

The protonation process in decarboxylation of OMP catalyzed by Orotidine 5'-Monophosphate Decarboxylase

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Abstract. In this article, the protonation processes before and after decarboxylation of orotidine 5'-monophosphate (OMP) catalyzed by orotidine 5'-monophosphate decarboxylase (ODCase) is investigated theoretically at both Hartree-Fock and density functional theory (DFT) levels. The results indicate that firstly O4 is protonated by residues of ODCase bridged by water molecules. Then, after decarboxylation, a fast stepwise protonation process happens. Additionally, the catalytic function of Lys-93 is also discussed. The protonation process plays an important role in the whole reaction.

1. INTRODUCTION

Orotidine 5'-monophosphate decarboxylase (ODCase, E. C. 4.1.1.23) catalyzes the decarboxylation of OMP with one of the largest rate enhancements by any enzyme, and it performs its task without the assistance of metals or small molecule cofactors [1]. Carboxylic acids tend to undergo decarboxylation in their anionic (-COO-) forms [2, 3]. The decarboxylation reaction catalyst assists in the delocalization of negative charge that accompanies the release of CO₂ from the substrate molecule. However, the substrate of ODCase lacks a p-orbital into which electrons can be delocalized [4]. For this, the decarboxylation of OMP remains a special mechanistic challenge to the enzyme after years' extensive experimental and theoretical studies [5-20].

Transferring a proton to the ring is the most simple and direct way to diffuse the negative charge. ¹³C and solvent isotope effects studies on the enzymatic reaction do indicate the presence of a proton-dependent step prior to decarboxylation in the reaction [21-23]. Theoretical studies also suggested protonation mechanisms 9, 10 in which protonation of the pyrimidine ring can lower the reaction barrier substantially. However, N1 nitrogen isotope effects [24] studies against mechanisms involving ring protonation, especially protonation at O2. Additionally, the even big challenge is that good candidates for a proton donor are not found in the enzyme structures [4, 11, 25, 26], Lys-93 (yeast numbering) is used to be regarded as the best candidates for proton donor based on the experimental facts that it is one of the charged network residues conserved in ODCase and the K93A mutation leads to a drastic loss of activity of ODCase [27, 28]. Unfortunately, it is not either. Lys-93 might play an important role in other functions which is also deserved to be studied.

In this work, we set out to design several possible protonation models before and after the decarboxylation and then to discuss the role of protonation processes played in the reactions of OMP.



2. Theoretical basis

All calculations are performed at both Hartree-Fork and density functional theory (DFT) levels, using the GAUSSIAN 03 packages [29]. Full geometry optimization of all stationary points are carried out by B3LYP [30, 31] and 6-31+G** basis set for decarboxylation barrier calculations. PKa [32] and protonation barriers calculations are performed at HF/6-31+G** level. Then frequencies calculations are also carried out at the same optimization level. Solvent effects calculations are carried out by the PCM model [33-36] using a dielectric constant of 4.0. The environment (residues of ODCase) around the substrate is treated as a homogenous medium with a dielectric constant of 4.0 throughout calculations. And in all models, the substrate OMP is modeled by deprotonated 1-methylorotate. Lysine residue is modeled by methylamine.

3. Results and discussion

Proton transfer processes before decarboxylation. Recently, new crystal structures have been presented [37, 38] that clarify further the binding of the OMP. In these structures, hydrogen bonding to O2/O4 is figured out much more detailed. A water molecule is also found near O4. And basing on another fact of chains of water molecules [39, 40] existing in the crystal structures, we suggest protonated residues of ODCase might be the source of proton donor and O2/O4 of OMP might be protonated by them bridged by the water molecules before decarboxylation (shown in figure 1).

