Heme oxygenase-1 affects cytochrome P450 function through the formation of heteromeric complexes: Interactions between CYP1A2 and heme oxygenase-1

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Heme oxygenase 1 (HO-1) and the cytochromes P450 (P450s) are endoplasmic reticulum–bound enzymes that rely on the same protein, NADPH-cytochrome P450 reductase (POR), to provide the electrons necessary for substrate metabolism. Although the HO-1 and P450 systems are interconnected owing to their common electron donor, they generally have been studied separately. As the expressions of both HO-1 and P450s are affected by xenobiotic exposure, changes in HO-1 expression can potentially affect P450 function and, conversely, changes in P450 expression can influence HO-1. The goal of this study was to examine interactions between the P450 and HO-1 systems. Using bioluminescence resonance energy transfer (BRET), HO-1 formed HO-1•P450 complexes with CYP1A2, CYP1A1, and CYP2D6, but not all P450s. Studies then focused on the HO-1–CYP1A2 interaction. CYP1A2 formed a physical complex with HO-1 that was stable in the presence of POR. As expected, both HO-1 and CYP1A2 formed BRET-detectable complexes with POR. The POR•CYP1A2 complex was readily disrupted by the addition of HO-1, whereas the POR•HO-1 complex was not significantly affected by the addition of CYP1A2. Interestingly, enzyme activities did not follow this pattern. BRET data suggested substantial inhibition of CYP1A2-mediated 7-ethoxyresorufin de-ethylation in the presence of HO-1, whereas its activity was actually stimulated at subsaturating POR. In contrast, HO-1–mediated heme metabolism was inhibited at subsaturating POR. These results indicate that HO-1 and CYP1A2 form a stable complex and have mutual effects on the catalytic behavior of both proteins that cannot be explained by a simple competition for POR.

Cytochrome P450 (EC 1.14.14.1) is a heme-containing, membrane-bound protein that catalyzes the oxygenation of a wide variety of exogenous and endogenous compounds (1, 2). Generally, metabolism by P450 converts lipid-soluble substrates to more water-soluble products and thereby facilitates their elimination. However, in many instances, metabolism by P450 can generate reactive intermediates that lead to toxicity, mutagenesis, and/or carcinogenesis (2, 3). Additionally, the standard monooxygenase reaction can be “uncoupled,” leading to generation of superoxide and H₂O₂ (4).

Heme oxygenase 1 (EC 1.14.14.18; HO-1) is responsible for the first step of heme degradation, converting the substrate to biliverdin (5, 6). This enzyme protects cells from oxidative damage and is induced by agents that are related to oxidative stress, including many xenobiotics, such as aspirin, statins, niacin, etc. (7–9). HO-1 levels are also influenced by disease states including inflammation, diabetes, hepatic injury, infectious disease, and cancer (6–8). A feature common to these conditions is oxidative stress, where HO-1 induction leads to the elimination of pro-oxidants (heme) and the generation of antioxidants (e.g., bilirubin and CO).

There is evidence that HO-1 and some P450s are capable of forming homomeric complexes that can affect function. HO-1 (10, 11), CYP1A2 (12), CYP2C2 (13), CYP2E1 (12, 14), and CYP3A4 (15, 16) were reported to form homomeric complexes which, in several cases, affected enzyme catalysis. The formation of heteromeric complexes also produces significant effects on P450 function. Such complexes have been reported for CYP1A2–CYP2B4, CYP1A2–CYP2E1, CYP2C9–CYP3A4, CYP1A1–CYP3A2, CYP2C9–CYP2D6, CYP2E1–CYP2B4, CYP2D6–CYP2E1, CYP3A4–CYP2E1, and CYP2C9–CYP3A4 (15, 17–30).

Heme oxygenase and P450s do not function alone but require an interaction with NADPH-cytochrome P450 reductase (POR) (31), which, in most tissues, is found in limiting concentrations (32). Although the HO-1 and P450 systems are interconnected by their common electron donor, the systems generally have been studied separately. The existence of both physical and functional P450•P450 interactions has been well established, whereas potential interactions between HO-1 and the P450s have attracted relatively little attention. Still, evidence that P450s and HO-1 can affect each other’s function does exist. Anwar-Mohamed et al. (33) showed that the arsenite-induced inhibition of CYP1A1, CYP1A2, CYP3A23, and CYP3A2 in rat hepatocytes was at least partially mediated by induction of HO-1, but the mechanism for these effects was not investigated.

