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A bioinformatics characterization of the human glycogen phosphorylase surface binding regions

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Abstract

This study performs a structural bioinformatics characterization of the glycogen phosphorylase protein in humans, revealing a high sequence and structure homology between the liver and muscle types. These enzymes can exist as inactive monomers or tetramers, but they are biologically active as homo-dimers. The two proteins have many allosteric sites and their known ligands are small molecules with both hydrophobic and hydrophylic regions. The ligands bind to cavities situated into one or both chains of the homo-dimer, interacting with the two chains by electrostatic and hydrophobic interactions. Knowledge of the structural features of identified effectors of glycogen phosporylase could be of help to design new allosteric inhibitors of this protein, with positive effect both for type 2 diabetes treatment and for preventing muscle glycogenolysis.

Keywords: sequence comparison, cavity prediction, surface fractality

Introduction

Muscle glycogen is the primary carbohydrate fuel for most types of physical exercises and it is degraded fast during high intensity exercises. Glycogen phosporylase (GP) is a key enzyme that regulates muscle glycogen degradation during glycogenolysis, because catalyses the degradative phosphorylation of glycogen to glucose-1-phosphate (G-1-P), the initial step in glycolysis [1]

$$(\text{Glycogen})_n + P_i \leftrightarrow (\text{Glycogen})_{n-1} + \text{glucose-1-phosphate}$$

From a structural point of view, GP can be a dimer or a tetramer form, but it is found to be mostly in the dimer forms *in vivo*, with two interconvertible forms in the dimer, GPa and GPb [1]. GP is an allosteric enzyme and has two conformations: an inactive (T state) or an active (R state) state, the transition between them being done by the phosphorylation of SER 14 or by the ATP binding [2]. The GPb is predominantly in T state and GPa is predominantly in R state. In resting muscle, GP is in the T state and it is inhibited by this state ligands, G-1-P, ADP and ATP [2] and it is activated by the AMP and certain analogues of 5-AMP.

Being an allosteric enzyme, GP is controlled by allosteric effectors interacting at specific sites and favoring a particular conformation and it is already known that it contains at least 6 potentially regulatory sites [3].

Because of its role in the glycogen metabolism, this protein can be interesting for bioinformatics and drug design studies concerning finding compounds able to prevent

unwanted glycogenolysis under high glucose concentrations and that may be relevant for the diabetes control. The glycogen itself, AMP, ADP, ATP, G-1-P and purine compounds (caffeine, nucleosides, flavonoids) are already known as GP effectors [4]. Also, many pharmacological inhibitors of GP have been developed in order to attenuate hyperglycemia associated with type 2 diabetes [5]. Major problem is that these inhibitors do not show specificity and they also may act to the muscle type GP, limiting muscle glycogen degradation and having muscular and cardiac effects [5]. So, it could be of high interest to find some GP inhibitors with high specificity for liver type GP. From this point of view, the bioinformatics structural characterization of the muscle type GP by comparison to the liver type GP as well as of their known effectors could be of great importance for further drug design studies, concerning GP inhibitors for diabetes treatment.

The key factors in protein-ligand interactions are the shape and chemical properties of protein's surface. Usually it is irregular, contains many clefts and groves of varying sizes and shapes, with particular importance in certain types of interactions and the surface properties of GP protein are the subject of this study.

Materials and Methods

There are three types of glycogen phosphorylase enzyme in humans: the brain, the liver and the muscle type. The liver type is involved in type 2 diabetes disease and the muscle type is associated to physical effort. An initial step in our analysis was the sequence alignment of the two types of GP in order to analyze if there are significant differences at this level. The sequence alignment was made using the free on-line accessible tool [6].

In addition to the sequence analysis structures superimposition using PyMol software was performed [7]. In order to obtain structural information concerning these proteins and their effectors the Protein Data Bank was used (http://www.rcsb.org/pdb/) [8] and the following structural files have been considered: 1z8d [9] for the muscle type of GP and 1em6 [10], 1fc0, 1l5q, 1l7x, 1xoi, 2zb2, 2ati, 3ceh and 3dd1 for the liver type.

Usually for ligand binding to a protein, its surface properties are important, especially the global and the local surface roughness added to the cavities sizes, shapes and polarities [11]. Also, these properties are sensitive to the conformational changes arising in proteins upon ligand binding [12]. There is a free accessible on-line tool to predict the protein surface cavities, Fpocket [13]. We have used it in order to obtain the cavities of GP molecule.

