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Orotidine Monophosphate Decarboxylase

A Mechanistic Dialogue

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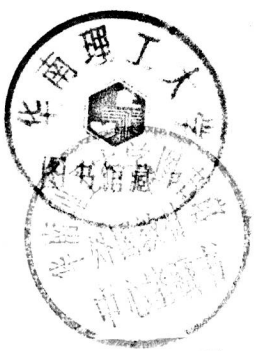

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238

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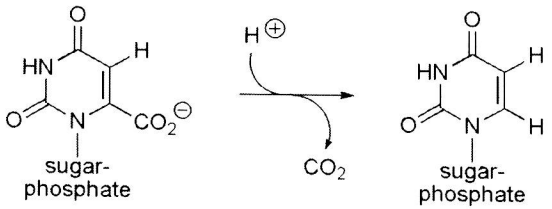
Preface

How can an enzyme that apparently does not utilize cofactors or covalent intermediates be one of the most proficient enzymes known? This is the mystery of orotidine monophosphate decarboxylase (ODCase). In this volume, experts in the field of enzyme catalysis describe their efforts to understand this puzzling enzyme.

The reaction catalyzed by ODCase is ostensibly quite simple—the decarboxylation of orotate ribose monophosphate (OMP) to produce uracil ribose monophosphate (see below). The problem with this reaction is that direct decarboxylation would lead to an anion whose lone pair of electrons is not aligned with any π-system that could lead to stabilization through delocalization. Various proposals have been put forth to overcome this apparent obstacle. These include selective stabilization of the transition state for direct decarboxylation by noncovalent interactions with ODCase, selective destabilization of the reactant by repulsive noncovalent interactions that are reduced or removed during direct decarboxylation, pre-protonation of the reactant on one of its carbonyl oxygens or alkene carbons such that decarboxylation would lead to a stabilized ylide, and concerted protonation-decarboxylation which would avoid the formation of a discrete uracil anion. The validity of these mechanistic proposals is analyzed from various viewpoints in this volume.



Houk and coworkers provide a survey of the known biochemical, structural, and computational studies on ODCase. In particular, they examine what recent theoretical studies have discovered about the origins of catalysis in ODCase, including the possibility that dynamic effects and/or iminium ion formation might actually be important contributors to ODCase's proficiency.

Wu and Pai provide a thorough examination of the molecular structure of ODCase, based on X-ray crystallographic studies of wild-type and mutant ODCases bound with various inhibitors. The implications of these structural studies are discussed, with an emphasis on the possibility that the conformational dynamics of the enzyme may be the key to catalysis.

Miller provides a synthesis of results from recent structural studies, computations, and biochemical experiments on mutant ODCases and truncated substrates. His detailed analysis of specific ODCase-substrate interactions is aimed at quantifying their importance and proposing roles for each in catalysis.

Smiley provides an analysis of the available structural and biochemical data, and based on these, proposes an unusual binding mode for OMP in ODCase. The implications of this binding mode for catalysis are discussed in detail.

Lundberg, Blomberg, and Siegbahn show how modern quantum chemistry has been applied to the ODCase problem. They describe results from quantum mechanical calculations on large models of OMP and the active site residues in ODCase that surround it, and provide a critical evaluation of many of the proposed mechanisms for catalysis.

Gao and coworkers describe how combined quantum mechanics/molecular mechanics computations have also been applied to the ODCase problem. In particular, he focuses on the importance of strain (in both the substrate and enzyme) to catalysis of direct decarboxylation.

Overall, this volume shows how many different mechanistic tools—biochemical kinetics, X-ray crystallography, state-of-the-art computations—have been brought to bear in parallel and occasionally in concert on the problem of understanding the origins of catalysis by ODCase. Still, it is clear that a consensus has not yet been reached as to the catalytic mechanism. In fact, while very few mechanistic proposals have been disproven, many new mechanistic proposals have arisen. For example, as pointed out by several of our authors, dynamic effects may prove to be essential for catalysis in this peculiar enzyme. We hope that the diverse chemistry discussed in this volume will inspire not only additional calculations and experiments, but also new viewpoints from which the mystery of ODCase's catalytic mechanism may be unraveled.

