Rapid Conformational Dynamics of Cytochrome P450 2E1 in a Natural Biological Membrane Environment†

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ABSTRACT: Among the members of the cytochrome P450 superfamily, P450 2E1 is most often associated with the production of reactive oxygen species and subsequent cellular toxicity. We sought to identify a structural basis for this distinguishing feature of P450 2E1 by examining its carbon monoxide binding kinetics as a probe of conformation/dynamics. We employed liver microsomes from wild-type and P450 2E1 knockout mice in order to characterize this P450 in a natural membrane environment. The CO binding kinetics of the P450s of wild-type microsomes had a rapid component that was absent in the knockout microsomes. Data analysis using the maximum entropy method (MEM) correspondingly identified two distinct kinetic components in the wild-type microsomes and only one component in the knockout microsomes. The rapid kinetic component in wild-type microsomes was attributed to endogenous P450 2E1, while the slower component was derived from the remaining P450s. In addition, rapid binding kinetics and a single component were also observed for human P450 2E1 in a baculovirus expression system, in the absence of other P450s. Binding kinetics of both mouse and human P450 2E1 were slowed in the presence of ethanol, a modulator of this P450. The unusually rapid CO binding kinetics of P450 2E1 indicate that it is more dynamically mobile than other P450s and thus able to more readily interconvert among alternate conformations. This suggests that conformational switching during the catalytic cycle may promote substrate release from a short-lived binding site, allowing activated oxygen to attack other targets with toxic consequences.

The cytochrome P450s (P450s)[1] comprise a ubiquitous superfamily of heme protein enzymes that oxidatively metabolize a wide variety of endogenous and exogenous compounds (*1*, *2*). The catalytic mechanism includes the controlled activation and modulation of the reactivity of molecular oxygen (*3*). During the catalytic cycle, substrate binding induces a change in the spin state of the heme iron, which facilitates electron transfer from an electron donor such as NADPH−cytochrome P450 reductase. The ferrous form of the enzyme binds molecular oxygen and proceeds through a series of steps that result in molecular oxygen scission, with one atom of oxygen leaving as a water molecule while the other atom forms an activated complex with the heme iron in the active site. The activated intermediate then attacks the adjacent substrate, most often leading to oxygen addition (hydroxylation) although numerous other reaction types are possible (*3*, *4*).

In mammals, the P450s have evolved to become the primary interface for the metabolism of drugs and other xenobiotics (*5*, *6*). While the P450s involved in biological pathways tend to have more traditional "one substrate → one product" reaction schemes, the P450s involved in xenobiotic metabolism, most prominently those of the liver, tend to have many different and structurally dissimilar substrates that are often converted into multiple products. P450-mediated metabolism of xenobiotics typically results in the introduction or unmasking of sites for subsequent biotransformation reactions, leading to a decreased lipid solubility and facilitation of excretion. The robust nature of the reactions catalyzed by the cytochrome P450 superfamily allows for the metabolism of a myriad of structurally distinct chemical entities and is the basis of its ability to metabolize the many xenobiotics to which mammals are exposed. This capability is augmented by the induction of cytochrome P450s, often by the xenobiotics which they metabolize (*7*). This is critical as the majority of the clinically prescribed drugs and other xenobiotics must undergo at least one step of P450-mediated biotransformation during the elimination process. The liver is the most prominent site of xenobiotic and drug biotransformation as it has the highest content of P450 and is dynamically responsive to these chemical challenges.

P450 2E1 is one of the most conserved xenobiotic-metabolizing P450s in mammals (*8*). The conservation most likely arises from the roles members of its subfamily play

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[1] Abbreviations: ΔA_{450}, absorbance change at 450 nm; CYP2E1 +/+, wild-type mice; CYP2E1 −/−, CYP2E1 knockout mice; *k*, rate distribution; MEM, maximum entropy method; $NADP^+$/NADPH, oxidized/reduced nicotinamide adenine dinucleotide phosphate; P450 or CYP, cytochrome P450; N(t), fraction of CO unbound; *P*(*k*), relative probability distribution of *k* values; C57/SV129, strain of the mice used.

