Supporting Information

Kinetic Solvent Isotope Effect in Human P450 CYP17A1 Mediated Androgen Formation: Evidence for a Reactive Peroxoanion Intermediate

Journal: Journal of the American Chemical Society

Corresponding Author: Stephen G. Sligar

Other Authors: Michael C. Gregory, Ilia G. Denisov, Yelena V. Grinkova, Yogan Khatri, Stephen G. Sligar

University of Illinois at Urbana Champaign

505 S Goodwin

Urbana, Illinois 61801

E-mail: s-sligar@illinois.edu

Phone: 217.344.4539

Fax: 217.265.4073

Detailed Methods

Protein Expression, Purification, and Nanodisc Incorporation

Protocols for the expression of CYP17A1, cytochrome b_5, and cytochrome P450 oxidoreductase (CPR) have been previously reported.[1-3] Nanodiscs containing human CYP17A1 were prepared as documented previously.[4]

Pregnenolone Hydroxylase Quantitation

Pregnenolone hydroxylase activity was quantitated using radiolabeled [7-^{3}H] pregnenolone. For preparation of deuterated buffers, the pH reading was corrected by the method of Glasoe and Long.[5] Substrate (8.5 nmol, 0.8 μCi) was incubated at 37°C with CYP 17A1 Nanodiscs (62 pmol), CPR (248 pmol) and, cytochrome b_5 (235 pmol) in 0.25 ml of 0.1 M K-phosphate buffer pH/pD 7.4 containing 50 mM NaCl for 10 minutes before the reaction was initiated by addition of 120 nmol NADPH. The incubation continued for 15 minutes and the reaction was terminated by addition of 2 mL of dichloromethane. After phase separation the organic layer was dried, reconstituted in 30 μL of methanol, and loaded onto a TLC plate (EMD TLC Silica gel 60 F254, 20X20 cm) which was run in a chloroform: ethyl acetate: water mix (80:20:1). Five-point calibration with known amounts of radioactivity was performed for each plate. The plate was imaged using BAS-IP TR 2025 imaging plate and Storm 840 imaging system. The data were processed using ImageJ software to obtain percent conversion.

OH-PREG Lyase Quantitation

OH-Pregnenolone lyase activity was quantitated in a reconstituted system containing 208 pmol CYP17A1, and a four-fold molar excess of cytochrome b_5

and CPR in 1 mL of 0.1 M potassium phosphate buffer, pH/pD 7.4 containing 50 mM NaCl and 50 μM OH-PREG (Sigma). All reactions were carried out in a stirred cuvette held at 37°C and monitored at 340 nm after initiation by the addition of 600 nmol NADPH. Reactions were quenched after 20 minutes by addition of 50 μL 8.9 N H_2SO_4 and were stored at -80 °C until extraction.

Solutions were thawed and 800 μL was delivered to a test tube and steroid product extracted via double extraction with 2 mL dichloromethane after addition of the progesterone internal standard. Extracted steroids were dried under a stream of N_2 and reconstituted in 1.5 mL 80% MeOH. The resultant lipemic mixture was purified via a second extraction using 750 μL pentane and the MeOH/steroid layer was removed and evaporated to dryness under vacuum. Steroids were reconstituted in cyclohexane and analyzed on an Agilent 6890 GC-FID equipped with a 15 m DB-1 column, 530 μm I.D., 1.5 μm film thickness (J & W Scientific). Injector temperature and volume were 250°C and 3 μL. Nitrogen carrier gas was set to 2.2 mL/min and the oven temperature was held at 100°C for 1.5 minutes and ramped to 275°C at 30°C/min and held for 13 minutes.

References:

(1) Gregory, M.; Mak, P. J.; Sligar, S. G.; Kincaid, J. R. *Angewandte Chemie* **2013**, *52*, 5342.
(2) Shen, A. L.; Porter, T. D.; Wilson, T. E.; Kasper, C. B. *J Biol Chem* **1989**, *264*, 7584.
(3) Mulrooney, S. B.; Waskell, L. *Protein Expr Purif* **2000**, *19*, 173.
(4) Luthra, A.; Gregory, M.; Grinkova, Y. V.; Denisov, I. G.; Sligar, S. G. *Methods in molecular biology* **2013**, *987*, 115.
(5) Glasoe, P. K.; Long, F. A. *The Journal of Physical Chemistry* **1960**, *64*, 188.