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Glycerol-3-Phosphate Dehydrogenase Isozymes: A Computational Approach to Their Metabolic Roles in the Flight Muscles of Triatoma Infestans

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Abstract: *Glycerol-3-phosphate dehydrogenase (GPDH) isoenzymes play different roles in Triatoma infestans flight metabolism and only differ in the C-terminal end. We studied with computational models their dynamics and interactions. Homology-modeling dynamics showed models close to minimal energy with amino acid distribution similar to crystallized structures. Radius of gyration decrease because the models presented well fitted folded structures with solvation free energy of non-polar groups increased with temperature. Heat maps suggested the need to form dimeric structures to preserve activity or that temperature could be an activity modulator. Modeled dimeric structures were similar to crystallized references and didn´t involve the C-terminals in their conformation. Docking studies of the C-terminals showed interaction between GPDH-1 and actin co-localized with glycolytic enzymes. GPDH-2 peptide could bind to model membranes and be substrate for lysine-acetyltransferases. Consequently, C-terminals could determine the sub-cellular localization and the metabolic functions in lipid biosynthesis or ATP generation.*

Keywords: *Docking, GPDH Isoforms, Localization, Molecular Modeling and Dynamics, Triatoma infestans_ -*

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I. Introduction

Chagas disease is one of the most serious human parasitic disease of Latin America with 12 million persons infected and 90 million living in endemic´s area [1]. The hematophagous insect Triatoma infestans (T. infestans) (Hemiptera; Reduviidae), is one of the main vectors between latitudes 10°S and 46°S. This insect has a nymphal period with five stages, each ending in a molt or ecdysis. Wings appear after the last molt, of the fifth instar nymphs to adult. Flying is important for dispersion and re-infestation of houses after insecticide spraying [2, 3]. The Glycerol-3-phosphate dehydrogenase (NAD^+-2-oxidorreductase, EC 1.1.1.8), present in all eukaryotic organisms, is homodimeric, soluble-cytosolic, and NAD-dependent enzyme that plays an important role in the metabolism of insect flight muscles[4, 5]. Several molecular forms showed specific tissue distributions during development [6]. In insects GPDH-1, is involved in the α-glycerophosphate cycle in flight muscle, and GPDH-2 and GPDH-3 provide precursors for lipid biosynthesis in gonads, fat bodies, abdomen, and thoracic muscles of larvae and nymphs [6, 7]. In T. infestans, GPDH total activity increases 30-fold in adult flight muscles [7]. Stroppa et al characterized two GPDH transcripts in T. infestans corresponding to GPDH-1 (Genebank: EU139315.1) and GPDH-2 (Genebank: EU139316.1). Both cDNAs differ only at the 3´end, and encode in the C-terminal of the deduced amino acid sequence for five or nine amino acids, respectively. This study, also demonstrated that GPDH isoforms presented a pattern of temporal expression, sex and tissue-specific, that changed in relation to temperature and the amount of intake [8].

In this work we report modeling and dynamics studies of the GPDH isozymes. Since flying is crucial for the spreading of this insect vector of Chagas disease and that the GPDH isozymes are particularly important in the flight muscle´s metabolism, these studies could be relevant to get a better understanding of how few different amino acids in the C-terminal ends of their sequences are determinant to accomplish their different metabolic functions and to indicate their sub-cellular localization.

II: Methodology

The novo and template-based isozymes´ modeling were performed on Phyre2 Web Server (http://sbg.bio.ic.ac.uk/phyre2) [9]. The models were analyzed and sorted by score values, and the pdb files were visualized using USFC Chimera and VMD software. Homology models were optimized through molecular dynamics simulations to understand their behavior in a physiological system. Initially, the structures were studied at fixed particle number, volume and energy (NVE), and root mean square deviations (RMSD) form the

initial structure, heat absorbing regions (heat maps) and Ramachandran plots were obtained with NAMD software [10]. Physiological system was simulated adding water, counter ions added just to neutralize the proteins, protonation state at pH 7.0 was set by PROPKA and temperature and pressure were maintained at 298 or 310 K and 1 bar. Once equilibrated, the models were heated to insect temperature and allowed to unrestricted movement with fixed pressure (NPT). Two sets of simulation were run independently with NAMD and GROMACS Simulation package with Gromos 96 forcefield and Berendsen thermostat [11, 12, 13]. Protein structure was monitored with RMSD tool and RMSD visualization tool from VMD and with the Gromacs complements for RMSD by amino acid, gyration radius (average radio from protein principal axis) and hydrophobic surface accessible to polar solvent.

