Inhibition of Orotidine 5'-Monophosphate Decarboxylase and Its Therapeutic Potential

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Abstract: Orotidine 5'-monophosphate decarboxylase (ODCase) is among the most proficient enzymes, and catalyzes the decarboxylation of OMP to UMP. An overview of ODCase and various proposals for its catalytic mechanism of decarboxylation are briefly presented here. A number of inhibitors of ODCase and new developments in the X-ray structures of ODCases from different species are discussed in the context of their therapeutic potential against cancer and infectious diseases. Latest discoveries in the inhibition of ODCase, for example using the novel C6 substitutions on the uridine, open new doors for drug discovery targeting parasitic diseases such as malaria.

Key Words: Orotidine 5'-monophosphate decarboxylase, de novo nucleotide synthesis, pyrimidine nucleotides, drug design.

1. INTRODUCTION

Orotidine 5'-monophosphate decarboxylase (ODCase, E.C. 4.1.1.23) catalyzes the decarboxylation of orotidine 5'-monophosphate (OMP, **1**) to uridine 5'-monophosphate (UMP, **2**, Fig. **1**). In the *de novo* synthesis of pyrimidine nucleotides, aspartic acid is the starting material that undergoes five transformations culminating in the final step of decarboxylation for the synthesis of UMP. This ribonucleotide is an essential building block for the synthesis of other pyrimidine nucleotides such as uridine 5'-triphosphate (UTP), cytidine 5'-triphosphate (CTP), thymidine 5'-triphosphate (TTP) and 2'-deoxycytidine 5'-triphosphate (dCTP). Due to the essential role of these nucleotides in a variety of cellular processes and disease conditions, inhibitors of the enzymes of these pathways are effective as drugs for the treatment of cancer, inflammatory disorders, and various infections.

When one ponders at this decarboxylation reaction carefully, OMP undergoes spontaneous decarboxylation to UMP with a half-life of about 78 million years [1]. However, ODCase accelerates this process by over 17 orders of magnitude in its active site, to a half-life of about 18 milliseconds, in order to provide UMP in reasonable quantities for further processing into nucleotides and nucleic acids in the cell [2]. This enzyme is quite remarkable because it carries out the decarboxylation reaction without the help of any cofactors, metals, or involvement of covalent intermediates [3-8]. Most of the decarboxylases in nature, however, use either a cofactor or covalent intermediates during the process of decarboxylation [9,10].

In humans, ODCase is a part of the bifunctional enzyme called UMP synthase [11]. In *archea*, bacteria and parasites, ODCase is a monofunctional enzyme, and is primarily active as a dimeric unit [12,13]. Depending on the therapeutic application, a number of features of ODCase could be used for drug development. For instance, in some species *de novo* nucleotide synthesis is essential as they lack the salvage pathway. The latter pathway is present in mammalian cells, including in humans, in addition to the *de novo* pathway. For example, *Plasmodia* such as *P. falciparum* and *P. vivax* are exclusively dependent on the *de novo* synthesis for pyrimidine nucleotides due to the absence of the salvage pathway [14]. Thus, the selective inhibition of ODCases from the pathogenic organisms could be a strategy for the development of drug candidates targeting malaria. One could also take advantage of the selectivity of various inhibitors to an ODCase in the pathogenic organism for therapeutic design. This requires understanding the structural and inhibitory properties of ligands to ODCases from different species.

A number of inhibitors have been considered over the years to investigate the mechanism of decarboxylation by ODCase, as well as their potential as therapeutic agents [15-20]. These include barbituric acid ribonucleoside 5'-monophosphate (BMP, **3**), 6-azauridine 5'-monophosphate (**4**), pyrazofurin 5'-pyrazofurin (**5**), xanthosine 5'-monophosphate (XMP, **6**), 6-thiocarboxamidouridine 5'-monophosphate (**7**), and 6- iodouridine 5'-monophosphate (6-iodo-UMP, **9**), as well as their nucleoside forms (Fig. **2**).

Here, we briefly review various interesting mechanisms of decarboxylation proposed by different groups based on

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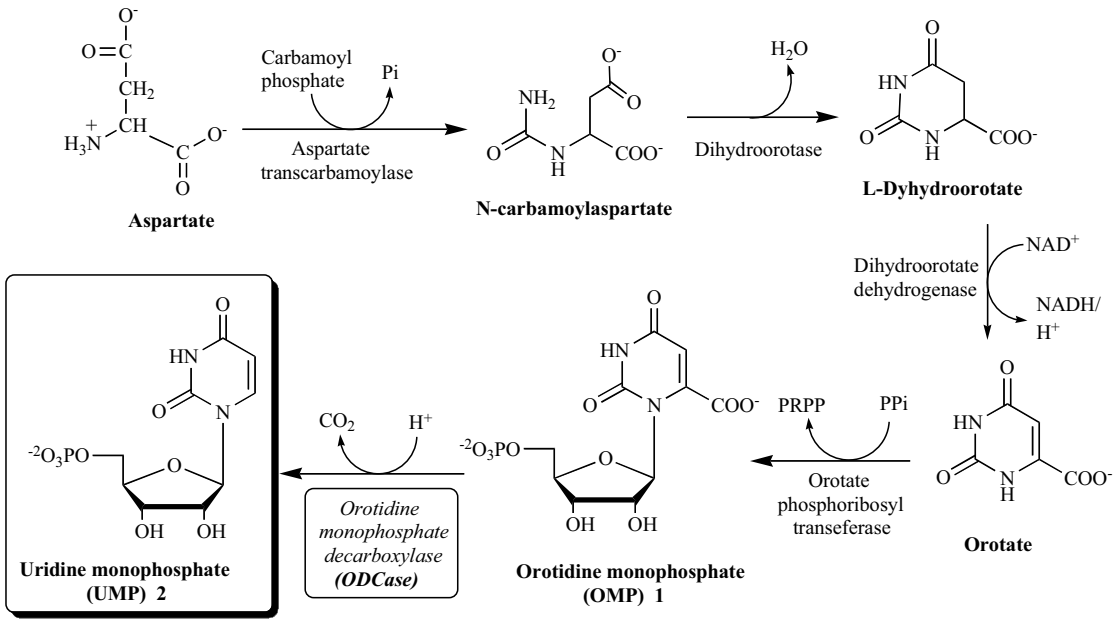

