Communication

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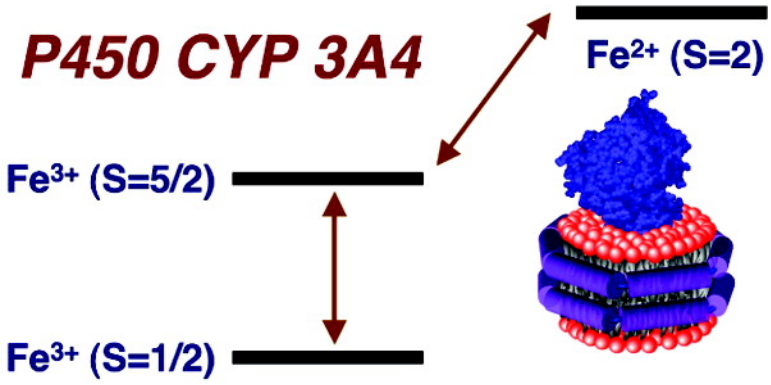

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Redox Potential Control by Drug Binding to Cytochrome P450 3A4

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Human cytochrome P450s play the most critical role in drug metabolism. There are multiple P450s in the liver, and the exact control of redox potential by drug binding to the cytochrome P450s is not well understood. For an effective physiological function of the numerous human P450s, understanding the mechanism of control of redox events on substrate binding is critical. Hepatic cytochrome P450 3A4 (CYP3A4) is a membrane bound cytochrome P450 involved in the metabolization of almost 50% of currently available drug molecules in humans.[1] The control of redox potential via drug binding to CYP3A4, studied herein, offers an elegant means of linking the utilization of pyridine nucleotide derived reducing equivalents to efficient substrate metabolism and thus avoiding the production of unwanted reduced oxygen species that could be deleterious to the organism.

For most soluble bacterial P450s, redox measurements have shown a correlation between the substrate induced shifts in the ferric spin state of the heme iron and the redox potential of the protein.[2,3] In fact, the basic thermodynamic spin-state control of redox potential has been shown to be true for many heme proteins.[2,4] However, numerous studies on the redox potential control by substrate binding to membrane bound CYP proteins (in detergents and liposomes) have claimed that the redox potential of membrane bound cytochrome P450s show little if any dependence on the binding of substrates,[5] the mitochondrial CYP11A1 being an exception.[6] In particular, previous studies on detergent solubilized CYP3A4 have suggested that there is no change in redox potential of the protein on binding substrates such as testosterone and ethylmorphine, although a change in the ferric spin equilibrium is observed.[5b] These studies have led to the speculations that substrate binding, spin state, and redox potential, which have been shown to be linked for soluble bacterial P450s, is not applicable to the important CYPs involved in drug metabolism.

In this Communication, we measure the solution redox potential of CYP3A4 in a native-like membrane bilayer mimic (Nanodisc). The Nanodisc provides an ideal environment for generating active, soluble, and monodisperse integral membrane proteins and avoids potential aggregation that could make redox potential measurements difficult.[7] We show that the redox potential of CYP3A4 in Nanodiscs can be correlated with the spin equilibrium constants of the ferric heme protein, analogous to that observed for CYP101, yielding a coherent understanding of metabolic regulation in the cytochrome P450s.

The assembly of CYP3A4 in Nanodiscs yields a 1:1 ratio of CYP3A4 to the nanobilayer and represents a homogeneous and monomeric population.[8] Previously, it has been shown that bromocriptine (BC) and testosterone (TST) binding to CYP3A4-Nanodiscs induce a >90% spin conversion to the high spin ($S = {}^5/_2$) state[8a] compared to an incomplete high spin conversion in various reconstituted or detergent solubilized systems.[9] Other substrates, such as erythromycin (ERY), yield only 22% high-spin content when bound to CYP3A4-Nanodiscs (Supporting Information). Hence, the change in redox potential on binding these substrates can be used to define the linkage of substrate binding, ferric spin state, and overall redox potential change in membrane bound cytochrome CYP3A4.