Docking studies were hosted by three servers: Hex Protein Docking Server (based on energy and cluster size), the PatchDock server (with geometric shape complementarity based scoring) and Docking2 from Rosie the Rosetta online server (for energy based local docking) [14, 15, 16]. The sequences of both GPDH isoforms also were submitted to MCPep Server software to score the possible conformations and configurations of both C-terminal peptides and their interaction with model membranes, and to ASEB Web Server for KAT (Specific Acetylation Site Prediction) [17, 18].

III. Results And Discussion

The homology model showed monomers composed by two domains: N-terminal NAD-binding domain and C-terminal catalytic domain (Fig. 1a). The NAD-binding domain (residues 4–193) contains a canonical structure of six parallel β-sheets, each flanked with α-helices and with an extra pair of β-sheets in antiparallel locations, is a part of the Rossmann fold, a structure motif that binds dinucleotides (NAD). The first βαβ unit contains the conserved NAD-binding motif GxGxxG, conserved in all NAD-dependent GPDHs (Fig. 1 a, b) [19]. The catalytic domain (residues 193–355/8) presents exclusively ten α-helices, three of them particularly long (residues: 193–220; 225-243 and 299-313). The two domains are linked by two-residue loop. The Ramachandran plot of GPDH-1 showed 92.957 % of the amino acids in the allowed region (175 were in α-helix, 6 in random helix and 61 in β-turn), and for GPDH-2 the 95.25 % of the amino acids fall in the allowed regions (177 were in α-helix, 6 in random helix and 67 in β-turn). RMSD vs time averaged values in NVE were 1.2 Å for GPDH-1 and 0.55Å for GPDH-2, while in NPT RMSD by amino acid were 1.6 Å and 0.51 Å respectively, indicating that the homology models were close to minimal energy structure, and that GPDH-2 was more stable.