Fig. (1). *De Novo* biosynthesis of UMP from aspartic acid.

various chemical/biochemical rationale, structural insights gleaned from several X-ray crystal structures of ODCases and their complexes with ligands, inhibitors targeting ODCase and the biological activities of various inhibitors as potential therapeutic agents. While this mini-review is not a comprehensive literature account on all aspects of ODCase inhibitors, important and relevant literature related to the inhibition of ODCase are discussed to provide the most up to date overview.

2. PROPOSED MECHANISMS OF DECARBOXYLATION BY ODCASE

Overall, ODCase achieves the removal of one carbon dioxide molecule from the C6 position of OMP (**1**), and the addition of one proton to the C6 position, generating UMP (**2**). While the reaction could be a simple transformation, it is quite intriguing when one considers the remarkable chemical stability of OMP along with the catalytic machinery in the

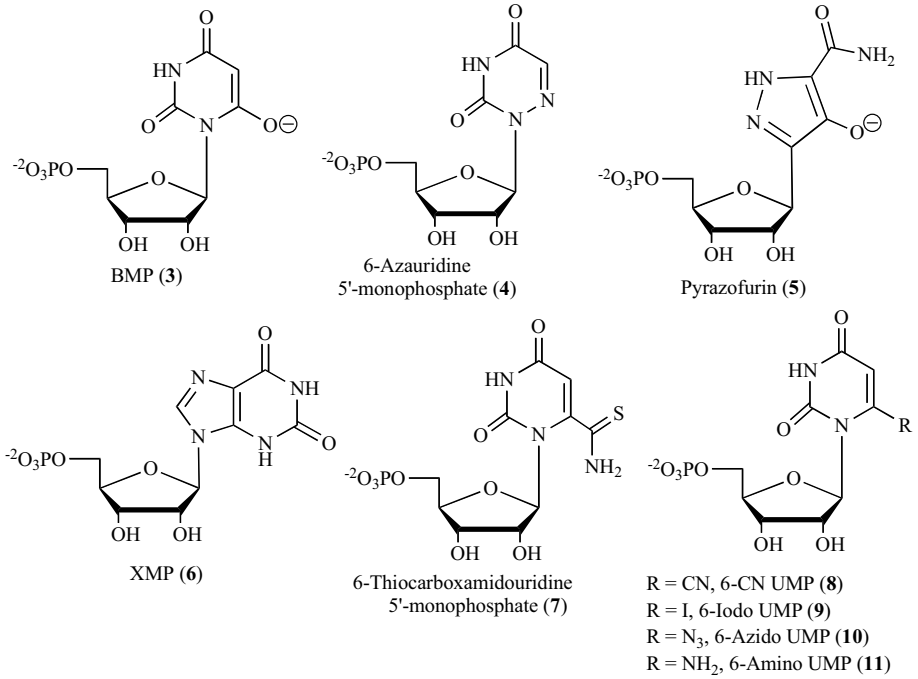

Fig. (2). Structures of select inhibitors of ODCase.

enzyme active site. Consequently, the mechanism of this catalytic reaction has been a subject of numerous debates over the years, and it is not exaggerating to state that there is no consensus even today on one single mechanism of decarboxylation by ODCase.

As early as in 1970's, Beak and Siegel explored the mechanism of decarboxylation by ODCase experimentally [21,22]. Based on the studies on the decarboxylation of the model compound 1,3-dimethyl-orotate at elevated temperatures, they proposed a mechanism of decarboxylation of OMP *via* a zwitterionic intermediate (Fig. **3A**). The first step in this mechanism is the protonation of the C2 carbonyl of the uracil moiety leading to a nitrogen ylide (**II**), which acts as an electron-withdrawing group to facilitate the elimination of carboxylate group. Upon the loss of carbon dioxide, species **III** is generated which is a dipole-stabilized carbanion, and this was suggested as a key feature in the transition-state. A deprotonation followed by protonation of the positions O2 and C6, respectively, will yield the product UMP (Fig. **3A**). During the 1990s, mutagenic studies by Smiley and Jones indicated that Lys93 in the yeast ODCase is critical for its catalytic activity, and its mutation into a cysteine residue results in the loss of enzyme activity [23]. The enzyme activity was partially recovered when the cysteine mutant was treated with 2-bromoethylamine, as anticipated. Based on this observation, it was proposed that the side chain of this cationic amino acid might act as the proton donor in the zwitterion mechanism proposed by Beak and Siegel, providing additional support to this mechanism. Rishavy and Cleland in 2000 reported that the N-isotope effect studies on ODCase did not show any bond order changes taking place at N1 [24]. Phillips and Lee argued that this lack of change in the bond order does not necessarily mean that there is no change in the bond order in N1, or rule out the possibility that the other positions in the pyrimidine ring may be involved [25]. This appears to be different from the zwitterion mechanism because the latter called for changes in the bond order at N1. This questioned the enolization (species **II** for-

