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CHARACTERIZATION OF 6-MERCAPTOPURINE BINDING SITE ON HUMAN α_1-ACID GLYCOPROTEIN (OROSOMUCOID) USING MOLECULAR DOCKING*

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6-Mercaptopurine (Purinethol, 6-MP), synthetic analogue of the natural purine bases adenine and hypoxanthine, is a drug used in the maintenance therapy of acute lymphatic (lymphocytic, lymphoblastic) leukemia. 6-MP has also been used in immunosuppressive therapy and in the treatment of steroid-unresponsive inflammatory bowel disease (1). The interaction of 6-MP with serum proteins has not been characterized in detail. Little is known of the binding of the 6-MP with human serum albumin and α_1-acid glycoprotein (AGP), which plays an important role in the binding of numerous drugs and their transport, mainly as a complex to regions remote from the site of administration. Clinical studies have shown that the absorption of an oral dose of 6-MP in humans is incomplete and variable, and associated with a half-life of 20 to 45 min in plasma, where its binding to serum proteins is averaging approximately 30% of the administered dose (2).

The 6-MP is a highly biologically active organic compound and therefore the phenomenon of tautomerism in its structure has been the subject of several investigations (3–6). It is very important from clinical perspective to understand the mechanism of action of 6-MP with various biomolecules. The 6-MP molecule may exist in eight tautomeric forms. The protropic tautomerism can occur in imidazole ring (N(7)H, N(9) N(7), N(9)H) and pyrimidine ring (N(1)H, N(3) $\leftrightarrow$ N(1), N(3)H), and thione-thiol tautomerism can occur in pyrimidine ring (–N(1)H, C(6)=S $\leftrightarrow$ N(1)=, C(6)SH) (3). Thus, two types of equilibria can be observed: thione $\leftrightarrow$ thiol and N(7)H $\leftrightarrow$ N(9)H. The thione forms dominate over the thiol forms in aqueous solution. The predominant species in aqueous solutions is thione-N(7) tautomer (6-MP (1,7)). However, the remaining tautomers can also exist in appreciable amounts at room temperature (5). Additionally, in solution at physiological pH 7.4, 6-MP occurs as a mixture of the neutral and anionic form (40.3% ionized form) owing to the fact that its pK_a value is 7.72. The 1-NH group is more acidic than the NH-group in the imidazole ring and therefore is expected to ionize first (4).

The computational simulation by molecular docking procedure may be used for the prediction of interactions between ligand and protein. However, the issue of considering tautomerism in small molecules in ligand–protein interactions is often disregarded and commonly omitted. Tautomeric forms of a molecule may differ in physical–chemical properties and the complementarity pattern towards the targeted protein. Hence, it may have significant impact on the prediction of the ligand binding using docking technique (7).

In this work, the interaction of 6-MP with α_1-acid glycoprotein (AGP) was studied by molecular docking. In order to gain more detailed information about the mechanism of the 6-MP binding to AGP, the docking procedure was performed at physiological pH 7.4 accounting for three tautomeric species of 6-MP and its ionized form.

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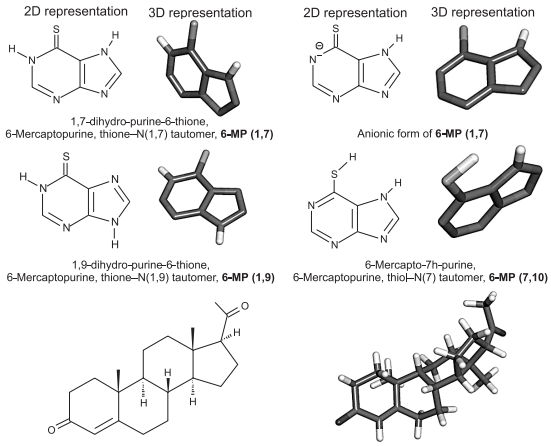

Figure 1. Chemical structures of the 6-thiopurines and progesterone

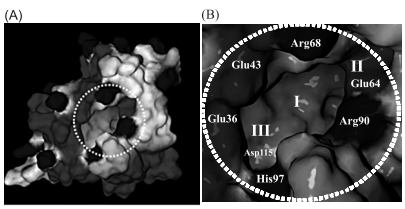

Figure 2. (A) Three-dimensional structure of AGP represented by electrostatic surface, with its ligand-binding site (the binding site is marked with a white circle); (B) The enlarged area corresponds to the ligand-binding site in the AGP structure. The lightest-grey and the darkest-grey regions correspond to the extreme values of the negative and positive electrostatic potential