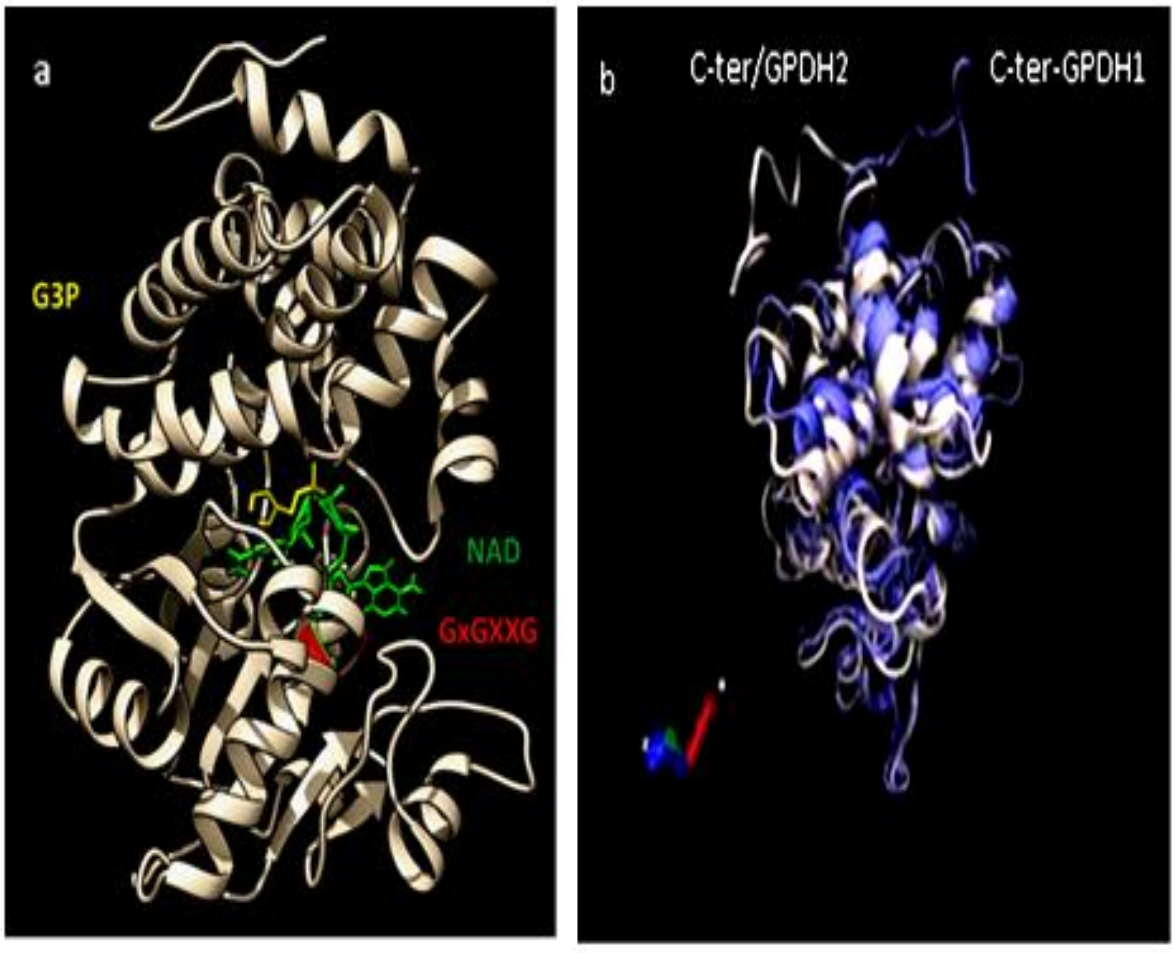
In NPT ensemble (run on GROMACS), the radius of gyration descended from 2.05 to 2.035 ± 0.005 nm for GPDH-1 and from 2.07 to 2.035 nm for GPDH-2 during transition to more fitted folded structures in line with hydrophobic solvent accessible surface area reduction from 102 to 94 ± 2 nm^2 in GPDH-1 and from 107 to 97± 2 nm^2 in GPDH-2, despite a heating processes that could expose non-polar residues. Hiding non-polar residues decreases free energy of hydration driven protein folding (ΔG_f) avoiding water molecules forming highly ordered structures (less entropy) to escape from non-polar phase [20]. The models obtained were optimal because heating at physiological temperature lead to a better fit folded state hiding the non-polar groups. Heat maps showed that N-terminal, the C-terminal ends (six or nine final amino acids) and NAD-binding domain absorbed more heat (Fig. 1c). The fact that the NAD-binding domain is a heat sensitive region could indicate the need of a dimeric structure to preserve activity and that temperature could be an important modulator [19, 21]. Dimers generated in three servers were: both proteins make contact along the mayor axis, with the N- and C-terminal domains facing similar domains of the counterpart and the NAD-binding cavity facing outwards; both monomers with the C-terminal domain of one facing to the N-terminus of the other (anti-parallel) and the NAD-binding cavity facing outwards. Both structures were of equal biological importance since they were assigned -588.176 and 589.441 rosetta energy units (reu) respectively. A third structure with -586.94 reu had both monomers facing each other only by their C-terminal domains (Fig. 1d), in close similarity to GPDH from Leishmania mexicana that was crystallized with twofold symmetry, T. infestans´ GPDH C-terminal coils were not involved in the dimeric interactions.

