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Modest catalysis of the decarboxylation of orotate by hydrogen bonding: a theoretical model for orotidine-5′-monophosphate decarboxylase

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Abstract

As a model for interactions present in the active site of orotidine-5′-monophosphate decarboxylase (ODCase), the effect of hydrogen bonds to the carbonyl groups (O-2 and O-4) of orotic acid and its decarboxylation product was probed with ab initio calculations. We have found that the transition state/carbanion intermediate is a better proton receptor and therefore, the hydrogen bonds can be a modest source of catalysis. Comparison of the calculated data with results from site-directed mutagenesis provides some insights into the polarity of the active site.

1. Introduction

Orotidine-5′-monophosphate decarboxylase (ODCase)[1] catalyzes the final step in the biosynthesis of the nucleotide uridine 5′-monophosphate (UMP). In the reaction, orotidine-5′-monophosphate (OMP) is decarboxylated to UMP (Scheme 1). The mechanism of ODCase has been the target of significant research interest due to its catalytic proficiency and the lack of cofactors or structural features to stabilize the carbanion intermediate [1–3]. A number of mechanistic proposals with novel

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[1] *Abbreviations used*: ODCase, orotidine-5′-monophosphate decarboxylase; OMP, orotidine-5′-monophosphate; UMP, uridine 5′-monophosphate; COSMO, conductor-like screening solvation model.

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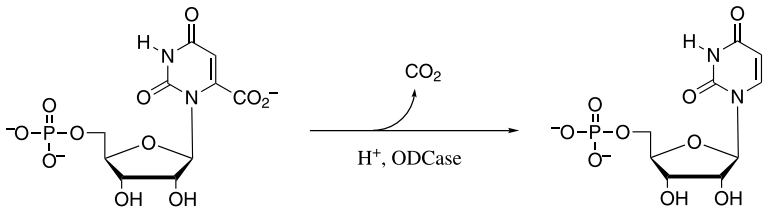


Scheme 1.

features have been forwarded, such as the involvement of a ylide or a carbene intermediate, protonation of C-5, covalent catalysis, and catalysis by destabilization of the substrate [4–9].

Recent crystallographic studies have revealed some interesting features of ODCase [10–13]. An aspartate residue was found to be adjacent to C-6 of the pyrimidine group of the bound product or other inhibitors in the structures, providing some support for the concept of substrate destabilization. On the other hand, the structures do not reveal any basic residues adjacent to the two carbonyl groups (O-2 and O-4) on the pyrimidine ring. This observation suggests that the ylide or carbene mechanism is unlikely since the mechanisms require proton transfer to either the O-2 or O-4 group [4,5]. However, both carbonyl oxygens are hydrogen-bonded to amide groups in the active site of ODCase [10–13].

Proton transfer to either O-2 or O-4 will preferentially stabilize the carbanion intermediate of the decarboxylation reaction as proposed in the ylide and carbene mechanisms [4,5]. The activation barrier is reduced as a result and rate acceleration is thus achieved. It is conceivable that the hydrogen bonds to the intermediate might be stronger than to the substrate and have the same catalytic effect as a proton transfer to these oxygens, though not as profound. We thought that it would be useful to investigate the specific roles played by the hydrogen bonds to O-2 and O-4. Although elegant modeling studies on the entire active site have been carried out [13–15], the present study separates out the possible contribution by these hydrogen bonding interactions.