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in metabolizing ketones generated during normal cellular processes. Accordingly, P450 2E1 substrates include acetone and ethanol, which are also two of its prominent inducers. A distinguishing feature of this P450 is its frequent association with liver damage and hepatotoxicity (*9*−*14*), which is thought to result from damage caused by the generation of reactive oxygen species (*15*−*19*). The tendency of P450 2E1 to uncouple during the catalytic cycle is strongly correlated with reactive species generation and in vivo cellular damage and apoptosis (*20*). The consequences of uncoupling have thus been examined from different perspectives, such as identification of modified proteins or lipids and effects on signaling pathways. However, little is known of the structural basis for the special propensity of P450 2E1 (relative to other P450s) to generate reactive oxygen species. We thus sought to identify a structural feature that would distinguish P450 2E1 from other P450s and which might help to explain its enhanced tendency to generate activated oxygen that does not transfer to the substrate. Gaining further insight into the fundamental early steps in catalysis will facilitate development of approaches to preempt drug and xenobiotic interactions that result in undesirable products and side reactions.

We used laser flash photolysis of CO as a probe ligand to examine P450 conformational and structural dynamics (*21*−*25*). P450 was examined in natural biological milieus rather than as the purified form that may yield artifactual results. The maximum entropy method (MEM) (*26*−*28*) was used to derive the kinetic landscape of liver microsomes from wild-type and P450 2E1 knockout mice and human P450 2E1 expressed in a baculovirus system. We identified a rapid CO binding species that is directly attributable to P450 2E1 and whose conformational dynamics are distinctly more rapid than that of the other P450s. Its enhanced flexibility suggests an active site lacking the requisite rigidity to stably orient substrate near the activated oxygen for efficient transfer, resulting in escape of activated oxygen and subsequent generation of undesirable products.

MATERIALS AND METHODS

Tissue Sources and Preparation. C57/SV129 wild-type and C57/SV129 *CYP2E1* −/− (knockout) mice (*11*) were kindly provided by Dr. Frank J. Gonzalez at the National Cancer Institute, National Institutes of Health. The mice were sacrificed by carbon dioxide asphyxiation and the livers immediately excised, rinsed with 100 mM phosphate buffer and 100 mM KCl, pH 7.4, and flash frozen in liquid nitrogen until used. Microsomes were prepared after brief tissue disruption with a polytron followed by homogenization using 10 strokes with a Teflon/glass motor driven tissue homogenizer at 4 °C. Differential centrifugation was used to isolate the microsomal fraction. The microsomes were stored at −80 °C in 0.1 M potassium phosphate and 20% glycerol, pH 7.4. Typically, samples were stored at 4−6 μM P450 concentration as calculated using $\epsilon_{450-490} = 91\ \mathrm{mM^{-1}\ cm^{-1}}$ for the carbon monoxide adduct (*29*). Human P450 2E1 was expressed in SF9 insect cells using recombinant baculovirus and prepared as previously described (*30*). P450 2E1 containing baculosomes was purchased from BD Gentest, Woburn, MA. Protein concentrations were measured by the bicinchoninic acid method (*31*).

Laser Flash Photolysis. The laser flash photolysis technique was used to measure the rebinding kinetics of the probe ligand, carbon monoxide, to the heme in the active center of the P450s. This was accomplished using a specially designed laser flash photolysis system (*32*). The instrument was designed to accurately detect small absorbance transients resulting from photolysis of P450-containing, turbid microsomal suspensions. Procedures for maximizing signal/noise ratios and preventing signal aliasing were addressed in the instrumental design to ensure that the kinetic measurements were reflective of the intended biological phenomena. Briefly, 0.5 μM total P450 was typically used under pseudo-first-order conditions (ligand/P450 ratio > 10). Experiments were conducted at 23 °C in 100 mM sodium phosphate (pH 7.4) containing 20% glycerol in a total volume of 1.2 mL. Samples were allowed to incubate at 23 °C for 20 min, and 20 μM CO and dithionite (a few grains) were added just prior to photolysis. Photodissociation of the heme−CO complex was initiated by a 0.6 μs laser pulse from a dye laser and the kinetics of rebinding monitored at 450 nm.