To test if C-terminal peptides might determine different sub-cellular localizations, C-terminal peptides of T. infestans GPDH isozymes, Drosophila´s GPDH-1B (P13706-2, UniProtKB) and aldolase (1FBA: A|PDBID) sequences were docked in silico with PatchDock to Drosophila actin (1RDW:X|PDB ID) modeled within Swiss Model Workspace [22] (Fig 2). Among the best scores were two situations, a) the binding of T. infestans C-terminal of GPDH-1, the C-terminal of GPDH-1B and aldolase from Drosophila to a wide cavity in the actin monomer nearby to actins' ATP-binding site (between the α-helix curl, from valine 201 to glutamic 205, and the β-strand that begins with leucine 65 threonine 66); and b) T. infestans´ C-terminal of GPDH-2 that was placed near to actin polymerizing region (serine 368 to phenylalanine 375). These results support the hypothesis that GPDH-1 may be more efficient binding to actin and locally reoxidizing $NADH_2$ from glycolysis. Wojtas et al demonstrated in D. melanogaster the co-localization of GPDH with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase at the Z-discs and M-lines [23]. Transgenetic Drosophila with GPDH-1B lacking C-terminal three amino acids (Q-N-L) in thoracic muscles, were unable to fly and GPDH, GAPDH and

aldolase remained unbound to myofibrils while preserving the enzymatic activities [23, 24]. The docking study with titin-myomesin complex (present at Z-discs and M-lines) showed unspecific binding for both T. infestans isoforms but in GPDH-1 the C-terminal residues were free, while GPDH-2 had the C-terminal residues buried into the docking site (Fig 3a) GPDH-2 C terminal lysine content made it candidate to bind membranes so both C-terminal were analyzed in MCPep server that returns the △G of partition to model plasma membranes. The score revealed that while the C-terminal of GPDH-1 remained perpendicular in water phase, GPDH-2 peptide was partially immersed or in parallel to the membrane plane (Fig 3 b, c, d) suggesting its preference for lipid vacuoles [7, 25] and a central role in lipid metabolism. In this line of evidence, exploratory Steered Molecular Dynamics [26] results showed GPDH-2 alone retains the bound state. Both GPDH 1 and 2 from T.infestans were placed at 4 Å from a DOPC membrane in 0,15 M NaCl and pulled away from the membrane at 10 Å/2.5 ns applying force on amino acid 349. GPDH-1 was fully displaced while GPDH-2 conserved Lysine 357 in the proximity of the membrane. These behaviors were reflected in the total energy profile for the last amino acids, 353-355 with increasing total energy for GPDH-1 and Lysine 357 remaining almost constant for GPDH-2, through the whole trajectory (Fig 4 a and b).

The presence of three lysine residues in the C-terminal coil of GPDH-2 opened the possibility of differential regulation by acetylation. Lysine 353 of GPDH-2 scored as the best acetylation site (CBP/p300 > GCN5/PCAF; KAT) in ASEB Web Server). Recent studies have revealed that acetylation modulates nuclear proteins but also cytoplasmic proteins, including GPDH and many metabolic enzymes.