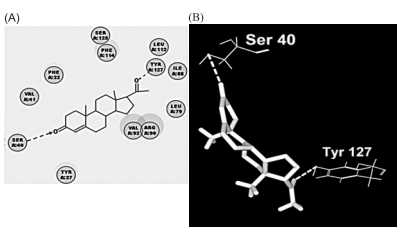

Figure 3. The interaction model between PRG and AGP. (A) Schematic (2D) representation of PRG–AGP interaction in the binding site (CAV) of the protein; only residues around 6 Å of the ligand are displayed; (B) The predicted hydrogen bonds between PRG and amino acid residues at the active site of AGP. PRG is shown in a stick and amino acids are shown in a thin stick; the hydrogen bonds are represented using dashed lines

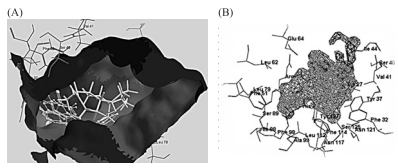

Figure 4. (A) Hydrophobic surface representation of the binding site, with PRG and 6-MP molecules located inside the binding site; (B) Graphical representation of the orientation of the PRG–binding site (CAV) in AGP structure; only residues around 6 Å of the CAV are displayed. PRG is shown in a stick and 6-MP molecules are shown in a ball and stick model; the CAV is depicted in black mesh

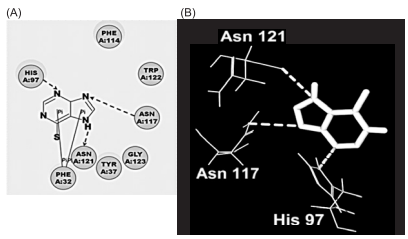

Figure 5. The interaction model between 6-MP (1,7) and AGP. (A) Schematic (2D) representation of 6-MP (1,7)–AGP interaction in the binding site (CAV) of the protein; only residues around 6 Å of the ligand are displayed; (B) The predicted hydrogen bonds between 6-MP (1,7) and amino acid residues at the active site of AGP. 6-MP (1,7) is shown in a stick and amino acids are shown in a thin stick representation; the hydrogen bonds are represented using dashed lines

EXPERIMENTAL

Protein and ligand structures

The X-ray structure of the AGP (PDB ID: 3BX6) was downloaded from the Protein Data Bank (PDB) database (8). Before experiment, protein was elaborated and the incomplete amino acid residues were fixed and optimized. The chemistry of the protein was corrected for missing hydrogen atoms consistent with respective protonation states at pH 7.4. The His, Arg and Lys were protonated, while those of Asp and Glu were deprotonated. The protein was treated as a rigid body and the polar hydrogen atoms were added. The two dimensional (2D) structures of

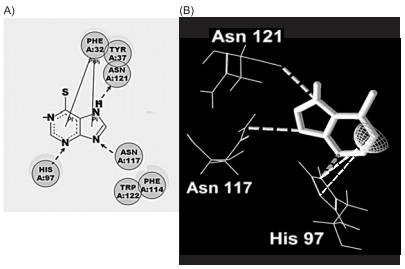

Figure 6. The interaction model between anion of 6-MP (1,7) and AGP. (A) Schematic (2D) representation of anion of 6-MP (1,7)–AGP interactions in the binding pocket (CAV) of the protein; only residues around 6 Å of the ligand are displayed; (B) The predicted hydrogen bonds between the anion of 6-MP (1,7) and amino acid residues at the active site of AGP.
The anion of 6-MP (1,7) is shown in a stick and amino acids are shown in a thin stick representation; the hydrogen bonds are represented using dashed lines; the electrostatic interaction is visualized as partial sphere correspond to favorable interaction oriented in the direction of the interaction and as thin dashed line.

