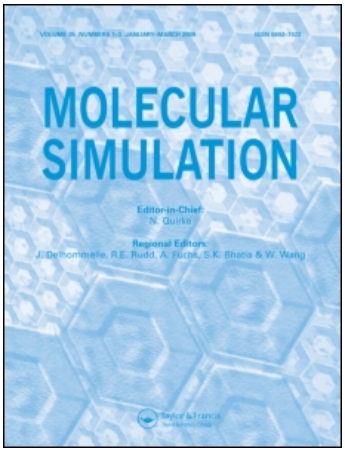

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Interactions of urocanic acid with bovine serum albumin and the influence of pH on binding affinities: a docking simulation study

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Interactions of urocanic acid with bovine serum albumin and the influence of pH on binding affinities: a docking simulation study

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In the present study, the interactions of urocanic acid (UA) with bovine serum albumins (BSA) at pH 5.0 and 7.4 were investigated by means of docking simulations. The binding modes of *trans*- and *cis*-UA to BSA at pH 5.0 and 7.4 were analysed. In addition, the theoretically predicted binding abilities of zwitterion and anion of UA with BSA are in good agreement with the experimental results. Through comparison with the binding patterns, we revealed that the stronger interactions of UA anion with BSA relative to the zwitterion primarily result from: (1) the increased number of hydrogen bonds between UA anion and BSA; (2) the attractive electrostatic interaction between the deprotoned carboxyl group in UA anion and Arg433 in comparison with the repulsion between the imidazole moiety in zwitterion and the same residue in BSA. This provides a rational explanation for the experimental finding that the binding of UA to BSA at pH 7.4 is much stronger than at pH 5.0.

Keywords: urocanic acid; bovine serum albumins; binding affinity; docking simulations

1. Introduction

Urocanic acid (UA, Figure 1), which is known as a deamination metabolite of histidine, is one of the primary chromophores in the stratum corneum of human skin [1,2]. UA is generated initially as a *trans* isomer (*trans*-UA). Upon ultraviolet light irradiation, it converts to the *cis* isomer [1–3]. Serum albumins are the most abundant plasma carrier in the circulatory system and can bind a variety of endogenous and exogenous substances. Therefore, it is of significance to study the interactions of UA with serum albumins. Previous experimental studies have investigated the binding of UA to bovine serum albumins (BSA) at various levels of pH [4,5]. It was found that UA can readily bind to BSA and the binding pocket locates at subdomain IIIA (Figure 2) [5]. However, the binding modes of UA to BSA remain to be elucidated. In addition, more effort is necessary to explain the experimental finding that the binding of UA to BSA at pH 7.4 is much stronger than at pH 5.0 [5]. Therefore, in the present work, the interactions of UA with BSA at pH 7.4 and 5.0 were investigated by means of docking simulations.

2. Theoretical methods

At pH 5.0 and 7.4, UA predominantly exists as zwitterion and anion, respectively (Figure 1) [2,3,6]. Thus, both forms are considered during the docking calculations.

A BLAST search in the protein data bank (PDB) [7] with a BSA sequence [Swissprot sequence ALBU_BOVIN (P02769)] revealed 75% identity with human serum albumin (HSA). As the structure of BSA is unavailable in PDB, structure coordinates for HSA in PDB file 1N5U [8] were employed to construct the BSA model by using the homology modelling module of Insight II software [9]. To take the pH effect on the protonation state of the protein residues into account, hydrogens were first added to the constructed model by employing the Biopolymer module of Insight II software with the pH value set to 5.0 and 7.4, respectively. Then, molecular dynamics equilibration was performed with the consistent-valence force field [10–12]. All the simulated structures were immersed in two layers (20 Å for the inner and 15 Å for the outer) of explicit water molecules. The inner layer was dynamic, while the outer layer was static and served as a solvent boundary to prevent the escape of the inner layer water molecules. The models were minimised by 500 conjugate gradient steps, heated from 2 to 300 K during 35 ps at a temperature increment of 50 K per 5 ps, then the constant temperature and pressure algorithm was applied at 300 K for 50 ps. The velocity verlet integrator was used with an integration step of 2 fs. The models were stable during the simulations with the equilibrium root mean square deviation value of 1.09 Å at pH 5.0 and 1.15 Å at pH 7.4 to the input structures, respectively, indicating that

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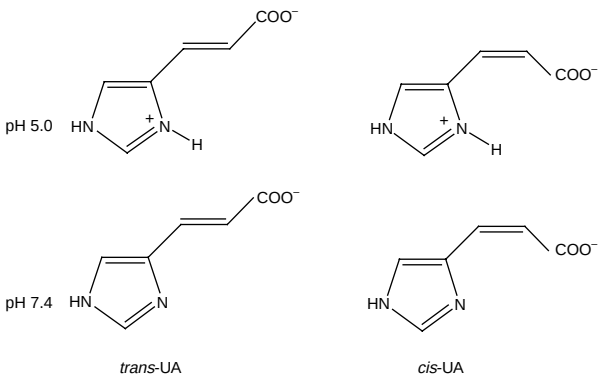

Figure 1. Chemical structures of *trans*- and *cis*-UA at pH 5.0 and 7.4.