IV. Figures And Tables



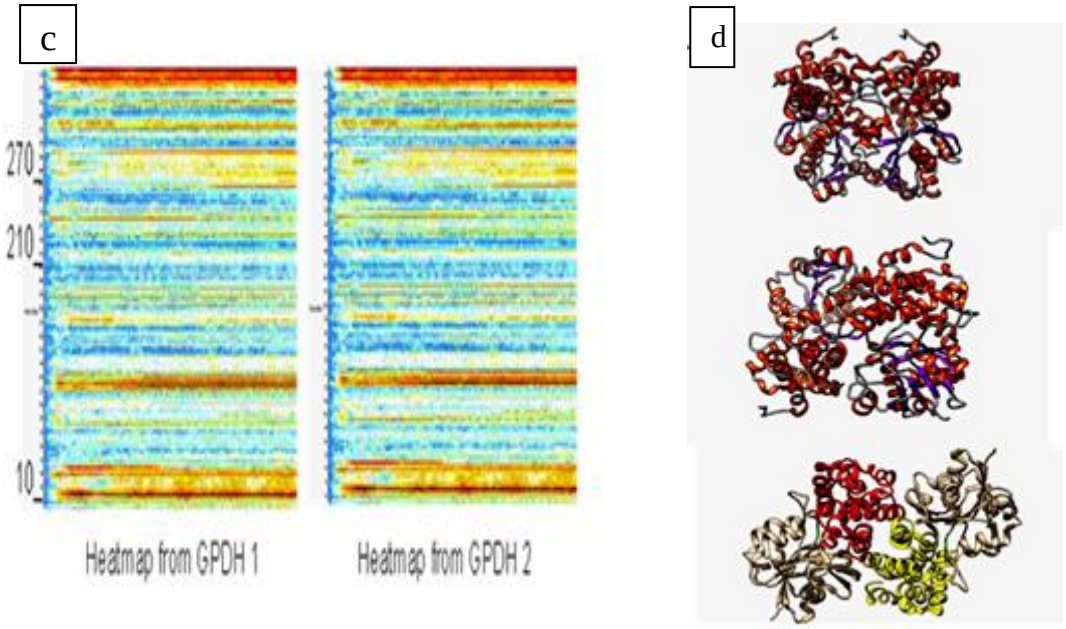


Figure 1. Features of Triatoma infestans´ GPDH-1 and GPDH-2 isoforms models: a) GPDH-1 model showing best glycerol-3-phosphate and NAD-binding configurations, GxGxxG consensus peptide and conserved β sheet domain. b) View of GPDH-1 and GPDH-2 showing their high similarity and exposing the different configuration of their C-terminal ends, c) Heat-maps of GPDH-1 and GPDH-2, hot regions are indicated in orange. The position of certain amino acids is indicated; d) Best scored structures for the dimers.

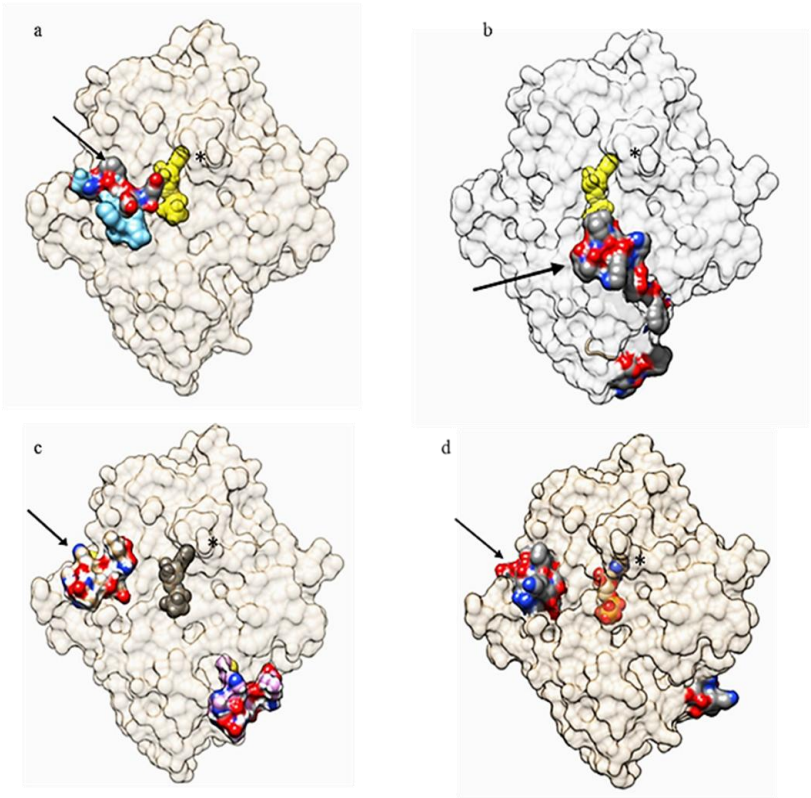


Figure 2. Docking of the C-terminal peptides from different proteins the interaction with Beta-actin. a) GPDH-1 peptide from T. infestans, b) GPDH-2 peptide from T. infestans c) GPDH-1B C-terminal peptide experimentally found to be necessary for the interaction with flying muscle fiber in Drosophila, d) Aldolase peptide from D. melanogaster that was experimentally shown to bind actin through a pocket of low score. The best and lowest score positions are depicted for each peptide and the arrow points to the best scored position. ATP (*) is depicted in the center of actin monomer.