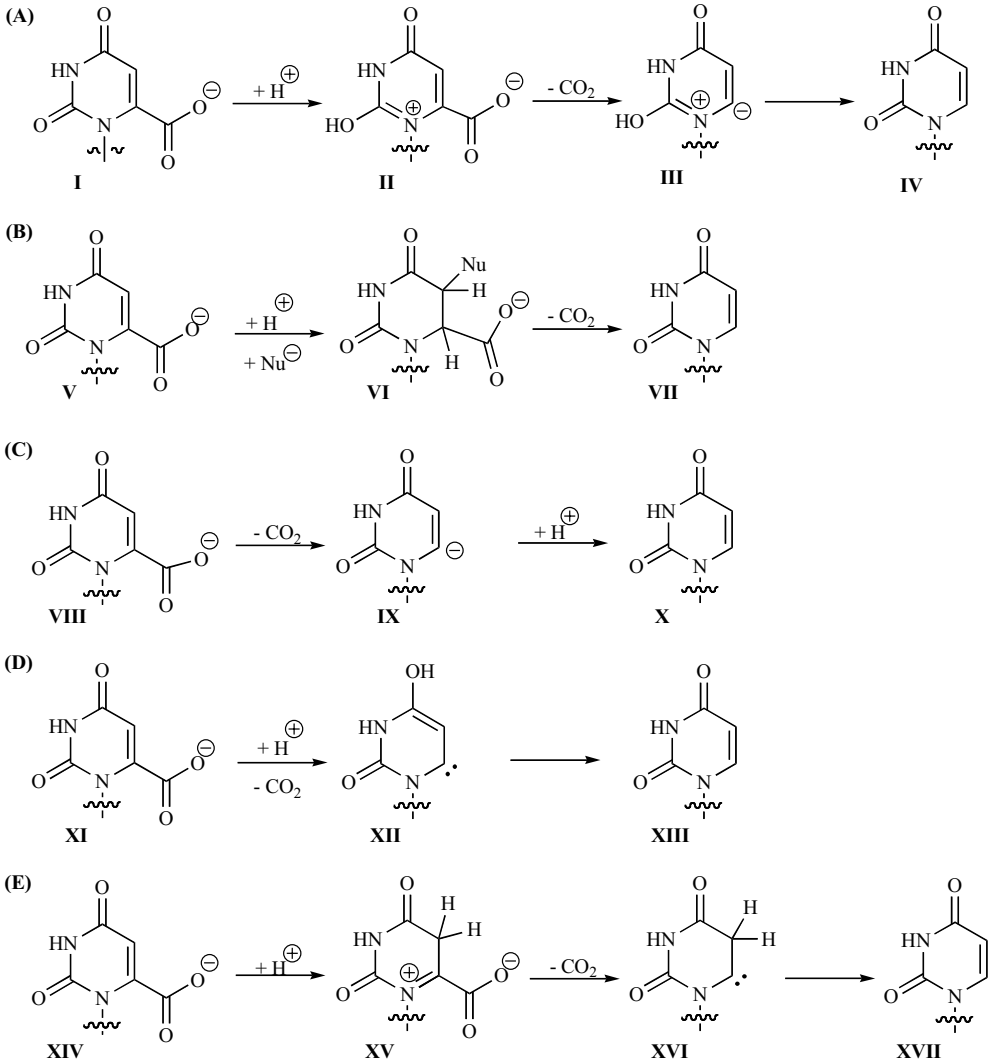

Fig. (3). Proposed mechanisms of decarboxylation by ODCase. (A) Zwitterion mechanism, (B) Nucleophilic addition mechanism, (C) Direct decarboxylation mechanism, (D) Carbene mechanism. (E) C5-Protonation.

mation) to give rise to the quaternary nitrogen at N1 for the zwitterion formation. Instead, a carbanion intermediate was suggested which may be stabilized by electrostatic interactions with Lys93. It was further suggested that the driving force for this reaction came from charge repulsion between Asp91 and the carboxyl moiety of OMP [24].

The second mechanism, proposed by Silverman and Groziak, involved Michael addition on the pyrimidine ring followed by decarboxylation (Fig. **3B**) [26]. According to this mechanism, first an active site nucleophile would attack the C5 position of the α,β-unsaturated carboxylic acid of the orotidine moiety, facilitated by an enzyme-mediated proton donation, leading to species **VI**. This would result in a change in the geometry at C5 from trigonal sp2 to a tetrahedral sp3 center. This is followed by the decarboxylation/elimination of the *trans* intermediate and the release of the product UMP. However, a subsequent report by Acheson *et al.* did not detect any addition of nucleophiles at the C5 position of BMP, which is a very potent inhibitor of ODCase [27]. This and other studies confirmed that the C5 position of the pyrimidine moiety does not undergo significant changes in its geometry before or during decarboxylation. This questioned the validity of the mechanism *via* nucleophilic addition, and thus did not gain wide acceptance.

