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Methyl syringate docking into albumin structure

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Human serum albumin is known as a major transport protein and is capable of binding a great variety of drugs and metabolites. Experimental studies have indicated that methyl syringate (MS) binds strong to human serum albumin (HSA) and bovine serum albumin (BSA). Docking studies determined two sites for MS and its radical where these species dock with the highest affinity at about –7.5 kcal/mol. High HSA/BSA homology and later docking studies showed that the most acceptable site in HSA responsible for strong MS complexation can be Site-I in domain II of HSA.

Key words: human serum albumin, bovine serum albumin, methyl syringate, docking

INTRODUCTION

Human serum albumin (HSA) is a major transport protein for unesterified fatty acids, but it is also capable of binding an extraordinarily diverse range of metabolites, drugs and organic compounds [1, 2]. The remarkable binding properties of albumin account for the central role it can play in both the efficacy and rate of the delivery of drugs. The association of metabolites, drugs and organic compounds with serum albumins has been investigated by a variety of methods such as UV-Vis, CD, NMR, calorimetry and X-ray techniques [3]. X-ray crystallographic analysis revealed complexation sites for some aromatic compounds like triiodobenzoic acid (TIB) and warfarin (WR) [4, 5] as well as for fatty acids [1]. An unliganded structure of HSA was revealed as well [6]. Tertiary structures of HSA with myristic acid and aromatic drug, were determined by Petitpas et al. at a resolution of 2.50 Å. The data showed WR complexation in HSA domain II with coincidence to TIB complexation in domain II. That site was called Site-I [2].

During investigations of peroxidase- and laccase-catalyzed oxidation of phenols, an unexpected stabilization of enzymes by albumins was found [7, 8]. To explain this effect, the interactions of albumins with methylsyringate (MS) were explored by using absorbance and fluorescence spectroscopy, and a strong complexation with albumins was revealed. At pH 6.0 and 25 °C the dissociation constant of the MS complex with albumine was 15.2 ± 5.4 μM and 18.7 ± 0.4 μM with BSA and HSA respectively. The number of binding sites was 1.06 ± 0.04 and 0.58 ± 0.01 for the same albumins. The association of MS with albumins was so strong that the complex showed the absorbance of the dissociated (phenolate) form of MS.

The aim of the computational studies presented here was to explore the bindings of these compounds and their radical forms into albumin structure by using molecular docking calculations. As far as we could determine, no previous experimental or computational studies have addressed this question.

METHODS

X-ray structures of unliganded HSA (1AO6) [7] and liganded HSA with WR [5] and TIB/myristate [1] were downloaded from Protein Data Bank (PDB).

Ab initio calculations of ligand structures (Fig. 1) and their charges were performed on a 3–21G basis set and Hartree–Fock theory with Gaussian 98W [9].

Automated flexible docking simulations were performed by AutoDock 3.0.5 [10–12]. All non-protein compounds were removed from the protein structures. Atomic interaction energy grid maps for HSA were calculated with 0.25 Å grid spacing and 120

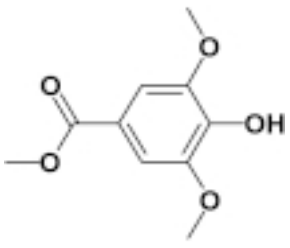

Fig. 1. The structure of MS

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grid points forming 30 Å cubic boxes centered on TIB and WR binding pockets. The boxes covered the whole binding pockets of WR and TIB and the space beyond. The electrostatic interaction energy grid used a distance-dependent dielectric function of Mehler–Solmajer [13]. The docking was accomplished using the Lamarckian genetic algorithm. The number of individuals in the population was set up to 50. The maximum number of generations was 27000. The number of the top individuals guaranteed to survive into the next generation was 1. The crossover rate and the mutation rate were 0.02 and 0.80, respectively. The local pseudo-Solis & Wets search was introduced with standard rules. The flexibility of methoxy and hydroxyl groups in ligands was estimated during the docking process.