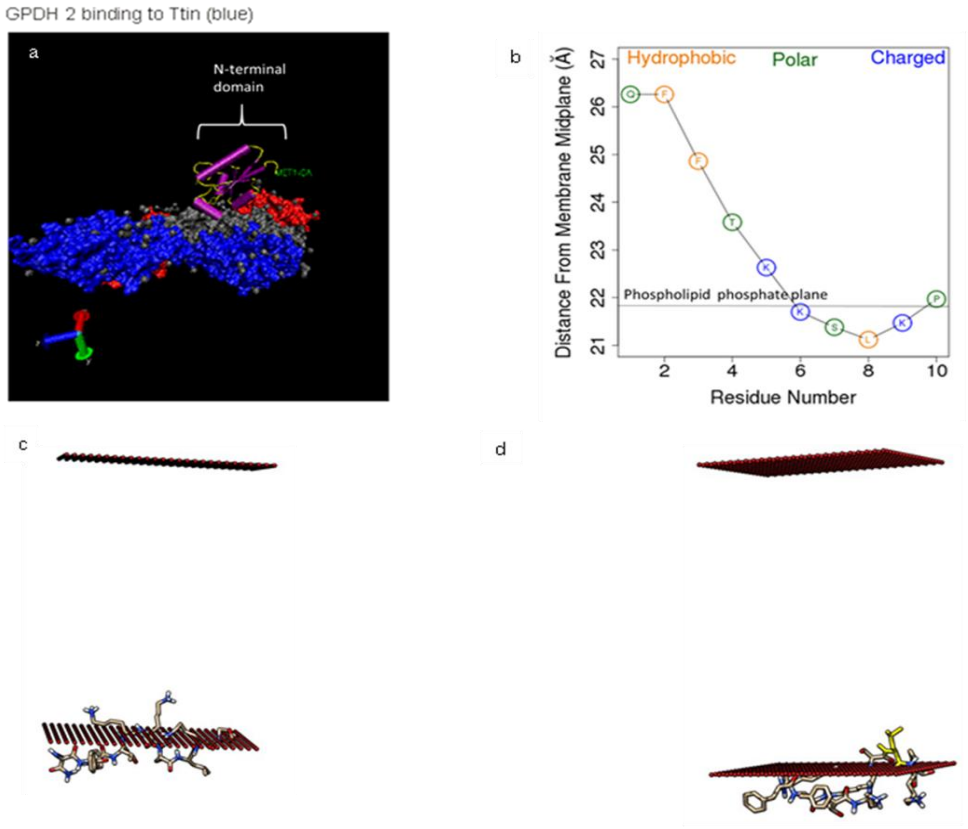


Figure 3. Docking Models: a) GPDH-2 interaction with titin-miomesin complex is unspecific but in mid-score configurations the C-terminal peptide is fully buried. b) Distance profile by amino acid for the GPDH-2 peptide near a modeled membrane c) & d) two of the best scored structures showing that GPDH-2 peptide is partially immersed in the modeled membrane.