In 2000, the "direct decarboxylation" mechanism was proposed based on the three-dimensional structure of ODCase from two different groups (Fig. **3C**) [32,39]. The charge repulsion between the C6 carboxylate group of OMP, and the carboxyl group of the side chain of $Asp71^{Ec}$ (^{Ec} indicates *E. coli* ODCase numbering) was proposed to be critical to drive the conversion of OMP to UMP [39]. In this mechanism, the decarboxylation of OMP was initiated by charge repulsion, followed by a proton transfer from $Lys73^{Ec}$ to the C6 position of UMP. Three-dimensional structures of ODCase suggested a strong network of charged residues around the C6 position that has the potential to destabilize the ground state and stabilize the transition state for the decarboxylation of OMP. The authors concluded that the enhancement in the rate of the reaction was attributed to either ground-state destabilization (GSD) and/or the transition-state stabilization (TSS) [38]. This mechanism is also supported by Warshel *et al.* based on computational models, and suggested that the rate enhancement is due to transition state stabilization (TSS) rather than ground-state destabilization (GSD) [28]. By far, this mechanism appears to be the most reasonable one among all proposals and accumulating evidence is adding more support.

A mechanism of decarboxylation proceeding through a carbene formation was proposed by Lee and Houk in 1997 using high-level computational analyses (Fig. **3D**) [29]. A proton transfer to O4 was proposed as the key step during the formation of C6 carbene moiety **XII**. Upon the determination of the ODCase crystal structures, however, this mechanism was very strongly objected to, and the three-dimensional structures did not demonstrate the presence of an appropriate proton donor to O4 [30].

More recently, Kollman and co-workers explored another possible mechanism using molecular dynamics simulations, which involved the protonation of OMP at the C5 position by a Lys residue in close proximity (Fig. **3E**) [31]. In this mechanism, C5 undergoes a hybridization change from sp2 to sp3, but such a structural change has not been supported by any existing evidence. These computations also suggested a protonation at C5 is favored over that at the C6 position (Fig. **3E**, species **XV)**. The authors argued that the absence of experimental evidence for hybridization changes at C5 might be due to the electrostatic attraction between the hydrogen at C5 and the negatively charged carboxyl moiety. Thus, the carboxylate group could constrain the C5-H5 bond in such a way that its vibrational frequency and out-of-plane bending motion are much higher than usual, thereby compensating for any inverse isotope effect.

These mechanistic proposals have shed some light onto and led to novel hypotheses on the mechanism of decarboxylation by ODCase. However, none of these proposals has been able to answer the mechanistic questions around this enzyme unambiguously, and this continues to be a matter of debate. Such studies are important because the design of new molecules could take advantage of the mechanistic details for selective and efficient drug discovery.

3. ODCASES FROM DIFFERENT SPECIES

Except in viruses, ODCase is present in all species. ODCases from at least eleven different organisms are crystallized to date including *archea*, parasites, mammals, and bacteria [32-40]. Regardless of the origin of the enzyme, the arrangement of the active site is found to be highly conserved between all these species, confirming that the architecture of the active site geometry has to be preserved for its decarboxylation activity. The biologically active form of ODCase is a dimer of two identical subunits (in both monofunctional and bifunctional enzymes). Each monomer consists of a triosephosphate isomerase (TIM barrel fold) made of eight β-strands and eleven α-helices for *Bacillus subtilis* and *Escherichia coli*, and eight β-strands and nine α-helices for *S. cerevisiae* and *M. thermoautotrophicum* (Fig. **4**). The comparison of the crystal structures of the free and ligand-

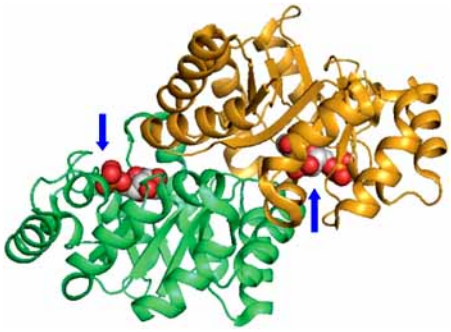

Fig. (4). X-ray crystal structure of the dimeric unit of the complex of UMP (**2**) with ODCase from *B. subtilis* at 2.4 Å resolution [36]. The active sites on each monomer, highlighted by arrows in blue, are located at the end of the TIM barrel, and near the interface of two monomers. Two monomers are rendered by a ribbon representation and one monomer is colored green and the other is in orange. UMP molecules are shown in space-filling representation, color-coded according to atom-type. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

bound enzyme revealed considerable conformational flexibility in some of the loops of the protein chain.

