Study of orotidine 5′-monophosphate decarboxylase in complex with the top three OMP, BMP, and PMP ligands by molecular dynamics simulation

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Catalytic mechanism of orotidine 5′-monophosphate decarboxylase (OMPDC), one of the nature most proficient enzymes which provides large rate enhancement, has not been fully understood yet. A series of 30 ns molecular dynamics (MD) simulations were run on X-ray structure of the OMPDC from Saccharomyces cerevisiae in its free form as well as in complex with different ligands, namely 1-(5′-phospho-D-ribofuranosyl) barbituric acid (BMP), orotidine 5′-monophosphate (OMP), and 6-phosphonoouridine 5′-monophosphate (PMP). The importance of this biological system is justified both by its high rate enhancement and its potential use as a target in chemotherapy. This work focuses on comparing two physicochemical states of the enzyme (protonated and deprotonated Asp91) and three ligands (substrate OMP, inhibitor, and transition state analog BMP and substrate analog PMP). Detailed analysis of the active site geometry and its interactions is properly put in context by extensive comparison with relevant experimental works. Our overall results show that in terms of hydrogen bond occupancy, electrostatic interactions, dihedral angles, active site configuration, and movement of loops, notable differences among different complexes are observed. Comparison of the results obtained from these simulations provides some detailed structural data for the complexes, the enzyme, and the ligands, as well as useful insights into the inhibition mechanism of the OMPDC enzyme. Furthermore, these simulations are applied to clarify the ambiguous mechanism of the OMPDC enzyme, and imply that the substrate destabilization and transition state stabilization contribute to the mechanism of action of the most proficient enzyme, OMPDC.

Keywords: molecular dynamics; enzyme–ligand interaction; substrate destabilization; transition state stabilization; orotidine 5′-monophosphate decarboxylase

Introduction

The enzyme orotidine 5′-monophosphate decarboxylase (OMPDC/ODCase/ODC; EC 4.1.1.23) catalyzes the biosynthesis of an important precursor in the production of DNA and RNA macromolecules, i.e. uridine 5′-monophosphate (UMP), through the de novo pathway that generates the product from the simple building blocks (Yablonski, Pasek, Han, Jones, & Traut, 1996) as shown in Figure 1. OMPDC has also been recognized as a target for anticancer drugs because of its primary role in DNA metabolism (Meza-Avina et al., 2010). OMPDC generates pyrimidine bases just through a de novo synthesis pathway, rather than through a salvage pathway that recycles pyrimidine nucleosides (Gero & O’Sullivan, 1990). Taking part in such significant pathways has made OMPDC a promising target in pharmacology, such that its inhibitors have been applied against a range of targets including West Nile virus (Morrey, Smee, Sidwell, & Tseng, 2002), malaria causative parasite, and RNA viruses such as poxviruses and flaviviruses. Some of these inhibitors could also be the most effective drugs against some eukaryotic organisms such as Plasmodium falciparum (Bello et al., 2008; Poduch et al., 2006).

The fold of OMPDC enzyme is a (β/α)8 barrel where the ligand binding site is located at the interface of the dimer (A and B subunits) in the opening end of each barrel of the monomer and requires residues from both subunits for its activity (Wise, Yew, Babbitt, Gerlt, & Tseng, 2002), malaria causative parasite, and RNA viruses such as poxviruses and flaviviruses. Some of these inhibitors could also be the most effective drugs against some eukaryotic organisms such as Plasmodium falciparum (Bello et al., 2008; Poduch et al., 2006).

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OMPDC as loop 3 (Miller, Hassell, Wolfenden, Milburn, & Short, 2000). In this paper, residues from the B subunit will be marked with an asterisk.

This dimeric enzyme is special because of being among the nature most proficient enzymes (Wood et al., 2010) and provides the largest rate enhancement achieved for any enzyme by a factor of $10^{17}$ (Lee, Chong, Chodera, & Kollman, 2001). In water, orotidine 5‘-monophosphate (OMP) spontaneously decarboxylates to uridine 5‘-monophosphate (UMP) with a half-life of 78 million years (Radzicka & Wolfenden, 1995). Surprisingly, at the OMPDC active site, the same reaction is catalyzed with a half-life of 18 ms (Bell & Jones, 1991; Miller et al., 2000). No metals or other cofactors involve in this conspicuous catalytic effectiveness and this efficiency depends on noncovalent binding forces (Miller & Wolfenden, 2002).