Davis and Piscataway, March 2004 Dean Tantillo and Jeehiun Lee

Contents

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What Have Theory and Crystallography Revealed About the Mechanism of Catalysis by Orotidine Monophosphate Decarboxylase?

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Abstract In 1995, Wolfenden and Radzicka showed that orotidine 5′-monophosphate decarboxylase (ODCase) was the most proficient enzyme. Since then, the mechanism of catalysis has been widely debated. The recent appearance of crystal structures for ODCase has led not to a definitive picture of catalysis as might be expected, but to even more conjecture concerning the mechanism. In addition, the many theoretical studies on ODCase have caused opinions to diverge, rather than converge, about the mechanism. This review summarizes the mechanistic, crystallographic, and computational evidence for

the mechanism of ODCase, and offers a critical evaluation of the various mechanisms based upon this evidence.

Keywords Quantum mechanics · Density functional theory · ODCase · Uracil · Decarboxylation

Abbreviations

ODCase	Orotidine 5′-monophosphate decarboxylase
OMP	Orotidine 5′-monophosphate
UMP	Uridine 5′-monophosphate
BMP	Barbituric acid ribosyl 5′-monophosphate
6-azaUMP	6-azauridylate 5′-monophosphate
XMP	Xanthosine 5′-monophosphate
CMP	Cytidine 5′-monophosphate
CPMD	Car-Parrinello molecular dynamics

1 Introduction

The mechanism of the enzymatic decarboxylation of orotidine 5′-monophosphate (OMP) to uridine 5′-monophosphate (UMP) (see Fig. 1) is an intriguing problem for which many solutions have been offered. Even before 1995 when Wolfenden and Radzicka declared OMP decarboxylase (ODCase) to be the "most proficient enzyme" [1], several different mechanisms had been proposed. Since that time, other mechanisms have been advocated. Curiously, the appearance of crystal structures for various wild-type and mutant ODCases has led not to a definitive picture of catalysis, but to even more conjecture and controversy concerning the mechanism.

This article summarizes the mechanistic, crystallographic, and computational evidence for the mechanism of decarboxylation of OMP by the family of orotidine 5′-monophosphate decarboxylase enzymes, and offers a critical evaluation of the various mechanisms based upon this evidence.

Figure 2 summarizes the nine different types of mechanisms proposed for this reaction and the original proponents of each. There is some overlap,

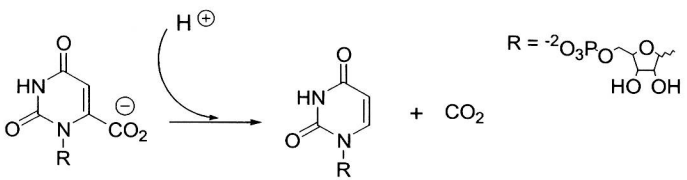


Fig. 1 Conversion of orotidine monophosphate to uridine monophosphate

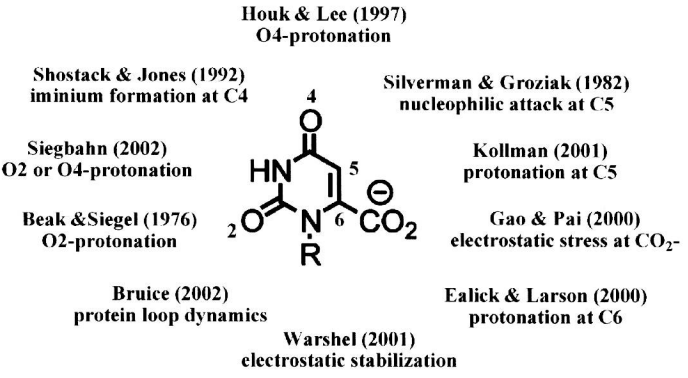


Fig. 2 Summary of mechanisms proposed for action of ODCase

and more than one researcher has backed one of the other mechanisms. Nevertheless, consensus has not been achieved.