Spectropotentiometric titrations of CYP3A4-Nanodiscs employed Dutton's method in the presence of standard mediators.[11] Figure 1A shows that on reduction, for both the substrate-free and -bound oxidized CYP3A4-Nanodiscs, there are transitions in both Soret and Q-band region. The fraction of the reduced protein as calculated from the spectral components is plotted against the applied potential in Figure 1C. From Figure 1D (Nernst plot),[10] the midpoint potential of the protein is calculated and the values are reported in Table 1.

The binding of the Type I substrates TST and BC leads to a significant increase in the high spin content, accompanied by an increase in redox potential of ca. +80 mV. The nanoscale bilayer environment allows clean measurement of redox potentials of the membrane protein owing to increased stability of the monomeric CYP3A4 and the absence of aggregation or presence of detergents which can alter the physical state of the CYP3A4 heme protein.

Key factors leading to the difference in redox potentials of the substrate-free and -bound P450 include the difference in ligand field stabilization energy between low-spin (LS) and high-spin (HS) ferric heme, the conformational change in the protein, and the change in the local dielectric[12] around the heme. In CYP3A4, the coordination of water at the sixth distal ligand position can stabilize the LS hexacoordinated state of the ferric heme. As water is a weak ligand for the ferrous heme, the reduced ferrous CYP3A4 is predominantly in the HS five-coordinated state. On substrate binding the water molecule coordinated to ferric LS can be displaced, leading to the formation of five-coordinated ferric HS iron.[13] This makes it easier for the substrate-bound protein to be reduced, as the free energy difference between substrate-bound five-coordinated ferric HS and five-coordinated ferrous HS is lower than substrate-free ferric LS and ferrous HS. This is manifested in an increase in the redox potential of CYP3A4 on binding substrates. The stronger the ability of the substrate to perturb the water ligation to the ferric heme, the more pronounced is the spin-state shift resulting in an increase in the redox potential.

We found a linear free-energy relationship between the formal potential ($E^{o\prime}$) observed versus the free-energy change $\Delta G = RT \ln K_{SPIN}$ of the ferric spin state equilibrium for both CYP3A4 and CYP 101[2c] (Figure 2) where K_{SPIN} is the ratio of the high-spin and low-spin fraction of the ferric protein. Interestingly, CYP3A4 in Nanodiscs displays the same slope as that previously determined for the soluble CYP101, but with a uniform shift to higher redox potential, demonstrating that the modulation of heme redox potential through effect on the ferric spin equilibrium is of similar origin for both proteins.

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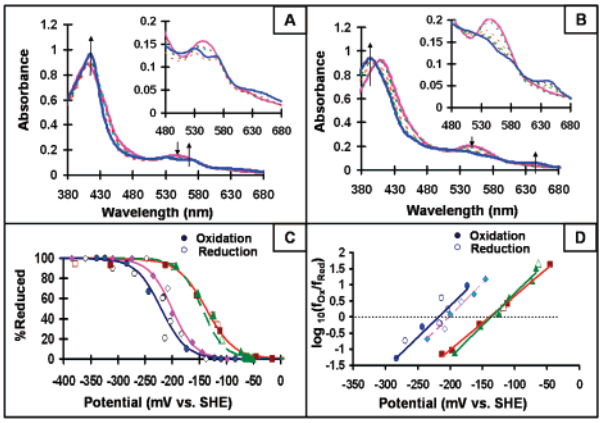