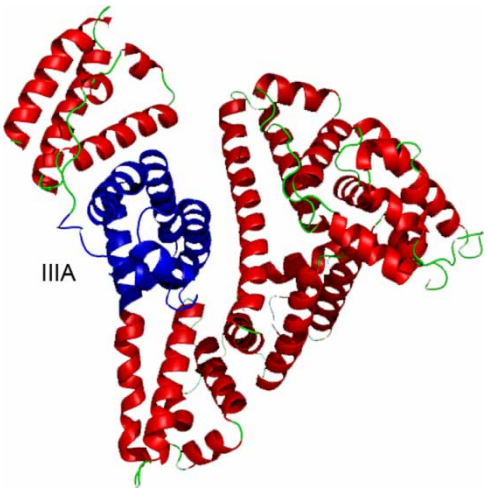

Figure 2. Cartoon structure of BSA. Subdomain IIIA is highlighted in blue.

the simulation has been converged and the energy of the system has been kept stable. The equilibrated models were used as the starting points of the docking calculations.

As it has been revealed that the binding of UA is located in subdomain IIIA of BSA (Figure 2), this pocket

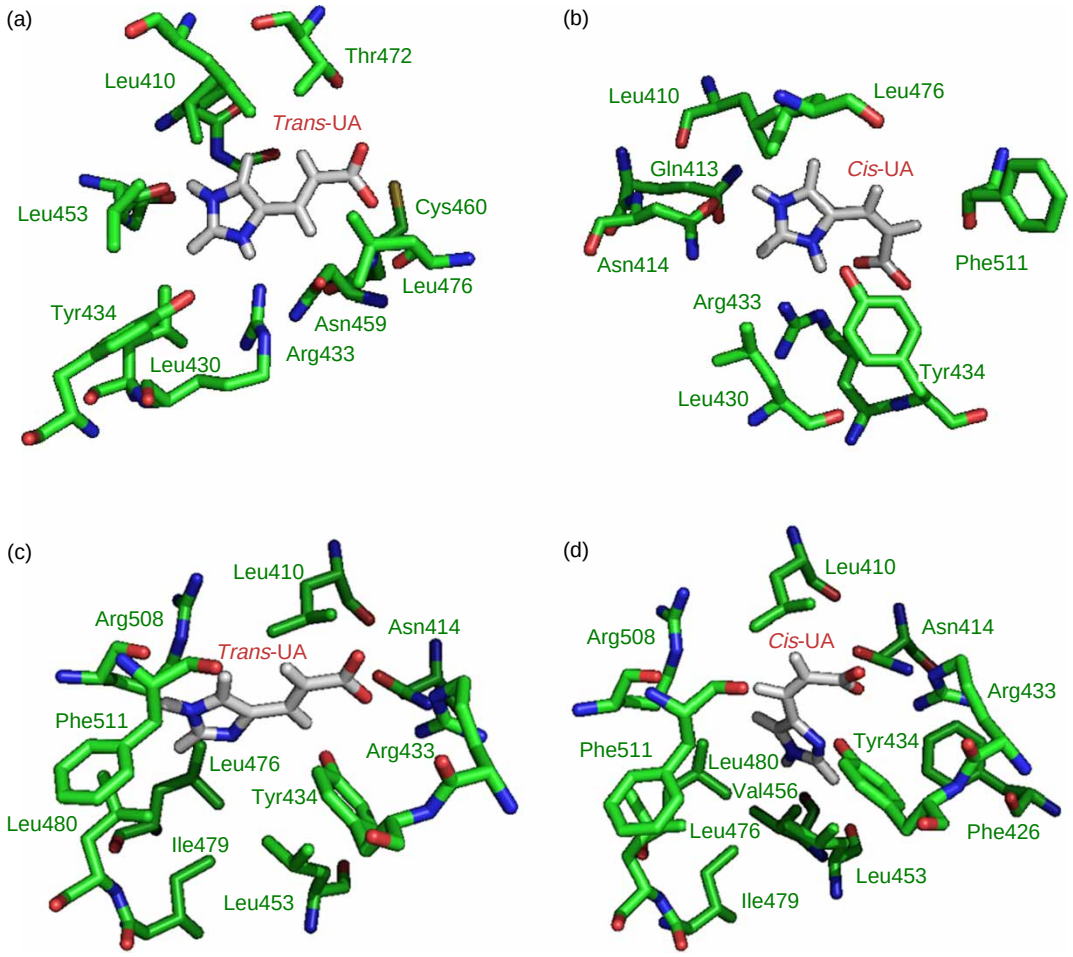

Figure 3. Close-up views of the binding modes of BSA with *trans*-UA (zwitterion) (a), *cis*-UA (zwitterion) (b), *trans*-UA (anion) (c) and *cis*-UA (anion) (d).