Figure 3 compares the proficiencies ($k_{cat}/K_M/k_{un}$) of ODCase, several other enzyme decarboxylases [2], and some antibody decarboxylases [3]. The proficiencies of the decarboxylase enzymes, including a variety of amino acid decarboxylases, are nearly equal. Many decarboxylases employ iminium intermediates formed by reaction of an amino acid with a cofactor such as pyruvoyl or pyridoxal, or by reaction of a β-keto ester with an active-site lysine residue. These intermediates have been found to be so reactive that the

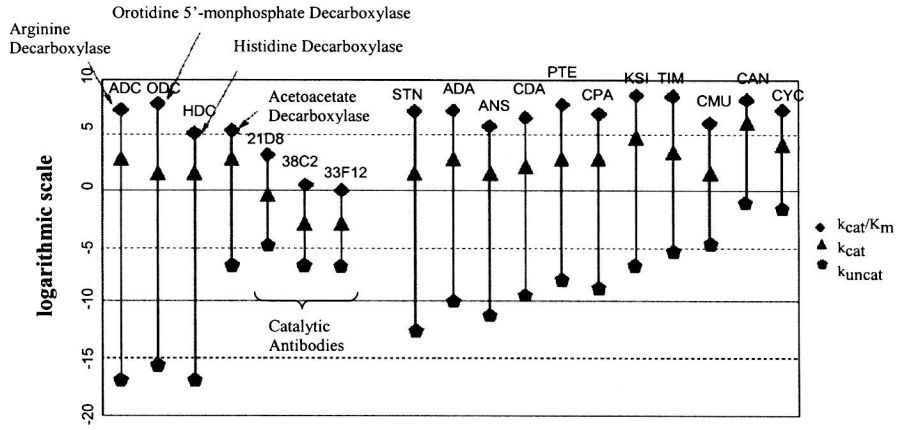


Fig. 3 A modified "Wolfenden plot" showing k_{cat}/K_M, k_{cat}, and k_{uncat} for a selection of enzymes and antibodies: Staphylococcal nuclease (*STN*), adenosine deaminase (*ADA*), cytidine deaminase (*CDA*), alanylalanine transpeptidase (*PTE*), carboxypeptidase A (*CPA*), ketosteroid isomerase (*KSI*), triose phosphate isomerase (*TIM*), chorismate mutase (*CMU*), carbonic anhydrase (*CAN*), cyclophilin (*CYC*)

rate of formation of the iminium intermediate becomes competitive with the rate of decarboxylation [4].

For ODCase, non-covalent mechanisms have often been proposed, as reflected in three of the mechanisms shown in Fig. 2. This is the crux of the attention showered on ODCase: how can this enzyme achieve its rate acceleration without the use of cofactors, metals, or acid-base catalysis? From Wolfenden's measurements of the uncatalyzed reaction of 1-methylorotic acid in water, he calculated the rate enhancement (k_{cat}/k_{un}) in the enzyme to be 1.4×10^{17}, corresponding to a reduction of $\Delta G^{\ddagger}$ of 24 kcal/mol [1]. He also reported the catalytic proficiency to be 2×10^{23}, meaning that the enzyme–transition state complex is an impressive 32 kcal/mol more stable than the free enzyme and transition state in water (i.e., the effective binding free energy of the transition state out of water is 32 kcal/mol) [1]! The experimental free energy of activation is 15 kcal/mol for this decarboxylation in ODCase.