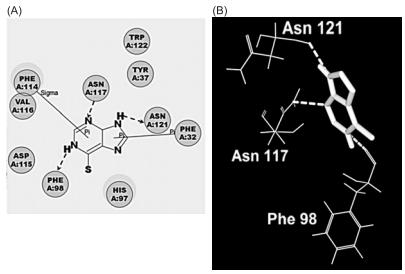

Figure 7. The interaction model between 6-MP (1,9) and AGP. (A) Schematic (2D) representation of 6-MP (1,9)–AGP interactions in the binding site (CAV) of the protein; only residues around 6 Å of the ligand are displayed; (B) The predicted hydrogen bonds between 6-MP (1,9) and amino acid residues at the active site of AGP. 6-MP (1,9) is shown in a stick and amino acids are shown in a thin stick representation; the hydrogen bonds are represented using dashed lines

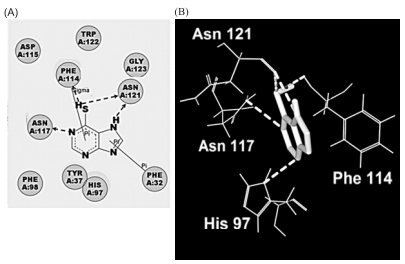

Figure 8. The interaction model between 6-MP (7,10) and AGP. (A) Schematic (2D) representation of 6-MP (7,10)–AGP interactions in the binding site (CAV) of the protein; only residues around 6 Å of the ligand are displayed; (B) The predicted hydrogen bonding between 6-MP (7,10) and amino acid residues at the active site of AGP. 6-MP (7,10) is shown in a stick and amino acids are shown in a thin stick representation; the hydrogen bonds are represented using dashed lines

6-MP were obtained using the ChemDraw Std Cambridge Soft 2002 v.7.0.1 computer program. 2D to three dimensional (3D) representations were converted by the use of CS Chem3D Ultra Molecular Modeling and Analysis 2001 CambridgeSoft v.7.0.0 software and were energy-minimized using semiempirical (AM1) method implemented in the same software, and imported to MVD as .mol file. The 3D structure of progesterone (PRG) was retrieved from Protein Data Bank database (PDB ID: 2HZQ – apolipoprotein D complex with progesterone (9)). All initial conformations of ligands used in the docking experiment were once more energy-minimized using the method implemented in MVD. The chemical 2D and 3D structures of all ligands are shown in Figure 1.

Molecular docking

Molecular docking studies were performed using the Molegro Virtual Docker (MVD) 2010.4.2.0 computer program and results were elaborated using Molegro Molecular Viever (MMV) 2011.2.2.0 computer program. Initially, the PRG, which was used as a standard ligand that specifically bind to AGP, was docked to the binding site constrained by the residues of Ser40, Leu79 and Tyr127. Next, the binding pocket (hereafter referred to as cavity, CAV) was detected automatically using the grid-based cavity prediction algorithm. The 6-MP molecules were docked to the obtained cavity. During the docking experiments, the crystallographic water molecules were removed from the protein. For each complex 10 independent runs were conducted, each of these runs was returning to a single final solution (pose). The resulting conformations were clustered and only negative the lowest-energy representation from each cluster was returned when the docking run was completed; the similar poses were removed keeping the best-scoring one. The cluster was ranked comparing conformation of the lowest binding energy in each cluster. The first pose representing estimated the lowest binding free energy was selected for analysis of the docking results (10, 11). The best docking complex solution (pose from MVD as a .pdb files) was also analyzed according to the potential of intermolecular interactions using the LPC/CSU server (12), which is used to analyze ligand–protein contacts in .pdb files. The .pdb files were also used to create a ligand interaction network as 2D diagram using Discovery Studio v3.1.011157 Visualizer (Accelrys Software Inc.) computer program.

Table 1. Best poses 6-MP molecules and progesterone (PRG) for docking to AGP.

Ligand	Interaction energy between the ligand and protein (arbitrary unit)					
	MolDock Score	E-Inter[1]	Steric	H-Bond	Electrostatic interaction	
					Short-range (r < 4.5 Å)	Long-range (r > 4.5 Å)
6-MP (1,7)	–65.80	–69.48	–64.22	–5.25	0.0	0.0
Anion of 6-MP (1,7)	–73.24	–77.48	–69.27	–8.66	–1.26	1.87
6-MP (1,9)	–73.00	–76.28	–70.31	–5.97	0.0	0.0
6-MP (7,10)	–74.33	–78.20	–69.14	–9.06	0.0	0.0
PRG	–108.51	–98.92	–95.44	–3.48	0.0	0.0

[1] E-Inter – the ligand protein energy interaction

Table 2. Hydrogen bond and electrostatic interactions between ligand molecule and amino acid residues of the active site on AGP.