2. Materials and methods

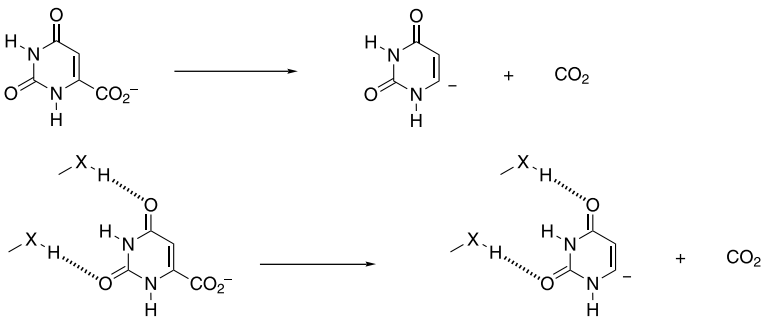
All models were built using *MacSpartan* and optimized at the AM1 level. The coordinates were imported into *Gaussian 94* or *98* for HF, MP2, and zero-point energy calculations. Geometries were optimized at the HF/6-31 + G(d) level and frequencies were calculated at the same level. Finally, single point MP2 calculations were carried out on the HF geometries using the 6-31 + G(d,p) basis set (MP2/6-31 + G(d,p)//HF/6-31 + G(d)). Condensed phase calculations were performed using a conductor-like screening solvation model (COSMO) that treats the solvent as a polarizable, continuous medium. The geometries were taken from the gas phase HF/6-31 + G(d) optimizations. All condensed phase calculations were done using Gaussian 98 at the MP2/6-31 + G(d,p) level.

3. Results and discussion

The absence of proton-donating residues in the vicinity of pyrimidine carbonyl groups makes the ylide or the carbene mechanism unlikely. Thorough modeling studies on the active site as a whole have provided insights on the mechanism [13–15]. However, the specific roles played by hydrogen bonds to the substrate oxygens have not been fully studied [16]. It is conceivable that these hydrogen bonds become stronger in the carbanion intermediate. It has been demonstrated that the proton affinity of the carbanion intermediate is much greater than that of the substrate [5]. So, it is plausible that stronger hydrogen bonds with the intermediate will result in the preferential binding of the reaction intermediate and thus catalysis of the decarboxylation reaction.

The enthalpies of the reactions shown in Scheme 2 were calculated at the MP2/6-31 + G(d,p)//HF/6-31 + G(d) level. It should be pointed out that our previous work has demonstrated that the transition state for the decarboxylation reaction has the same energy as the carbanion, i.e., there is no barrier aside from the endothermicity of the process [8,9]. Therefore, the calculated enthalpies of the reactions are essentially equal to the activation enthalpies of the reactions. In the second reaction, two hydrogen bonds with O-2 and O-4 were included and the O–H distances were optimized without constraint. The enthalpy of the uncatalyzed reaction (i.e., without hydrogen bonding) was found to be 44.6 kcal/mol, in excellent agreement with a previously reported computational result [5].

The reductions in activation enthalpy ($\Delta\Delta H^{\ddagger}$) and the corresponding rate acceleration (k_{cat}/k_{noncat}) at 298 K caused by four donors (water, methanol, formamide, and formic acid) are listed in Table 1. The hydrogen bonds to O-2 and O-4 are indeed stronger for the carbanion intermediate since the activation enthalpy is reduced in the hydrogen-bonded systems. The amount of reduction is dependent on the proton-donating capacity of the hydrogen bond donor group. The better the proton donor, the stronger the hydrogen bond, and thus, the larger the reduction in the activation enthalpy ($\Delta\Delta H^{\ddagger}$). As a measure of their donating ability, the gas phase proton affinities of their conjugate bases are 390.3, 382.0, 359.8, and 345.3 kcal/mol



Scheme 2.

Table 1
Extent of catalysis by hydrogen bonds to O-2 and O-4 in the gas-phase

H-bond donor	$\Delta\Delta H^{\ddagger}$ (kcal/mol)	k_{cat}/k_{noncat}
Uncatalyzed	0.0	
HO–H	3.2	2.2×10^2
CH_3O–H	2.7	95
HC(O)–N–H_2	6.6	7.4×10^4
HC(O)–O–H	7.2	2.0×10^5

(available from the NIST Chemistry WebBook http://webbook.nist.gov/chemistry/), respectively.

It is apparent from the data that the dipole moment of the proton-donor also plays a significant role. For example, the larger dipole moment of water can compensate for its weaker acidity and so it acts as a better hydrogen-bond donating group than methanol in this system. It is also conceivable that dipole moments of active site residues could be oriented in a way that selectively stabilizes an intermediate or the transition state in the active site and therefore, as suggested by Warshel [17], the potential contribution of dipoles to enzymatic catalysis should not be overlooked.