2 Mechanistic Investigations of ODCase

The lack of evidence for the involvement of cofactors in ODCase catalyzed decarboxylation prompted Beak and Siegel to propose a novel mechanism, which was supported by experiments on model systems [5]. They showed that the decarboxylation of 1,3-dimethylorotic acid in sulfolane follows two different pH-dependent pathways. In neutral solution, decarboxylation is initiated by zwitterion formation. This led to the proposal of a zwitterion intermediate in the ODCase catalyzed reaction in which the O2 of the pyrimidine ring is protonated, promoting the release of CO_2.

Various covalent mechanisms have also been postulated. Silverman and Groziak used model systems to support the Michael addition of an active-site nucleophile to the C5 position of OMP followed by decarboxylation [6]. This mechanism was later discarded when kinetic isotope effect experiments indicated that there was no change in bond order between C5 and C6 during decarboxylation [7].

As noted above, many decarboxylases are known to exploit Schiff base formation in the active-site as a source of catalysis. Shostack and Jones explored this possibility in the case of ODCase [8]. They found that when the enzymatic reaction is performed in ^{18}O water, the product does not incorporate ^{18}O from bulk solvent. For this reason, a covalent iminium mechanism for ODCase was abandoned, in spite of its attractive similarities to other decarboxylase mechanisms.

Besides structural characterization described below, various other experimental techniques have been employed to probe the nature of the

ODCase active site. Wolfenden performed atomic absorption spectroscopy and initially concluded that two atoms of zinc were present per active ODCase monomer [9]. This finding was later withdrawn when atomic and x-ray absorption spectroscopy did not detect zinc in active ODCase samples [10].

Mutagenesis experiments were also used to determine the importance of active-site residues involved in binding and catalysis. Mutation of either Lys93 or Asp91 (yeast numbering) to alanine was shown to reduce activity by more than 10^5, and to drastically reduce substrate binding [11]. The substitution of alanine at Lys59 or Asp96 reduced the activity by a comparable amount, but the mutant retained some substrate affinity.

Likewise, a series of mutagenesis and substrate truncation experiments indicated the importance of phosphate binding to the complexation of substrate and to catalysis [11, 12]. Single mutations of several active-site residues that are known to interact with the phosphoryl group reduced the value of k_{cat}/K_m by factors of up to 7,300-fold. The value of k_{cat}/K_m was reduced by more than twelve orders of magnitude when the phosphoribosyl group of the OMP substrate was removed. The magnitude of this interaction has been used to argue in favor of substrate destabilization as a source of catalysis, whereby the favorable interaction of the phosphoryl group compensates energetically for the destabilization of the reactive portion of the substrate (discussed in detail in Sect. 4). More recent mutagenesis experiments indicate that favorable interactions between the enzyme and substrate phosphoryl group increase as the substrate approaches the transition state, reinforcing the importance of transition state stabilization in catalysis [13].

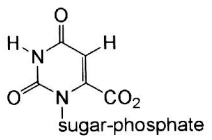
3
Crystallographic Studies

3.1
Initial Reports

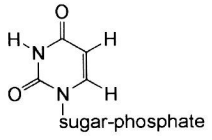
To date, more than twenty X-ray crystal structures of ODCase have been reported, all by the groups of Ealick, Pai, Larsen, and Short and Wolfenden (see Table 1) [14–21]. The first six of these were reviewed previously by Houk, Lee, and coworkers [22] and Begley, Appleby, and Ealick [23]. As noted by these reviewers, the active-site structure of ODCase is extremely similar in all of the reported structures, boasting an Asp-Lys-Asp-Lys tetrad in the vicinity of the 6-position of the pyrimidine ring of bound inhibitors defined above, a group of uncharged hydrogen bond donors and acceptors that interact with the 2-, 3-, and 4-positions of the pyrimidine ring, and several hydrophobic residues that interact with the faces of the ring (Table 2). A representative structure is shown in Fig. 4.