The known abundance of P450•P450 interactions and the observed effects the interactions on enzyme function suggest...
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interactions between HO-1 and P450s may also influence catalytic activities. The goal of this study was to examine the potential for interactions between the HO-1 and P450 systems, focusing on CYP1A2. The results show that the presence of HO-1 has a significant impact on P450 function that cannot be explained by a simple mass-action competition between HO-1 and P450 for limiting reducing equivalents. The results also show that HO-1 and CYP1A2 form a stable complex both in reconstituted systems and in natural membranes, suggesting that the change in both CYP1A2 and HO-1 function is the result of this complex.

Results

Effect of HO-1 on complex formation with different P450 enzymes

With the knowledge that P450•P450 interactions have been associated with changes in protein activity, the potential for physical interactions between HO-1 and P450s was examined. In an effort to determine if interactions between different P450s and HO-1 were selective for the individual enzymes, an initial search was performed with different green fluorescent protein (GFP)–tagged P450s and Rluc-tagged HO-1. Because full-length HO-1 has a naturally occurring C-terminal membrane-binding region, the Rluc–HO-1 fusion protein was engineered with an N-terminal Rluc tag. Conversely, P450s and POR (having N-terminal membrane domains) were labeled on the C terminus. This was done to minimize association of the tags with the membrane-binding regions of the proteins, allowing them to interact with the endoplasmic reticulum. HEK 293T/17 cells were transfected with the proteins at a high GFP-to-Rluc ratio to ensure that the measured signal approximated bioluminescence resonance energy transfer (BRET)max for each pair. Cells were incubated for 48 h post transfection to optimize protein expression to allow for the detection of lower-affinity P450–HO-1 interactions. Under these conditions, higher BRET signals were observed where HO-1 was paired with CYP1A1, CYP2D6, and CYP1A2, with much weaker responses observed with CYP2A6 and CYP2C9 (Fig. 1). Based on these data, the HO-1–CYP1A2 reaction was examined for further study. These results suggest that interactions between HO-1 and P450s are specific for the particular P450 enzyme present.

Characterization of physical interactions among CYP1A2, HO-1, and POR

We then focused on CYP1A2 because of the high BRET signal generated when paired with HO-1 in the initial screening experiment (Fig. 1), and the known ability of CYP1A2 to form complexes with other P450s (17–21, 26, 34). The goal of this study was to determine whether CYP1A2 and HO-1 formed a specific BRET-detectable complex and to see if the complex was stable in the presence of POR. This was accomplished by measuring complex formation for each of the three potential binary interactions among CYP1A2–HO-1, HO-1–POR, and CYP1A2–POR in the presence and absence of the third protein. Expression vectors for CYP1A2-GFP and Rluc-HO-1 were transfected into HEK 293T/17 cells at a range of GFP-to-Rluc ratios. These data follow saturation curves that were fit to a hyperbolic function. The maximum BRET value (BRETmax) correlates with the fraction of Rluc-tagged proteins interacting with GFP-tagged proteins and serves as an indicator of protein complex formation. Adjustments to the DNA levels were made to ensure that measured differences in BRETmax could not be attributed to differences in protein expression levels. Specifically, when a difference in BRETmax was observed, total tagged protein expression for each point of the lower curve was between 1.0 and 1.2x the average total tagged protein expression of the higher curve (Fig. 2B).

The first goal was to establish that HO-1 and CYP1A2 formed a stable complex and to determine if the complex could be disrupted by the presence of POR. Figure 2A shows that the CYP1A2-GFP–Rluc-HO-1 BRET pair generated a saturation curve that was consistent with the formation of a specific CYP1A2•HO-1 complex. Transfection of unlabeled POR to the HEK293T cells showed no significant disruption of this complex, suggesting that the CYP1A2•HO-1 complex was stable. Figure 2B shows that the total amount of protein expression was similar in both the absence and presence of cotransfected, unlabeled POR.

