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Molecular Dynamics Simulation of Interactions in Glycolytic Enzymes

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Abstract—Two glycolytic enzymes, phosphoglycerate mutase (PGM) and enolase from *Saccharomyces cerevisiae*, have been chosen to detect complex formation and possible channeling, using molecular dynamics simulation. The enzymes were separated by 10 Å distance and placed in a water-filled box of size 173 × 173 × 173 Å. Three different orientations have been investigated. The two initial 3-phosphoglycerate substrate molecules near the active centers of the initial structure of PGM have been replaced with final product (2-phosphoglycerate) molecules, and 150 mM NaCl together with three Mg^{2+} ions have been added to the system to observe post-catalytic activity under near-physiological conditions. Analysis of interaction energies and conformation changes for 3 nsec simulation indicates that PGM and enolase do show binding affinity between their near active regions, which is necessary for channeling to occur. Interaction of the C-terminal residues Ala239 and Val240 of PGM (which partially "cap" the 2-phosphoglycerate) with enolase also favors the existence of channeling.

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Glycolytic enzymes are responsible for conversion of glucose to pyruvate, which is subsequently converted into lactate or ethanol or included in the Krebs cycle. Of special interest is the formation of dynamic multi-enzyme complexes between glycolytic enzymes, where regulatory mechanisms may be applied. Metabolite channeling by means of a direct transfer mechanism can be defined as a phenomenon where a pair of enzymes that catalyze consecutive reactions form a bi-enzyme complex, and the product is transferred from the active center of one enzyme to the active center of next enzyme as its substrate without equilibrating with the bulk solution [1]. The advantages of channeling that can occur in different metabolic pathways as a result of static and dynamic multi-enzyme formations have been widely discussed [2] and some results have been previously reported indicating the possibility of channeling in glycolysis [3]. The interaction itself is highly sensitive to the physiological status of the environment, which has been confirmed on the bases of analysis of the interrelation between the influence of ionic strength and pH on the binding activity of glycolytic enzymes [4, 5]. Due to high concentration of glycolytic enzymes in cytoplasm (for example, in yeast [6, 7]) the average distance between enzyme molecules appears much smaller than the size of the molecule [8]. This suggests that glycolytic enzymes do interact and may form complexes. On the other hand, due to the fact that the concentration of enzymes in cytoplasm can be much greater than the concentration of metabolites, the idea of physiological significance of enzymes acting as buffers for metabolites has been reviewed [9]. Consequently, the probability of free diffusion of a substrate diminishes due to the interacting nature of enzymes and possible supramolecular organization.

In the present communication, an attempt is made to show interaction and estimate channeling possibility between phosphoglycerate mutase (PGM) (EC 5.4.2.1) and enolase (EC 4.2.1.11) from *Saccharomyces cerevisiae* using a new approach of molecular dynamics (MD) simulation.

PGM in yeast is responsible for reversible transfer of a phosphate group from the third carbon of 3-phosphoglycerate (3PG) to the second carbon atom, forming 2-phosphoglycerate (2PG). *Saccharomyces cerevisiae* enolase is a homodimer that catalyzes the dehydration of 2-

Abbreviations: MD) molecular dynamics; 2PG) 2-phosphoglycerate; 3PG) 3-phosphoglycerate; PGM) phosphoglycerate mutase.

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D-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway and the reverse reaction in gluconeogenesis. There are several publications on *in vitro* enolase–mutase complex formation [10, 11]. A number of methods have been used to prove the interaction, its specificity, and to obtain qualitative parameters. Because of the weak and dynamic nature of the interaction, the results were not very consistent. Major criticism was related to methods used for detection of complex formation. For example, the method based on the detection of fluorescence anisotropy requires enzyme with covalently linked fluorescence dyes, which can change the interaction behavior of the molecule [11]. The major aim of the present paper is to re-examine complex formation using MD simulation.