Table 1 X-ray crystal structures of ODCase, listed in the order reported

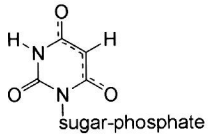
Date	Ligand bound	Mutation(s)	Organism	Resolution (Å)	Group
2000	UMP	none	*B. subtilis*	2.4	Ealick [14]
2000	none	none	*S. cerevisiae*	2.1	Short & Wolfenden [15]
2000	BMP	none	*S. cerevisiae*	2.4	Short & Wolfenden [15]
2000	none	none	*M. thermoautotrophicum*	1.8	Pai [16]
2000	6-azaUMP	none	*M. thermoautotrophicum*	1.5	Pai [16]
2000	BMP	none	*E. coli*	2.5	Larsen [17]
2001	BMP	All Met to SeMet	*E. coli*	3	Larsen [18]
2002	6-azaUMP	Lys42Ala	*M. thermoautotrophicum*	1.5	Pai [19]
2002	6-azaUMP	Asp70Gly	*M. thermoautotrophicum*	1.5	Pai [19]
2002	UMP	Asp70Ala	*M. thermoautotrophicum*	1.5	Pai [19]
2002	6-azaUMP	Asp70Asn	*M. thermoautotrophicum*	1.5	Pai [19]
2002	UMP	Lys72Ala	*M. thermoautotrophicum*	1.5	Pai [19]
2002	OMP	Asp70Ala,Lys72Ala	*M. thermoautotrophicum*	1.5	Pai [19]
2002	6-azaUMP	Asp75BAsn	*M. thermoautotrophicum*	1.5	Pai [19]
2002	6-azaUMP	Ser127Ala	*M. thermoautotrophicum*	1.6	Pai [19]
2002	6-azaUMP	Gln185Ala	*M. thermoautotrophicum*	1.5	Pai [19]
2002	none	none	*E. coli*	2.5	Larsen [20]
2002	UMP	none	*M. thermoautotrophicum*	1.5	Pai [21]
2002	BMP	none	*M. thermoautotrophicum*	1.6	Pai [21]
2002	XMP	none	*M. thermoautotrophicum*	1.9	Pai [21]
2002	CMP	none	*M. thermoautotrophicum*	1.9	Pai [21]
2002	6-azaUMP	Arg203Ala,184–87 deleted	*M. thermoautotrophicum*	1.9	Pai [21]



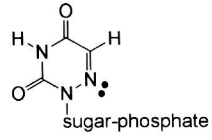
OMP



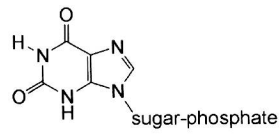
UMP



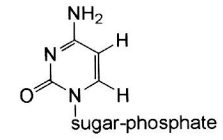
BMP



6-azaUMP



XMP



CMP

Although no structures of ODCase complexed with its natural substrate (OMP) were available at the time of these reports, several structures complexed to related compounds were described. These compounds (Chart 1) were UMP (the ultimate product of decarboxylation), BMP (a transition state analog in which a negatively charged oxygen atom replaces the carboxylate group of OMP), and 6-azaUMP (a transition state analog in which a nitrogen atom and its lone pair replace the C6-CO^- substructure of OMP and mimic the C6 anion thought to be produced upon OMP decarboxylation).

Table 2 Active-site residues in the pyrimidine-binding region

B. subtilis	*S. cerevisiae*	*M. thermoautotrophicum*	*E. coli*
Asp-Lys-Asp-Lys tetrad			
Lys33	Lys59	Lys42	Lys44
Asp60	Asp91	Asp70	Asp71
Lys62	Lys93	Lys72	Lys73
Asp65b	Asp96b	Asp75b	Asp76b
Uncharged hydrogen bond donors and acceptors			
Gln 194	Gln215	Gln185	Gln201
Thr123	Ser154	Ser127	Thr131
Hydrophobic residues			
Ile66b	Ile97b	Ile76b	Ile77b
Pro182	Pro202	Pro180	Pro189
Leu122	Leu153	Met126	Leu130
Val160	Ile183	Val155	Val167