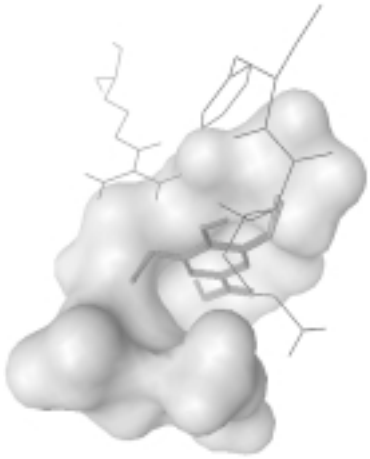

Fig. 2. MS and MSr dockings at Max-1 site of HSA. Tiny line structures are amino acids at close contacts with MS and MSr. Other close amino acids are covered by pseudo-solvatation surface

RESULTS

Calculations of MS and its radical (MSr) docking in HSA revealed two distinct sites (*Max-1* and *Max-2*) with maximal docking energies in the center of heart-shaped HSA (Table 1). For both ligands His464, Glu465, Val462, Cys461, Asn458, Lys205, Phe206, Arg484, Cys477 Thr478, Thr474, Val473, Ala201 and Pro468 surrounded the *Max-1* centre, whereas the *Max-2* centre was built by Phe149, Gln196, Ala191, Ser193, Ala194, Lys190, His146, Ala455, Pro147, Leu198, Tyr148, Arg197, Asn458, Gln459, Asp108 and Asn109.

Table 1. **Eergies of ligands in *Max-1* and *Max-2* of HSA**

Ligand	Docking energy, kcal/mol	
	Max-1	*Max-2*
MS	–7.5 – –7.9	–7.3 – –7.6
MS-r	–7.4 – –7.6	–7.4

The calculations of MS docking as well as its oxidation product (MSr) revealed that both compounds complexate in the same *Max-1* site (Fig. 2). The energy of the radical docking, however, was similar to that of phenol. That means that the role of hydrogen bonding in the ligand docking is insignificant. Possibly, the main factor that determines ligand docking in the *Max-1* site is hydrophobic interaction. The dockings of MS and MS-r in *Max-2* site revealed a little higher docking energies.

The distinct experimental differences between BSA and HSA cannot be explained by a possible complexation in *Max-1* and *Max-2* because of a high BSA–HSA a.a. homology and big differences in experimental data. Both albumins have an identical a.a. composition in the *Max-1* site and almost identical in the *Max-2* site. The difference by two a.a. does not inflict changes in the enviroment of the *Max-2* cavity, as their side-chain radicals do not interact with the cavity. Therefore, these determined loci cannot inflict the clear and significant differences in experimental findings of kinetic and spectroscopic measurements [7, 8].

Experimental crystallographic studies determined some sites for drug and drug-analog binding in HSA [1, 5, 6] with the aromatic nature of ligands. The crystallographic experiments showed that TIB binds to domains I and II. WR binds also in domain II in the same cavity as TIB and that site is called Site-I [2]. Those binding sites are burried deeper than the determined loci *Max-1* and *Max-2* and contain His residues which could be essential for stabilization of our phenolic ligands. In addition, His serves as a perfect proton acceptor.

The core of the TIB-binding pocket is a.a. Ile142, His146, Phe149, Leu154, Phe157, Tyr161, Arg186, Gly189 and Lys190. The docking results are tabulated in Table 2. MS and MSr dockings in the TIB binding cavity of domain I are similar. As is shown in Fig. 3, aromatic fragments of MS and MSr are almost "frozen" in the cavity. The hydroxyl groups of MS and MSr are located near His146. Negligible changes in spatial position between MS and MSr reveal the lack of H-bonding influence stabilizing MS and MSr structures is the cavity.