There has already been an attempt to investigate the interaction of glycolytic enzymes using different methods of computer simulation. For example, the possibility of substrate channeling between the glycolytic enzymes fructose-1,6-biphosphate aldolase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) has been checked using Brownian dynamics [12]. This showed a small preference (by a factor of two) of glyceraldehyde 3-phosphate (GAP) channeling from aldolase to GAPDH when compared to substrate binding from solution, though the difference was not large enough to consider the results as strong theoretical evidence of channeling between proteins.

The MD simulation performed for PGM and enolase presented in this paper has some advantages in comparison with Brownian dynamics; the complete structure of enzymes including hydrogen atoms is taken into account with inclusion of explicit water molecules, allowing hydrophobic interactions and conformation changes of proteins and 2PG substrates. NaCl (150 mM) has also been added to each system to run the simulation at near physiological conditions as it is well known that interaction between glycolytic enzymes strongly depends on the ionic strength [4]; a particularly significant increase in retention for PGM and enolase has been shown under high ionic strength conditions.

MATERIALS AND METHODS

Molecular dynamics (MD) has been used to investigate the interaction and possible substrate channeling between PGM and enolase. X-Ray structures have been taken from the RCSB Protein Data Bank [13] for PGM (1QHF) [14] and enolase (2ONE) [15], where both enzymes are in dimeric forms. The CHARMM program [16] has been used for energy minimization, MD, and analysis.

The two 3PGs in the initial structure of PGM have been modified to 2PGs (with an evaluation version of the Hyperchem 7.5 program [17]) to observe the post-catalytic interaction of PGM and enolase and possible transfer of 2PG between them. Then, the 2PG and phosphoenolpyruvate have been removed from the initial structure of enolase to free the active regions of the enzyme. Three orientations of PGM and enolase, which would cover different favorable configurations, have been investigated for interaction. For all cases, the active centers of both enzymes were oriented to face each other to test the possibility of direct transfer of metabolite. Each of two 2PGs of PGM had a charge equal to −3e in units of electron charge. Negative charges were distributed on one of the oxygen atoms of the first carbon atom and the other −2e were distributed among two oxygen atoms of the phosphate group. Each subunit of PGM had a charge equal to +3e. The active center His8 of each subunit was chosen to be in the protonated state having charge +1e. Enolase was assigned to −8e (−4e for each subunit). Three Mg^{2+} ions were distributed near the active center of enolase to record the influence of metals on binding and catalytic activity.

Na^+ and Cl^- ions were added with concentration 150 mM to match the physiological condition and bring the total charge of the system to zero. The enzyme molecules were separated by 10 Å distance from each other to fit in the interaction cutoff distance of length 14 Å, which would guarantee initial interaction between surface residues of the enzymes. The proteins were placed into a cubic water-filled box of size 173 × 173 × 173 Å. Explicit water molecules of type TIP3 were used for all orientations (about 50,000 water molecules for each orientation). In each of three orientations, the average number of atoms participating in the system was 160,000. Leapfrog molecular dynamics was used to detect complex formations. This step followed an energy minimization step, to bring potential energy of the system below $-4 \cdot 10^5$ kcal/mol. Minimization and dynamics were performed on a cluster (ArmCluster) using 16 nodes (3.06 GHz Xeon dual processor, Linux RH9.0). A total of 71,424 CPU hours were used to simulate 3 nsec dynamics.

RESULTS AND DISCUSSION

His8 and His159 are located at the active centers of PGM and enolase, respectively. The two 2PGs were initially positioned near His8 of PGM. To determine the interacting picture and the possibility of channeling, three orientations have been analyzed. The initial orientations for all three structures are shown in Fig. 1 [18]. For orientation “a” (Fig. 1a), the active centers of both subunits of enolase are oriented toward the active centers (where 2PGs reside) of the subunits of PGM (relative spatial vicinity of active sites of the enzymes is shown with dotted lines). In orientation “b” (Fig. 1b), enolase has only one active center oriented toward active center of

