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Complexation of syringic acid esters with human serum albumin

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Syringic acid esters are phenol compounds that bind with albumins forming dissociated phenols. To explain this phenomenon, docking calculations of nine syringic acid esters were performed in three areas of human serum albumin: triiodobenzoic acid binding site in domain I and in Sites I and II. It was shown that compounds can bind in all these sites. Alkyl radical of the esters make contacts with hydrophobic amino acids, electrostatic bonds are formed with histidines and other amino acids. The best proton-accepting sites have been found in domains I and II.

Key words: docking, human serum albumin, complexation, syringic acid ester

INTRODUCTION

Human serum albumin (HSA) is the most abundant plasma transporter of many ligands: fatty acids, hormones, metabolites, heme, etc. [1, 2]. HSA is also able to bind various aromatic compounds in some distinct sites. Syringic acid esters are phenol compounds and typical laccase substrates [3]. Laccase is a blue copper enzyme that catalyzes the transfer of four electrons from various organic substrates to reduce dioxygen to water. Laccase is applied in many industrial processes: lignin degradation, dye or stain bleaching, etc. To increase the efficiency of these industrial processes mediators are added. Various organic redox compounds have been suggested as mediators for laccase-catalyzed reactions. Syringic acid methyl ester (MS) shows promising results as a laccase mediator due to its suitable redox potential [3]. MS undergoes a single electron oxidation by laccase-catalyzed reaction and forms a phenoxy radical. The conversion scheme of MS-assisted target molecules can be written as follows:

$$\text{Laccase}_{ox} + \text{MS} \rightarrow \text{Laccase}_{red} + \text{MS}^{\bullet} + \text{H}^{+}, \quad (1)$$

$$\text{Laccase}_{red} + \text{O}_2 + 4\text{H}^{+} \rightarrow \text{Laccase}_{ox} + 2\text{H}_2\text{O}, \quad (2)$$

$$\text{MS}^{\bullet} + \text{target molecule} + \text{H}^{+} \rightarrow \text{Product} + \text{MS}. \quad (3)$$

The yield of the mediator-assisted catalysis depends on the enzyme turnover capacity (TC). An inactivation of laccase was noted during the oxidation of laccase-catalyzed MS, resulting in limitation of the enzyme TC. Moreover, some compounds, among them albumins, can prevent laccase inactivation and increase the reaction yield [3]. Since inactivation of oxidoreductases is a common phenomenon concomitant with oxidation of phenol substances, the laccase inactivation during mediator oxidation is of general interest in the applications of these enzymes. Therefore, finding the reasons for laccase inactivation may allow to improve the industrial processes in presence of mediators.

To explain albumin action, the absorbance spectra of MS were analyzed in an albumin-free solution at different pH (Fig. 1) [3]. The spectra were the same at pH 3.0 and 6.0, but the peak shifted from 274 to 323 nm at pH 10 (Fig. 1) [3]. The shifting of absorbance maximum to a longer wavelength at alkaline pH was attributed to MS dissociation:

$$\text{Ph–OH} \leftrightarrow \text{PhO}^{-} + \text{H}^{+}, \quad (4)$$

where Ph–OH corresponds to the non-dissociated form of MS. PhO^{-} formed at alkaline pH is absorbed at a longer wavelength in comparison to non-dissociated phenol. In presence of albumins a similar spectrum was generated at pH 6.0 [3], indicating

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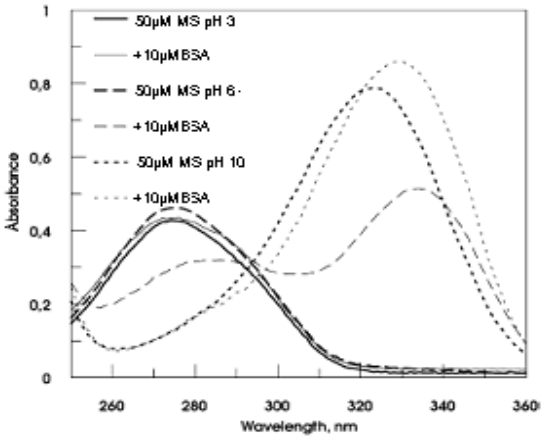

Fig. 1. Absorbance of MS at different pH and in presence of albumins: 0.05 mM MS (solid bold) with 0.01 mM BSA (solid) in 20 mM citrate buffer, pH 3.0; 0.05 mM MS (broken bold) with 0.01 mM BSA (broken) in 20 mM acetate buffer, pH 6.0; 0.05 mM MS (dot bold) and with 0.01 mM BSA (dot) in 20 mM carbonate buffer, pH 10.0 [3]

that MS formed a complex with albumin and that MS was dissociated in the complex; this can explain the shifted absorption peak at pH 6.0.