The crystal structure of *B. subtilis* ODCase complexed with UMP at 2.4 Å resolution revealed that the active site is located at the end of the TIM barrel corresponding to the carboxy-terminals of the β-strands and the amino terminals of the α-helices, and near the interface of two monomers (Fig. **4**) [36]. In the binding site of the 5'-monophosphate group there is an extensive network of ten hydrogen bonds that are accepted by the phosphate with Arg185 (two hydrogen bonds), Gln194^{Bs} (one) (^{Bs} refers to the numbering of *B. subtilis* ODCase), Gly214^{Bs} (one), Arg215^{Bs} (three), and one hydrogen bond by each of the three water molecules (Fig. **5A**). In the ribosyl moiety, there are two hydrogen bonds, one each between the 2'-hydroxyl group and Asp65'^{Bs}, and the 3'-hydroxyl group and Asp11^{Bs} (residue numbers with "prime" refer to the residues from the second monomer). This extensive hydrogen bonding network and the resulting binding energy between the enzyme and the ligand were implicated as the driving force to place the pyrimidine base into the active site pocket [36].

In the pyrimidine-binding region, there is one hydrogen bond between O2 and the amino group of Gln194^{Bs}, which is also involved in a hydrogen bond with one of the phosphate

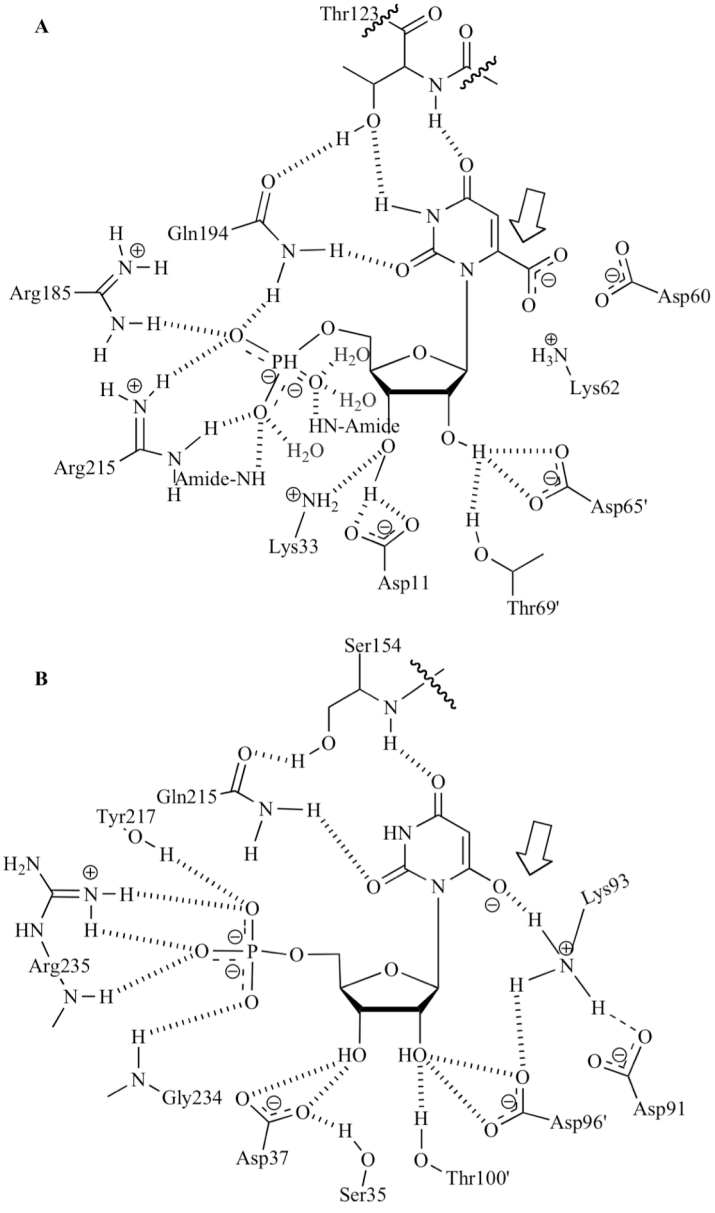

Fig. (5). (A) Schematic representation of the hypothetical interactions between OMP and ODCase from *B. subtilis*, based on the structure of the complex with UMP [36]. Position C6 where the scissile bond is located is highlighted by an arrow. (B) Schematic representation of the interactions between ODCase from *S. cerevisiae* and its inhibitor BMP [37,39]. Location of the deprotonated hydroxyl moiety at C6 is highlighted by the arrow, and this structural feature renders the molecule a high-affinity inhibitor to ODCase.

oxygens. Two hydrogen bonds are due to the side chain and the backbone carbonyl moiety of Thr123^{Bs} with the O4 and N3 atoms of the uracil base, respectively. The X-ray structure of UMP bound to ODCase indicates the possible location for the carboxylate binding of the substrate, OMP. One could also assume that the orotidine base might bind in the same conformation as the uracil base, as seen in the UMP-ODCase complex. The carboxylate pocket is formed by the side chains of Lys33^{Bs}, Asp60^{Bs}, and Lys62^{Bs} of one monomer, and Asp65'^{Bs} of the second monomer in the dimeric unit (indicated by the "prime", Fig **5A**). It is hypothesized that the carboxylate group of OMP may point directly at Asp60^{Bs} during the ground-state destabilization process, and an additional pocket that could accommodate the departure of the carbon dioxide molecule near the C5 position of the pyrimidine base [36]. This latter pocket consists of various hydrophobic contacts, including the side chains of Val19^{Bs}, Leu122^{Bs}, Pro182^{Bs}, Val160^{Bs}, Val212^{Bs}, and the methylene groups of Lys33^{Bs} and Lys62^{Bs}. The departing CO_2 molecule may be retained here until the product UMP is released [36].