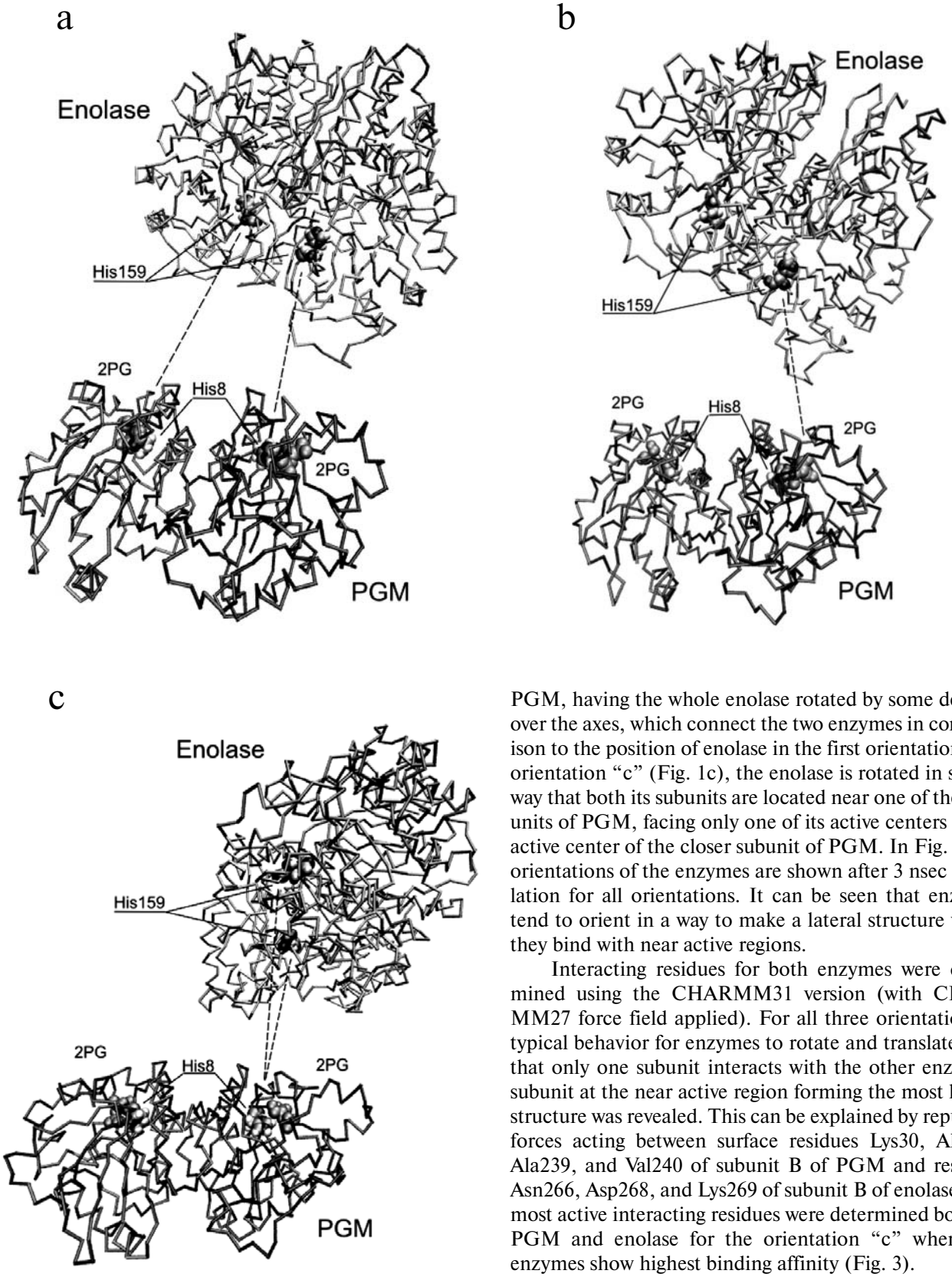

Fig. 1. Initial configurations of enzymes for all orientations.