The specificity of complex formation was also illustrated by measuring the BRET response as a function of total protein expression (Fig. 2C). If these complexes were not specific and simply the result of protein crowding, a straight line with a positive slope going through the origin would be expected (35). These results also demonstrate that over a large range of transfection levels, the maximal BRET response is relatively insensitive to changes in levels of transfected DNA. Taken together, these results indicate that HO-1 and CYP1A2 form a
specific complex when cotransfected into living cells and that the complex is stable in the presence of added POR.

Next, the effect of HO-1 on CYP1A2-POR complex formation was examined. As expected, CYP1A2 and POR formed an efficient BRET complex (Fig. 3A). Cotransfection of untagged HO-1 alongside the CYP1A2-GFP–POR-Rluc BRET pair led to a greater than 70% decrease in BRET$_{\text{max}}$. Again, levels of the proteins in the BRET pair were similar both in the absence and presence of unlabeled HO-1 (Fig. 3B), indicating that the decrease in BRET signal was not simply due to a decrease in protein expression but due to disruption of the POR–CYP1A2 complex. The disruption of the POR–CYP1A2 complex is consistent with HO-1 preferentially binding to POR when all three proteins are present.

In the complementary experiment, the effect of unlabeled CYP1A2 on the POR–HO-1 BRET pair was examined. Again, the expected complex between HO-1 and POR was generated (Fig. 4). Surprisingly, cotransfection of unlabeled CYP1A2 did not significantly affect formation of the POR–HO-1 complex. Again, these results are consistent with a CYP1A2–HO-1 complex where POR selectively associates with its HO-1 moiety.

CYP1A2 is known to form homomeric complexes in membranes, which was shown by both BRET and chemical cross-linking (12). As this homomeric BRET complex is known to be disrupted by the cotransfection of unlabeled POR, the potential of unlabeled HO-1 to disrupt the CYP1A2–CYP1A2 BRET pair was examined (Fig. 5). The results clearly show that HO-1 can disrupt the CYP1A2–CYP1A2 BRET pair.

**Effect of HO-1 on CYP1A2 activity**

The presence of a stable CYP1A2–HO-1 complex leads to the question of what effect this complex might have on P450
and HO-1 activities. To investigate this, CYP1A2-mediated 7-ethoxyresorufin deethylation (EROD) was measured as a function of POR concentration where the proteins were reconstituted into L-α-dilauroyl-sn-glycero-3-phosphocholine (DLPC). In the absence of HO-1, CYP1A2 produced a sigmoidal response as a function of POR concentration (Fig. 6). This corroborates our previous reports and is consistent with the tendency of CYP1A2 to form a homomeric complex (Fig. 5 and (12)). Interestingly, in the presence of HO-1, EROD appeared to be stimulated at subsaturating POR but moderately inhibited at higher POR concentrations, showing a V\text{max} that was about 20% lower when HO-1 was present in the reaction.

**Effect of CYP1A2 on HO-1 activity**

In the converse experiment, the effect of CYP1A2 on HO-1-mediated heme metabolism was examined (Fig. 7). As previously reported (36, 37), in the binary reconstituted system containing HO-1 and POR, very tight binding between the proteins was observed having a \( K_m \) of about 0.001 μM. However, addition of CYP1A2 caused an inhibition of HO-1 activities at subsaturating POR. The apparent \( K_m^{\text{POR-HO-1}} \) increased from less than 0.001 μM to 0.024 ± 0.006 μM in the presence of CYP1A2.

**Discussion**

The P450s and HO-1 share a necessary binding partner in POR. P450s are inducible by a variety of endobiotic and xenobiotic agents (1, 2). HO-1 also is inducible by endogenous and foreign compounds as well as by oxidative stress (38). POR is often expressed at much lower levels than are the P450s, leading to conditions where they must compete for limiting POR (39, 40); however, the environment can be dramatically changed by HO-1. In the uninduced state, HO-1 levels are lower than those of both POR and total P450; however, when induced, HO-1 concentrations can exceed the levels of both proteins (41). These factors contribute to the potential for P450s, HO-1, and POR to interact differently based on the relative levels of each of the proteins. Thus, different induction states might change how these interactions manifest in terms of enzyme activities.

