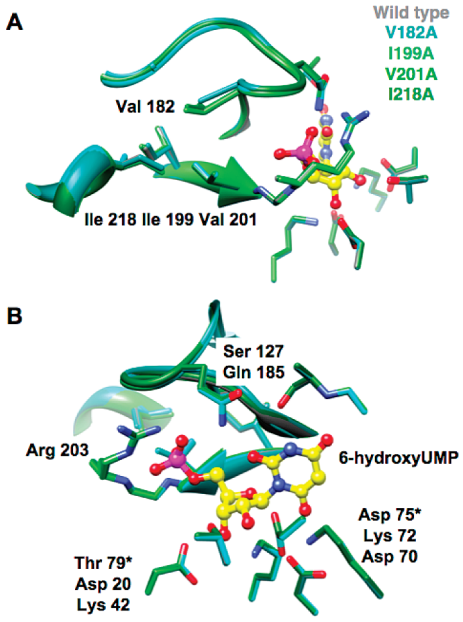
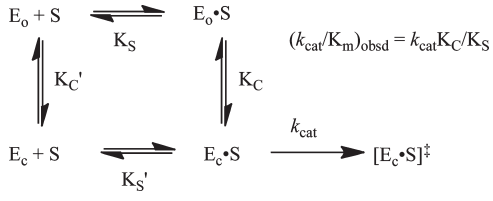


FIGURE 2: Superpositions of the 6-hydroxyUMP-liganded structures of wild-type MtOMPDC and the single mutants in the hydrophobic cluster: (A) hydrophobic cluster and (B) active sites.

Scheme 2



Instead, the effects can be explained by decreased stabilities of the closed conformation in which the substrate is destabilized (*9*) and the anionic intermediate is stabilized. A consistent model (Scheme 2) is one in which an equilibrium of open (E_o) and closed (E_c) conformations exists in the absence or presence of the substrate (K_c' or K_c; $K_c' \ll 1$). The substitutions destabilize E_c (decrease K_c and K_c'), so k_{cat}/K_m is decreased (the energy difference between E_o + S and $[E_c \cdot S]^{\ddagger}$ is increased). However, the invariance of k_{cat} establishes that the substitutions do not alter the reactivitiy of $E_c \cdot S$ (the energy difference between $E_c \cdot S$ and $[E_c \cdot S]^{\ddagger}$). The energy to form $E_c \cdot S$ from E_o and S is derived from (1) interactions of the 5′-phosphate group with its binding motif (IBE; vide infra) and (2) an increased concentration of

OMP to form $E_c \cdot S$ (K_c/K_s or, equivalently, K_c'/K_s', although the former is expected to be the relevant pathway).

We also used the two-part $EO \cdot HP_i$ substrate. The values of k_{cat}/K_m for EO are decreased relative to that for the wild type (Table 1); these can be explained by decreased populations of E_c (Scheme 2), assuming that EO, without a 5′-phosphate group, is unable to promote the transition from E_o to E_c. The values of k_{cat}/K_m for OMP and k_{cat}/K_m for EO allow calculation of the IBE for the 5′-phosphate group of OMP (Table 1).

HP_i activates the mutants as judged by the values of the third-order rate constant, $(k_{cat}/K_m)_{EO \cdot HP_i}/K_d$. The equivalent changes ($\Delta\Delta G^{\ddagger}$) in k_{cat}/K_m for both OMP and EO and the third-order rate constant indicate that all three measure the effects of the substitutions on the values of K_c.

The values of the IBEs for the 5′-phosphate group for the mutants are the same as that for the wild type, establishing that decarboxylation in the $E_c \cdot S$ complex occurs with equivalent amounts of ground state destabilization (*9*) and transition state stabilization (as also reflected by the invariant values of k_{cat}). The IBEs provide further support for the role of the remote hydrophobic cluster in stabilizing E_c relative to E_o but do not directly participating in catalysis.

Our experiments implicate a structurally conserved hydrophobic cluster, Val 182, Ile 199, Val 201, and Ile 218 in MtOMPDC, in stabilizing the closed conformation required for catalysis. Its identification provides evidence that structural elements distal from the active site, in addition to the proximal active site loop that closes to clamp the substrate, are required for OMPDC's extraordinary catalytic efficiency and proficiency.

SUPPORTING INFORMATION AVAILABLE

Descriptions of the experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Wood, B. M., Chan, K. K., Amyes, T. L., Richard, J. P., and Gerlt, J. A. (2009) *Biochemistry 48*, 5510–5517.
2. Radzicka, A., and Wolfenden, R. (1995) *Science 267*, 90–93.
3. Toth, K., Amyes, T. L., Wood, B. M., Chan, K., Gerlt, J. A., and Richard, J. P. (2007) *J. Am. Chem. Soc. 129*, 12946–12947.
4. Amyes, T. L., Wood, B. M., Chan, K., Gerlt, J. A., and Richard, J. P. (2008) *J. Am. Chem. Soc. 130*, 1574–1575.
5. Miller, B. G., Hassell, A. M., Wolfenden, R., Milburn, M. V., and Short, S. A. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 2011–2016.
6. Appleby, T. C., Kinsland, C., Begley, T. P., and Ealick, S. E. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 2005–2010.
7. Harris, P., Navarro Poulsen, J. C., Jensen, K. F., and Larsen, S. (2000) *Biochemistry 39*, 4217–4224.
8. Wu, N., Mo, Y., Gao, J., and Pai, E. F. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 2017–2022.
9. Chan, K. K., Wood, B. M., Fedorov, A. A., Fedorov, E. V., Imker, H. J., Amyes, T. L., Richard, J. P., Almo, S. C., and Gerlt, J. A. (2009) *Biochemistry 48*, 5518–5531.
10. Sievers, A., and Wolfenden, R. (2005) *Bioorg. Chem. 33*, 45–52.
11. Amyes, T. L., Richard, J. P., and Tait, J. J. (2005) *J. Am. Chem. Soc. 127*, 15708–15709.
12. Toth, K., Amyes, T. L., Wood, B. M., Chan, K. K., Gerlt, J. A., and Richard, J. P. (2009) *Biochemistry 48*, 8006–8013.
13. Jencks, W. P. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol. 43*, 219–410.
14. Barnett, S. A., Amyes, T. L., Wood, B. M., Gerlt, J. A., and Richard, J. P. (2008) *Biochemistry 47*, 7785–7787.
15. Harris, P., Poulsen, J. C., Jensen, K. F., and Larsen, S. (2002) *J. Mol. Biol. 318*, 1019–1029.