Data Analysis. Origin Pro 7.0 (OriginLab, Inc., Northhampton, MA) was used for averaging, fitting, and graphical representations of data sets. Laser flash photolysis data were analyzed by using the maximum entropy method (MEM) (*26*−*28*) through the MemSys kernel (Maximum Entropy Data Consultants, U.K.). The MEM is a probability-based statistical method that makes minimal assumptions about the data. It is superior to classical multiexponential analysis because low signal/noise ratios do not obscure the salient trends in the analysis. Raw absorbance transients ($\Delta A_{450\mathrm{nm}}$ versus time) were converted to fraction unbound [$N(t)$] versus time by normalizing to the point of maximum photolysis (maximum ΔA). Data in this format were appropriate for analysis via the MEM and were searched in log k space to identify the most plausible distribution pattern. Typically, 250 bins were searched in log k (s^{-1}) space to yield the probability distribution $P(k)$ versus k. With completely noiseless data, the positions and heights of the distributions solved by the MEM are identical to the discrete species identified via classical multiexponential analysis. However, with high experimental noise and the nonorthogonal nature of exponentials, the MEM solutions are typically more informative and less ambiguous than those obtained by multiexponential analysis. The signal/noise ratios with mammalian cytochrome P450s in microsomes are much lower than those obtained when using soluble heme proteins as much of the incident light is lost due to turbidity-induced scatter. Although the design of our laser photolysis system dramatically minimized these effects, lower signal/noise ratios greatly affected classical fitting approaches while the MEM was minimally affected and relatively insensitive to the noise in the data sets. The MEM results are depicted as plots of $P(k)$ vs k. The peaks depicted in this type of analysis represent the kinetic components present in the data. The MEM solves for the most plausible distribution of rates that describes the data by maximizing the entropy function while minimizing the χ^2 values in an iterative fashion.

Western Blotting. Polyacrylamide gel electrophoresis of samples was conducted using Pierce Ready Gels and running buffer (Pierce, Rockford, IL). Proteins were electrophoresed at 125 mA/gel for 35−45 min in a Mini-Protean 3 (Bio-Rad Laboratories, Hercules, CA). Western blotting was performed using the Towbin buffer system (*33*). Polyacrylamide gels were equilibrated in Tris/SDS/methanol for 10 min and then

transferred to polyvinylidene difluoride (PVDF) membranes using the Mini-Transblot electrophoretic transfer system (Bio-Rad Laboratories, Hercules, CA). Transfer was at 300 mA for 4 h followed by 90 mA overnight. Blots were washed with 1× PBST, blocked for 1 h with 3% non-fat dry milk, and incubated with primary antibody for 1 h in 3% non-fat dry milk. A rabbit polyclonal anti-human P450 2E1 antibody (Chemicon International, Temecula, CA) was used to specifically detect both mouse and human P450 2E1. Blots were visualized with CL Xposure X-ray film (Pierce, Rockford, IL) via chemiluminscent detection of a horseradish peroxidase-conjugated goat anti-rabbit IgG with the West Dura detection kit (Pierce, Rockford, IL). X-ray film was developed using a M35A X-OMAT processor (Eastman Kodak, Rochester, NY).