PGM, having the whole enolase rotated by some degrees over the axes, which connect the two enzymes in comparison to the position of enolase in the first orientation. For orientation "c" (Fig. 1c), the enolase is rotated in such a way that both its subunits are located near one of the subunits of PGM, facing only one of its active centers to the active center of the closer subunit of PGM. In Fig. 2, the orientations of the enzymes are shown after 3 nsec simulation for all orientations. It can be seen that enzymes tend to orient in a way to make a lateral structure where they bind with near active regions.

Interacting residues for both enzymes were determined using the CHARMM31 version (with CHARMM27 force field applied). For all three orientations, a typical behavior for enzymes to rotate and translate such that only one subunit interacts with the other enzyme's subunit at the near active region forming the most lateral structure was revealed. This can be explained by repulsion forces acting between surface residues Lys30, Ala237, Ala239, and Val240 of subunit B of PGM and residues Asn266, Asp268, and Lys269 of subunit B of enolase. The most active interacting residues were determined both for PGM and enolase for the orientation "c" where the enzymes show highest binding affinity (Fig. 3).

Tables 1 and 2 bring cross reference of the most active interacting residues (whose interaction energy is greater than 1.0 kcal/mol absolute) for PGM and eno-

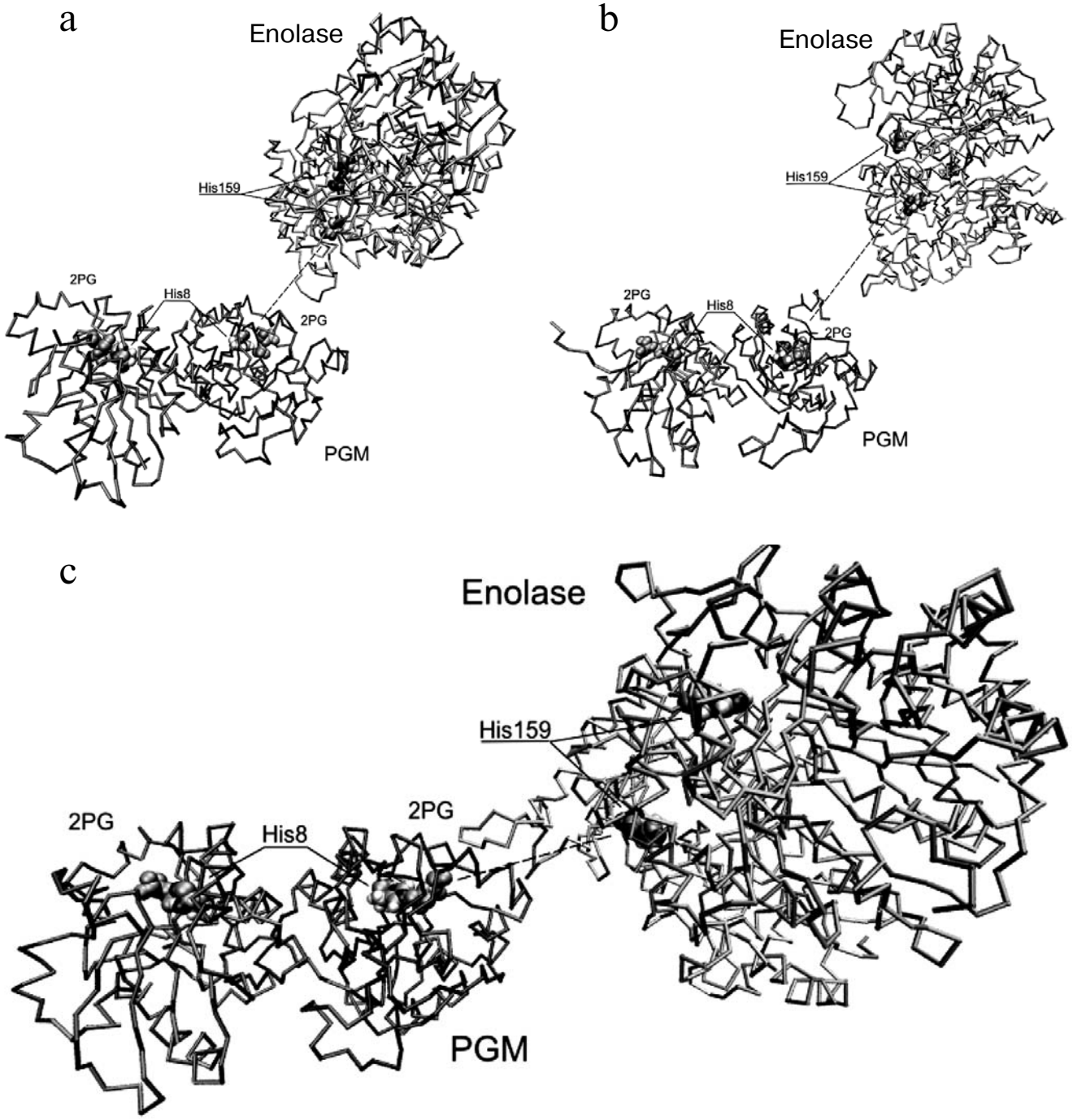

Fig. 2. Interaction pictures of enzymes after 3 nsec simulation for all orientations.

lase, respectively, after 1.5 nsec simulation. The interaction energy presented is the sum of electrostatic and van der Waals energies.