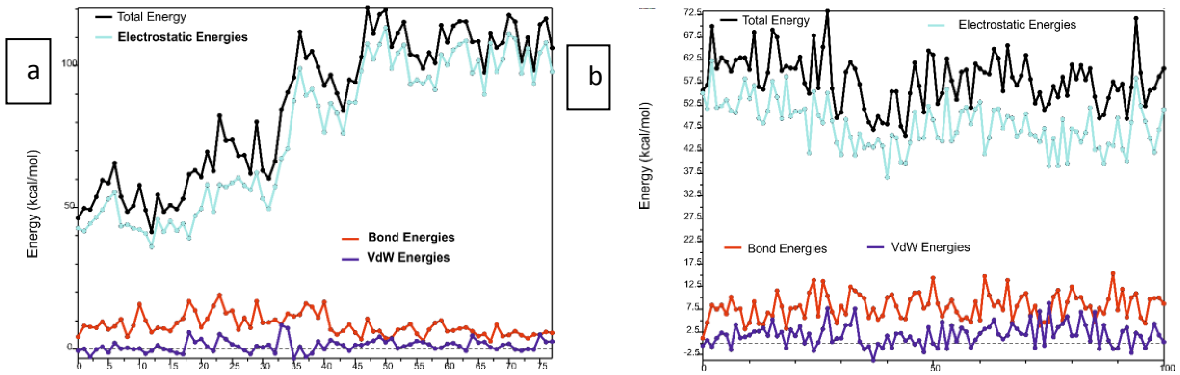


Figure 4: Energy profile during SMD of C-terminal final amino acids of a) GPDH-1 aa 353-355, and GPDH-2 aa 357. Both protein models were in silico placed in a 0.15 M NaCl solution with amino acids 349 to the last ones of the C-terminal peptides at 4 Å from DOPC modeled membrane. After equilibration a force was applied to amino acid 349 of each model to pull them away from the membrane. GPDH-1 amino acids 353-355 increased total energy because membrane interaction was lost while amino acid 357 of GPDH-2 conserved an almost constant energy through all the time the force was applied since interaction with the model membrane was also conserved.

V. Conclusion

These results are in accord with previous experimental results and provide support for the hypothesis that the difference of C-terminal coils among both isoforms is not merely a residual splicing mechanism. Thus GPDH-1 preferentially binding to actin, co-localizing with glycolytic enzymes might contribute with ATP generation to support flight activity, and GPDH-2 with a stable binding to membranes like those of vacuoles or lipid droplets, could take part in lipid biosynthesis and be regulated by acetylation.