However, many functional sites in proteins are not concave and they cannot be identified by developed computational techniques and in this situation, other criteria must be applied, such as surface roughness. As a measure of the protein surface roughness, the surface fractal dimension was successfully used in literature [14]. So, we calculated the local and global surface fractal dimensions for the two proteins in complexes with effectors using the on-line tool GETAREA [15]. The surface fractal dimension was determined using the scaling law between the surface area *(SA)* of the protein and the radius of the rolling probe molecule *(R)*

$$SA \sim R^{2-D_s}$$

from the slope of the double logarithmical plot of *SA* versus *R* [16]. Surface area of the protein has been calculated using the probe radii of 1, 1.2, 1.4, 1.6, 1.8 and 2 Å respectively. In the case of dimmers, the surface fractal dimension has been calculated for the monomeric subunit A.

Results and Discussions

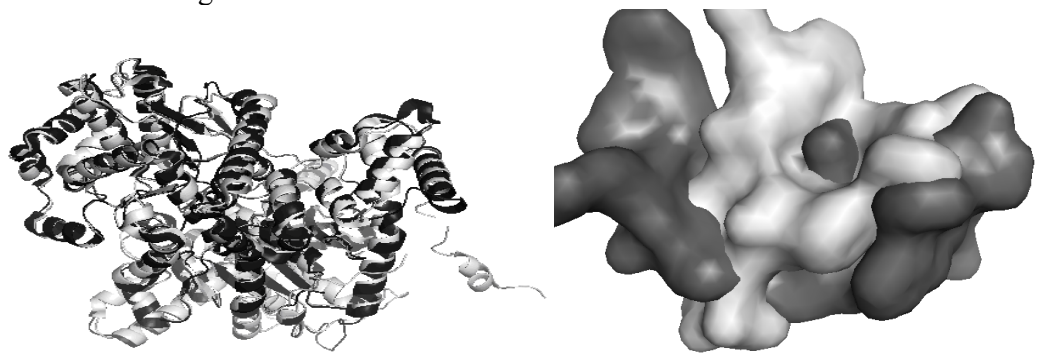
The sequence similarity between the muscle and liver types of GP is 79% indicating a very high sequence homology. The alignment result is presented in Figure 1.

Fig 1. Sequence alignment of the muscle and liver types of glycogen phosphorylase

There are only two small regions presenting low sequence similarity between the two types of human GP: the region 319–326 (which is highlighted in Figure 1) and the ending region of C-terminal domain, 835-end. In the spatial structures of the two types of GPs the 319-326 regions are unstructured and situated at the protein surfaces.

The superimposition of the A chain structures for the liver (1EM6) and muscle type (1Z8D) GP is presented in Figure 2.a. The high degree of the sequence similarity between the two types of GP is also reflected in the structural homology. Even if every structure has distinct ligands, the structural homology reflects that their binding only causes small local

rearrangements in the protein structures. It is not surprising if we take into account the small size of registered ligands and the fact that they usually bind into small cavities situated at the protein surface [11]. Zooms into the spatial structures of the two proteins in the regions 319-326 are shown in Figure 2.b. We noticed that there is a significant difference in the shape of the surfaces of two regions.

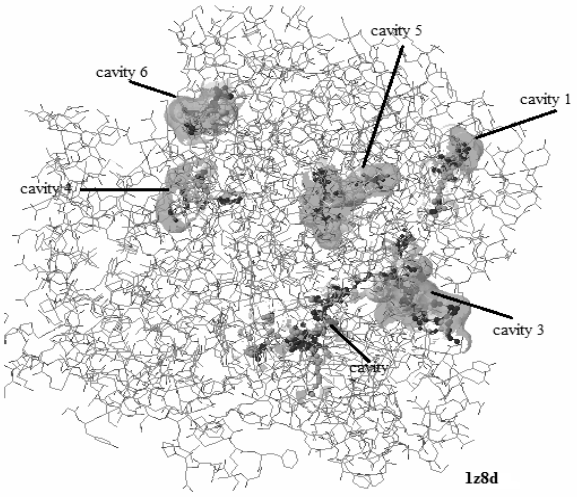


(a) (b)

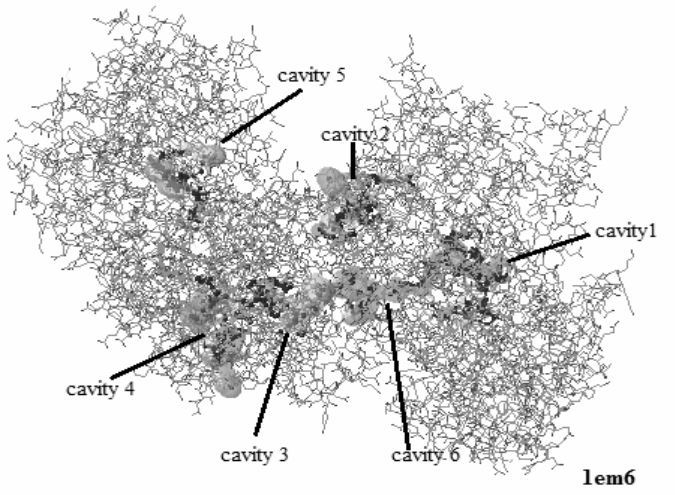
Fig. 2. The superimposition of the structures of muscle (light grey) and liver (dark grey) types of human glycogen phosphorylase (a) and zoom into the region 316-326 for the two superimposed proteins (b)