The main task of the current work was to investigate the syringic acid esters dockings into human serum albumin, with the aim to elucidate and explain the experimental facts of a strong interaction of the esters.

METHODS

Ab initio calculations of the structure and charges of syringic acid esters were performed on a 3-21G basis set and Hartree–Fock theory with Gaussian 98W.

The syringic acid esters were: methyl (MS), ethyl (ES), propyl (PS), isopropyl (IPS), butyl (BS), hexyl (HS), octyl (OS) and decyl (DS) (Fig. 2). The dissociated form of syringic acid (SY) was considered, too. For docking simulations the rotation of methoxy, hydroxyl groups and alkyl tails was considered as well. The crystal data on native HSA (1AO6) were downloaded from Protein Data Bank. Automated flexible docking simulations were performed by AutoDock 3.0.5 [4–6]. All non-protein compounds were removed from the structures during docking calculations of syringic acid esters. Atomic interaction energy grid maps for HSA were calculated with 0.25 Å grid spacing and 120 grid points forming 30 Å cubic boxes centered on tri-iodobenzoic acid binding pocket in domain I and on Sudlow's site I and II. The space of the cubic boxes covered the binding pockets of HSA and the space beyond. The electrostatic interaction energy grid used a distance-dependent dielectric function of Mehler and Solmajer [7]. The docking was accomplished using the Lamarckian genetic algorithm. The number of individuals in a population was set up to 50. The maximum number of energy evaluations was 250 000 and the maximum number of generation was 27 000. The number of the top individuals that were guaranteed to survive into the next generation was one. The crossover rate and the mutation rate were 0.02 and 0.80, respectively. A total of 100 automated runs were calculated for each compound. Cluster tolerance was set up to 1 Å. A local pseudo-Solis & Wets search was performed. The number of iterations for the search was 300, and the probability of performing a local search on an individual was 0.06.

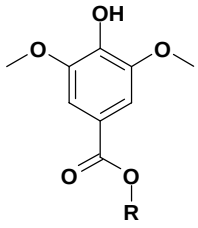

Fig. 2. Structure of compounds used in docking experiments. R represents alkyl radicals of syringic acid esters

RESULTS AND DISCUSSION

The structure of the compounds is shown in Fig. 2. The docking calculations of syringic acid esters were performed in three areas of albumin (sub-domains IB, IIA and IIIA) It is known that iodobenzoic acid (aromatic drug analog) binds in sub-domain IB of HAS forming Tib- binding site [1]. The other two sites are known as Sudlow's drug binding sites I and II (sub-domain IIA and IIIA, respectively). Sites I and II are warfarin and diazepam binding sites, respectively [8], also capable of binding various benzopyranes [9]. Site I complexates a tri-iodobenzoic acid molecule as well [1]. A presumption that the most reliable amino acid (a.a.) to accept a proton could be histidine was made, due to its capacity to trap a proton and become positively charged (pK_a 6.0). The probability to trap a proton by other basic a.a. (lysine and arginine) is low due to high pK_a values, 10.53 and 12.48 respectively. During our docking calculations the interaction of syringic acid and it esters with histidines and other tyrosines was indicated (Figs. 3–7).

The Tib-site in domain IB is formed mainly by Ile 142, His 146, Leu 154, Phe157, Tyr 161, Arg 186 and Lys 190. In this cavity the interaction of ethers with His 146 was noted (Fig. 3). Almost all ligands in the current study are in a similar orientation in the binding pocket, except IPS and DS.