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References

[1]. World Health Organization, 2013. Geneva: WHO. Chagas disease (American trypanosomiasis). WHO Media centre: Fact sheet N° 340.
[2]. Carcavallo RU, Jurberg J, Galindez Giron I, Lent H, 1997. Atlas of Chagas' Disease Vector in the Americas, Rio de Janeiro: Editora Fiocruz.
[3]. MC Cecere, RE Gürtler, DM Canale, R Chuit, JE Cohen. Effects of partial housing improvement and insecticide spraying on the reinfestation dynamics of Triatoma infestans in rural northwestern Argentina. Acta Trop 84, 2004, 101-116.
[4]. GC Bewley, JL Cook. Molecular structure, developmental regulation, and evolution of the gene encoding glycerol-3-phosphate dehydrogenase isozymes in Drosophila melanogaster. Isozyme 3, 1990, 341-374.
[5]. SJ O´ Brien and RJ MacIntyre. The alpha-glycerophosphate cycle in Drosophila melanogaster. Genet Aspects Genet. 71, 1972, 127–138.
[6]. JL Cook, GC Bewley, JB Shaffer. Drosophila α-glycerol-3-phosphate dehydrogenase isozymes are generated by alternate pathways of RNA processing resulting in different carboxyl-terminal amino acid sequence. J Biol Chem 263, 1988, 10858-10864.
[7]. P Scaraffia, S Remedi, C Maldonado, A Aoki and NM Gerez de Burgos. Comparative enzymatic and ultrastructural changes in thoracic muscles of Triatomine insects during the last stage of metamorphosis. Biochem Physiol 116, 1997, 173–179.
[8]. MM Stroppa, C Carriazo, N Soria, R Pereira, NM Gerez de Burgos. Differential tissue and flight developmental expression of glycerol-3-phosphate dehydrogenase isozymes in the Chagas disease vector Triatoma infestans. Am J Trop Med Hyg 79, 2008, 28-35.
[9]. LA Kelley and JE Sternberg. Protein structure prediction on the web: a case study using the Phyre server. Nature Protocols 4, 2009, 363 – 371.
[10]. JC Phillips, R Braun, W Wang, J Gumbart, E Tajkhorshid, E Villa, C Chipot, RD Skeel, L Kale, and K Schulten. Scalable molecular dynamics with NAMD. J Comput Chem 26, 2005, 1781-1802.
[11]. SD Van Der, L Erik, H Berk, G Gerrit, EM Alan, JCB Herman. GROMACS: fast, flexible, and free. J Comput Chem 26, 2005, 1701-1718.
[12]. LD Schuler, X Daura, WF Van Gunsteren. An Improved GROMOS96 Force Field for Aliphatic Hydrocarbons in the Condensed Phase. J Comput Chem 22 (11), 2001, 1205-1218.
[13]. B Hess, H Bekker, HJC Berendsen, JGEM Fraaije. LINCS: a linear constraint solver for molecular simulations. J Chem Theory Comput 18, 1997, 1463–1472.
[14]. G Macindoe, L Mavridis, V Venkatraman, M Devignes., DW Ritchie. HexServer: an FFT-based protein docking server powered by graphics processors. Nucl Acids Res 38, 2010, W445-W449.
[15]. D Schneidman-Duhovny, Y Inbar, R Nussinov, HJ Wolfson. PatchDock and SymmDock: servers for rigid and symmetric docking. Nucl Acids Res 33, 2005, W363-367.
[16]. S Chaudhury, M Berrondo, BD Weitzner, P Muthu, H Bergman, JJ Gray. Benchmarking and analysis of protein docking performance in Rosetta v3.2. PLoS One 6(8), 2011e22477. doi: 10.1371/journal.pone.0022477. Epub 2011 Aug 2. Online
[17]. Y Gofman, T Haliloglu, and N Ben-Tal. Monte Carlo simulations of peptide-membrane interactions with the MCPep web-server**.** Nucleic Acids Res 40(W1), 2012, W358-W363**.**
[18]. L Wang, Y Du, M Lu, T Li. ASEB: a web server for KAT-specific acetylation site prediction. Nucleic Acids Res 40 (Web Server issue), 2012, W376-9. doi: 10.1093/nar/gks437
[19]. S Marche, PA Michels, FR Opperdoes. Comparative study of Leishmania mexicana and Trypanosoma brucei NAD-dependent glycerol-3-phosphate dehydrogenase. Molec Biochem Parasitol 106, 2000, 83–91
[20]. SL Taylor, R Evans and C P Royall. Temperature as an external field for colloid–polymer mixtures: 'quenching' by heating and 'melting' by cooling. J Phys Condens Matter 24, 2012, 464128 (8pp).
[21]. LG Neven. Physiological responses of insects to heat. Postharvest Biol and Tech 21, 2000 103-111.
[22]. T Schwede, J Kopp, N Guex, MC Peitsch. SWISS-MODEL: an automated protein homology-modeling server. Nucleic Acid Res 31, 2003, 3381–3385.
[23]. K Wojtas, N Slepecky, L von Kalm, D Sullivan. Flight muscle function in Drosophila requires co-localization of glycolytic enzymes. Mol Biol Cell. 8, 1997, 1665–1675.
[24]. DT Sullivan, R MacIntyre, N Fuda, J Fiori, J Barrilla and L Ramizel. Analysis of glycolytic enzyme co-localization in Drosophila flight muscle. J Exp Biol, 206, 2003, 2031-2038.
[25]. PY Scaraffia , C Maldonado , A Aoki , NM Gerez De Burgos. Comparative study of enzymes in testes and ovaries from adult Dipetalogaster maximus (Uhler) and triatoma infestans (Klug) (Hemiptera: Reduviidae). Correlation with fine structural organization. Eur J Histochem 45(3), 2001, 295-303.
[26]. D.L Guzmán., J.T Roland., H Keer., Y.P Kong., T Ritz, A Yee,. and Z.Guan. Using steered molecular dynamics simulations and single-molecule force spectroscopy to guide the rational design of biomimetic modular polymeric materials. Polymer (Guildf) ; 49(18), 2008, 3892–3901

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