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Molecular basis for *cis*-urocanic acid as a 5-HT_{2A} receptor agonist

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ABSTRACT

The underlying mechanisms of urocanic acid (UA) to induce immune suppression remain elusive until the recent finding that *cis*-UA acts via the serotonin, 5-hydroxytryptamine (5-HT) receptor subtype 5-HT_{2A}. In the present study, the interactions of *cis*-UA to 5-HT_{2A} receptor were explored and compared with those of 5-HT to the same receptor using computational docking. Similar binding modes were observed for *cis*-UA and 5-HT with 5-HT_{2A} receptor and the former possessed relatively higher binding affinity, which may account for *cis*-UA being a serotonin receptor agonist. Moreover, the molecular basis for the distinct binding affinities between the *trans*- and *cis*-UA with 5-HT_{2A} receptor was also provided.

As a deamination metabolite of histidine, urocanic acid (UA, Fig. 1) is one of the primary skin chromophores in the stratum corneum.[1,2] UA is generated initially as *trans*-isomer (*trans*-UA), which then may convert to *cis*-isomer (*cis*-UA) upon ultraviolet (UV) exposure.[1–3]

As we know, UV radiation can induce skin cancer and suppress the immune response. For induction of immune suppression, UV radiation must be absorbed by an epidermal receptor. UA was found as a UV photoreceptor[4] and its ability to induce immune suppression has been widely reported.[5,6] However, its underlying mechanisms of action remain elusive.[7] Recently, significant progress has been made with the finding that *cis*-UA may act via the serotonin, 5-hydroxytryptamine (5-HT) receptor.[8] Among the various 5-HT receptor subtypes, 5-HT_{2A} is the one involved in the UA-induced immune suppression and in vitro study showed that *cis*-UA binds to this receptor with relatively high affinity.[8] However, more effort is needed to elucidate the binding modes of UA to 5-HT_{2A} receptor and compare with those of 5-HT, which will be helpful to understand the molecular basis for *cis*-UA being a 5-HT receptor agonist. In addition, it is interesting to explore why *cis*-UA binds to 5-HT_{2A} receptor with relatively high affinity while *trans*-UA shows almost no binding.[8] Therefore, in the present work, the interactions of *cis*-UA, 5-HT as well as *trans*-UA with 5-HT_{2A} receptor were investigated by means of docking simulations.

A BLAST search in the Protein Data Bank (PDB) with human 5-HT_{2A} receptor sequence revealed no significant identity. However, among the retrieved results, bovine rhodopsin, which also belongs to G protein-coupled receptors (GPCRs), was included. Further alignment between the 5-HT_{2A} receptor sequence and that of bovine rhodopsin indicated it is an unambiguous alignment in the transmembrane helical regions. Thus, according to previous study,[9] the region of the second extracellular loop in the alignment was modified by hand to properly align the cysteine residues of the disulfide linkage. Then, structure coordinates for bovine rhodopsin (PDB code 1U19:A)[10] was used to construct the 5-HT_{2A} receptor model by employing the MODELLER module of Insight II software.[11] To generate structurally diverse receptor conformations, all residues within 12 Å of the bound retinal ligand in the template rhodopsin structure were mutated to alanine. The third intracellular loop was modeled simply as a poly-Gly chain whose backbone coordinates were taken from the structure of rhodopsin. The N and C-termini were truncated in this model.

First of all, hydrogens were added to the constructed model at pH 7.0 by employing the Biopolymer module of Insight II software. Then, molecular dynamics (MD) equilibration was performed with the consistent-valence force field (CVFF)[12–14] on a SGI Origin 350 server. The model was firstly minimized by 1000 conjugate gradient steps for equilibration, heated from 2 K to 300 K during 35 psec at temperature increment of 50 K per 5 psec. Then, the constant temperature and pressure algorithm was applied at 300 K for 200 psec. The velocity verlet integrator was used with an integration step of 2 fsec. Moreover, the feasibility of modeled structure (Fig. 2) of 5-HT_{2A} receptor was evaluated by Verify3D, which calculated structural compatibility scores based on 3D–1D profiles. The predicted structure of 5-HT_{2A} receptor had an acceptable 3D–1D self-compatibility score, beyond the incorrect fold score threshold indicating that the sequence of human 5-HT_{2A} receptor is energetically compatible with the structural environment of the model throughout the structure.[15]

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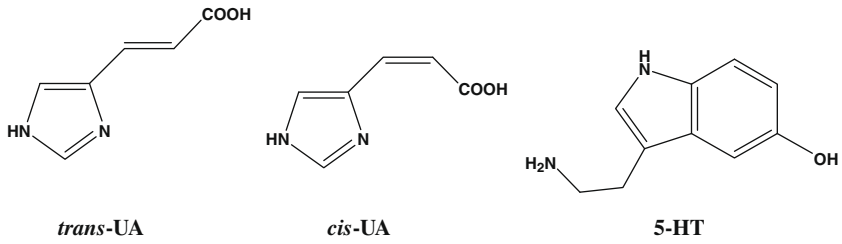

Figure 1. Chemical structures of *trans*-UA, *cis*-UA and 5-HT.