Ligand atom	Protein atom	Distance (Å)	Ligand atom	Protein atom	Distance (Å)
6-MP (1,7)			6-MP (1,9)		
N(3) acceptor	His97 (ND1 sc[a])	3.02	N(1)H donor	Phe98 (O bb)	2.59
N(7)H donor	Asn121 (O bb[b])	2.75	N(3) acceptor	Asn117 (ND2 sc)	2.55
N(9) acceptor	Asn117 (O bb)	3.10	N(9)H donor	Asn121 (O bb)	2.78
anion of 6-MP (1,7)			6-MP (7,10)		
N(1) ES	His97 (ND1 sc)	3.87	N(1) acceptor	Asn117 (ND2 sc)	3.17
N(1) ES	His97 (ND2 sc)	5.35	N(3) acceptor	His97 (ND1 sc)	2.84
N(3) acceptor	His97 (ND1 sc)	3.04	N(7)H donor	Asn121 (O bb)	3.12
N(7)H donor	Asn121 (O bb)	2.81	S(10)H donor	Phe114 (O bb)	2.60
N(9) acceptor	Asn117 (ND2 sc)	3.10	S(10)H donor	Asn121 (O bb)	2.93
PRG					
O(22) acceptor	Ser40 (OH sc)	3.06	O(23) acceptor	Tyr127 (OH sc)	2.88

[a] sc – side-chain functional group of the amino acid; [b] backbone (bb); ES – electrostatic interaction between charged atoms.

RESULTS AND DISCUSSION

AGP is the main transport protein for most basic compounds and the hydrophobic forces play important role in the binding of these drugs to AGP. However, certain acidic drugs may also interact with AGP by hydrophobic bonds with a high affinity. Despite the fact that the AGP plasma concentration is much lower than human serum albumin concentration, the association constants of some acidic drugs with AGP are so high that one may suggest that binding to AGP will contribute considerably to the total plasma binding of these drugs (13, 14). AGP consists of single polypeptide chain of 183 amino, containing three Trp residues, from which one residue, Trp160, is at the surface of the protein and two, Trp25 and Trp122, are located in the protein matrix (15). AGP has one common, wide drug binding site consisting of a basic ligand binding subsite, acidic ligand binding subsite and steroid hormone binding site, which significantly overlap. Ligand binding–pocket consists of three lobes I, II and III. The main lobe I is big and completely nonpolar, and it provides hydrophobic contacts to accommodate a ligand molecule. On each of its sides, there are smaller lobes II and III, both negatively charged due to residues Glu64 and Asp115 presence. The opening to the cleft-like cavity is flanked with two positively charged side chains, Arg68 and Arg90 (16). The location of the ligand binding site in AGP structure and structural characterization of this binding–site is presented in Figure 2.

Table 3. Classification of the potential interactions types detected between ligands and AGP residues in the best docking pose solution from the MVD program obtained using the LPC/CSU server.

Residue	Type of contact				
	6-MP (1,7)	Anion of 6-MP (1,7)	6-MP (1,9)	6-MP (7,10)	PRG
Phe32	Ar	Ar	Ar	Ar	Ar; Ph
Tyr37	Ph; Ar;	Ph; Ar	Ar	Ar	Ar; Ph; AA*
Ser40					HB
Val41	Ph	Ph			Ph
Ile44					Ph
Phe51					Ph
Leu62					Ph
Glu64					other
Leu79					Ph
Ile88					Ph
Ser89					Ph
Arg90			Ph		Ph; HH*
Val92	Ph; HH*	Ph; HH*	Ph; HH*	HH*	Ph
His97	HB; HH*	HB; Ar; HH*	Ar; Ph; HH*	HB; HH*	Ph; HH*
Phe98	other	other	HB;	Ph	other
Ala99			Ph		Ph
Leu112					Ph; HH*
Phe114	Ph; Ar; HH*	Ar; Ph; HH*	Ph; Ar; HH*	HB; Ph; Ar; HH*	Ph
Val116			Ph	Ph	
Asn117	HB	HB	HB; Ph	HB; HH*; Ph	
Asn121	HB; Ph	HB; Ph	HB	HB	
Trp122				other	
Ser125					HH*
Tyr127					HB

HB – hydrogen bonds; Ph – hydrophobic contact; Ar – aromatic–aromatic contact; HH – hydrophilic–hydrophobic contact; AA – acceptor–acceptor contact; * non attractive contacts.

