The X-ray Crystal Structure of the Human Monooxygenase Cytochrome P450 3A5-Ritonavir Complex Reveals Active Site Differences between P450s 3A4 and 3A5

Mei-Hui Hsu, Uzen Savas, and Eric F. Johnson
Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037
Molecular Pharmacology

Supplemental Figures:
S1: PCR primers used in this study.
S2: Ritonavir binding studies.
S3: Helix I conformations observed for 3A4 and 3A5 ritonavir complexes and substrate-free 3A4.
S4: Schisantherin E docked in the Active Site of 3A5 and poised for O-demethylation.
S5: Divergent pairs of amino acid side-chains in the helix F through helix G region of 3A4 and 3A5 and interacting portions of the C-terminal loop, helix A' and helix A.
(A) PCR primers

**N termini**

N1: 5’ggattccatATGGCTCTATATGGGACCCGTACACATGGACTTTTTTAAGAGACTGGGAATT 3’
      NdeI M A L Y G T R T H G L F K R L G I

N2: 5’ggattccatATGGGCTCGTACACATGGACTTTTTTAAGAGACTGGGAATTCCAGGG 3’
      NdeI M A R T H G L F K R L G I P G

**C termini**

C1: 5’ctagtctagatcatTTCTCCACTTAGGTTCCATCTCTTTGAATC 3’
      XbaI H H H H E G S L T G D R S D

C2: 5’cggattccgaagcttttaggtggtggtTCCATCTCTTTGAATCCACCTTTTAGAACAATGGGTCTCTCAGTGGCTCTTTG 3’
      HindIII H H H H G D R S D V K L V I P K

(B) Primer sets used for the constructs generated:

<table>
<thead>
<tr>
<th>Construct</th>
<th>Upper</th>
<th>Primer sets</th>
<th>Lower</th>
</tr>
</thead>
<tbody>
<tr>
<td>dH</td>
<td>N1</td>
<td>C1</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>N1</td>
<td>C2</td>
<td></td>
</tr>
<tr>
<td>N2C2</td>
<td>N2</td>
<td>C2</td>
<td></td>
</tr>
</tbody>
</table>

**Figure S1. PCR primers used in this study.** (A) Four PCR primers were used for the generation of 3A5 constructs. The restriction sites, NdeI for the N terminal primers and HindIII or XbaI for the C terminal primers, for cloning are underlined. The nucleotides for the four histidine residues in the C terminal primers are indicated with double underline. Stop codons in the C terminal primers are indicated as small bold italic letters. The capital letters in the primers represent the original or modified nucleotides in the CYP3A5 cDNA and the corresponding amino acid residues are indicated bellowed. (B) The combinations of PCR primer sets used in generating 3A5 constructs.
Figure S2. Ritonavir binding studies.
The binding of ritonavir to purified 3A5C2 (5.08 µM) was monitored by the reduction in the intensity of the absorption spectrum at 416 nm and the increase of intensity at 421 nm. After a 3 min incubation following each addition of ritonavir (in methanol), the spectra were recorded at ambient temperature. (A) Spectra of 3A5 in the absence of the ligand (red line) and after additions of ritonavir are shown. Incremental increases in the concentration of ritonavir of 1 (green), 2 (blue), 2.5 (cyan), 3 (magenta), 4 (yellow), 5 (gray), 6 (rose), 8 (lime), 15 (royal blue), 22 (sky blue) and 32 µM (violet) shift the Soret absorption maximum to higher wavelengths indicative of a low spin ferric heme, whereas the intensity of the Q-band at 567 nm diminishes. (B) Difference spectra were calculated by subtracting the spectrum obtained in the absence of the ligand from each of the spectra recorded following the addition of the ligand. (C) The concentration dependence of the absorbance difference between the peak and trough of the difference spectra for the Soret band were fit to the quadratic form of the one-site binding equation by non-linear regression as described in Experimental Procedures. The estimated dissociation constant is 0.051 µM with a maximum absorbance change of 0.218 for the experiment shown. The mean and standard error for values of the dissociation constant obtained from 3 replicate experiments are 0.051 ± 0.005 µM.
**Figure S3.** A: The conformation of the central portion of helix I of the 3A5 ritonavir complex (yellow) is similar to that of the 3A4 ritonavir complex PDB:3NXU (cyan). The middle portion of helix I is bowed outward for the 3A5 and 3A4 ritonavir complexes relative to the 3A4 substrate-free structure PDB:1TQN (magenta). B: A 2mFo-DFc composite omit map contoured at 1σ around the central portion of helix I defines the conformation of 3A5 ritonavir complex for the central region of helix I. Oxygen, nitrogen, sulfur, and iron are colored red, blue, yellow-orange, and orange, respectively.
S4: Schisantherin E docked in the active site of 3A5 and poised for O-demethylation. The selectivity for O-demethylation of Schisantherin E (slate blue carbons) relative to related lignans is dependent on a hydroxyl group and the benzoate ester moiety [Wu et al. (2017). A Naturally Occurring Isoform-Specific Probe for Highly Selective and Sensitive Detection of Human Cytochrome P450 3A5. J Med Chem 60: 3804]. In this docking pose, the critical hydroxyl group accepts an H-bond from Arg-106 (3.1Å) and donates an H-bond to Glu-374 (2.9Å). The esterified benzoate group binds in a pocket formed by L107, F210, F213, L240 and F241 in the structure of 3A5. The methyl group is positioned 4.2Å from the heme iron and is poised for hydrogen abstraction. Arg-106 and Glu-374 reside in more distant positions in 3A4, and F210 facilitates opening the pocket for the benzoate ester in 3A5. Autodock Vina was used to generate poses in the active site, and the pose was selected based on the distance of the site of metabolism from the heme iron. This pose differs from that reported for a homology model of 3A5 by Wu et al. 2017.
Figure S5. Divergent pairs of amino acid side-chains in the helix F through helix G region of 3A4 (cyan) and 3A5 (yellow) and interacting portions of the C-terminal loop, helix A’ and helix A. The divergent pairs of amino acid side-chains are depicted as sticks. When only one side-chain is shown, the corresponding amino acid in the other enzyme is a glycine.