To examine the validity of this model, we carried out PKa calculations by the PCM mode using a dielectric constant of 4.0 to clarify the possibility that O2/O4 is protonated by amino acid (in Figure 1). All PKa calculations are conducted using the following equations [32].

$$pK_a(HA) = \frac{\Delta G_{aq}}{2.303 \times RT} \quad (1)$$

$$\Delta G_{aq} = \Delta G + G_{aq}(H^+) \quad (2)$$

$\Delta G_{aq}(H^+)$ can be derived from experiment. According to eq1 and 2, the larger the ΔG , the larger the PKa, the easier it is protonated. Here only relative PKa value is needed and ΔG is enough for data analysis. Additionally, In bio-environment (PH \approx 7), only basic (histidine, lysine, arginine) amino residues exist in their protonated states. Among them, Lys is the most readily one to lost an H $^+$ and is suitable to be selected as the proton donor in our PKa calculations. ΔG calculated is 259.5, 271.5 and 267.0 kcal/mol for O2, O4 and Lys respectively. This result indicates that O4 is easier than O2 to be protonated by Lys.

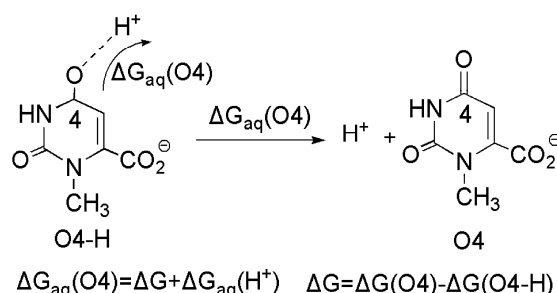


Fig. 1. OMP O4-deprotonation model for PKa calculations.

The reaction barriers of proton transfer between O2/O4 and Lys are figured out using PCM model. Computed barriers of the protonation process are schematically given in Table 1 (shown in Figure 2). In the figure, reactant energy of O4 path is defined as 0 kcal/mol. As shown, barrier of O2 protonation path is 23.49 kcal/mol, which is 20.58 kcal/mol higher than that of O4 path. It indicates that proton

transfer from Lys to O4 is faster than O2, i.e., O4 is easier than O2 to be protonated, which correlated well with trend that PKa calculation indicated above.

Table 1. The reaction barriers of proton transfer between O2/O4 and Lys.

	$E_R(\text{kal/mol})$	$E_{TS}(\text{kal/mol})$	$E_a(\text{kal/mol})$
O2	10	33.49	23.49
O4	0	2.91	2.91

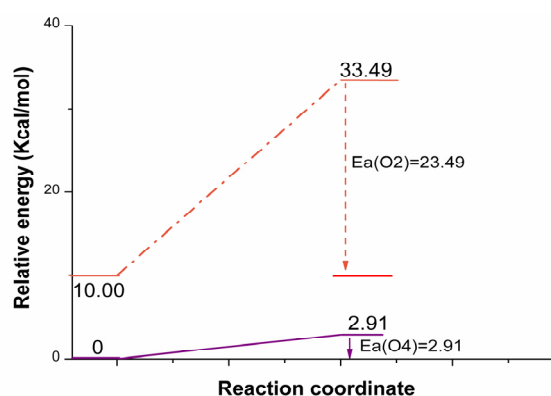


Fig. 2. Relative energy profiles for O2 (orange dashed line) and O4 (purple solid line) protonation process.

According to the protonation at O4 suggested above, two possible protonation models for OMP after decarboxylation are designed (shown in Figure 3). (1) Concerted protonation. H^+ transfer from Lys93 to C6— and from O4 to Lys93 occur in concert. (2) Stepwise protonation. First, Lys93 protonates C6— of OMP, and then O4—H transfers H^+ to Lys93.