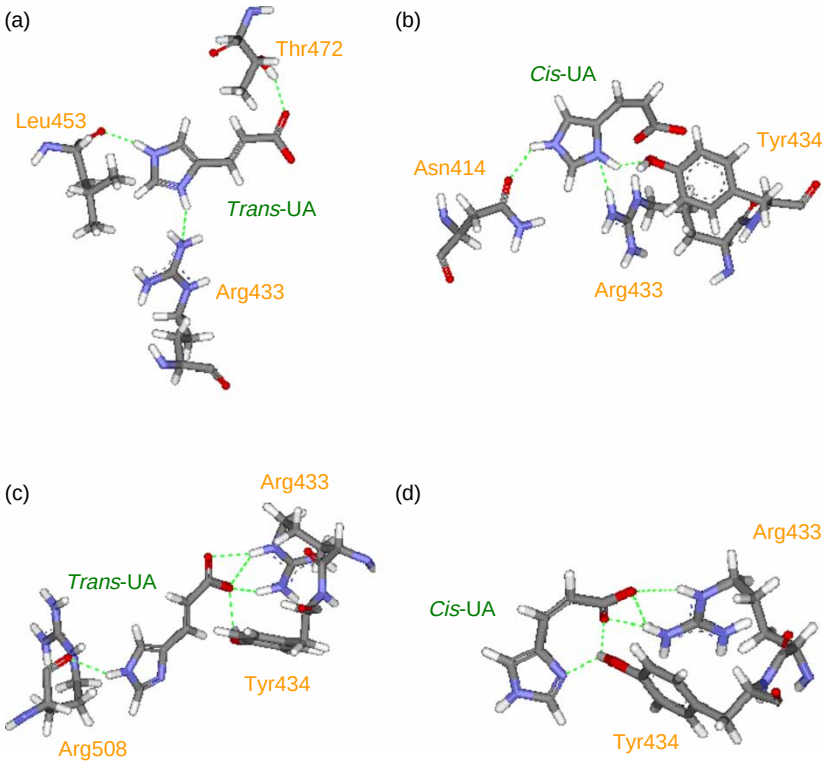

Figure 4. Close-up views of hydrogen bonds formed between BSA and *trans*-UA (zwitterion) (a), *cis*-UA (zwitterion) (b), *trans*-UA (anion) (c) and *cis*-UA (anion) (d). The hydrogen bonds are marked in green dotted lines.

was utilised for the present docking simulations [5]. The program FlexX [13] of SYBYL 7.0 [14] was used to explore the interactions of UA with BSA. Standard parameters were employed during the docking calculations. The Ludi module of Insight II was used to estimate the binding affinities of the four systems [15]. The Ludi score derived by the program is empirically related to the binding constant K_i: Ludi score $= 100 \log K_i$. Then, ΔG is calculated according to the equation $\Delta G = -2.303 RT \log K_i$.

Table 1. Hydrogen bonds formed between UA (zwitterion and anion) and BSA.

Complex	Donor	Acceptor	Distance (Å)	Angle (°)
pH 5				
BSA/*trans*-UA (zwitterion)	UA:H5	BSA:453:O	1.78	160.94
	BSA:472:HG1	UA:O9	2.17	137.26
	UA:H3	BSA:433:N	2.79	158.48
BSA/*cis*-UA (zwitterion)	UA:H3	BSA:434:O	2.19	161.50
	BSA:433:HH22	UA:N3	2.22	173.30
	UA:H5	BSA:414:OD1	1.94	167.90
pH 7.4				
BSA/*trans*-UA (anion)	UA:N5H	BSA:508:O	2.85	139.80
	BSA:433:HE	UA:O9	2.16	167.27
	BSA:434:HH	UA:O10	1.90	138.20
	BSA:433:HE	UA:O10	2.14	130.86
	BSA:433:HH22	UA:O10	1.56	152.65
BSA/*cis*-UA (anion)	434:HH	UA:O10	2.07	134.10
	434:HH	UA:N3	1.85	131.30
	433:HH22	UA:O9	1.74	149.95
	433:HE	UA:O10	2.09	152.11
	433:HH22	UA:O10	2.29	143.58