The results in Table 1 demonstrate that hydrogen bonding can be one of the ways that ODCase catalyzes the decarboxylation of OMP. The catalysis provided by formamide (7.4×10^4-fold rate acceleration) is noteworthy since this is the kind of functional group commonly seen in enzyme catalysis in general and ODCase in particular [10–13]. Further, analysis of catalysis by formamide has revealed that the effect of hydrogen bonds to O-2 and O-4 is more or less additive. Hydrogen bonding by formamide to O-2 causes a 3.9 kcal/mol reduction in $\Delta H^{\ddagger}$ while hydrogen bonding to O-4 causes a 3.0 cal/mol reduction.

The dielectric constant of solvents can have a large effect on the strength of hydrogen bonds [18]. Therefore, the $\Delta\Delta H^{\ddagger}$ of the reaction was examined in different solvent environments using the COSMO continuum model. When the dielectric constant is changed to 2.0, 4.9, 10.36 or 46.7, $\Delta\Delta H^{\ddagger}$ (as compared to uncatalyzed reactions in the same media) is reduced rapidly when formamide is the hydrogen bonding donor (Table 2). However, $\Delta\Delta H^{\ddagger}$ is not as sensitive to dielectric constant and remains significant when a stronger hydrogen bonding donor (e.g., formic acid) is used. With formamide, a highly polar species that is a weak donor, the dielectric medium simulated by the continuum model is able to cancel out its effect by providing a polar

Table 2
Catalysis in various media using formamide and formic acid as hydrogen bonding donors

Dielectric constant	$\Delta\Delta H^{y}$ (kcal/mol)	
	HC(O)–NH_2	HC(O)–OH
1	6.6	7.2
2.0	2.6	5.7
4.9	1.8	4.9
10.36	0.7	4.6
46.7	0.1	4.4

medium for the reaction. Formic acid is less polar than formamide, but is a better hydrogen bond donor. Apparently the specific hydrogen bonding interaction found in the solvate provides added stabilization that cannot be reproduced by the application of a polar medium (*i.e.*, the continuum model). Thus, a more polar active site can greatly diminish the catalytic impact of the hydrogen bonds, depending on the strength of the donor. It should be pointed out that some hydrogen bonds are especially strong, even in aqueous solution [19–21]. Furthermore, enzymes might manipulate the environment of the active site to provide a pre-organized polar environment that is more conducive towards hydrogen bonding [17]. Therefore, hydrogen bonds to O-2 and O-4 could preferentially stabilize the carbanion intermediate and thus, could be a component in the acceleration of the reaction.

Some measure of the importance of hydrogen bonding in the active site of ODCase can be gleaned from site-directed mutagenesis studies. Mutation of Gln-215 of the yeast ODCase, which forms a hydrogen bond with O-2, to alanine has been accomplished and the contribution of this residue to the catalysis was determined to be about 1 kcal/mol [16]. Unfortunately, mutagenesis studies on the residue that hydrogen bonds to O-4 could not be carried out since the hydrogen bond donor is a backbone amide in ODCase and cannot be eliminated [10–13]. Nevertheless, comparison of the calculated $\Delta\Delta H^{\ddagger}$ values for media of various dielectric constants with the results from mutagenesis studies may be able to provide a measure of the polarity of the active site. For example, the result from the Q215A mutation of the yeast ODCase (see above) suggests an effective active site dielectric constant about 5–10 when formamide is used as a model for the hydrogen bonding donor.

Our results have demonstrated that hydrogen bonding to pyrimidine carbonyl groups (O-2 and O-4) could be a component in the catalytic proficiency of ODCase. The data also indicate that comparison of results from mutagenesis studies with those from calculations modeling media of various dielectric constants may provide a way of estimating the effective polarity of the active site.

Acknowledgments

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