By considering model compounds, kinetic isotope experiments and X-ray crystal structures of the OMPDC enzyme, numerous mechanisms have been suggested to describe the enzyme proficiency (Lee, 2001). Also, for understanding the catalytic mechanism of OMPDC, different kinds of the UMP and OMP derivatives with substitutions on C6 position have been used extensively in the kinetic analysis and structural studies of OMPDC (Bello et al., 2008). It is noteworthy that most of these derivatives show inhibitory effects in kinetic analysis and could be considered as a lead compound for designing new inhibitors. Among them, 1-(5‘-phospho-D-ribofuranosyl) barbituric acid, also known as 6-hydroxy-UMP (BMP), is extensively recognized as an effective inhibitor of OMPDC (Poduch et al., 2006).

Due to the significance of OMPDC enzyme in treatment of the aforementioned diseases, we perform a computational study of the complexes of OMPDC with its inhibitor (BMP), its substrate (OMP) and with its substrate analog (PMP). An enzyme may perform its function by lowering the activation energy by creating a microenvironment where the substrate bound to enzyme is distorted into the transition state form; this transition state is in turn stabilized by opposite charge distribution (Fersht, 1985). In this work, these two aspects are discussed in the context of OMPDC mechanism of action.

Several different mechanisms have been proposed to explain OMPDC mechanism. The suggested mechanisms so far for OMPDC enzyme include protonation at O2 (Beak & Siegel, 1976), nucleophilic attack at C5 (Silverman & Groziak, 1982), formation of iminium ion at C4 (Zhou et al., 2001), protonation at O4 (Lee & Houk, 1997), electrostatic stress at COO⁻ (Wu et al., 2000), protonation at C6 (Appleby, Kinsland, Begley, & Ealick, 2000), protonation at C5 (Kollman et al., 2000), electrostatic stabilization of vinyl carbanion intermediate (Warshel, Strajbl, Villa, & Florian, 2000), and O2 or O4 protonation (Siegbahn, 2002). There are some overlaps among these mentioned mechanisms and some of them have been backed by more than one research group. On the other hand, there are some refuting data against some of the proposed mechanisms after conducting more rigorous experiments. For example, nucleophilic attack at C5 (Silverman & Groziak, 1982) was later discarded when kinetic isotope effect experiments indicated that there was no change in bond order between C5 and C6 during decarboxylation (Acheson, Bell, Jones, & Wolfenden, 1990). Or in the case of the formation of iminium ion at C4 (Zhou et al., 2001), when the enzymatic reaction is performed in Water-18O, the product does not incorporate 18O from bulk solvent, so a covalent iminium mechanism for ODCase was abandoned, in spite of its attractive similarities to mechanism of other decarboxylases (Shostak & Me, 1992). Obtaining X-ray crystal structures of OMPDC revealed that there is not any acidic residue in the neighborhood of the carbonyl groups at the 2- or 4-position of the pyrimidine ring which challenged the validity of protonation of one or the other of these carbonyls (Beak & Siegel, 1976; Lee & Houk, 1997) for stabilizing the charge that accumulates on OMPs pyrimidine ring during decarboxylation. The crystallographic results also showed that no nucleophilic residue exists nearby C5. Hence, a covalent catalysis mechanism, to involve a bond between a protein residue and position C5 of the substrate base, can be ruled out. Eventually, researcher who applied computational methods such as quantum mechanics for the C5 and C6 protonation proposed mechanisms acknowledged the uncertainties of their calculations because of

![Figure 1](https://i.imgur.com/3z5Z5Z5.png)
approximations employed in representing the enzyme active site (Lee, 2001).

It is worth to mention that finally transition state stabilization (TSS) or ground state destabilization (GSD) is underlain and involved in all of the aforementioned mechanisms. In our simulations we considered substrate–OMPDC, transition state analog–OMPDC and substrate analog–OMPDC complexes. By comparison of obtained results of complexes of these ligands and corresponding free ligands and enzyme and also comparing the different complexes with each other, our data provided some clues in favor of both GSD (substrate distortion) through electrostatic forces between COO$_2^-$ and anionic Asp91 in initial course of the reaction and TSS mechanism via formation of hydrogen bond between O4 and Ser 127 followed formation of vinyl carbanion intermediate.