The simplest interaction model is one where HO-1 and CYP1A2 do not form a complex, and the proteins simply compete for POR based on their relative affinities. We know
that HO-1 has an extremely high affinity for POR ($K_m^{POR-HO-1} \sim 0.001 \text{ } \mu\text{M}$) (37, 42) & (Fig. 7), whereas the apparent affinity of CYP1A2 for POR is $\sim 60$ times lower ($K_m^{POR-CYP1A2} \sim 0.055 \text{ } \mu\text{M}$) (Fig. 6) & (17–19). These data allow us to predict that when all three proteins are present, HO-1 should outcompete CYP1A2 for the available POR and that CYP1A2 activity should be dramatically inhibited by the addition of equimolar HO-1, which is consistent with the BRET responses observed (Figs. 3 and 4). However, the BRET results also clearly establish that a CYP1A2-HO-1 complex is produced (Fig. 2), eliminating the possibility that these proteins exist in the membrane exclusively as monomers.

A second possibility is that the CYP1A2-HO-1 complex forms but formation of this complex does not change the ability of either moiety to bind with POR. According to this model, an HO-1-CYP1A2 complex would be evident (Fig. 2), the POR-CYP1A2 BRET complex would be significantly disrupted by the presence of unlabeled HO-1, and the high-affinity POR-HO-1 complex would be minimally affected by unlabeled CYP1A2. The BRET results (Figs. 3 and 4) are consistent with this mechanism.

The BRET measurements of physical complex formation are consistent with those of a CYP1A2-HO-1 complex that is capable of forming a high-affinity POR-HO-1 complex. This result is corroborated by the significant inhibition of POR-CYP1A2 by HO-1 and the inability of CYP1A2 to disrupt the POR-HO-1 complex. Based on BRET studies alone, a substantial inhibition of CYP1A2-mediated EROD activity would be expected in the presence of HO-1. However, when EROD was measured and POR was limiting, CYP1A2-mediated activity actually increased in the presence of HO-1 despite BRET data showing significant depression of the POR-CYP1A2 complex. CYP1A2-mediated EROD was only inhibited at higher POR concentrations. When CYP1A2 and POR were examined in a simple binary system, CYP1A2 activities exhibited sigmoidal kinetics as a function of the POR concentration (Fig. 6), which was more pronounced at higher [CYP1A2] (12, 43). In a previous report, we proposed that CYP1A2 forms a homomeric CYP1A2•CYP1A2 complex that is less active (12). Higher concentrations of POR disrupt this complex and allow the formation of a functional POR•CYP1A2 complex. In a similar manner, the binding of HO-1 may disrupt the inhibitory CYP1A2•CYP1A2 complex, allowing generation of functional POR•CYP1A2 at low POR concentrations. Although part of the increased EROD could be rationalized by the disruption of an inhibitory CYP1A2•CYP1A2 complex, it does not reconcile with the dramatic disruption of the POR•CYP1A2 complex by HO-1 (Fig. 3). In other words, if HO-1 is present and disrupts the CYP1A2•CYP1A2 complex, it would be replaced by the CYP1A2•HO-1 complex, where the HO-1 moiety would preferentially bind POR. At higher POR concentrations, inhibition of EROD was observed, suggestive of a conformationally mediated alteration in the maximum rate of EROD catalysis upon formation of the CYP1A2•HO-1 complex. CYP1A2 also was shown to affect HO-1-mediated heme metabolism (Fig. 7). Based on the BRET studies alone, HO-1 activities were expected to be negligibly affected by the presence of CYP1A2, particularly at subsaturating POR (Fig. 4). Although the maximal response was not affected at excess POR concentrations, HO-1-mediated catalysis was inhibited by CYP1A2 at subsaturating POR. This inhibition of HO-1-mediated heme catalysis at subsaturating POR would be unexpected unless formation of the CYP1A2•HO-1 complex conformationally affects the catalytic characteristics of the component enzymes.
**Interactions between CYP1A2 and HO-1**