P450 Activity Assays. P450 2E1 activity was measured using a *p*-nitrophenol hydroxylation assay (*34*, *35*). Activity of the liver microsome preparations was compared to that of a commercial baculosome sample containing human P450 2E1 and reductase (Gentest, Woburn, MA) using assay conditions suggested by the manufacturer. Reaction mixtures consisted of 1.3 mM $NADP^+$, 3.3 mM glucose 6-phosphate, 3.3 mM $MgCl_2$, 0.002 unit of glucose-6-phosphate dehydrogenase, and 10−100 pmol of P450. For the mouse liver samples, endogenous NADPH−cytochrome P450 reductase was the electron donor. Reactions were incubated for 30 min at 37 °C. Reactions were stopped by the addition of $^1/_5$ volume of 20% trichloroacetic acid followed by centrifugation for 2 min at 10000*g*. Two volumes of the resulting supernatant was added to one volume of 2 M NaOH and the 4-nitrocatechol content determined via absorbance spectroscopy using a Cary 100 scanning spectrophotometer (Varian, Palo Alto, CA), using $\epsilon = 10.28\ mM^{-1}\ cm^{-1}$ for 4-nitrocatechol (*34*). Control baculosomes (without P450 2E1) and rat brain microsomes were used as negative controls.

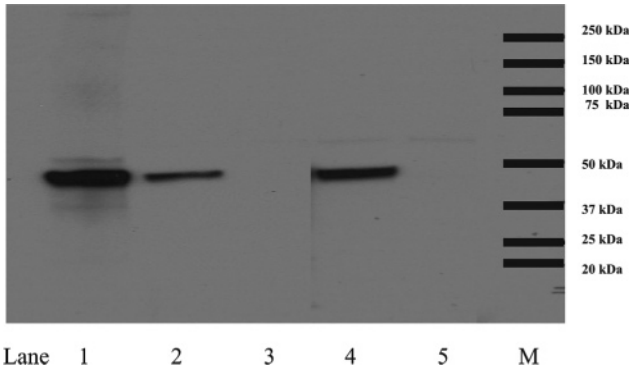

FIGURE 1: Western blot analysis of wild-type and P450 2E1 knockout mice microsomes. Rabbit anti-human P450 2E1 antibody was used for immunodetection. Lanes: 1, baculosomes from insect cells expressing human P450 2E1 (2.5 pmol of P450 2E1); 2 and 4, wild-type microsomes (10 and 25 pmol of total P450); 3 and 5, knockout microsomes (10 and 25 pmol of total P450); M, molecular mass markers.

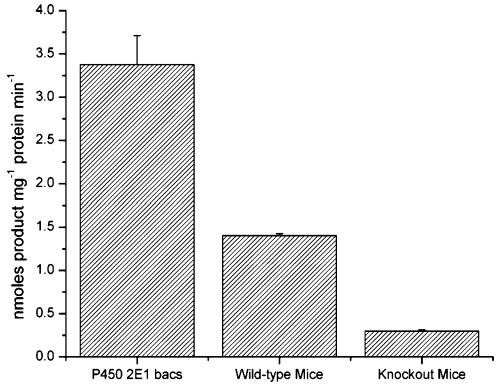

FIGURE 2: Comparison of the *p*-nitrophenol hydroxylase activity of wild-type and knockout mouse microsomes. Assays were conducted under similar conditions for a human P450 2E1 baculosome control, wild-type mouse microsomes, and P450 2E1 knockout mouse microsomes.

RESULTS

Characterization of Mouse Microsomes. To examine P450s in a natural membrane environment, we first examined microsomes from the livers of genetically manipulated mice. The strains used were C57/SV129 either with an intact endogenous *CYP2E1* gene (wild-type) or with the gene disrupted (knockout). This system affords a unique opportunity to investigate the properties of P450 2E1 in the background of other hepatic P450s. Microsomes prepared from the two mouse strains were electrophoresed, transferred to membranes, and probed with a P450 2E1-specific antibody. P450 2E1 was found in the liver microsomes from wild-type but not knockout mice (Figure 1). In the wild-type mice (lanes 2 and 4), a band migrating with an apparent molecular mass of ∼50 kDa is readily apparent. The corresponding knockout lanes (lanes 3 and 5) lack a signal in that region. In the control lane (lane 1), the antibody identifies P450 2E1 from baculosomes prepared from insect cells expressing recombinant human cytochrome P450 2E1. These results verify the expected phenotypic consequences of the P450 2E1 knockout mice and confirm similar molecular masses of the mouse and human P450 2E1.

