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Minireview

OMP decarboxylase—An enigma persists ☆

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Abstract

In 1995, Radzicka and Wolfenden reported that the rate enhancement produced by orotidine 5′-phosphate decarboxylase (ODCase) approaches 10^{17}, making this enzyme the most effective pure protein catalyst known in Nature [A. Radzicka, R. Wolfenden, Science 267 (1995) 90–93]. Over the last 12 years, there have been many hypotheses put forward to explain that impressive effect. In this perspective, we provide a summary of the reaction pathways under consideration for ODCase, highlight the supporting and refuting data, and suggest experiments designed to further test each of the candidate pathways.
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1. Introduction

Raised to be a farmwife, but born to be a star—those were the words used recently [2] to describe the late opera singer Birgit Nilsson. The same sentiment could apply to the enzyme orotidine 5′-phosphate decarboxylase (ODCase). For 50 years following its discovery, ODCase remained in obscurity largely because it was viewed only in the context of nucleotide metabolism, where it catalyzes the final reaction in the *de novo* biosynthesis of UMP (Fig. 1a). The big breakthrough for this enzyme finally arrived in 1995 in the form

☆ Ref. [1].

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of a simple comparison between the speed of orotidine 5′-phosphate (OMP) decarboxylation in the presence and in the absence of added ODCase [3].

At the active site of yeast ODCase, the reaction in Fig. 1a was observed to proceed with a half-time of 18 ms. By contrast, that same chemical transformation was estimated by Arrhenius extrapolation to occur spontaneously, under otherwise identical conditions of solvent and temperature, with a half-time of 78,000,000 years! Dividing the rate constant for spontaneous transformation (k_{non}) by the enzyme's turnover number (k_{cat}) places the rate enhancement produced by ODCase at an imponderable value of 10^{17}. No other pure protein catalyst is known that surpasses this mark. To generate its rate acceleration, ODCase associates weakly with the substrate in the ground state ($K_m = 10^{-6}$ M), but then tightens its grip as the altered substrate approaches the chemical transition state, yielding a complex with an estimated dissociation constant, K_{tx}, of $<10^{-24}$ M. This "proficient enzyme" jolted many biochemists' assumptions about the catalytic potential of enzymes, and so inspired a still-running quest to explain how ODCase works.

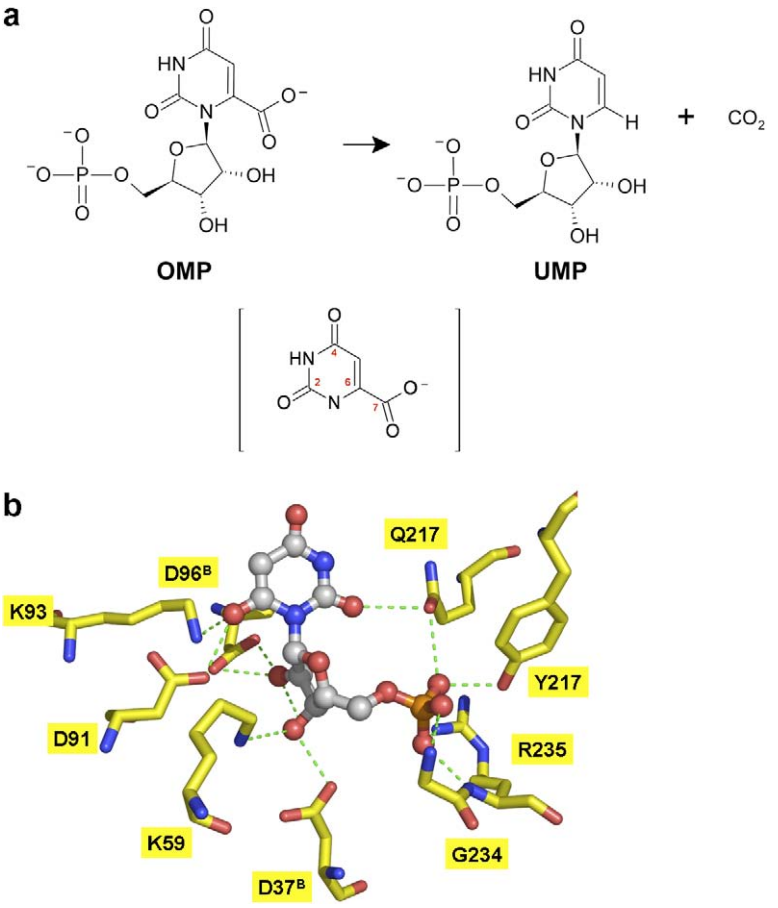

Fig. 1. (a) The reaction catalyzed by OMP decarboxylase and pyrimidine numbering scheme. (b) The structure of the yeast ODCase active site in complex with 6-hydroxyUMP, a transition state analogue inhibitor.

Despite the difficulty of its reaction, ODCase functions without a metal or any other type of cofactor [4]. The 10^{17}-fold rate enhancement is derived solely from interactions between proteinogenic residues and bound OMP. In early 2000, atomic details of those interactions were revealed in quadruplicate, with the simultaneous publication of X-ray structures of ODCase from *Bacillus subtilis*, *Escherichia coli*, *Methanobacterium thermoautotrophicum*, and *Saccharomyces cerevisiae* [5]. Structures of the yeast enzyme in complex with a transition state analogue inhibitor indicated that the site of decarboxylation is formed from an evolutionary conserved tetrad of alternating lysine and aspartate side chains (Fig. 1b). Results of point mutation experiments later indicated that the integrity of this charged network must be maintained for efficient catalysis [6,7]. Comparison of the apo and ligand-bound forms of the enzyme also showed distinct open and closed forms of ODCase. Achieving the catalytically competent, closed form, with the charged network poised for catalysis, depends upon the anionic 5′-phosphoryl group of OMP contributing 15 kcal/mol of intrinsic binding energy [8,9]. That effect might seem unusually pronounced for a group separated from the site of decarboxylation by ~9 Å. Nonetheless, the action of several other enzymes depends similarly on interactions with remote groups of their respective substrate, including cytidine deaminase [10], arginine deiminase [11], isoleucyl tRNA synthetase [12] and triosephosphate isomerase [13].