Figure 1. The redox titration of CYP3A4 in Nanodiscs is conducted in 100 mM phosphate buffer pH 7.4 at 25 °C for substrate-free and substrate-bound protein. (A) For substrate-free CYP3A4, the direction of the arrows indicate that the absorption maxima at 548 and 408 nm decreases and at 569 and 415 nm increases during the course of oxidative titration. (B) For substrate bound CYP3A4, the absorption maxima at 548 and 408 nm decreases while that at 645 and 391 nm increases during the course of the oxidative titration. The changes in the absorption maxima were used to calculate the percentage of reduced protein. The potential is measured at a 4,4′-dithio-dipyridine modified gold electrode. (C) The percentage of reduced cytochrome CYP3A4 in Nanodiscs vs the electrode potential is plotted for the (i) substrate-free (circles), (ii) erythromycin-saturated (diamonds), (iii) bromocriptine-saturated (triangles), and (iv) testosterone-saturated (squares) forms. The oxidative and reductive cycles of redox potentiometry are shown as closed and open data points. (D) The Nernst plot (25 °C) for single electron redox function is reported for the substrate-free CYP3A4 and substrate-bound forms.[10] The zero intercept gives $E^{\circ\prime}$, the redox potential of the protein (Table 1), and the slope divided by 59 mV yields n, the number of electrons involved in the reaction which is 0.8−1 for the measurements indicated.

Table 1. The Formal Potential ($E^{\circ\prime}$) and Substrate Induced ($\Delta E^{\circ\prime}$) Shifts for CYP3A4-Nanodisc Binding to the Different Substrates as Determined from the Nernst Plots Shown in Figure 1D

enzyme state	high spin	$E^{\circ\prime}$ (mV)	$\Delta E^{\circ\prime}$ (mV)
substrate free	11%	−220 ± 10	
erythromycin	22%	−210 ± 10	+10 ± 5
testosterone	92%	−140 ± 5	+80 ± 10
bromocriptine	93%	−137 ± 5	+83 ± 10

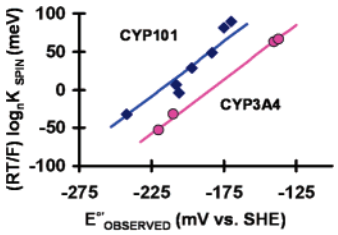

Figure 2. Free-energy correlation between the CYP3A4 and CYP101 ferric spin-state equilibrium and the observed redox potential.

The local dielectric constant can also play an important role in determining the absolute redox potential of the heme protein as ferrous heme is neutral compared to a ferric heme which has a net positive charge.[12] The redox potential values measured for CYP3A4 in Nanodisc are relatively more positive compared to those reported for soluble CYP101, Figure 2. This right shift in the linear free-energy plot reflects the more nonpolar environment of the heme center in the membrane. Such effects have been discussed in the literature for other heme proteins.[12b,c]

In summary, we show that spectropotentiometry of membrane bound cytochrome P450s can be successfully achieved using the Nanodisc system. Multiple redox cycling of CYP3A4-Nanodiscs is reversible, without any conversion to the inactive P420 form. We report a significant increase in CYP3A4 redox potential upon binding some Type I substrates and show a common linkage between substrate binding, the ferric spin state equilibrium, and overall redox potential that is shared by both soluble and membrane bound P450s. These linkages can reflect a substrate dependent control of metabolism, providing a means for avoiding unwanted production of toxic reduced oxygen species in the absence of substrate.