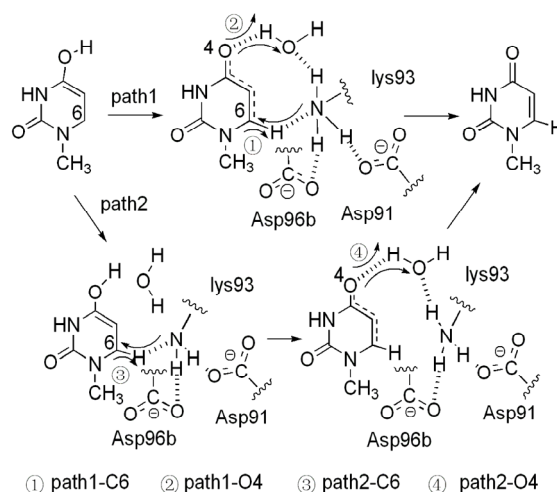


Fig. 3. Two possible proton transfer paths model after OMP decarboxylation: path1, concerted protonation; path2, stepwise protonation.

For path 1 and 2, ΔG calculations were also performed with the same method used above. Calculated ΔG (①, ②, ③, ④ in Figure 3) is in Table 2.

Table 2 ΔG of Lys93, O4 and C6 of path 1 and 2.

ΔG (kal/mol)	O4	Lys93	C6
Path 1	295.3	255.7	287.5
Path 2	240.4	255.7	287.5

Fortunately, There do exist the trends of interest that $\Delta G_{O4} < \Delta G_{Lys} < \Delta G_{C6}$ in path 2, i.e., $PKa_{O4} < PKa_{Lys} < PKa_{C6}$. However, in path 1, ΔG of path1-O4 is 295.3kal/mol which is much larger than that of Lys93, 255.7kal/mol. The result indicates that Lys93 is protonated by O4-H is very impossible via path1. It is stepwise not concerted protonation path that proton transfers via. In this protonation model, Lys93 plays an important role in this protonation path and just functions as catalyst. It is proton donor in the first step, and in next step it is recycled. This can explain experimental K93A mutations study results [27, 28] well.

In path 2, the large ΔG difference between Lys93 and C6 as well as between O4 and Lys93 indicates a very possible fast proton transfer process as above PKa results indicated. This result well supports recent finding that the enzyme catalyzes the exchange of the hydrogen at C6 of UMP [41].

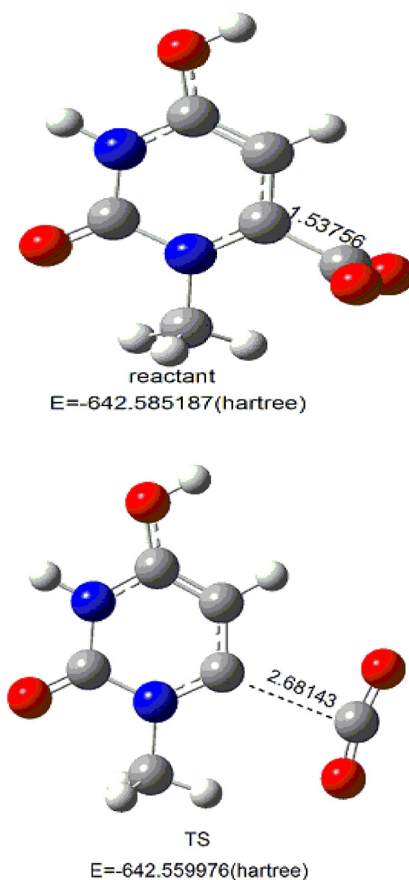


Fig. 4. Optimized structures and energy of reactant and transition state.

The carbene mechanism cannot be discounted on the basis of energetics [42]. In this part, the decarboxylation barrier of OMP with O4 protonation mechanism is also calculated by PCM model

using a dielectric constant of 4.0. Optimized structures of reactant and the transition state are shown in figure 4. The calculated energy barrier is 15.8kcal/mol, which agrees very well with the experimental value [15].

4. Conclusions

In general, protonation processes play an important role in the OMP decarboxylation reactions. First, O4 is protonated by residues of ODCase bridged by water molecules. Then, after decarboxylation, a proton is transferred to C6- catalyzed by Lys93. Additionally, the decarboxylation barrier of O4 mechanism is calculated to agree well with the experiment. At last, these results may indicate that O4 mechanism might be the most likely mechanism for OMP decarboxylation.

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