The functional activity of P450 2E1 was also assessed via its ability to hydroxylate *p*-nitrophenol. This assay is considered relatively selective for mouse P450 2E1, although other human and rat P450s can also hydroxylate *p*-nitrophenol (*35*). The specific activity of wild-type liver microsomes was ∼50% that of baculosomes containing human P450 2E1 (Figure 2). The specific activity of the knockout microsomes was 5-fold lower than that of wild-type microsomes. The low activity of the former presumably derives from metabolism of *p*-nitrophenol by endogenous mouse P450s other than P450 2E1, since P450 2E1 protein was undetectable in immunoblots of these microsomes (Figure 1). The results thus indicate that the wild-type mouse microsomes have similar activity to the P450 2E1-containing baculosomes while knockout mice microsomes had very little activity. Thus, in terms of both P450 protein content and functional enzyme, the knockout mice lack P450 2E1, which is consistent with previous studies (*11*, *35*).

CO Binding Measurements and Kinetic Landscape of Cytochrome P450s. The laser flash photolysis technique was applied to measure the CO binding kinetics of P450s in wild-type and knockout microsomes. Representative ligand binding curves (Figure 3A) show that the wild-type time course returns to baseline faster than the knockout sample. A rapidly decaying component is particularly evident at the earliest times in the wild-type microsomes that is absent in the knockout microsomes. We subjected the data to MEM

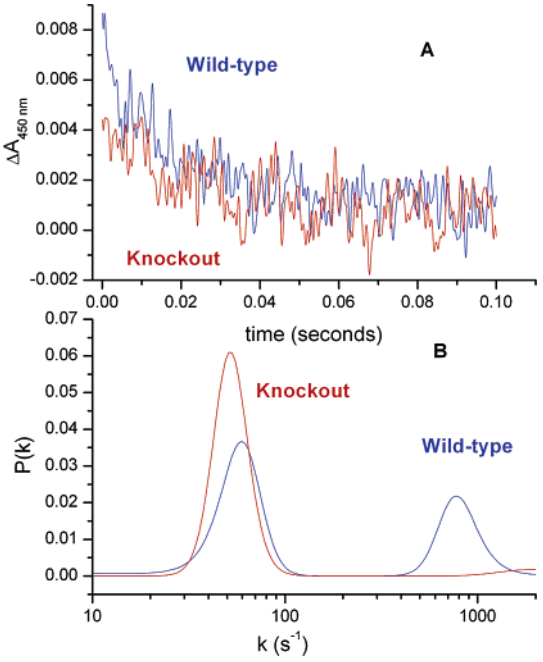

FIGURE 3: Kinetics of CO binding for wild-type (blue lines) and knockout (red lines) mouse microsomes. The raw absorbance versus time traces (A) and MEM analysis profiles, $P(k)$ vs k, (B) are shown.