These results provide important information regarding the interactions between the HO-1 and P450 systems but also raise many important questions. First, complex formation between HO-1 and different P450s is specific; not all P450s appear to form such complexes (Fig. 1). Second, the BRET response using CYP1A2-GFP and Rluc-HO-1 shows the expected saturation as the ratio of the GFP-containing proteins to Rluc-containing proteins is increased (Fig. 2). Interestingly, this complex is not significantly affected by the addition of POR, which indicates that when both proteins are present, they act not as monomers or homomeric complexes but as a ternary (or higher order) complex. Third, the functional POR·CYP1A2 BRET complex is affected by HO-1; however, the POR·HO-1 complex is not affected by CYP1A2. Finally, the activities of both CYP1A2-mediated EROD and HO-1-mediated heme metabolism are influenced by the presence of the other protein in ways that cannot be explained by a simple mass-action competition for limiting POR. The data at this point suggest multiple effects on the oligomerization/conformation of the enzymes in the ternary systems. A more detailed kinetic analysis will be required to identify the specific modifications taking place.

**Experimental procedures**

**Materials**

Dulbecco’s Modified Eagle’s Medium (DMEM), phosphate buffered saline (PBS), fetal bovine serum (FBS), and Lipo-fectamine 2000 were purchased from Invitrogen (Eugene, OR). The plasmids used to generate BRET vectors (pGFP2-N1, amine 2000 were purchased from Invitrogen (Eugene, OR). Buffered saline (PBS), fetal bovine serum (FBS), and Lipofectamine 2000 were purchased from Invitrogen. 3-Ethoxyresorufin was purchased from Anaspec (Fremont, CA), and cytochrome c was obtained from Sigma (St Louis, MO). 3-Phosphoglycerate kinase 1 (3-PGK) expression vector (pEF-BOS, Invitrogen) was digested with SacI and 3-PGK and used to drive expression from the CMV promoter. Porcine rhodamine B was purchased from Sigma (St Louis, MO). Antibiotic-antimycotic solution was purchased from Invitrogen (Eugene, OR). Fetal bovine serum (FBS) was purchased from Invitrogen. 3-Phosphoglycerate kinase 1 (3-PGK) expression vector (pEF-BOS, Invitrogen) was digested with SacI and 3-PGK and used to drive expression from the CMV promoter. Porcine rhodamine B was purchased from Sigma (St Louis, MO).

**Generation of BRET vectors**

HO-1, rabbit CYP1A2, and POR were wild-type proteins without any modifications to their amino acid sequences. To generate the BRET expression vectors, linear cDNA coding for full-length, wild-type CYP1A2 (NM_001171121) and POR (NM_001367562) was amplified from existing bacterial expression vectors with PCR using primers to introduce restriction sites that immediately flanked the full-length gene, excluding the stop codon. The restriction sites (with the 5’ site listed first) were EcoRI and BamHI for CYP1A2 and EcoRI and HindIII for POR. These PCR products were then ligated into the empty vectors (pGFP2-N1 and pRluc-N2 for the P450s, pGFP2-N3 and pRluc-N1 for POR) after each was digested by the indicated pair of restriction enzymes (New England Biolabs, Inc; Ipswich, MA). The multiple cloning sites of the vectors are upstream of the GFP or Rluc tag. This information is summarized in Table 1. This orientation was chosen to ensure that the heterologous protein tags were less likely to interfere with the ability of the membrane-binding regions of the proteins to insert into the ER membrane. Due to the restriction sites used, the BRET vectors code for a 5 to 20 amino acid sequence between the 3’ of the inserted gene and the start codon for the GFP or Rluc tag. To generate vectors for the expression of untagged, wild-type CYP1A2, HO-1, and POR, site-directed mutagenesis was performed to create a stop codon immediately 3’ to the main protein sequence. Site-directed mutagenesis was performed using the QuikChange II kit from Agilent Technologies (Santa Clara, CA). All PCR amplification and mutagenesis primers were purchased from Integrated DNA Technologies (Coralville, IA). Insertion of cDNA and mutagenesis were confirmed by sequencing (ACGT; Germantown, MD).

Vectors coding for human CYP1A1 (vector: pCMVSPORT6; accession number: BC023019), CYP2A6 (pCR-BluntIII-TOPO; BC067430.1), CYP2C9 (pCR-BluntIII-TOPO; BC125054.1), and CYP2D6 (pDNK-Dual; BC067432.1) were purchased from GE Healthcare/Dharmacon (Lafayette, CO). The pPORhWT vector that served as a source of human POR cDNA was a generous gift of Dr Bettie Sue Masters (44). Human HO-1 cDNA was derived from the pGEX-4T-2 vector as described previously (37).