A special advantage of this study is that, in addition to detailed structural information, the dynamics are also taken into consideration. Thus, comparing the interactions of these three types of ligands with OMPDC gives more comprehensive information about ligand binding, types of bonds involved in the binding of the ligands to its active site, geometrical position of ligands in the active site, as well as the unique changes in the configuration of active site during the simulation of each complex. Furthermore, the results provide some clues for developing better strategies for inhibiting this enzyme and also some suggestions for its mechanism of action.

**Methods**

Coordinates of OMPDC dimer were obtained from chains A and B of OMPDC crystal structure from *Saccharomyces cerevisiae* (PDB code 1DQX, 2.4 Å resolution) in complex with BMP, i.e. the inhibitor and the transition state analog of OMPDC. This complex mostly resembles the geometry of OMPDC–OMP in its transition state in all of these complexes crystallized so far. The substrate (OMP) and the substrate analog, 6-phosphonouridine 5'-monophosphate (PMP), were considered as ligands (Figure 2). Since the OMPDC inhibitors which contain negative charges at their C6 position show greater binding affinities over neutral ones (Levine, Brody, & Westheimer, 1980), we chose the ligands with negative charge in our simulations.

By carefully modifying the hydroxyl substituent at C6 atom of pyrimidine ring in OMPDC–BMP complex performed by ChemBioOffice software, the other OMPDC–OMP and OMPDC–PMP complexes were generated. OMPDC is catalytically active as a dimer, so all residues (1-534) of the monomers A and B were included in the model. The active site of each subunit is surrounded by two loops from the same subunit, loop 1 (L1, 151-165) and loop 2 (L2, 207-218), as well as one loop from the other subunit, loop 3 (L3, 95*-103*).

It has been shown that the two active sites are catalytically independent (Porter & Short, 2000). In the models, the ligands were considered in the active sites of both monomers A and B of the enzyme. The results for the two active sites were comparable and similar for the two monomers; this implied reproducibility of the results. Therefore, just the results of the site A are reported, and only this active site will be discussed in all the simulations. Asp91 in the active site was considered to be in two states, i.e. protonated and negatively charged, in the six enzyme–ligand complexes and two ligand-free enzymes. All histidines were protonated at their δ-nitrogen atoms.

All the MD simulations were carried out using the AMBER 10.0 package. Each system was solvated by using a cubic box of TIP3P water molecules. Periodic boundary conditions and particle mesh Ewald (PME) method were employed in all of the simulations (Darden & Pedersen, 1993). During each simulation, all bonds in which the hydrogen atom was present were considered fixed and all other bonds were constrained to their equilibrium values by applying the SHAKE algorithm (Ryckaert, Ciccotti, & Berendsen, 1977). The force fields parameters for ligands were taken from Lee et al. (2001).

Partial charges for the ligands were obtained by the RESP method (Bayly, Cieplak, Cornell, & Kollman, 1993). Phosphoribosyl and base fragments were defined by splitting the ligand at the glucosidal bond (C1'-N1) and capping the resultant fragments with formamide and methyl groups, respectively (Lee et al. 2001). Then, electrostatic potentials for these fragments were computed through HF/6-31++G(d,p) calculation by Gaussian 03W package. Atomic charges were obtained by fitting each fragment independently, so that the net charge of the
phosphoribosyl and PMP fragments was \(-2\), while the net charge for the base fragment was \(-1\) (for OMP, BMP); also, the capping groups were constrained to have a zero net charge.

A cut-off radius of 12 Å was used for the proteins and complexes, whereas the cut-off for the free ligands simulations was set to 10 Å. Each of minimization and equilibration phases were performed in two stages. In the first stage, ions and all water molecules were minimized for 1000 cycles of steepest descent followed by 1000 cycles of conjugate gradient minimization. Afterward, the whole systems were minimized for a total of 5000 cycles without restraint wherein 2000 cycles of steepest descent were followed by 3000 cycles of conjugate gradient minimization. In the second stage, the systems were equilibrated for 2000 ps while the temperature was raised from 0 to 300 K, and then equilibration was performed without a restraint for 100 ps while the temperature was kept at 300 K. Sampling of reasonable configurations was conducted by running a 30 ns simulation with a 2 fs time step at 300 K and 1 atm pressure. In previous works on OMPDC, a 5-8 ns production run time has been used for sampling the conformational space (Raugei, Cascella, & Carloni, 2004), so this 30 ns production run seemed to be sufficient. A constant temperature was maintained by applying the Langevin algorithm, while the pressure was controlled by the isotropic position scaling protocol used in AMBER (Case et al., 2005).