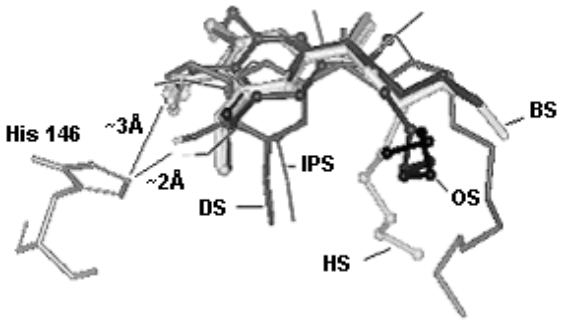

Fig. 3. The docking of SY, MS, ES, PS, IPS, BS, HS, OS and DS in Tib-site in sub-domain IB close to His 146

The hydroxyl groups of esters form electrostatic interactions with nitrogen of His 146 in imidazole ring (Fig. 3). The average distance between nitrogen and hydroxyl groups of ligands is ~3Å, except for IPS and DS where the average distance is ~2Å. (Fig. 3). Alkyl residues of esters are surrounded mostly by hydrophobic amino acids (Leu 115, Ile 142, Leu 154, Phe 157, Gly 189). The esters with the largest hydrophobic residues possess the lowest docking energies. The dissociated form of syringic acid has the lowest docking energy in comparison with the rest (Table). This trend was noticed in all explored areas of the protein. It could be explained by a small molecule of SY, not having a hydrophobic chain. It shows that the main force holding the substrates in binding pockets is hydrophobic properties of ethers. Long-chain esters (BS, HS, OS and DS) do not form big clusters, *i.e*. only a few structures bind in a similar orientation and interact with His 146.

Site I is a very hydrophobic pocket formed mainly by Leu 219, Phe 223, Leu 238, Val 241, His 242, Ala 261, Ile 264 and Ala 291. In this locus the interaction of three compounds (SY, MS and IPS) with His 242 and Lys 199 was noted (Fig. 4). All three structures point their hydroxyl groups to nitrogen in imidazole ring (the average distance is ~3Å). The orientation of SY and MS in this complexation pocket is very similar, unlike IPS whose aromatic ring accommodates perpendicularly to the others. Other esters (except SY and MS) also bind in this pocket, but not so close as to His 242. All of them have almost identical orientation (Fig. 5). In this pocket alkyl residues contact with hydrophobic a.a. (Leu 219, Leu 234, Leu 260, Ala 261, Ile 264, Ile 290 and Ala291). Docking energies of substrates in this cavity are lower than in Tib-site (Table). In this cavity, the same trends as in Tib-site were noted: long-chain esters possess a lower doc-

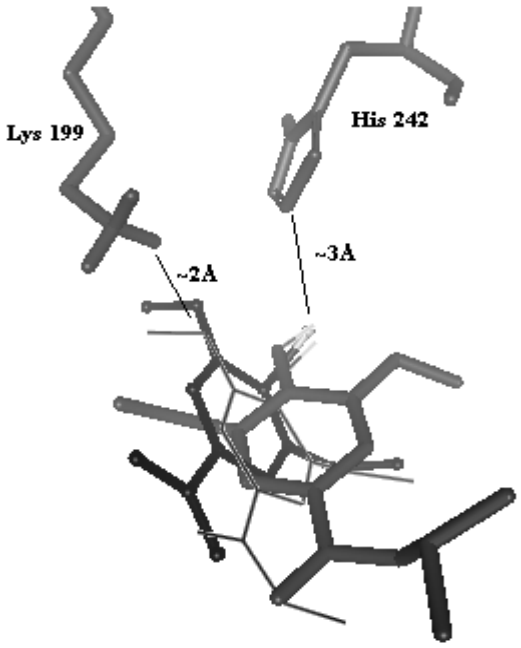

Fig. 4. Interaction of SY, MS and IPS with His 242 and Lys 199 in sub-domain IIA

Table 1. **Docking energies (kcal/mol) and the percentage of structures in clusters of syringic acid and its esters in the strongest interaction sites of HSA**