The X-ray crystal structure of *S. cerevisiae* ODCase with 6-hydroxyuridine 5'-monophosphate (BMP) at a resolution of 2.4 Å was determined in 2000 and this compound is the most potent inhibitor known for ODCase today [37]. Most of the interactions of BMP are identical to with UMP in the phosphoribosyl moiety region as well as the urea region of the pyrimidine base. Additionally, hydrogen bond interactions of the deprotonated O6 moiety with protonated Lys93^{Sc} are noted (^{Sc} denotes *S. cerevisiae* ODCase numbering; Fig. **5B**). Larsen and co-workers reported the crystal structure of *E. coli* ODCase co-crystallized with BMP at a 2.5 Å resolution [39]. The structure of this complex is almost identical to that from *S. cerevisiae* with some minor differences in the active site region.

Pai and co-workers determined the three-dimensional structure of the complex of 6-aza-UMP bound to ODCase from *M. thermoautotrophicum* at 1.5 Å resolution (Fig. **6**) [33,34,38]. Structural features of this *archeal* ODCase are similar to those of other complexes of ODCases (*vide supra*). There is a network of charged residues, Lys42-Asp70-Lys72-Asp75' (*M. thermoautotrophicum* numbering) between the ribose ring, and the pyrimidine base, providing functional roles for binding, catalysis and the product release. The phosphate group is ensconced in the pocket created by the side chain from Arg203^{Mt}, and the loop formed by the residues 180^{Mt}-190^{Mt}. One water molecule bridges the 5'-phosphate group and the C2 carbonyl group of 6-aza-UMP (Fig. **6**). Each of the hydroxyl groups on the ribose are held with two hydrogen bonds to the enzyme. Asp20^{Mt} and Lys42^{Mt} interact with 3'-hydroxyl, and Asp75'^{Mt} and Thr79'^{Mt} to the 2'-hydroxyl moiety. The ribose ring is in the 2'-*endo* conformation and the pyrimidine base in the *syn* conformation. While all other interactions are similar to the above complexes, N6 is involved in hydrogen bonding with Lys72^{Mt}, similar to the 6-hydroxyl moiety in BMP [38].

In the past three years, many other structures of ODCase have become available, some of which are worth mentioning here. ODCase structures from the pathogenic parasite *Plasmodia* have been recently resolved (PDB IDs: 2aqw, 2f84, 2ffc, 2fds, 2guu) and from *Pyrococcus horikoshii* OT3 (PDB IDs: 2cz5, 2czd, 2cze) have been determined as well, including the co-crystal structures with several inhibitors. In 2007, three structures of the ODCase domain of the human UMP synthase were reported (PDB IDs: 2eaw, 2p1f and 2jgy). These intensified structural investigations and understanding of various three-dimensional structures of ODCases will certainly aid new drug design.

4. INHIBITORS OF ODCASE AND THEIR THERAPEUTIC POTENTIAL

In the past two decades, several groups have investigated inhibitors of ODCase and their potential in drug development. Inhibitors of ODCase in general have a wide range of

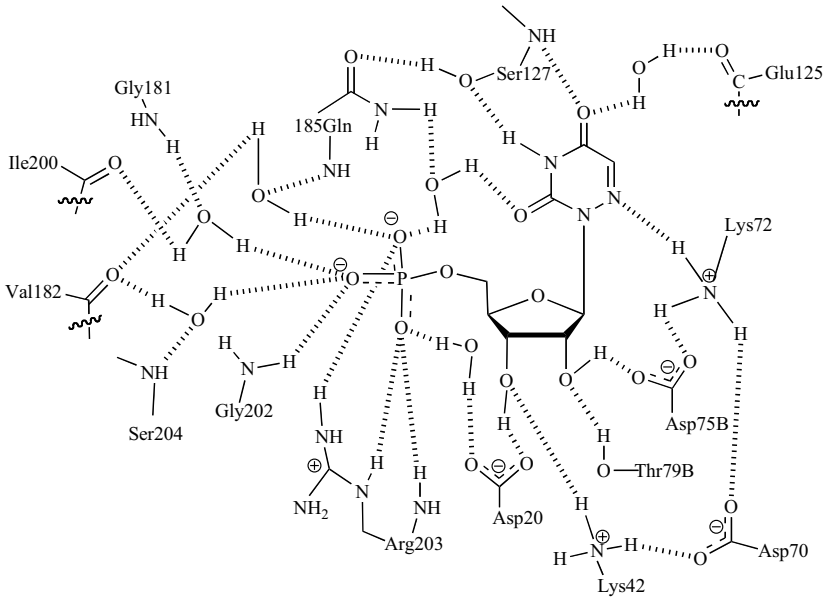

Fig. (6). Schematic representation of 6-aza-UMP bound to the active site of ODCase from *M. thermoautotrophicum* [38].

therapeutic applications including malaria, various bacterial infections, RNA viral infections and cancer. One of the potential problems targeting ODCase for drug development is that it is present in all species, including in humans. However, in higher species such as humans, pyrimidines are obtained *via* the *de novo* pathway in which ODCase is involved, as well as the salvage pathway. Thus, selective inhibition of ODCase could lead to the inhibition of those pathogenic cellular processes where *de novo* pyrimidine biosynthesis is essential [30]. For example, in *Plasmodia* species such as *P. falciparum* and *P. vivax*, this has allowed a strategy leading to the specific design of drugs against malaria [14-20]. Here, a brief account of various inhibitors in the context of ODCase structural interactions, and the potential of select inhibitors as therapeutic agents are presented.