2. Mechanistic proposals

One popular proposal put forth for the ODCase mechanism, which has found limited support in crystallographic studies [14], involves activation of the bound substrate's carboxyl moiety via electrostatic stress. It is envisaged that binding of the substrate's critical phosphoryl group drives into juxtaposition the labile carboxylate group and a negatively charged active site aspartate residue (Asp-91 in yeast ODCase). With that clash of like charges, the free energy of the ground state complex is raised closer to the chemical transition state. The proposal is reminiscent of Lumry's suggestion that enzymes distort substrates in a manner similar to an ancient torture device that tore its victims in two [15]. Results of computational analyses predict that electrostatic stress furnishes the majority of the catalytic driving force for OMP decarboxylation. Several independent experimental and computational studies, along with careful thermodynamic considerations, have nevertheless provided circumstantial evidence that questions the significance of electrostatic stress as a catalytic device for ODCase [16,17]. Most importantly, the yeast enzyme does not exhibit a measurable affinity for a substrate analogue in which the labile carboxylate group is replaced by a cationic substituent. One possibility for future studies could involve chemical rescue, in which the substrate and the catalytically dead D91A variant of the yeast enzyme are incubated together with aspartate-mimicking anions, such as acetate, in an attempt to restore enzymatic activity. Faced with a positive result, i.e. OMP decarboxylation, one would be hard-pressed to explain how a freely diffusing anion could be lured into an active site where it is subjected to electrostatic repulsion alone.

Also being examined are enzymatic mechanisms in which protonation of OMP at the pyrimidine O4 or O2 atom occurs prior to decarboxylation, leading to formation of an ylide involving the N1 atom [18]. Consistent with those proposals are the findings of a proton inventory study indicating the existence of an isotope sensitive rate-limiting step [19]. Results of carefully constructed experiments in the 1970s involving low molecular weight model compounds also support protonation as the favoured pathway, at least for those

non-enzymatic reactions [20]. There are arguments against protonation, however, the most serious of which seems to be the absence of a suitably positioned proton donor, with the exception of amide groups, in the crystal structures of ligand-bound ODCase. In addition, results of ^{15}N kinetic isotope experiments effectively exclude ylide generation as a rate-limiting step in the ODCase reaction [21]. If protonation of the keto O4 atom of OMP were an essential catalytic step, a truncated form of the substrate in which the O4 atom is replaced with a hydrogen atom should be inert toward enzymatic decarboxylation. To our knowledge, this chemical mutagenesis experiment has not yet been attempted. A similar test could be employed to probe the role of protonation of OMP at the O2 atom during enzymatic decarboxylation.

Doubts about the viability of a protonated intermediate, as well as the absence of electron stabilizing cofactors, prompted several investigators to question the very existence of negative charge at the transition state in the ODCase reaction. An alternative pathway that was proposed by Begley and co-workers involves the synchronous addition of a proton at the C-6 atom as the C-6–C-7 bond ruptures, bypassing formation of a discrete carbanion [5]. That possibility, while seemingly at odds with the enzyme's preference for nucleotide inhibitors possessing electronegative substituents at the C-6 position [7], finds precedent in the chemically challenging reaction catalyzed by organomercurial lyase (EC# 4.99.1.2) . For further analysis, it would be useful to extend the pilot experiments measuring the inhibitory activity of 5,6-dihydroOMP, which seems to possess the geometry and charge distribution expected for an intermediate on an SE2-like pathway, and thus should be bound tightly by ODCase. Preliminary results, however, suggest that this derivative is bound by ODCase with affinity no stronger than the substrate itself [7].

Finally there is the idea that ODCase action may be explained by simple electrostatic attraction. Here, formation of the putative C-6 carbanion creates favourable dipole interactions with a cationic, active site lysine residue [22]. There are many examples in the literature illustrating the profound effect that complimentary electrostatic interactions can have on chemical reactivity, but all that we are aware of fall orders of magnitude short of the 10^{17}-fold acceleration generated by ODCase. A prediction made by proponents of the electrostatic attraction theory is that the lysine residue thought to pair with the carbanion will have a perturbed pK_a value from its value in solution, so as to optimize its potential as an H-bond donor. It is not unreasonable to suspect anomalous equilibriums of active site residues because the solvation behaviour of the ODCase active site differs considerably from that of bulk water [23]. One approach to investigating the lysine pK_a issue directly could begin by covalently reactivating a catalytically dead K93C variant enzyme via treatment with ^{13}C enriched bromoethylamine [24]. Subjecting the reactivated enzyme to NMR analysis, should in theory allow determination of the state of protonation of the active-site lysine residue as a function of pH as well as in the presence and absence of tight binding inhibitors.

3. Conclusion

The chemical pathway for OMP decarboxylation at the active site of ODCase is in a state of mechanistic limbo. A sensible next step might involve putting each proposal outlined above through a deliberate attempt at experimental falsification – not unlike the process that sorted lysozyme's long-debated mechanism [25]. The mechanism that finally emerges for the ODCase reaction will supply the anticipated explanation as to how this

catalyst manages to discriminate so effectively between the ground-state and transition-state species. That capacity, which is intrinsic to the staggering rate acceleration produced by ODCase, constitutes the most extreme example of molecular recognition yet known in biology.

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