Compound	Domain I		Domain II				Domain III			
	H 146		H 242				T 452		T 411	
	E	%	E	%	E	%	E	%	E	%
SY	–7.2	49	–7.4	31	–	–	–8.2	16	–7.5	47
MS	–8.4	38	–8.3	19	–	–	–9.2	21	–8.4	17
ES	–9.2	37	–	–	–8.6	66	–10.3	17	–9.7	78
PS	–9.9	63	–	–	–9.5	92	–10.8	13	–10.3	100
IPS	–9.2	6	–7.7	5	–9.0	90	–9.9	16	–10.0	0
BS	–10.4	53	–	–	–10.3	92	–11.3	9	–10.2	0
HS	–11.6	33	–	–	–12.0	87	–13.2	17	–	–
OS	–12.1	9	–	–	–13.1	67	–13.0	9	–	–
DS	–12.3	3	–	–	–13.1	100	–13.3	4	–	–

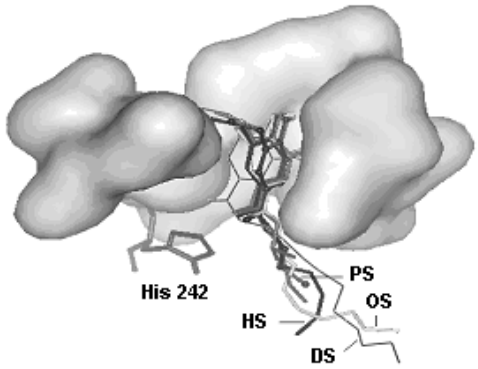

Fig. 5. Interaction of esters in Site I of HSA

king energy and *vice versa*. SY has the lowest docking energy.

The environment in Site II is formed mainly by Leu 387, Cys 392, Phe 395, Phe 403, Leu 407, Arg 410, Tyr 411, Leu 430, Cys 438, Arg 445 and Leu 453. In domain III we noted two big clusters where the compounds bind and have low docking energies (Figs. 6, 7, Table). The first of them is situated at a distance of ~12Å from Site II, in a split between domains I and III. All esters (except DS) in this site point hydroxyl groups towards the oxygen of Tyr 452 residue (Fig. 6). The average distance of this bond is ~2 Å (SY, ES) and ~2.5–3 Å (the rest). DS does not point his hydroxyl group towards Tyr 452 and does not make electrostatic bounds with any a.a. (Fig. 6). Hydrogen of Tyr 452 hydroxyl group interacts with the oxygen atom of Asn 429 peptide bond (distance 1.6 Å) (Fig. 6), allowing to suggest a strong H-bonding proton-accepting center. Despite the fact that this area of HSA is situated in the interspace between two domains, the short-chain ethers (MS, PS, IPS and BS) are closely surrounded by hydrophobic a.a (Ala 191, Ala 194, Val 455, Val 456 and hydrophobic portion of Lys

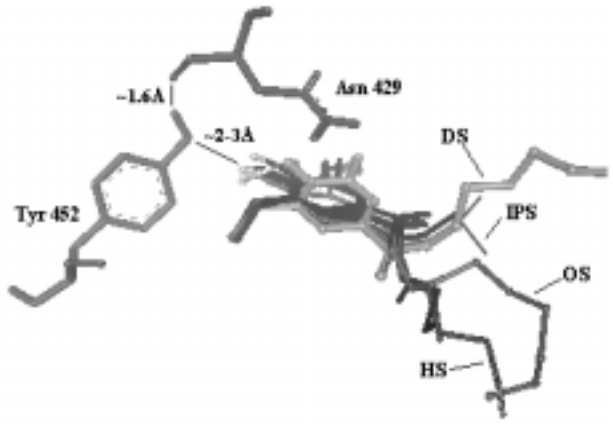

Fig. 6. Esters binding in domain III close to Tyr 452. Interaction of hydroxyl group of Tyr 452 with Asn 429 is shown by a line

190). However, alkyl-residues of the rest structures bulge into solution.

The second locus is situated inside the sub-domain IIIA, in Site II. Esters having shorter methylene chains complexate close to Tyr 411. They point hydroxyl groups towards the oxygen of Tyr 411 residue (distance ~2 Å). All compounds in this complexation site have low docking energies (Table). Though IPS and BS also complexate there, they do not point hydroxyl groups towards Tyr 411. HS, OS and DS does not complexate in this cavity at all (Fig. 7).