Results and discussion

To provide a better understanding of the phenomena which occurred during the 11 simulations mentioned in the “Materials and Methods” section, herein we put the findings into several subsection: we first evaluate the MD simulations to ensure they have reached stable steady state. Then, we separately study the conformational changes of the active site and the ligands, as well as the dihedral angles of the latter, to shed light on the interactions involved in the enzyme mechanism of action. Afterwards, we discuss the hydrogen bonds formed between the ligands and the active site residues of OMPDC, and show the loop movements later. We then clarify the role of water molecules in the simulations, particularly their role in the different OMPDC forms in complex with ligands. Finally, the fluctuations of residues and the flexibility of active site are discussed.

Validation of MD simulations

In total, 11 MD simulations were run: five of which considered the three free ligands and two states of the protein (protonated Asp91 [OMPDC(ASH)] and negatively charged Asp91 [OMPDC(ASP)]) while the other six simulations were run on OMPDC–ligand complexes.

Figure 3 demonstrates the RMSD values of the protein structure relative to the reference structure. The RMSDs of the main simulations confirm that they have reached a stable steady state at the end of each simulation.

Conformational changes of the active site

The RMSD values indicate that the enzyme structure was dramatically affected by each of its ligands.
Surprisingly, in all of the free ligands, changes in the RMSD appeared to be the outcome of changes in the dihedral angle of O4′-C4′-C5′-O5′ (Figure 4).

An average structure was computed and used for comparison of the different complexes and enzymes, and then the global RMSFs were calculated as listed in Table 1 of supplementary data. Comparison of the global RMSFs relative to their initial structures (column 2 of the table) explains why the bound systems have different RMSFs relative to the corresponding unbound systems; this behavior is obvious in both negative and protonated states of Asp91 in the OMPDC–PMP complexes. These data imply that OMPDC structure was affected by ligand binding. The clear differences between the global RMSF values of the negative and protonated states of Asp91 in each OMPDC–ligand complex indicate that, for Asp91, whether or not being negative affects the configurational changes of these complexes.

**Conformational changes of ligands**

Table 2 of supplementary data compares the calculated RMSD values of the ligands in the active site with those of the free ligands in the aqueous solvent. These data indicate that binding of the ligands to the OMPDC active site occurs along with very little RMSD changes.

Average conformations of the ligands bound to the active site compared to the average conformations of the corresponding free ligands in water show no significant geometrical differences. This is in good agreement with what has been suggested previously, i.e. that there is no external strain by the enzyme residues (Hur & Bruice, 2002). On the other hand, our result may also support a role for Asp91 in the substrate destabilization (Chan et al., 2009) due to slightly larger RMSD changes which occur in OMPDC(ASP) compared to OMPDC(ASH).

As has been shown in Table 2 of supplementary data, the ligands of BMP and OMP–enzyme complexes experience less changes in both protonation states of Asp91, in comparison with the ligands in PMP–enzyme complexes, i.e. 1.50 and 0.51 Å in OMPDC(ASP)–PMP and OMPDC(ASH)–PMP, respectively. This trend is also apparent in the global Ca RMSFs values. This might be the result of more negative charge and a larger substituent on the C6 of PMP–enzyme complexes compared to the other OMPDC–ligand complexes, which in turn might cause more clashes and repulsive forces in the PMP–enzyme complexes.

Figure 4. RMSD for free ligands in aqueous solution (left) and their correlated dihedral angles (right).
Conformation of BMP in both states of OMPDC–BMP complexes is more stable than OMP and PMP. This indicates that the dihedral angle located between the C6 substituent group and the glycosidic bond and the dihedral angle between the C6 substituent group and the pyrimidine ring plane, represented in Table 3 of supplementary data and 4 of supplementary data respectively, for the bound BMP are close to the corresponding angles of BMP in aqueous solution. This is a predictable result because BMP is a transition state analog of OMP, and therefore those types of forces and strains that induce distortion on substrate do not affect the transition state analogs. Furthermore, the obvious observed difference between the dihedral angels of OMP in the free and bound states is in good accordance with the substrate destabilization mechanism for OMPDC enzyme (Thirumalairajan, Mahaney, & Bearne, 2010).