Rational drug design targeting an enzyme for therapeutic purposes requires a good understanding of the complex interactions between the enzyme and the substrate or inhibitors at the atomic level. In this context, ODCase is known to bind to a variety of nucleotide derivatives, such as its product UMP, BMP, 6-aza-UMP, XMP, 6-cyano-UMP, and CMP (Fig. **2**) [15,17,35]. The enzyme inhibition assay method using isothermal titration calorimetry (ITC) and developed by our group has opened new doors towards the discovery and analysis of new inhibitors [19]. While it is not intended here to extrapolate on ITC techniques, it is worth mentioning in the context of inhibitor discovery because a reliable and accurate biological assay is an important tool. Until now, a UV-based technique was the method of choice to monitor the enzymatic decarboxylation of OMP [41]. However, the overlapping absorption spectra of substrate, product, and inhibitors of ODCase make the use of this method highly problematic. A sensitive and reliable enzyme assay using radiolabeled substrate has been used by many laboratories, but the lack of readily available radiolabeled substrate and its cost are definite drawbacks for this assay. ITC is free of many of the limitations posed by other techniques because it only depends on changes in heat energy, an inherent property of any biochemical reaction [41-44].

Interestingly, the structures, kinetic profiles as well as affinity towards various inhibitors are noticeably different for ODCase enzymes from different sources including yeast, *E. coli*, mouse, *P. falciparum* and humans [19]. This may offer an opportunity to develop ODCase-specific inhibitors that may selectively target the disease-causing pathogen with limited side effects to patients. In other words, a sufficiently large therapeutic index could be achieved. No such drug has been found to date and most ODCase inhibitors that have been evaluated in clinical trials were abandoned due to unacceptably high toxicity, which may be explained by their lack of selectivity [15].

Additional structure-activity relationship studies and mechanistic investigations are warranted to uncover the "mystery" surrounding the high performance catalysis of this fascinating enzyme, and especially to be able to exploit the differences of ODCases from different species. Theoretical, crystallographic and inhibition studies of the mechanism of catalysis by ODCase have mainly stressed the importance of a negative charge or electron-rich domain around the C6 position. The presence of the phosphoryl group, although spatially distant from the decarboxylation site, contributes a factor of 10^{11} to the value of k_{cat}/K_m observed for OMP [6,7,45]. While the substitution of O4 of the uracil moiety by a thio group in the substrate OMP has limited effect on its affinity for the ODCase active site, substitution of O2 with a thio moiety results in weak binding. Surprisingly, 2-thio-OMP does not undergo decarboxylation either. This striking difference has been attributed to the inability of 2-thio-OMP to bind in the "right" *syn* conformation required for ODCase activation [46]. Cytidine 5'-monophosphate (CMP) is also known to adopt the *anti* conformation in solution, which perhaps is not the preferred conformation for binding to ODCase [47]. Both CMP and its 6-carboxylate derivative were found to have weak binding to ODCase. In fact, the carboxylate derivative of CMP was found to be a very poor substrate for ODCase (10^5 folds less active than OMP) [46].

Other derivatives that have been evaluated as ODCase inhibitors include 6-aza-UMP (**4**), pyrazofurin 5'-monophosphate (**5**), 6-thiocarboxamidouridine 5'-monophosphate (**7**) and 6-amino-UMP (**11**). All known inactivators of ODCase bind at the active site through a network of hydrogen bonds and electrostatic forces, with a major contribution for binding from the 5'-phosphoryl moiety [7,28,32,38]. The only exceptions known today are 6-cyano-UMP, which is enzymatically converted into BMP within the ODCase active site, and 6-iodo-UMP, which covalently binds to the active site through Nε of the catalytic lysine residue in the center of the active site and C6 of the uracil ring [20,35].

A well-known inhibitor in the literature, 6-azauracil and its nucleoside derivative, 6-azauridine are converted into 6-azauridine 5'-monophosphate (**4**) within the cell. Compound **4** inhibits ODCase from yeast with an inhibition constant (K_i) 0.51 μM [48]. 6-Azauracil has shown anticancer activity against a number of experimental tumors [49]. Another most actively studied compound is pyrazofurin (3-β-D-ribofuranosyl-4-hydroxypyrazole-5-carboxamide, previously designated as Pyranomicin). This compound is one of three pharmacologically active C-nucleosides isolated from *Streptomyces candidus* [50,51]. Mammalian cells readily take up pyrazofurin and transform it into its 5'-monophosphate derivative (**5**), a potent inhibitor of ODCase with an inhibition constant (K_i) of 5 nM [52]. Studies showed that pyrazofurin does inhibit *Plasmodium falciparum in vitro* [18, 53]. Clinical trials have shown that pyrazofurin has anticancer activity but its use is limited by its toxicity to patients [54]. Pyrazofurin in its nucleoside form inhibits malarial OPRTase (enzyme that catalyzes the transformation of Orotate→Orotidine 5'-monophosphate), while its 5'-monophosphate derivative **5** inhibits ODCase (OMP→UMP) [55]. The 5'-monophosphate derivative of barbiturate ribonucleoside (**3**) is a very potent inhibitor of ODCase (K_i=8.8x10^{-12} M against yeast ODCase) [48]. The 5'-monophosphate derivatives of allopurinol, oxipurinol, and xanthine, with the ribose linked at the 3- or 9-position of the purine ring, are also good inhibitors of ODCase [56].