Table 2. Theoretically estimated FlexX scores of UA with BSA at pH 5.0 and 7.4.

	pH 5.0		pH 7.4	
Complex	BSA/*trans*-UA (zwitterion)	BSA/*cis*-UA (zwitterion)	BSA/*trans*-UA (anion)	BSA/*cis*-UA (anion)
Score	−15.5	−15.3	−24.3	−25.9
K_i[a]	280 ± 110	–	3400	6900
K_i[b]	380	209	4466	7762
ΔG[c]	−3.52	−3.16	−4.98	−5.31

[a] Experimental binding constants (M^{-1}) of UA to BSA from [5].
[b] Theoretical binding constants (M^{-1}) of UA to BSA.
[c] Theoretical ΔG (kcal/mol) for the binding of UA to BSA.

3. Results and discussion

To identify and characterise the binding sites of BSA, UA was centred to probe to a sphere of BSA with radius 5 Å. The residues included are depicted in Figure 3, from which, it can be seen that the binding patterns of UA are different at pH 5.0 and 7.4.

Close-up views of hydrogen bonds formed between BSA and UA at pH 5.0 and 7.4 are shown in Figure 4 and the characters of the hydrogen bonds formed between the ligand and receptor are listed in Table 1. Primarily, in the BSA/UA complex predicted by docking at pH 5.0, three hydrogen bonds form between residues of BSA and UA. In the BSA/trans-UA complex, Thr472 is with the deprotoned carboxyl group, Leu453 and Arg433 with the imidazole ring (Figure 4(a)). As to the BSA/*cis*-UA complex, all the three related residues, i.e. Asn414, Arg433 and Tyr434, are with the imidazole ring (Figure 4(b)). Moreover, at pH 7.4, five hydrogen bonds are observed between *trans*-UA and BSA, Arg433 and Tyr434 with the deprotoned carboxyl group, Arg508 with the imidazole ring as shown in Figure 4(c). In the BSA/*cis*-UA complex, five hydrogen bonds also form between the ligand and the receptor (Figure 4(d)) despite only two protein residues being involved, Arg433 with the deprotoned carboxyl group and Tyr434 with both the deprotoned carboxyl group and the imidazole ring. Given the same two residues (Arg433 and Tyr434) involved in the interactions of *trans*- and *cis*-UA with BSA, the general location of the binding site is similar in both cases.

To quantify the binding ability of UA to BSA, the FlexX scores calculated for the BSA/UA models at pH 5.0 and 7.4 are listed in Table 2. The lower the FlexX score the complex exhibits, the stronger the interactions exist between the ligand and the receptor. Therefore, according to the FlexX scores the binding of UA anion to BSA is much stronger in comparison with the zwitterion, which is in agreement with the experimental results that UA interacts more strongly with BSA at pH 7.4 than at pH 5.0 (Table 2) [5]. Moreover, at pH 7.4 *cis*-UA binds to BSA relatively stronger than *trans*-UA judging from the FlexX scores (Table 2), also in agreement with the experimentally determined higher binding affinity of the *cis* isomer ($3400\,M^{-1}$ for the *trans*-UA and $6900\,M^{-1}$ for the *cis*-UA) [5]. To make more direct comparisons of the binding affinities of the four systems, their respective ΔG have been estimated and listed in Table 2. It can be seen that the binding of UA to BSA is more favourable at pH 7.4 according to the relatively lower ΔG than that at pH 5.0. Moreover, the theoretical K_i is close to the experimental value, which verifies the methodology.

Through comparison of the binding modes of UA in zwitterionic and anion forms with BSA, it was revealed that the number of hydrogen bonds formed between the anion of UA and BSA at pH 7.4 is larger relative to the zwitterion at pH 5.0 (3 vs. 5, Table 1). In addition, as shown in Figure 3(a) and (b), Arg433 is close to the imidazole moiety in the BSA/UA complex at pH 5.0. Thus, the repulsion between the positive residue and the positively charged imidazole moiety in the zwitterion of UA will weaken the interactions between the ligand and receptor to a certain extent at pH 5.0. In comparison, at pH 7.4, the attractive interaction between Arg433 and the deprotoned carboxyl group will enhance the binding between UA and BSA [Figure 3(c) and (d)]. The opposite effect of electrostatic interaction may account in part for the experimentally observed increased binding affinity for UA anion with BSA.

In summary, the binding modes of both the *cis*- and *trans*-UA to BSA and the influence of pH on the binding affinities were explored by theoretical simulations. Based on the theoretical models, it can be inferred that the number of hydrogen bonds as well as the opposite effect of electrostatic interaction between the ligand and receptor may be the principal causes of different binding affinities displayed by UA anion and zwitterion with BSA.

Acknowledgements

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