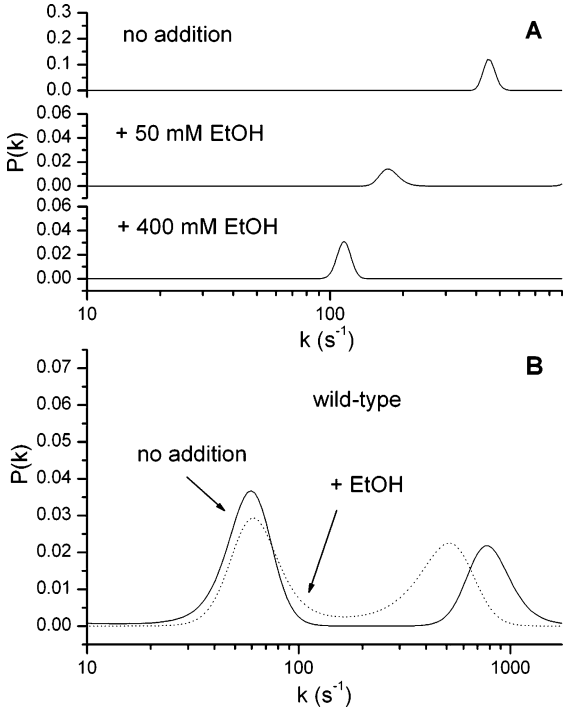

FIGURE 4: Effects of ethanol on the CO ligand binding of P450 2E1. Maximum entropy method analysis was performed on baculovirus-expressed human P450 2E1 in the presence of 0, 50, and 400 mM ethanol (A). Similar analysis for wild-type mouse microsomes (B) is shown in the absence or presence of 400 mM ethanol, indicated by solid and dashed lines, respectively.

analysis, which extracts the most probable kinetic distribution with minimal initial assumptions. Figure 3B shows a distinct difference between the MEM profiles of these samples, as the wild-type exhibited two peaks while the knockout sample exhibited only one peak. Both the wild-type and knockout samples have similar peaks at 60 and 53 s^{-1}, respectively. The wild-type microsomes had a unique peak at approximately 775 s^{-1}, which is absent in the distribution for knockout microsomes. A similar rapid component is also observed in the single peak at 450 s^{-1} observed with expressed human P450 2E1 (Figure 4A). These results thus suggest that the rapid component in the wild-type mouse microsomes derives from endogenous mouse P450 2E1, while the peak at the lower rates derives from the remaining P450s.

Ethanol is both an inducer and substrate of P450 2E1. When 250 mM ethanol was added to the mouse microsomes, the rapid component in the wild-type samples was slowed from 775 to 520 s^{-1} (Figure 4B) while little effect was seen on the slower component. The ethanol effect is also seen with the concentration-dependent change in the human P450 2E1 distributions toward slower binding rates (Figure 4A). The selective ethanol effect on the rapid component in mouse microsomes further supports its assignment to P450 2E1.

DISCUSSION

P450 2E1 is one of the xenobiotic-metabolizing P450s found in mammalian liver. This isoform, unlike many of the other P450s involved in biotransformation, is well conserved in mammalian species (*8*). This conservation may be due to its role in metabolizing ketones, alcohols, and other small molecule endobiotics and xenobiotics. P450 2E1 is induced by a number of its substrates including acetone and ethanol (*36*). Distinctive from many other hepatic P450s, the mechanism of P450 2E1 induction is independent of transcriptional activation and involves changes in mRNA and protein stability (*7*, *37*).

P450 2E1 plays a prominent role in the production of reactive oxygen species and toxic metabolites (*15*, *16*, *38*) and is thought to contribute to alcohol-induced liver disease and toxicity as well as to the tissue damage that can occur in diabetes and obesity (*39*). The common theme among the aforementioned maladies is the elevation of P450 2E1 inducing agents, ethanol from the former case and ketones and other endogenous metabolic products in the latter. The mechanisms by which P450 2E1 exerts its deleterious effects have been examined from several perspectives. Targets of the reactive oxygen species have been identified and include proteins and membrane lipids. The consequences of these modifications have been studied and include alterations in signaling pathways. Although the effects of P450 2E1 associated toxicity have been demonstrated, little is known about the structural basis for these effects; i.e., does P450 2E1 have some property, that is especially prominent relative to other P450s, that enhances its ability to generate reactive oxygen species? Some insight into this problem may be achieved by first considering the relatively small in vivo lifetime of P450 2E1 (*40*, *41*). Although its degradative machinery is complex, protein stability is one factor that determines susceptibility to proteolysis. Some distinctive structural feature of this P450 may thus explain its instability. The relatively high uncoupling of catalysis has been invoked as one explanation for the instability (*41*, *42*). However, the origin of this inefficient coupling is unknown. We thus sought to identify a structural feature that might rationalize the inefficient transfer of activated oxygen to substrate. To minimize artifacts that might result from using P450s either in a purified state or in an artificial membrane reconstitution system, we only examined P450s in natural membrane environments.