The cavities predicted by Fpocket tool are ranked according to their ability to bind small molecules such as sugar molecules, cofactors or drug-like molecules, but their ranking does not reflect drugability. The properties of 6 predicted cavities and corresponding to the binding sites of some of the identified ligands for both the muscle [9] (a total of 43 cavities for 1z8d) and liver [10] (a total 47 cavities for 1em6) types of GP molecule are presented in the Table 1, and they are also visualized in Figures 3.a and 3.b. In Table 1 we show the volume, the amino acids that are involved and the hydrophobicity score (H) of every cavity. This hydrophobicity score is the mean of hydrophobicity scores of all residues in the cavity and reveal the overall hydrophobic or hydrophilic character of the cavity. The values presented in table 1 for the hydrophobicity score show that most of the cavities considered for analysis are hydrophobic.

The big number of identified cavities for glycogen phosphorylase may be explained by the fact that this protein presents internal channels that can be seen as a sum of small cavities. It was observed that the largest cleft of GP is made by a series of deep interconnected grooves in the exterior of the protein spanning most of its surface [17].



(a)



(b)

Fig. 3. Visualization of the predicted cavities (light gray surface) at the surface of the muscle (a) and liver type (b) glycogen phosphorylase. Prediction has been made using Fpocket tool and the involved amino acids are presented in dark gray.

The global surface fractal dimensions for the two molecules when they bind their effectors are presented in Table 2 and the dimensional properties of their ligands are presented in Table 3. We notice similar global fractal dimensions for all the investigated files being in good agreement with other published data concerning globular proteins showing surface fractal dimensions comprised in the interval (2.17–2.42) [16]. Such a result suggests similar folding mechanisms for the two proteins resulting in homolog structural properties.

Table 1. Properties of identified cavities of muscle and liver types of glycogenphosporylase

Protein	Cavity	Involved amino acids	V (Å ^{3})	H. score
Muscle type GP	Cavity 1	ARG 60, VAL 64, TRP 67, ILE 68, LYS 191, ARG 193, ASP 227, THR 228, PRO 229, THR 240	549.67	16.28
	Cavity 2	HIS 582, THR 585, LEU 586, ARG 589, ARG 592, GLU 593, LYS 596, PHE 597, PHE 598, VAL 599, ARG 601, GLU 737, ILE 781, GLN 784, GLU 785, SER 788, LYS 792,	675.88	7.12
	Cavity 3	Val 15, ARG 16, GLY 17, VAL 21, GLU 22, ASP 61, HIS 62, GLY 65, ARG 66, ARG 69, PRO 835, ALA 836	535.34	3.34
	Cavity 4	VAL 452, ASN 453, GLY 454, SER 460, LEU 463, LYS 464, PHE 468, PHE 479, GLN 480, ASN 481,	154.71	28.36
	Cavity 5	ASP 339, THR 340, THR 374, ASN 376, HIS 377, THR 378, PRO 381, ALA 383, LEU 384, GLU 385, ARG 386, ASN 440, ALA 442, ILE 467, PHE 468	356.65	14.22
	Cavity 6	PHE 163, GLY 164, PHE 166, GLU 177, GLU 178, ALA 179, ASP 180, VAL 278, LEU 279, PRO 281, PRO 611,	714.29	33.94
Liver type GP	Cavity 1	A: PHE 37, THR 38, VAL 40, B: ARG 60, ASP 61, LEU 63, VAL 64, TRP 67, PRO 188, TRP 189, GLU 190, LYS 191, ASP 227, PRO 229,	220.31	36.73
	Cavity2	A: GLY 260, ASP 261, TYR 262, ILE 263, B: PRO 281, ASN 282, ASP 283, ASN 284, PHE 285, GLU 287, GLU 382, HIS 571, GLU 572, ALA 610, PRO 611, GLY 612, TYR 613, ARG 770,	790.51	2.05
	Cavity3	A: ARG 93, LEU 95, GLN 96, ASN 97, GLU 120, GLU 123, GLU 124, GLU 126, ARG 490, LEU 494, CYS 495, PRO 497, ASN 541, LYS 544, SER 651, GLU 654, LYS 655,	591.22	-2.74
	Cavity4	B: ASN 496, LEU 499, LEU 534, ALA 535, VAL 537, LYS 538, GLN 539, ASN 541, LYS 542, ASP 661, ASN 684, GLY 685,	597.14	36.38