In domain III we noted the same trends as in previous ones: substrates having longer alkyl tails had a lower docking energy and *vice versa*. SY had the lowest docking energy and behaved like the majority of ligands with short methylene chains (Figs. 3–7, Table).

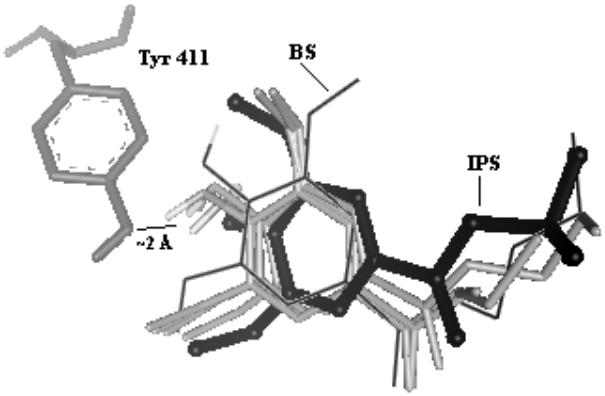

Fig. 7. SY, MS, ES, PS, IPS and BS complexation close to Tyr 411 in domain III

CONCLUSIONS

The present investigation shows that syringic acid ethers can strongly bind in all three investigated domains of HSA. All investigated substrates bind close to the proton acceptor (His 146) and have low docking energies in the Tib-site (Fig. 3, Table). In Site I only three ligands (SY, MS and PS) interact directly with histidine (Fig. 4). Other ligands bind in this site also and have low docking energies, but they have a different orientation and their hydroxyl groups do not make strong electrostatic bonds with any a.a. (Fig. 5). In domain III we noted two loci, where the substrates interacted with tyrosines (Tyr 411 and Tyr 452) and their hydroxyl groups formed strong H-bonds (Fig. 6–7). The dissociated form of syringic acid complexated in the same way as other esters having short hydrophobic chains, but had a considerably lower docking energy in all calculated areas of HSA. Substrates having

long alkyl chains (BS, HS, OS, and DS) had the lowest docking energy. Docking modelings showed that the main force holding substrates in the binding pockets is of a hydrophobic nature. We noted that a strong interaction with HSA tended to interact with short-chain ethers, because only a few structures of long-chain esters complexated in the same orientation.

Thus the best proton-accepting sites to explain the experimental facts of strong interaction could be Tib-site and Site I. In these sites bind all the compounds studied, they have low docking energies and interact with potential proton acceptors, *i.e.* histidines (Figs. 3–5, Table). Docking calculations showed that all ethers complexated in domain III also and the hydroxyl group had electrostatic interactions with Tyr 411, while Tyr 411 made a hydrogen bond with Asn 429 (Fig. 6). This cavity is situated in the interspace between domains I and III of the protein. Nevertheless, short-chain esters in this site are closely surrounded by hydrophobic a.a., while the very ends of long-chain esters bulge into solution. Thus, this cavity also could be responsible for the strong interaction between short-chain esters and HSA.

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ŽMOGAUS SERUMO ALBUMINO IR SIRINGO RŪGŠTIES ESTERIŲ SĄVEIKA

S a n t r a u k a

Eksperimentiškai nustatyta, kad siringo rūgšties esteriai komplektuojasi su žmogaus serumo albuminu (HSA- angl. *human serum albumin*) ir sudaro disocijuotą (fenolinę) siringato formą. Šiame darbe su programa AutoDock 3.0.5 atlikti devynių siringo rūgšties esterių komplektacijos modeliavimai trijose baltymo vietose: trijodobenzoinės rūgšties komplektacijos vietoje I domene, Site I ir Site II (II ir III domenai atitinkamai). Paaiškėjo, kad siringo rūgšties esteriai tirtuose albumino lokusuose jungiasi su žema komplektacijos energija bei sąveikauja su potencialiais protonų akceptoriais. Darbe nustatyta, kad geriausios protonų akcepcijos vietos yra Tib-site ir Site I, kur komplektuojasi visi tirti junginiai.