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Supporting Information Available: Description of the experimental methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Guengerich, F. P. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 1−17. (b) Guengerich, F. P. in *Cytochrome P450: Structure Mechanism, and Biochemistry*, 3rd ed.; Ortiz de Montellano, P. R., Ed.; Kluwer Academic/Plenum Publishers: New York, 2005; pp 377−530.
(2) (a) Sligar, S. G. *Biochemistry*, **1976**, *15*, 5399−5406. (b) Sligar, S. G.; Gunsalus, I. C. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1078−1082. (c) Fisher, M. T.; Sligar, S. G. *J. Am. Chem. Soc.* **1985**, *107*, 5018−5019.
(3) Johnson, D. L.; Conley, A. J.; Martin, L. L. *J. Mol. Endocrinol.* **2006**, *36*, 349−359.
(4) (a) Safo, M. K.; Nesset, M. J. M.; Walker, F. A.; Debrunner, P. G.; Scheidt, W. R. *J. Am. Chem. Soc.* **1997**, *119*, 9438−9448. (b) Nesset, M. J. M.; Shokhirev, N. V.; Enemark, P. D.; Jacobson, S. E.; Walker, F. A. *Inorg. Chem.* **1996**, *35*, 5188−5200.
(5) (a) Guengerich, F. P. *Biochemistry* **1983**, *22*, 2811−2820. (b) Yamazaki, H.; Johnson; W. W.; Ueng; Y.-F.; Shimada, T.; Guengerich, F. P. *J. Biol. Chem.* **1996**, *271*, 27438−27444. (c) Sligar, S. G.; Cinti, D. L.; Gibson, G. G.; Schenkman, J. B. *Biochem. Biophys. Res. Commun.* **1979**, *90*, 925−932.
(6) Light, D. R.; Orme-Johnson, N. R. *J. Biol. Chem.* **1981**, *256*, 343−350.
(7) Nath, A.; Atkins, W. M.; Sligar, S. G. *Biochemistry* **2007**, *46*, 2059−69.
(8) (a) Baas, B. J.; Denisov, I. G.; Sligar, S. G. *Arch. Biochem. Biophys.* **2004**, *430*, 218−228. (b) Denisov, I. G.; Grinkova, Y. V.; Baas, B. J.; Sligar, S. G. *J. Biol. Chem.* **2006**, *281*, 23313−23318. (c) Denisov, I. G.; Baas, B. J.; Grinkova, Y. V.; Sligar, S. G. *J. Biol. Chem.* **2007**, *282*, 7066−7076. (d) Denisov, I. G.; Grinkova, Y. V.; McLean, M. A.; Sligar, S. G. *J. Biol. Chem.* **2007**, *282*, 26865−26873.
(9) (a) Hosea, N. A.; Miller, G. P.; Guengerich, F. P. *Biochemistry* **2000**, *39*, 5929−5939. (b) Harlow, G. R.; Halpert, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6636−6641.
(10) Nernst equation $\Delta E = \Delta E^{\circ} + (2.303\ RT/n(F))\ \log_{10}(C_{OX}/C_{RED})$ Where ΔE is the observed potential, C_{OX} is the concentration of oxidized and C_{RED} is the concentration of the reduced species.
(11) Dutton, P. L. *Methods Enzymol.* **1978**, *54*, 411−435.
(12) (a) Kassner, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2263−2267. (b) Krishtalik, L. I.; Tae, G. S.; Cherepanov, D. A.; Cramer, W. A. *Biophys. J.* **1993**, *65*, 184−195. (c) De Biase, P. M.; Doctorovich, F.; Murgida, D. H.; Estrin, D. A. *Chem. Phys. Lett.* **2007**, *434*, 121−126. (d) Hervás, M.; Ortega, J. M.; De la Rosa, F. F.; Losada, M. *Biochem. Biophys. Res. Commun.* **1988**, *152*, 981−986.
(13) (a) Tsai, R.; Yu, C. A.; Gunsalus, I. C.; Peisach, J.; Blumberg, W.; Orme-Johnson, W. H.; Beinert, H. *Proc. Natl. Acad. Sci. U.S.A*, **1970**, *66*, 1157−1163. (b) Champion, P. M.; Lipscomb, J. D.; Munck, E.; Debrunner, P.; Gunsalus, I. C. *Biochemistry*, **1975**, *14*, 4151−4158 (c) Raag, R.; Poulos, T. L. *Biochemistry* **1989**, *28*, 917−922. (d) Denisov, I. G.; Makris, T. M.; Sligar, S. G.; Schlichting, I. *Chem. Rev.* **2005**, *105*, 2253−77.

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