The first column presents residues, which show particular interaction activity (second column) with the next enzyme (third column). The fourth column shows minimum distance between specific residues (first column) and the other enzyme (third column). For PGM the interacting residue regions are Arg7-Phe19, Leu27-Arg37, Lys97-Ser115, and Ala198-Ile209. For enolase, the binding region limits with residues Ser249-Ser270, which include one of the catalytic loops, Asp255-Asn266 [15]. As can be seen from Tables 1 and 2, Asp255 of enolase mainly interacts with Arg114 and Lys202 of PGM. The repulsion energy between residues Lys30 of PGM and Lys269 of enolase is almost completely compensated

Table 1. Most actively interacting residues of PGM and corresponding energies after 1.5 nsec simulation

Residue of second subunit of PGM	Total energy, kcal/mol	Residue of first subunit of enolase	Minimal distance, Å
Lys202	−57.16209	Asp255	1.8469
Arg114	−44.27127	Asp255	1.7824
Lys30	18.25827	Lys269	3.0241
Glu34	−12.05011	Lys269	5.3565
Glu106	−10.68117	Lys254	3.9185
Asn14	−3.66183	Lys269	5.0381
Gln10	3.14232	Lys269	4.042
Lys103	2.95263	Lys53	7.2733
Glu15	−2.51458	Lys269	6.3594

Table 2. Most active interacting residues of enolase and corresponding energies after 1.5 nsec simulation

Residue of first subunit of enolase	Total energy, kcal/mol	Residue of second subunit of PGM	Minimal distance, Å
Asp255	−106.79346	Arg114	1.7824
Lys254	−14.25917	Glu106	3.9185
Lys257	−5.88858	Gly236	4.3374
Lys53	2.90772	Lys103	7.2733
Asn264	−2.38028	Lys102	3.7911
Phe253	2.19422	Lys202	5.8679

Table 3. Most active interacting residues of PGM and corresponding energies after 3 nsec simulation

Residue of second subunit of PGM	Total energy, kcal/mol	Residue of first subunit of enolase	Minimal distance, Å
Lys202	−57.37946	Asp255	1.5856
Glu106	−55.31423	Lys254	1.6651
Arg114	−39.02517	Asp255	1.9391
Lys97	−33.77279	Asp268	3.7205
Arg113	−8.05999	Ser267	5.2245
Ala239	−8.02098	Asp268	2.5726
Lys30	7.26975	Lys269	5.6369
Leu101	−5.63718	Pro265	2.2435
Val240	−4.86187	Trp272	2.4647
Asn110	3.33713	Ser267	2.7142
Asn17	3.30155	Asp268	5.3846
Asn14	2.37279	Asp268	6.1267