The emergence of genetically manipulated mouse strains has allowed us to address precise questions about the role that a specific macromolecule plays in growth and development, homeostasis, and disease progression. In many cases, disrupting or knocking out a functional protein has no effect on the phenotype. Yet, at the structure/function level, there may be a number of consequences that are relevant to understanding macromolecular interactions. The ability to remove a single protein from an organism affords a unique opportunity to ascertain the importance of that protein, especially if one can probe its interactions in a functional milieu. We thus used a P450 2E1 knockout mouse strain, C57/SV129 −/− CYP2E1, in this study. The inactivation of the CYP2E1 gene was nonlethal, and there were no obvious effects on development (*11*). However, when challenged with drugs or other agents that are known to elicit P450 2E1-mediated toxicity, metabolic consequences were observed. For example, a challenge with the drug acetaminophen resulted in the generation of a toxic metabolite and extreme hepatotoxicity and necrosis in the wild-type mice while the knockout mice tolerated higher drug levels (*11*).

We examined two systems: (1) mouse P450 2E1 in wild-type and knockout mouse liver and (2) human P450 expressed in insect cells. The knockout system allowed us to assign a rapidly reacting kinetic component to P450 2E1. The rapid kinetics was also observed with the human P450 2E1 expressed in the baculovirus system. To further confirm that this component was indeed P450 2E1, we assessed the effect of ethanol, a known substrate, inducer, and effector for this P450. In both systems ethanol slowed the rate somewhat, although the rate was still much greater than that of the bulk P450s. The data thus show that P450 2E1 from two different species and in different membrane environments exhibits kinetics distinct from the bulk of P450s.

The rate and complexity of CO binding depends on the accessibility of the heme active site to CO that migrates from the surrounding solvent and thus reflects the subtleties of P450 conformational/structural dynamics. The CO binding rates are sensitive to both the local environment of the heme active site and global dynamic motions of the protein. The common rapidity of CO binding by mouse and human P450 2E1 indicates a commonality in their conformation/dynamics. This may be a manifestation of the functional conservation of members of this subfamily in mammals (*8*) that distinguishes P450 2E1 from other mammalian P450s. P450 2E1 binding kinetics appear to be monoexponential, unlike human P450 3A4 and P450 1A1 which exhibited more complex multiexponential binding kinetics (*22*, *23*). The presence of a lone conformer in human and mouse P450 2E1 and of multiple conformers in P450 3A4 and P450 1A1 suggests a functional role for conformational diversity that may be related to some of the differences in substrate interactions of these P450s.

Recent studies using high-pressure spectroscopy and fluorescence transfer techniques have also suggested that P450s may exist as a population of structurally distinct conformers whose populations can be modulated by substrate binding or interactions with other effectors (*43*−*45*). This conformational diversity may account for the atypical kinetics often observed with the P450s, especially in the case of P450 3A4 (*43*, *46*−*48*). In addition, several recently solved mammalian P450 crystal structures demonstrate both "open" and "closed" active site conformations which can be influenced by bound ligands (*49*−*52*). Computational solvent mapping furthermore finds active sites in the ligand-bound but not ligand-free structures of mammalian P450s (*53*). These findings imply that movement and flexibility of the active site are critical to the ability of mammalian P450s to recognize the many different and structurally dissimilar xenobiotics. This is consistent with the conformer model whereby a single P450 exists as a population of structurally and functionally discrete entities that are dynamically adaptable to the nature of the substrate present in the active site.