**Dihedral angles of the ligands**

Angles of the C6 substituent relative to the pyrimidine plane and the out-of-plane oxygen of the carboxylate are listed in Table 4 of supplementary data. According to the substrate destabilization mechanism, there is a clear difference in the dihedral angle of the C6 substituent and the pyrimidine ring plane between the free and bound states of OMP with the native negatively charged Asp91. This angle is 1.86° in OMPDC (ASP)–OMP complex and −8.92° in free OMP, so the carboxylate group of OMP bends by 10.78° relative to pyrimidine ring plane in the distorted (destabilized) OMP in the active site of OMPDC. This result agrees with the reported angle by Thirumalairajan and co-workers, that is 14°, through the investigation of the X-ray crystal structure of the OMPDC from *Methanobacterium thermoautotrophicum* (Thirumalairajan et al., 2010). This bending is due to repulsive interactions of Asp91 conserved residue.

Because of the structure and the van der Waals volume of the C6 substituent, the out-of-plane oxygens of carboxylate group of distorted OMP are orientated above and below the plane (Heinrich, Diederichsen, & Rudolph, 2009). Thirumalairajan et al. (2010) experimentally demonstrated that the phosphonate group of PMP mimics the out-of-plane oxygens of carboxylate in the distorted OMP, and at least one phosphonate oxygen is above and below the plane of the pyrimidine ring (Thirumalairajan et al., 2010). Our calculated results in Table 4 of supplementary data confirm their reported angles.

**Hydrogen bonds**

As provided in Table 5 of the supplementary data and in Figure 5, the different ligands form different hydrogen bonds (H bonds) with residues in the active site of OMPDC enzyme. Cut-off distance for the H bond calculation was set at 3.5 Å and the cut-off angle was set at 180°. H bonds with occupancies over 70% were considered.

In the negative Asp91 states, BMP forms H bonds with all of the three loops and OMP forms H bond just with the loop 1, while PMP forms no H bonds with the loops. Since BMP is an excellent inhibitor, it forms more H bonds with residues of the active site than OMP and PMP. However, in protonated Asp91 state, OMP forms H bonds with all of the three loops, whereas BMP forms H bonds with the loops 1 and 2, and PMP with the loops 1 and 3.

Data reported in Table 5 of supplementary data indicates that in the different complexes, different residues of the active site contribute to H bonds formation and consequently to binding of the ligands. Through binding of BMP, which is a potent inhibitor and transition state analog of OMPDC, the protein loops move and the ligand is almost completely surrounded, which in turn causes the formation of numerous favorable interactions between the active site-forming residues and the phosphoryl and ribofuranosyl groups and pyrimidine ring of the ligand (Miller et al., 2000). Our results demonstrate that in OMPDC–BMP complex, Lys93, Ser154, and Gln215 form crucial H bonds with 100% occupancy; also in OMPDC–OMP complex the Ser154 residue and in OMPDC–PMP complex the Lys93 residue form H bonds with 99% occupancy.

In both states of the BMP complexes, Ser154 in the active site forms H bonds with O4 of pyrimidine ring of the ligand, whereas in both OMP complexes Ser154 forms H bonds with both O4 and N3 of the ligand. OMPDC possibly takes advantage of the O4–Ser154 amide bond for the binding and stabilizing of the negative charge of C6. Analysis indicates that the resulted negative charge during catalysis is dispersed throughout the pyrimidine ring (Iiams et al., 2011). The Ser154 residue forms dual hydrogen bonds: (1) between the O4 atom of OMP and the Ser154 backbone amide, and (2) between the hydroxyl side chain of Ser154 and the N3 atom of the substrate, as well as to another highly conserved residue, Gln215, which leads to an arrangement between O2 and Arg235, which is an important phosphate binding residue. In such arrangement, Ser154 and Gln215 clamp the substrate into the active site (Iiams et al., 2011).