Table 4. Most active interacting residues of enolase and corresponding energies after 3 nsec simulation

Residue of first subunit of enolase	Total energy, kcal/mol	Residue of second subunit of PGM	Minimal distance, Å
Asp255	−101.59264	Lys202	1.5856
Lys254	−72.40668	Glu106	1.6651
Asp268	−27.90206	Ala239	2.5726
Asp259	15.17873	Glu106	4.7675
Ser267	−11.81999	Ala239	2.678
Lys257	8.57076	Val240	4.1658
Pro265	−8.07695	Leu101	2.2435
Asn264	4.95274	Glu106	4.1497
Phe262	4.88171	Glu106	4.4601
Lys263	−3.69621	Glu106	5.7195
Lys269	2.35698	Ala239	4.3985
Phe253	2.10925	Lys202	6.422

by attraction energy between Glu34 of PGM and the same Lys269 of enolase.

The Glu106 of PGM and Lys254 of enolase also show high binding activity. Lys269 was determined to be the most actively interacting residue with 2PG with interaction energy equal to −0.84136 kcal/mol and distance 6.86 Å after 1.5 nsec simulation.

From Tables 3 and 4, which show the same interaction data after 3 nsec simulation period, one can see that Glu106 of PGM and Lys254 of enolase change their position toward higher interacting energy region. The difference of interactions energies of Glu106 and Lys254 presented in Tables 3 and 4 mainly relates with repulsion energy between negatively charged Glu106 and Asp259. The C-terminal amino acids (Glu231-Val240) also show binding activity after 3 nsec simulation run. This tail partially covers the active center [14] and consequently "caps" the way for 2PG to be released (Fig. 3). Binding tendency between active centers of the enzymes for all orientations confirms the absence of bias to particular

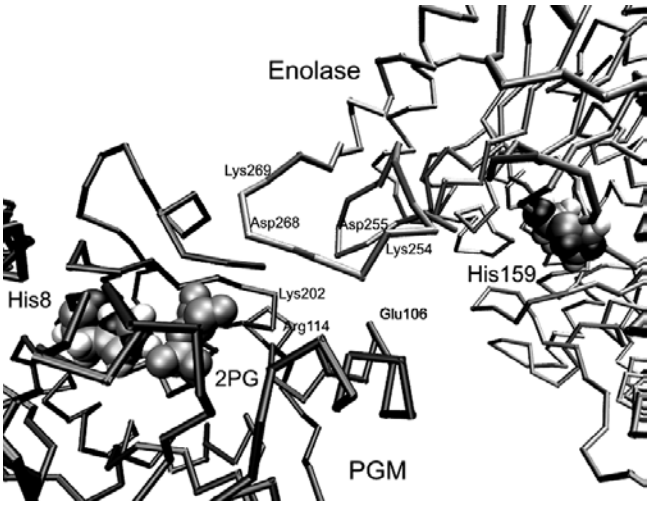

Fig. 3. Interacting residues of PGM and enolase together with 2PG and His of active centers for orientation "c" after 3 nsec simulation.

active center of PGM or enolase unless a preference appears due to conformation changes in quaternary structure. One possibility for enolase to attract the 2PG and initiate transfer is to move further apart its residues Asp268 and Asp255 to enlarge the "groove" and make positively charged Lys254 and Lys269 become main participants in electrostatic field formation around 2PG.

It has been shown that PGM and enolase at specific orientations tend to interact with their near catalytic regions, which is a necessary step for channeling to occur. Interaction of the C-terminal tail of PGM with enolase, which has been recorded after 3 nsec MD simulation, is also a required step for the 2PG to be released, which, though indirectly, favors the existence of channeling.

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