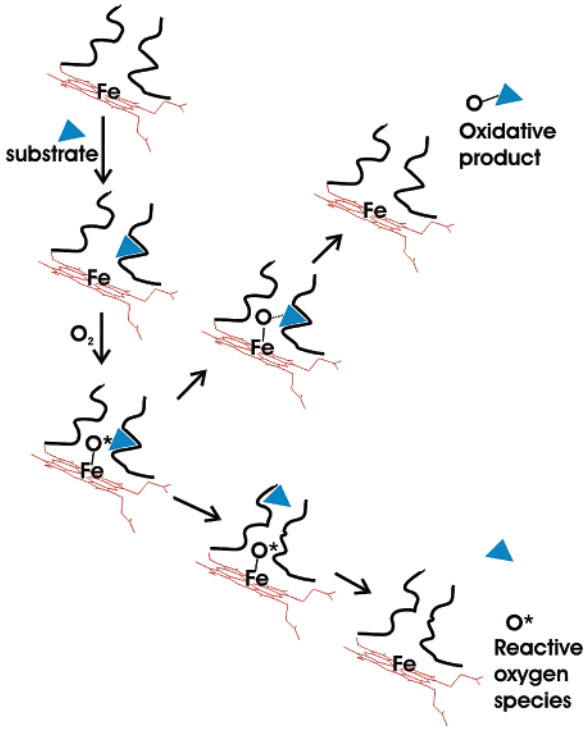

FIGURE 5: Scheme for oxidative uncoupling in P450 2E1 catalysis. Substrate first binds to the active site (top). Molecular oxygen is then activated (O*) at the heme iron center, positioned to attack the substrate. The upper branch depicts successful catalysis, where oxygenated product dissociates from the P450. Uncoupling is represented by the lower branch, in which the substrate binding site is not maintained, allowing substrate to migrate away from the activated oxygen. The latter then reacts with other targets.

The monophasic kinetics and single MEM peak of P450 2E1 may at first suggest that, in contrast to other P450s, it is conformationally homogeneous. However, in view of the rapid binding rate, the single peak may also derive from a flexibility that allows rapid interconversions between numerous, energetically similar structures in the conformational landscape. This interpretation is supported by resonance Raman spectra of human 2E1 which revealed low-temperature conformational heterogeneity at the active site of human P450 2E1 (*54*). The rapid kinetics we observed thus suggests that, at room temperature, these conformers interconvert rapidly relative to other P450s. In contrast, at low temperatures, the conformers do not have sufficient energy to overcome the energy barriers for interconversion and are thus resolvable. Our structural interpretation is supported by an enzyme kinetic study of ethanol oxidation which indicates that conformational changes occur during the catalytic cycle of P450 2E1 (*55*).

The observation of a dynamic, flexible P450 2E1 active site might account for its propensity to uncouple during

catalysis. A plausible explanation for uncoupling is shown in the scheme in Figure 5. First, substrate binds the active site and is followed by generation of activated oxygen at the heme iron. The next step in successful P450 catalysis entails transfer of activated oxygen to the substrate. However, in the highly flexible P450 2E1 active site, the substrate has the opportunity to dissociate from a transitory binding site and migrate away from the heme. In the absence of a substrate, the activated oxygen is released from the heme and reacts with another target, with potentially toxic consequences. This would provide a plausible rationale for the oft observed uncoupling of catalysis by P450 2E1 and subsequent damage by reactive oxygen species.

In summary, we have shown that the CO binding kinetics of mouse and human P450 2E1 are unusually rapid compared to other P450s. This indicates a dynamic mobility that is enhanced over other P450s, which facilitates interconvertibilty among alternate active site conformations. During catalysis, such a fluid active site would allow substrate release and permit activated oxygen to escape and engage in detrimental reactions. This model thus provides a link between P450 2E1 structure and its often observed special propensity to generate reactive oxygen species.

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