Table 2. **Ligand dockings in Domain I and Domain II of HSA**

Ligand	Docking energy, kcal/mol	
	Domain I	Domain II
MS	–7.3	–7.4
MS-r	–7.0	–7.2

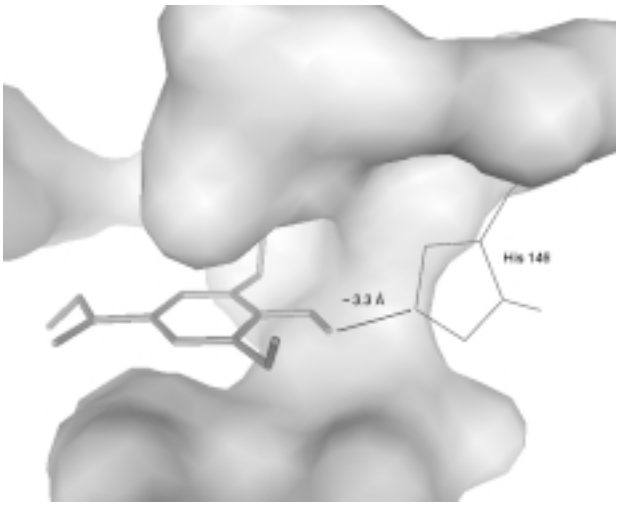

Fig. 3. MS docking at the TIB-binding site in Domain I of HSA. Tiny line structure is His146 and other close amino acids are covered by pseudo-solvatation surface. Numbers shows the distance

MS and MSr were docked in Site-I (domain II). This site consists of Tyr150, Lys199, Leu219, Phe223, Leu234, Leu238, Val241, His242, Arg257, Leu260, Ala261, Ile264, Ile290 and Ala291. Docking energies in domain II of HSA are greater by about 0.1–0.2 kcal/mol. MS was docked in Site-I with hydroxyl hydrogen at about 3.6 Å from His242 with –7.4 kcal/mol docking energy. However, MSr docking proceeds in the same place, but the hydroxyl groups of the radicals directly interact by H-bonding with Lys199 at about 1.7Å. At the same time Lys199 interacts with one methoxy oxygen atom. Therefore, MSr is stabilized with a strong electrostatic interaction and H-bonding. In both cases of docking with MS and MSr the carboxyl group is directed out of His242 and the aromatic ring is almost in the same position as if it would be the energetic minimum for the aromatic core of MS.

DISCUSSION

The results of docking reveal some common features of all docked ligands in HSA. First of all, the hydrophobic term is noticed to play significant role in stabilizing the aromatic core of ligands, as it would be in TIB binding pockets of domain I or II. Moreover, all radical forms of the ligand indicate almost identical docking energy within the domains. The differences are detected with protonated forms of ligand when the hydrogen position can tune docking energy in an appropriate way specific to each ligand as noted for the TIB binding pocket in domain I. No significant influence of H-bonding was detected.

Summarizing the results, the different experimental behavior of MS with BSA and HSA can be explained by MS complexation in Site-I. In addition, the Site-I has differences in a.a. composition, which can determine the different effect on MS complexation with successive spectroscopic differences.

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METILSIRINGATO KOMPLEKSACIJOS MODELIAVIMAS ALBUMINE

S a n t r a u k a

Žmogaus serumo albuminas yra vienas pagrindinių kraujo plazmos transportinių baltymų, galinčių prisijungti įvairius vaistus ir metabolitus. Eksperimentais nustatyta, kad metilsiringatas (MS) stipriai prisijungia žmogaus ir jaučio serumo albuminuose. Kompleksacijos skaičiavimais nustatytos dvi vietos žmogaus serumo albumine, kuriose MS ir jo radikalas sudaro didžiausio gimininguma kompleksą; vidutinė kompleksacijos energija yra apie 7,5 kkal/mol. Didelė žmogaus ir jaučio serumo albuminų amino rūgščių homologija ir tolimesni kompleksacijos modeliavimo tyrimai atskleidė, kad labiausiai metilsiringatui tinkama stiprios kompleksacijos vieta serumo albumine gali būti „Site-I“ vieta II albumino domene.