		TYR 791, PRO 794, TRP 797, ASN 798, VAL 801, ILE 805,		
	Cavity5	B: ARG 66, ARG 69, THR 70, HIS 73, TYR 74, LYS 77, PRO 79, LYS 80, GLY 151, LEU 152, ALA 153, ASN 235, ASN 236, THR 237, ASN 826, VAL 827, GLU 828, SER 830,	1173.34	8.82
	Cavity6	B: ILE 170, ASP 172, GLY 173, TYR 553, VAL 555, THR 624, ALA 627, ASP 628, ASN 631, VAL 642, ILE 643, PHE 644, GLU 646,	453.27	18.97

Table 2. Global surface fractal dimensions for muscle and liver types of GP in complex with their effectors.

PDB entry code	Global surface fractal dimension
1Z8D	2.39±0.02
1EM6	2.39±0.01
3DD1	2.40±0.01
1FC0	2.40±0.02
1L5Q	2.40±0.01
1L7X	2.40±0.01
1XOI	2.41±0.01
2ZB2	2.39±0.01
3CEH	2.41±0.02

Despite their similar global roughness, the two investigated GP proteins may have distinct local fractal properties. For the biggest identified cavities of muscle and liver types of human GP, the local surface fractal dimensions are 2.62±0.17 and 2.61±0.19, respectively. It means that cavities having different volumes may also have distinct fractal dimensions in comparison to the entire protein. This result, added to distinct hydrophobicity scores of cavities, explains the binding possibility of ligands possessing different geometric and physicochemical properties, such as presented in Table 3.

Table 3. Dimensional properties of registered inhibitors of the human GP

Inhibitor*	Volume ($Å^3$)	Accessible solvent surface ($Å^2$)
AMP	257.6	489.9
GLC	134.8	308.5
ADE	104.9	273.8
NBG	176.3	389.7
PLP	178.9	395.8
CP4	424.9	817.9
MPD	117.7	287.2
CFF	162.1	355.8
IHU	273.6	518.9
700	363.9	645.9
288	316.5	603.4
MES	155.3	348.6
MRD	118.5	284.9
A46	268.3	586.4

**A*MP – adenosine monophosphate, GLC alpha-D-glucose, ADE adenine, NBG 1-N-acetyl-beta-D- glucoseamine, PLP pyridoxal-5'-phosphate, CP4 bis [5-chloro-1H-indol-2-YL -carbonyl-aminoethyl]-ethylene-glycol, MPD (4R)-2-methylpentane- 2,4-diol, CFF cafeine, IHU N-(2-chloro-4- fluorbenzenoyl)-N'-5-hydroxy-2 methoxyphenyl)urea, 700 5-chloro- 1H-indol-2-carbonyl phenylalaninyl, 288 5-chloro-1H-indol -2-carboxylicacid-[cyclopentyl-(2-hydroxyethyl)-carbamoyl]-methyl}-amide, MES 2-(N-mofpholino)-ethanesulfonic acid, MRD(4S)-2-methyl-2,4-pentanediol, A46 5-chloro-N-{4-[(1R)-1,2- dihydroxyethyl]phenyl}-1h-indole-2-carboxamine,*

The analysis of the results presented in Table 3 reveals small ligands for GP protein. Also, analysis of the binding sites composition reveals that GLC, ADE, NBG, IHU, MES, CFF usually interact with hydrophobic residues and the other ligands interact both with charged and hydrophobic residues.

Conclusions

The present study performed a structural bioinformatics analysis of muscle and liver types of GP structures. Knowledge of the structural features of the protein and its surface in addition to those concerning the ligands sizes and properties could be a starting point for the design of new allosteric inhibitors with high specificity for liver GP.

We have identified a short region, 316-326, of muscle and liver types of GP, significantly differing in sequence. It is known that the 313-326 loop of muscle GP contributes to AMP binding being disordered and in the T state and ordered around AMP in R state [10]. This loop has a different conformation in liver GP that may explain why liver GP is not activated by AMP [18]. The significantly difference in the sequences of the region 316-323 of the two types of human GPs results in a more hydrophobic, less flexible and a higher percent of buried side chains region in liver type than in muscle type of GP. These differences may also be responsible for distinct kinetic properties [10] of the two types of GPs. The fact that the mentioned regions are disposed at the proteins surfaces opens the possibility to search for distinct cavities related to these regions, allowing specific drug-like ligands for every type of GP protein.

Mapping of the ligand-binding sites in GP protein identifies the hotspots for ligand interactions and the complementary information obtained within this study can be used for an efficient search for new possible allosteric effectors. This observation is in good agreement with the recently obtained results showing that ligand-binding cavity variations can complement information on protein similarity [11].

The results presented in this study added to those already existing in specific literature make GP as a promising target for further drug-design studies and discoveries.

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