Previous studies reported two possible binding sites for 5-HT_{2A} receptor ligands[16] and site 1 has been proposed as the binding site for agonists,[17,18] which was utilized for the present docking simulations. Moreover, at pH 7.0, UA exists mainly as monoanion form, which is used as the starting point for the calculations.[3,19] The program FlexX[20] of SYBYL 7.0[21] was used to explore the interactions of UA with BSA. The Ludi module of Insight II[11] was used to estimate the binding affinities. The Ludi score derived by the program is empirically related to the dissociation constant K_d: Ludi score = $-100 \log K_d$.

Figure 3 shows the binding sites of 5-HT and *cis*-UA with 5-HT_{2A} receptor. Given the five same residues (Asp155, Val156, Ser159, Phe222 and Phe339) involved in the interactions of the two ligands with 5-HT_{2A} receptor, the general locations of the binding sites are similar to each other. To depict the interactions contributed to the binding, the hydrogen bonds formed between the ligands (5-HT and *cis*-UA) with the receptor 5-HT_{2A} receptor are marked in green dotted line and the residues involved in hydrophobic interactions are labeled in olive (Fig. 3). Primarily, in the 5-HT_{2A}/5-HT complex predicted by docking, four hydrogen bonds are formed between the receptor and 5-HT, the protonated nitrogen of 5-HT side chain with both Asp155 and Ser159, the indole hydrogen with Phe339, the phenolic hydroxyl with Lys223 (Fig. 3a). The predicted results is in agreement with previous experimental findings that Asp155 and Ser159 are crucial for the binding of 5-HT with its receptor

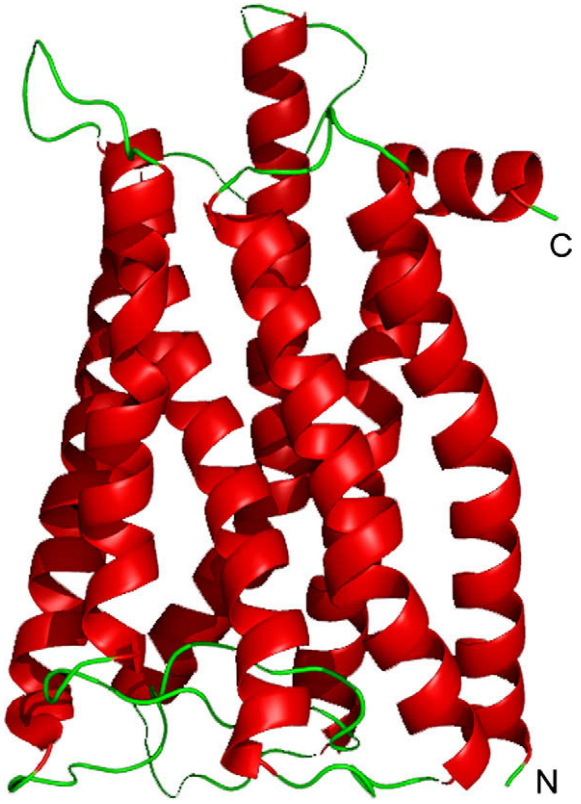

Figure 2. Cartoon structure of 5-HT_{2A} receptor generated using PyMol (http://www.pymol.org). Red: α-helix; green: random coil.