Structural studies of various OMPDCs reveal that O4 of the substrate always forms hydrogen bond to the backbone NH group of a conserved serine residue. Besides, the hydroxyl group side chain of the Ser residue forms a hydrogen bond with the proton on N3 atom of the substrate.

Recently, Iiams et al. (2011) kinetically characterized the S127A (corresponding to Ser154 in *S. cerevisiae*),
Figure 5. H bonds with occupancies higher than 70% in MD trajectories in (a) OMPDC(ASP)–BMP, (b) OMPDC(ASP)–OMP, (c) OMPDC(ASP)–PMP, (d) OMPDC(ASH)–BMP, (e) OMPDC(ASH)–OMP, and (f) OMPDC(ASH)–PMP (to avoid overlapping, orientation of the residues and loops have been slightly changed.).
S127G, and S127P mutants of the *Methanobacterium thermophilum* OMPDC for a better understanding of the functional role of this particular Ser residue (Iiams et al., 2011). S127P substitution replaces the backbone NH group of Ser with the Cγ atom of proline side chain. In their study, replacement of Ser127 with proline (S127P) made a large effect on $k_{cat}/K_M$ (a reduction of $2.5 \times 10^6$-fold) as well as an increase in $K_M$ and a decrease in $k_{cat}$, which suggests a role for Ser154 in TSS. Lee and Houk suggested that an anionic carbene resonance species is formed for the anionic intermediate in which the negative charge is delocalized to the O4 atom during catalysis (Lee & Houk, 1997). Houk and co-workers also noted that a hydrogen bond donor near the O4 atom of the substrate could play a considerable role in the reduction of $\Delta G^\ddagger$ through stabilization of the transition state/anionic intermediate, when the negative charge in the pyrimidine ring is delocalized on the O4 atom (Houk, Lee, Tantillo, Bahmanyar, & Hietbrink, 2001).

In our simulations we noticed that, in the case of BMP, the length of hydrogen bond between the backbone NH of Ser154 and O4 of pyrimidine ring (Figure 6) over time has a tendency towards getting shorter; however, in OMP this distance remains almost constant in the course of 30 ns simulation. We also compared the statistical values for this distance in 30 ns simulation for OMP and BMP. Table 1 indicates that for all of different statistical values, BMP has smaller values than OMP.

The shorter the length of a hydrogen bond is, the stronger it is, so we suggest that the strength of hydrogen bond to the backbone NH group of Ser154 increases as the negative charge is delocalized to the O4, supporting the hypothesis that the hydrogen bond between the backbone NH and O4 of the substrate is significant in reducing $\Delta G^\ddagger$ (Iiams et al., 2011; Shan & Herschlag, 1996).

**Closure of loop 2 and ordered β-hairpin**

Loop regions in all of the OMPDC–ligand complexes, except PMP complexes, exhibited less flexibility than their ligand-free forms in structural alignment analysis. This characteristic is illustrated in Figure 7 as per residue RMSF plots of the ligand-bound and ligand-free forms of OMPDC. Evidently, by placing PMP in the active site, the active site-forming loops show more flexibility than in the ligand-free state; for instance, loop 2 in OMPDC exhibited larger RMSFs. Hence, the loops around the active site and N or C terminal regions (next to residues 267 and 534) show more flexibility and are prone to dynamic activities which take place within the enzyme (Malabanan, Amyes, & Richard, 2010).

As shown in Figure 8, by changing OMPDC–OMP complex to OMPDC–intermediate, the loop 2 forms a β-hairpin along with some additional H bonds. This result is in accordance with Hur results (Hur & Bruice, 2002). This positional shift is accompanied with a change in position of residues in loop 2 and the subsequent conformational change in the unorganized loop 2, which in turn converts it to an ordered β-hairpin. These changes suggest an increase in intermediate stabilization due to formation of H bonds which maintain the ordered hairpin conformation in the OMPDC(ASP)–BMP complex (Hur & Bruice, 2002).