The obtained best docking energy results for PRG docked into the binding site of AGP (Fig. 3, Tabs. 1 and 2) were found to be in agreement with the computed model obtained by Schönfeld (16). A complex of a protein with PRG in Figure 4A showed that ligand molecule fits tightly into the binding site. The twelve amino acid residues within 6 Å distance of the docked complex may influence binding of PRG. The side-chains of Phe32, Tyr37, Ser40, Val41, Leu79, Ile88, Arg90, Val92, Leu112, Phe114, Ser125 and Tyr127 interact hydrophobically with ligand and the two hydrogen bonds forms between the keto groups of PRG and the Ser40 and Tyr127 hydroxyl groups (Figs. 3A and 3B). Arg90 was the only charged amino acid near the bound complex. Moreover, other apolar, polar and charged residues were also a part of the predicted binding pocket of PRG (Fig. 4B). To obtain the 6-MP–AGP complexes, docking of 6-MP into model structure of binding cavity of PRG was performed. The final solutions of this procedure are presented in Figures 4A, 5–8 and in Tables 1 and 2. The results from Table 1 were interpreted in terms that more negative are the values of predicted binding energy, the more thermodynamically favorable is binding itself. Hence, all the 6-MP–AGP complexes exhibited the lower interactions (E-Inter) than PRG–AGP complex. However, the H-bond energy interactions were higher for the former. The differences among Energy-Inter values of 6-MP with AGP were not large and the lowest energy was observed for 6-MP (7,10). The specific electrostatic interaction between

ionized 6-MP (1,7) and AGP was responsible for the increased negative energy in comparison with unionized 6-MP (1,7). When the models of 6-MP–AGP complexes were compared with model PRG–AGP (Fig. 3A), the 6-MP molecules were found to be located inside the CAV, but interactions with binding site were less extensive and 6-MP molecules were not as tightly inserted into the site as PRG was. The orientations of the four ligands were similar, in that they were located in lateral, more hydrophilic part of cavity (Figs. 4A, 5A–8A).

In the complexes formed between 6-MP molecules and AGP the amino acid residues of Asn and His influence the 6-MP binding affinity; all the 6-MP have a hydrogen bond interactions with Asn at position 117 and 121, and with His97 (except 6-MP (1,9)) (Figs. 5A,B–8A,B and Tab. 2). Additionally, His97 can be a target for interaction with the electronegative N or S atoms of ionized form of 6-MP (1,7) (Fig. 6B).

As the results listed in Table 3 show, the hydrophobic interactions play an important role in the binding of basic PRG to AGP. In the case of 6-MP, the interaction with hydrophilic residues located in binding site was preferred. In relation to hydrophobic interactions, only PRG showed interactions with amino acid residues in sequence 40–90 and 6-MP molecules do not interact with residues which were primarily responsible for binding of PRG.

On analyzing the effect of tautomerism of 6-MP within the 6-MP–AGP complexes it can be seen that involvement of sulfur atom of 6-MP in the binding with AGP was possible only in the case of thiol–N(7) tautomer, i.e., when sulfur atom was in –SH group. Hence, the thiol ↔ thione equilibrium may have significant impact on interaction of the 6-MP with AGP.

CONCLUSION

In this study, the interaction between 6-MP molecule and AGP was investigated by the use of computer-assisted molecular docking. The 6-MP molecules were docked to predicted site (CAV) where PRG may bind. It was observed that the binding site of basic PRG may be shared by acidic 6-MP. Hydrophobic interaction was essential in the binding of PRG to AGP. In contrast, the interactions between 6-MP and AGP were not exclusively hydrophobic in nature and several ionic and polar residues in the proximity of 6-MP–AGP complexes played important role in stabilizing these complexes. The amino acids sequence 97–121, including His97, Phe98, Asn117, Phe114 and Asn121, was crucial part of the binding site for 6-MP. It was observed that all the investigated tautomers of 6-MP and ionized form of 6-MP were able to form hydrogen bonds and interact hydrophobically with amino acid residues present in the binding site. However, is possible that the thiol–N(7) tautomer may form relatively most stable complex with AGP than the other tested tautomers.

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