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Computer simulation of complex formation between glycolytic enzymes

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Glycolysis is generally a process of conversion of glucose to pyruvate [12, 15]. This conversion plays a crucial role in both eukaryotic and prokaryotic cells. One of the main purposes of glycolysis is the energy provision within a cell. The dominating model of glycolysis is a chain based model where intermediate product of catalysis of first enzyme transfers to the second enzyme which in turn passes its product to the third one and the like (glycolytic pathway).

The interactions between glycolytic enzymes and structural framework of the cell have generated a considerable interest in the past decade. Enzymes-enzyme interactions occur in cytosol and mitochondria. As these dynamic interactions are relatively weak and transient, the reversible variation in their complexed states may present one mechanism of several by which regulation of catalytic activity may be achieved [10, 18].

Due to the huge number of atoms constituting enzymes and fast interaction times (order of nanoseconds) there no adequate experimental method exists to investigate their interaction and detect possibility of substrate channelling between active sites of enzymes. Channelling by means of direct transfer mechanism can be defined as a process of substrate transfer from active site of the first enzyme to the active site of the second enzyme without equilibrating with the bulk solution [12]. The advantages of channelling which can occur in different metabolic pathways as a result of static and dynamic multi-enzyme formations have been widely discussed [1].

Molecular dynamics (MD) simulation, being a relatively new method, has already proved its suitability in investigation of various biological systems. What distinguishes computer simulation in general from other forms of computation is the manner in which the computer is used; rather than merely performing a calculation, the computer becomes a virtual laboratory in which a system is studied – a numerical experiment. MD simulation is only one of the several possible simulation schemes among which are classical Monte Carlo [2], quantum-based techniques involving path-integral [4, 8] and Monte Carlo methods [16] and MD combined with electron density function theory [14, 17], as well as discrete approaches, such as cellular automata and the lattice-Boltzmann method [7].

The simplest form of MD, that of structureless particles, involve little more than Newton's second law. Because of the nature of the interatomic interaction, exemplified by the Lennard-Jones potential with a strongly repulsive core, atomic trajectories are unstable in the sense that an infinitesimal perturbation will grow at an exponential rate, and it is fruitless to seek more than moderate accuracy in the trajectories, even over limited periods of time. Thus a comparatively low-order numerical integration method often suffices; whether or not this adequate emerges from the results, but the reproducibility of MD measurements speaks for itself. In practice, the phenomena studied by MD simulation are those where relativistic effects are not observed and quantum effects can, if necessary, be incorporated as semi-classical corrections [9]. This fact has in no way diminished the power and effectiveness of the method [13].

The major aim of the present paper is to re-examine complex formation between phosphoglycerate mutase (PGM) [EC 5.4.2.1] and enolase [EC 4.2.1.11] taken from Saccharomyces cerevisiae using the approach of MD simulation. PGM in yeast is responsible for reversible transfer of phosphate group from the third carbon of 3-phosphoglycerate (3PG) to the second carbon atom forming 2-phosphoglycerate (2PG). S. cerevisiae enolase is a homodimer, which catalyzes the dehydration of 2-D-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway, and the reverse reaction in gluconeogenesis. The present MD simulation performed for PGM and enolase has those advantages comparison with previously performed brownian dynamics simulations [11] that the complete structure of enzymes including hydrogen atoms was taken into account with inclusion of explicit water molecules allowing hydrophobic interactions and conformation changes of proteins and 2PG substrates.

Materials and Methods

MD has been used to investigate interaction and possible substrate channelling between PGM and enolase. X-ray structures have been taken from RCSB Protein Data