Closure of the phosphate gripper loop 2 over the phosphodianion group of substrate is a key event in

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**Table 1.** Hydrogen bond distance (Å) between backbone NH of Ser154 and O4 during 30 ns simulation.

| Time (ns) | 0  | 5  | 10 | 15 | 20 | 25 | 30 |
|----------|----|----|----|----|----|----|----|
| OMPDC(ASP)–BMP | 2.79 | 2.71 | 2.63 | 2.52 | 2.36 | 2.24 | 2.01 |
| OMPDC(ASP)–OMP | 3.00 | 2.93 | 2.86 | 2.91 | 2.89 | 2.85 | 2.81 |

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Figure 7. Atomic positional fluctuations (Å) of Ca atoms in the ligand-bound enzymes (blue line) compared to the ligand-free enzymes (red dotted line) in OMPDC(ASP)–BMP, OMPDC(ASP)–OMP, OMPDC(ASP)–PMP, OMPDC(ASH)–BMP, OMPDC(ASH)–OMP, and OMPDC(ASH)–PMP complexes and related ligand-free structures from top to bottom.
Figure 8. Ordered and disordered hairpins in different complexes: negatively charged Asp91 in (a) OMPDC(ASP)–BMP, (b) OMPDC(ASP)–OMP, and (c) OMPDC(ASP)–PMP complexes and neutral charged Asp91 in (d) OMPDC(ASH)–BMP, (e) OMPDC(ASH)–OMP, and (f) OMPDC(ASH)–PMP complexes.
catalysis, which causes a large conformational change in OMPDC (Amyes & Richard, 2007). As Miller and co-workers showed, the ordered hairpin loop 2 in OMPDC(ASP)–BMP complex causes the formation of more interactions with the phosphoryl group of ligand (Miller et al., 2000). Our results, depicted in Figures 5 and 8, also indicate the presence of this hairpin with formation of more hydrogen bonds in OMPDC–BMP than OMPDC–OMP complex.

The reported interactions of the phosphodianion group of ligand with three key residues, namely Gln215, Tyr217, and Arg235 which involve in stabilization of closed form of the enzyme (Amyes et al., 2012), are comparable with our findings for the six complexes (Figure 5), so that the closed conformation of OMPDC–BMP complex is stabilized by hydrogen binding interactions of the three aforementioned residues. However, OMP shows no interaction with Tyr217, and PMP forms H bond only with Tyr217. The same interactions are seen in the two states of negatively charged and protonated Asp91 of each OMPDC complex as well. Also, the loops 1 and 2 form H bonds with each other, which leads to enveloping the pyrimidine ring of ligand (Malabanan et al., 2010) in BMP and OMP complexes, and in PMP complexes no H bonds is formed between these loops (Table 2).

**Water molecules in the active site**
The closure of loop 2 over the phosphodianion group of the substrate envelops the ligand within the enzyme, withdraws water molecules from the ligand environment in the active site, leads to some extra contacts between the enzyme and the ligand, and then effectively transfers the ligand from the bulk water to the active site (Herschlag, 1988; Jencks, 1975). A decrease in the number of water molecules results in a lower effective dielectric constant in the active site, and consequently an increase in TSS, either by forming additional electrostatic interactions with polar amino acid side chains, or by strengthening the existing hydrogen bonds and electrostatic interactions (Hu, Boone, & Yang, 2008).

In our simulations, the number of water molecules was less in the active site of OMPDC–BMP complex and more in OMPDC–OMP and significantly more in OMPDC–PMP during the simulation time. There was much more water molecules in all ASH states compared to the ASP states, in both the ligand-bound and ligand-free systems. This result agrees well with the previously reported results (Amyes & Richard, 2007), and indicates that this enzyme shows its open form (Eo) in complex with PMP where the active site is exposed to the bulk solvent, and its closed form (Ec) with BMP.

**Fluctuations of residues and flexibility of the active site**
Previous studies suggest that binding ability of OMPDC to substrate analogs with a bulky substituent at the C6 position is consistent with the idea of possessing a “commodious” active site (Thirumalairajan et al., 2010). It has been suggested that these bulky analogs may avoid unfavorable interactions, and bind via other “opportunistic interactions” (Chan et al., 2009).

