Supplemental Information

Substrate-specific allosteric effects on the enhancement of CYP17A1 lyase efficiency by cytochrome b_5

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Methods

Although low concentrations of $Cytb_5$ significantly stimulate the lyase activity of human P450 CYP17A1,[1] higher concentrations prevent formation of the P450-CPR complex and thus has been shown to inhibit the catalytic activity.[1-3] Considering the importance of controlling the stoichiometry of protein-protein interactions, and the aggregation states of the membrane proteins under investigation, we use the Nanodisc system.[3] Incorporation of monomeric CYP17A1 in the lipid bilayer of Nanodiscs provides a well characterized and stable preparation of fully functional enzyme, as described in previous studies.[3,5,6,8] The manganese protoporphyrin replaced cytochrome b_5 (Mn-$Cytb_5$) is used as an isostructural analog that is inactive in electron transfer due to the very rapid autoxidation of Mn(II) structure.[4] Importantly, the use of Mn-Cytb5 avoids spectroscopic interference with the iron P450 heme allowing improved sensitivity for documenting the vibrational modes of the iron-oxygen complex that reflect changes in the positioning of the substrate in the active site.

The expression and purification of CYP17A1 was performed as described.[5] Reconstitution of $Cytb_5$ with manganese protoporphyrin IX was performed as described.[3] Nanodiscs with CYP17A1 were assembled as documented.[5,6] Briefly, human CYP17A1 with histidine affinity tag and cytochrome b5 from rabbit, both containing full-length trans-membrane helical anchors, were

expressed in E.coli. Nanodiscs were assembled with purified CYP17A1 and His-tag cleaved MSP1D1 solubilized with POPC lipids and Triton X-100 with the final ratio of CYP17A1:MSP1D1:POPC:Triton 1:10:650:750. Following 30 min incubation on ice, self-assembly of Nanodiscs was initiated by addition of 1 g of Amberlite per 1 mL of reconstitution solution. After 5 hr incubation at 4° C, Amberlite was removed by filtration, and Nanodiscs with CYP17A1 were purified using Ni-NTA affinity column and then size-exclusion chromatography on Superdex 200 10/300 GL column (GE Life Sciences). Incorporation of Mn substituted Cytb5 (Mn-Cytb5) into purified CYP17A1 Nanodiscs was performed by direct addition of Mn-$Cytb_5$ to the reconstitution mixture in a 2-fold molar excess, as documented.[7-9]

Resonance Raman sample preparation

To better investigate effects of cytochrome b_5 on the H-bonding interactions in the CYP17A1 distal pocket, two substrates, 17α-hydroxyprogesterone (OH-PROG) or 17α-hydroxypregnenolone (OH-PREG) were incubated with CYP17A1 in Nanodiscs in the presence of a 2-fold excess Mn-$Cytb_5$. Cytochrome b5 is known to spontaneously anchor in a phospholipid bilayer due to its simple single trans-membrane helix. Samples for rR spectroscopy contained 250 μM CYP17A1 in Nanodiscs, 0.1 M potassium phosphate, pH 7.4, 0.2 M NaCl, and 400 μM of 17α-hydroxyprogesterone or 17α-hydroxypregnenolone (Sigma Aldrich). Solutions were prepared in distilled 30% (v/v) glycerol in H_2O buffer starting by degassing the samples by connecting the NMR tube to a vacuum line and refilling with argon gas. This process is repeated for three times to ensure complete deoxygenation. Samples were reduced under anaerobic conditions by titrating with a 0.9~1.0 fold molar excess of sodium dithionite in the presence of 6.25 μM methyl-viologen. Each sample was reduced at room temperature and then transferred to a dry ice-ethanol bath held at -20 °C where it was cooled for 2 minutes. Oxy-ferrous complexes were formed by bubbling $^{16}O_2$ or $^{18}O_2$ for 7 seconds, followed by rapid freezing in liquid N_2.

Resonance Raman Measurements

To determine the effect of $Cytb_5$ on the heme stretching modes of ferric CYP17A1, we employed Mn-$Cytb_5$. This was done to prevent interference from the heme of $Cytb_5$ as was previously reported by Mak and coworkers in rR studies of CYP2B4.[10] We acquired resonance Raman spectra after adding 2-fold excess of Mn-$Cytb_5$. The spectra of frozen dioxygenated

samples were obtained using the 413.1 nm excitation line from a Krypton ion laser (Coherent Innova Sabre Ion Laser), which effectively enhances the internal modes of the Fe-O-O fragment. Each oxygenated sample was measured for 4 hours with very low power (1 mW) maintained on the sample. The rR spectra of all samples were measured using a Spex 1269 spectrometer equipped with a Spec-10 LN liquid nitrogen-cooled detector (Princeton Instruments, NJ). All samples were measured in a spinning NMR tube to avoid local heating and protein degradation. The spectra were collected using a 180° backscattering geometry, and the laser beam was focused on the sample with a line image using a cylindrical lens. Spectra were calibrated with data acquired for fenchone and processed with Grams/32 AI software (Galactic Industries, Salem, NH).

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