Bank [3] for PGM (1QHF) [6] and enolase (2ONE) [19] where both enzymes are in dimeric forms. CHARMM program [5] has been used for energy minimization, MD and analysis. The two 3-phosphoglycerates in the initial structure of PGM have been modified to 2-phosphoglycerates to observe the post catalytic interaction of PGM and enolase and possible transfer of 2PG between them. On the other hand the 2-phosphoglycerate and phosphoenolpyruvate have been removed from initial structure of enolase to free the active regions of the enzyme. Three orientations of PGM and enolase have been investigated for interaction. For all cases the active sites of both enzymes have been oriented to face each other to test the possibility of direct transfer of metabolite between active sites. Each 2PG of PGM had a charge equal to -3e in units of electron charge. Negative charges have been distributed on one of the oxygen atoms of the first carbon atom and the other -2e have been distributed among two oxygen atoms of the phosphate group. Each subunit of PGM had a charge equal to +3e. The a/s His8 of each subunit has been chosen to be in protonation state having charge +1e. Enolase has been assigned to -8e (-4e for each subunit). Three Mg^{2+} ions have been distributed at near active sites of enolase to record the influence of metals on binding and catalytic activity. Ions of Na^- and Cl^+ have been added with concentration 150mM to fit the physiological condition and bring the total charge of the system to zero. Enzymes have been separated by 10 E distance from each other to fit in the interaction cutoff distance of length 14 E which would guarantee an initial interaction between surface residues of enzymes. Proteins have been placed into a cubic water box of size 173x173x173 Å. Explicit water molecules of type TIP3 have been used for all orientations (about 40 000 water molecules for each orientation). In each of three orientations the average number of atoms participating in the system has been equal to 140 000. Leapfrog MD has been used to detect complex formations. This step followed an energy minimization step, to bring potential energy of the system below $-4x10^5$ kcal/mol. Minimization and dynamics have been performed on a cluster (ArmCluster) using 16 nodes (3.06GHz Xeon dual processor, Linux RH9.0). 35712 CPU hours have been used to simulate 1.5ns dynamics.

Results and Discussion

Active sites for enzymes are His8 for PGM and His159 for enolase. To figure out the interacting picture and the possibility of channelling three orientations have been analyzed. For the first orientation the active sites (a/s) of both subunits of enolase are oriented toward a/s (where 2PGs reside) of PGM's subunits. In the second orientation enolase has only one a/s oriented toward a/s of PGM having the whole enolase rotated at some degree over the axis which connects two enzymes in comparison to the enolase in the first orientation. For the third orientation 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 a/s to the a/s of closer subunit of PGM. After 1.5ns simulation, enzymes in all orientations tend to form the most possible lateral configuration where regions at near a/s of both enzymes can interact.

The most active interacting residues have been determined both for PGM and enolase for the third orientation, where enzymes show highest binding affinity. Tables 1 and 2 bring cross reference of most active interacting residues (whose interaction energy is greater than 1.0 kcal/mol in absolute) for PGM and enolase respectively after 1.5ns simulation. The interaction energy presented is the sum of electrostatic and van der Waals energies.

Table 1

Most active interacting residues of PGM and corresponding energies after 1.5ns simulation

Residue of second subunit of PGM	Total energy in kcal/mol	Residue of first subunit of enolase	Min. dist. in E
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 1.5ns simulation

Residue of first subunit of enolase	Total energy in kcal/mol	Residue of second subunit of PGM	Min. dist. in E
Asp255	-106.79346	Arg144	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

The first column presents residues which show particular interaction activity (second column) with the next enzyme. The forth 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 [19]. As it 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 by attraction energy between Glu34 of PGM and the same Lys269. The Glu106 of PGM and Lys254 of enolase also show high binding activity. Lys269 has been determined as the most active interacting residue with 2PG with interaction energy equal to -0.84136 kcal/mol and distance 6.86 E after 1.5ns simulation. The C-terminal tail of PGM partially 'caps' the 2PG [6]. For the direct transfer to exist it is necessary that this amino acid tail reposition in a way to allow the 2PG to interact with water solution and enolase. Consequently the interaction of C-terminal tail with enolase residues is required for the channelling to occur.

The results show that PGM and enolase do interact with their near active site residues and the formation of bi-enzyme complex is a sign that substrate's direct transfer may exist between PGM and enolase.