The calculated Ca positional fluctuations for key residues of the active sites (Table 6 of supplementary data) confirm that the active site is very flexible. These residues are present in the carboxylate-binding pocket, the ribosyl ring-binding pocket, the 5′-phosphate-binding pocket, and the pyrimidine-capping loop of the active site. Our analysis for all of the OMPDCs in the simulations indicated that Asp91-Lys93 (from the carboxylate-binding pocket) and Lys59 (from the ribosyl-binding region) have the smallest fluctuation values (0.3–0.7 Å) and are almost perfectly superimposable when their averaged structures are aligned. However, Asp96* shows the largest fluctuation values (0.5–1.14 Å) (Table 6 of supplementary data). The relative rigidity of the carboxylate-binding pocket, which consists of Asp91 and the surrounding hydrophobic residues (Iiams et al., 2011), might be an evidence of the substrate destabilization mechanism, since the rigidity of binding sites that are involved in GSD is necessary for catalysis.

Our results show quantitatively that when the structure of the active sites in OMPDC–BMP and OMPDC–PMP are superimposed to the active site of OMPDC–OMP, rigid tetrad residues (Lys59, Asp91, Lys93, and Asp96*) in OMPDC preserve their positions in the process of converting the OMPDC–OMP complex to OMPDC–BMP and OMPDC–PMP, whereas positions of the ribosyl and phosphate-binding domains change

| Lys156 and Gly214 H-bond | OMPDC–BMP | OMPDC–OMP | OMPDC–PMP | OMPDC–BMP | OMPDC–OMP | OMPDC–PMP |
|--------------------------|------------|------------|------------|------------|------------|------------|
| Occupancy (%)            | 98         | 95         | 10.6       | 99.01      | 97         | 72.04      |
| Distance (Å)             | 2.78 (0.18)| 2.75 (0.2 )| 3.00 (0.21)| 2.9 (0.18) | 2.84 (0.22)| 3.00 (0.19)|
| Angle (dg)               | 17.43 (10.57)| 20.35 (11.38)| 27.88 (19.07)| 17.21 (9.98)| 22.260 (9.75)| 24.26 (16.91)|
| Life time                | 33 (56.7)  | 32.5 (63.3)| 6.9 (8.0)  | 85.9 (98.5)| 36.4 (96.2)| 17.6 (22.8)|
Table 3. RMSD (Å) of different parts of the OMPDC(ASP)–BMP and OMPDC(ASP)–PMP complexes relative to OMPDC (ASP)–OMP.

| Complex                  | Active site | Loop 2 | Phosphoribosyl binding residues | Tetrad |
|--------------------------|-------------|--------|---------------------------------|--------|
| OMPDC(ASP)–BMP           | 1.541       | 1.924  | 2.234                           | 0.149  |
| OMPDC(ASP)–PMP           | 1.703       | 1.068  | 2.686                           | 0.145  |

(Table 3), as previously stated. RMSD value of the phosphoribosyl-binding residues of the active site in OMPDC-BMP complex over corresponding OMPDC–OMP complex is 2.234 Å, which implies a large structural change in OMPDC after binding of OMP (Hur & Bruice, 2002).

Conclusions
This work aimed to investigate the characteristics of ligand interactions with OMPDC, in order to shed more light on the mechanism of its catalysis and inhibition. Therefore, we used short time MD simulations at nanosecond timescale.

According to Gerlt, Chan, and coworkers (Chan et al., 2009) two mechanisms, namely substrate destabilization and TSS, contribute to the catalytic mechanism of OMPDC together. The former is achieved by both electrostatic stress of Asp91 and a hydrophobic pocket on the carboxylate group of substrate, while the latter is due to H bond formation between the O4 of ligand and the NH backbone of Ser154. Our computational results possibly confirm the effects of substrate destabilization at beginning of the catalytic process, where breaking the covalent bond between the carboxylate group and the C6 atom happens, and then the transition state is stabilized through formation of particular hydrogen bonds. The existence and formation of these interactions require a specific milieu, and our results directly and indirectly could be indicative of the presence of such a microenvironment. In specific, our results indicate that complete closure of the loop 2 over the active site and formation of regular hairpin occur perfectly in OMPDC(ASP)–BMP, compared to OMPDC(ASP)–OMP, which could contribute to extrusion of water molecules from the active site and consequently lower the number of water molecules in the active site of OMPDC(ASP)–BMP. These circumstances lead to creation of a microenvironment with lower dielectric constant and increase the TSS, as supported by studies on triosephosphate isomerase (Malabanan et al., 2010), a structural homologue of OMPDC.

Supplementary data
The supplementary material for this paper is available online at http://dx.doi.org/10.1080/07391102.2014.881303.

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