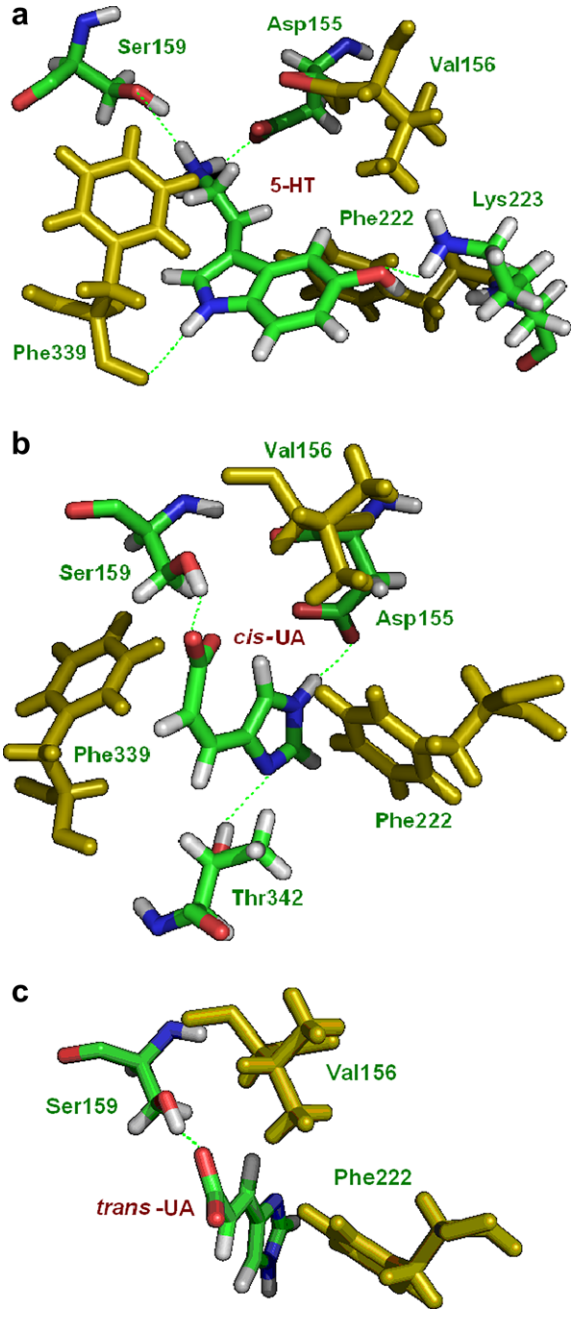

Figure 3. Close-up views of binding modes of 5-HT_{2A} receptor with 5-HT (a), *cis*-UA (b) and *trans*-UA (c). The hydrogen bonds are marked in green dotted lines and hydrophobic residues are labeled in olive.

5-HT_{2A} receptor.[22,23] As to the 5-HT_{2A}/*cis*-UA complex, three hydrogen bonds are observed between receptor and ligand, that is, Ser159 with the deprotoned carboxyl group, Asp155 and Thr342 with the imidazole ring (Fig. 3b). Although the residues Asp155 and Ser159 are both involved in the binding sites of 5-HT_{2A}/5-HT and 5-HT_{2A}/*cis*-UA, the detailed interaction patterns are different. Owing to the charge repulsion between the deprotoned carboxyl of *cis*-UA and that of Asp155, the imidazole ring orientated to Asp155 and form hydrogen bonds, while the deprotoned carboxyl of *cis*-UA located near Ser159. (Fig. 3a). Judging from the predicted structures, the similar binding modes of 5-HT and *cis*-UA to 5-HT_{2A} receptor is not based on their structure similarities as proposed in previous study (Fig. 1).[8] Moreover, the two ligands 5-HT and *cis*-UA make hydrophobic interactions with the same side chains of the protein active site, that is, Val156, Phe222 and Phe339 (Fig. 3a and b).

To quantify the binding abilities of *cis*-UA and 5-HT to 5-HT_{2A} receptor, the Ludi scores for the two complexes were calculated (Table 1). Then, according to the equation: Ludi score = $-100 \log K_d$, the binding affinity is estimated (Table 1). Firstly, the theoretically estimated K_d is about 9.1 nM for the 5-HT_{2A}/*cis*-UA complex, which is very close to the experimental value (4.6 nM).[8] The binding affinity of the 5-HT_{2A}/*cis*-UA is relatively higher than that of 5-HT_{2A}/5-HT. This is also consistent with the experimental result that the binding of *cis*-UA to 5-HT_{2A} receptor is favorable compared with 5-HT to 5-HT_{2A} receptor.[8,24]

In addition, previous study revealed that *trans*-UA shows almost no binding to 5-HT_{2A} receptor in comparison with the relatively higher binding affinity of *cis*-UA to the receptor.[8] As shown in Figure 3c, only one hydrogen bond is observed between 5-HT_{2A} receptor and *trans*-UA, Ser159 with the deprotoned carboxyl group. At the same time, comparing with the *cis*-UA, the hydrophobic interactions formed between *trans*-UA and surrounding residues are weakened (Fig. 3c). The theoretical K_d of the 5-HT_{2A}/*trans*-UA complex is about 81 μM, which is in agreement with the experimental finding that almost no binding is observed between *trans*-UA and the receptor.[8]

Table 1
Theoretically estimated Ludi scores and K_d of 5-HT, *cis*-UA and *trans*-UA with 5-HT_{2A} receptor

Complexes	Ludi scores	K_d
5-HT_{2A}/5-HT	747	34 nM
5-HT_{2A}/*cis*-UA	804	9.1 nM
5-HT_{2A}/*trans*-UA	409	81 μM

In summary, the computational docking studies found that the binding modes of *cis*-UA and 5-HT to 5-HT_{2A} receptor are very similar to each other and the two complexes also exhibit high binding affinities, which may be the principal molecular basis for *cis*-UA being a serotonin receptor agonist.

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