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Գլիկոլիտիկ ֆերմենտների կոմպլեքսագոյացման հետազոտումը համակարգչային մոդելավորման միջոցով

Դ.Է. Հակոբյան

Ուսումնասիրվել է գլիկոլիտիկ ֆերմենտների՝ ֆոսֆոգլիցերատ մուտազի (ՖԳՄ) և էնոլազի փոխազդեցությունը մոլեկուլային դինամիկ մոդելավորման միջոցով: Հետազոտվել են ֆերմենտների երեք տարբեր դիրքորոշումներ, որտեղ ֆերմենտների ակտիվ կենտրոններին կից ամինաթթվային հատվածները ուղղված են գլխավորապես իրար դեմ, գրանցելու համար հնարավոր թունելային անցումը: 1.5նվ մոդելավորման հետագծերի վերլուծությունը ցույց տվեց, որ ՖԳՄ-ը և էնոլազը հակված են փոխազդելու հենց իրենց ակտիվ կենտրոններին կից հատվածներով, դիրքորոշվելով այնպես, որ ստացվի հնարավորինս երկար կառուցվածք: Այս տիպի դիրքորոշման ժամանակ յուրաքանչյուր դիմերային ֆերմենտից միայն մեկ սուբմիավորն է փոխազդում մյուս ֆերմենտի հետ: Այսպիսի փոխազդեցության պատկերը խոսում է ի օգուտ մետաբոլիկ թունելի գոյության մասին:

Исследование комплексообразования гликолитических ферментов методом компьютерного моделирования

Д.Э. Акопян

Исследовано взаимодействие гликолитических ферментов фосфоглицератмутазы (ФГМ) и енолазы методом молекулярного динамического моделирования.

Проанализированы три разных расположения ферментов, где околокаталитические участки ориентированы преимущественно друг против друга, для выявления возможного метаболического туннеля. Анализ полученных траекторий, моделированных для периода 1,5нс, показал, что ФГМ и енолаза проявляют тенденцию взаимодействовать именно между своими околоактивными участками.

Ферменты склонны к комплексообразованию наиболее явно для тех ориентаций, при которых получается наиболее удлиненная структура, где взаимодействует только одна из субъединиц димера от каждого фермента. Такая схема взаимодействия говорит в пользу существования метаболического туннеля.

References

1. *Agius L., Sherratt H.S.A.* Channelling in Intermediary Metabolism. Portland Press, London, 1997.

2. *Allen M. P., Tildesley D. J.* Computer Simulation of Liquids, Oxford University Press, Oxford, 1987.

3. *Berman H.M. et al.* The protein data bank, Nucleic Acids Research, 2000, 28, p. 235. Website: http://www.rcsb.org/pdb/.
4. *Berne B. J., Thirumalai D.* Ann. Rev. Phys. Chem., 1986, 37, p. 401.
5. *Brooks B.R. et al.* J. Comp. Chem., 1983, 4, p. 187.
6. *Crowhurst G.S. et al.* J. Biol. Cryst., 1999, 55, p. 1822.
7. *Doolen G. D.* Lattice Gas Methods for PDEs, North-Holland, Amsterdam, 1991.
8. *Gillan M. J.* Computer Modelling of Fluid Polymers and Solids. Kluwer Academic Publishers, Dordrecht, 1990.
9. *Maitland G. C., et al.* Intermolecular Forces. Clarendon Press, Oxford, 1981.
10. *Masters C.J.* Curr. Top. Cell Regul., 1977, 12, p. 75.
11. *Ouporov I.V. et al.* J. Biophys., 2001, 80, p. 2527.
12. *Ovadi J.* J. Theor. Biol., 1991, 152, p. 1.
13. *Rapaport D. C.* The Art of Molecular Dynamics Simulation. Cambridge University Press, 1995.
14. *Remler D. K., Madden P. A.* Mol. Phys., 1990, 70, p. 921.
15. *Salerno C. et al.* Mechanism of Action and Regulation of Enzymes. Budapest, 1975.
16. *Schmidt K. E., Ceperley D.* Monte Carlo Methods in Condensed Matter Physics. Springer, Berlin, 1992.
17. *Tuckerman M. E., Parrinello M.,* J. Chem. Phys., 1994, 101, p. 1302.
18. *Wilson J. E.* Trends Biochem. Sci., 1978, 3, p. 124.
19. *Zhang E. et al.* Biochemistry, 1997, 36, p. 12526.