6-Cyano-UMP and 6-amino-UMP are novel inhibitors that were designed based on the substructure volumes in the substrate OMP (**1**) and BMP (**3**) [19,35]. These inhibitors of ODCase were designed based on the principles of bioisoster-

ism. 6-Aza-UMP (**4**) and 6-cyano-UMP (**8**) competitive inhibitors of ODCase, with inhibition constants (K_i) of 12.4 and 29 μM against *M. thermoautotrophicum* ODCase, respectively. 6-Amino-UMP (**10**) is a potent inhibitor of ODCase, with an inhibition constant of 840 nM [19]. These compounds follow the trend that good inhibitors of ODCase carry a negative charge or electron-rich group at the C6 position.

Fujihashi *et al.* in 2005 revealed that 6-cyano-UMP (**8**) is a pseudo-substrate to ODCase, and compound **8** is transformed into BMP (**3**) by ODCase from *M. thermoautotrophicum* [35]. This was later confirmed with ODCases from *P. falciparum* (Poduch and Kotra, unpublished results). Although this transformation is slow, and requires a water molecule (hence was proposed as a "pseudo-hydrolysis" process), BMP generated *in situ* in the active site of ODCase in turn inactivates ODCase with pico molar affinity. The substitution of the 6-cyano group by a hydroxyl moiety and its catalysis by ODCase at its active site was confirmed independently by X-ray crystallography, time-dependent enzyme assays, irreversible inhibition of ODCase, and high-resolution mass spectral analyses. According to these results, although the natural biochemical transformation of OMP to UMP is thought to proceed through a nucleophilic intermediate, the transformation of 6-cyano-UMP to BMP possibly includes an electrophilic center that could react with a nucleophilic water molecule [35]. 6-Cyano-UMP (**8**) itself is a moderate competitive inhibitor of ODCase with an inhibition constant (K_i) in the range of 20-30 μM [19].

Another interesting inhibitor of ODCase is the 6-iodouridine derivative **9**. 6-Iodouridine 5′-monophosphate (**9**) irreversibly inhibits the catalytic activities of ODCases from several species including *M. thermoautotrophicum*, *P. falciparum* and *P. vivax*. Mass spectral analysis of the enzyme-inhibitor complex confirms the covalent attachment of the inhibitor to ODCase accompanied by the loss of two protons and the iodo moiety [20]. The X-ray crystal structure, at 1.6 Å resolution, of the complex of **9** and ODCase clearly showed the covalent bond formation with the active site residue Lys42 (Fig. **7**) [20]. This compound is the first covalent inhibitor reported for ODCase, and it is a particularly noteworthy reaction considering that none of the active site residues was known to covalently participate in any biochemical or catalytic event. 6-Iodouridine (**9**) exhibited potent *in vitro* antiparasitic activities against *P. falciparum* including drug-resistant isolates [20]. Thus, this series of compounds are showing promising potential as antimalarial agents.

SUMMARY

ODCase has been an interesting enzyme to enzymologists for over two decades and its mechanism of decarboxylation of OMP is still an ongoing debate. Novel inhibitors discovered in the recent years and the availability of the X-ray crystal structures of ODCases from a variety of species will position this enzyme as a potential target for new drug development. Since the chemical mode of action of ODCase inhibitors could be complementary to other drugs targeting infectious diseases and cancer, and the possibility of modulating potential toxicity through selectivity or alternate biochemical pathways, these inhibitors might have potential clinical use.

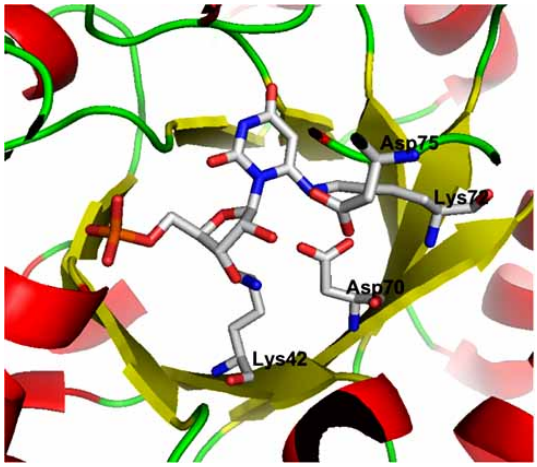

Fig. (7). X-ray crystal structure of the covalent complex formed between 6-iodo-UMP and ODCase from *M. thermoautotrophicum*, based on an electron density map calculated at 1.6 Å resolution [20]. The nucleotide and the key residues in the binding site of ODCase are rendered in a capped-stick representation. The enzyme is rendered according to secondary structure.

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