Because the C terminus of HO-1 binds to cellular membranes (in contrast with the P450s and POR) (45, 46), N-terminal tags were used to avoid interfering with membrane insertion. This involved using the pRluc-C3 and pGFP2-C2 vectors with 5′ SacI and 3′ Xhol restriction sites and designing primers that conserved the 3′ stop codon. In order to create a vector for unlabeled HO-1 expression, the coding sequence was excised from the pRluc-C3 vector again using the 5′ SacI site with a 3′ BamHI site downstream of the stop codon. The complete vector was then produced by ligating the HO-1 insert into a similarly digested pGFP2-N1 backbone.

**BRET assays**

For BRET assays, cells were first transfected with 1 to 3 μg of total DNA at different ratios of the GFP and Rluc constructs. The goal for these transfections was to generate a series of cells expressing the same total protein at a range of GFP-to-Rluc ratios to ensure that the BRET complexes were specific and not due to changes in protein expression levels. If this transfection strategy yielded approximately the same total protein expression across all conditions, the results were considered valid. In many cases, however, one or two additional trials were necessary to achieve constant levels of protein expression. After allowing at least 24 h of protein expression, cells were checked using fluorescence microscopy to ensure efficient expression of the GFP fusion proteins. Each transfection was performed with the following three controls: a GFP–Rluc fusion protein, the Rluc fusion protein alone, and cells transfected with only pUC19. Cells were harvested in 1 ml of PBS,
for each sample were then normalized to that of the GFP. The GFP and Rluc signals were determined photometrically by comparison with cells expressing a GFP fusion protein (which is assumed to have a 1:1 GFP-to-Rluc expression ratio), so that dividing the normalized GFP value by the normalized Rluc value yielded an approximation of the actual GFP-to-Rluc expression ratio. In each case, there were no significant differences when comparing the data in the absence and presence of the unlabeled protein.

Interactions between CYP1A2 and HO-1

Reconstituted systems were prepared as described previously (36). A 5 mM DLPC stock solution was prepared in 50 mM potassium phosphate (pH 7.25) with 20% (v/v) glycerol, 0.1 M NaCl, and 5 mM EDTA. This solution was then sonicated at room temperature in a water-bath sonicator for 20 min or until the solution clarified. To set up POR titration assays, purified recombinant CYP1A2, HO-1, or both were mixed with varying quantities of purified recombinant POR and preincubated for 2 h at room temperature in DLPC stock solution. During preincubation, enzyme concentration was kept as high as possible and (unless otherwise noted) the molar ratio of DLPC to the enzyme being assayed was kept at 160:1 (19).

HO-1 activity assay

To measure HO-1 activity, we used a coupled assay including a source of biliverdin reductase, which converts the biliverdin generated by HO-1–mediated heme catabolism into bilirubin. Reconstituted systems (RSs) were prepared and preincubated for 2 h at room temperature before the addition of other reaction components in 0.1 M potassium phosphate (pH 7.4). The substrate, heme, was added at 15 μM and rabbit liver cytosol (0.8 mg/ml protein) to provide biliverdin reductase. Catalase was added to a concentration of 0.25 U/μl in order to mitigate the effect of H2O2 accumulation on reaction linearity (36). All activity assays were allowed to preincubate at 37 °C for 3 min prior to initiation of the reaction by the addition of NADPH to a final concentration of 5 mM. Each reaction was performed at 37 °C in triplicate, in black, clear-bottom 96-well plates with a final volume of 0.1 ml. A SpectraMax M5 plate reader was used to monitor bilirubin formation using a real-time assay, measuring the difference in absorbance at 464 and 530 nm. The assay was monitored for 10 min, and the initial rates were measured from the slope of the linear portion of the curve. Enzyme activities were calculated using the delta extinction coefficient of 40 mM\(^{-1}\)cm\(^{-1}\) (47, 48).

7-Ethoxyresorufin-O-deethylase assay

For the measurement of CYP1A2 activity in reconstituted systems, we used 7-ethoxyresorufin (7-ER) as a substrate.
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CYP1A2 converts 7-ER into the fluorescent product resorufin. After preincubation for 2 h at room temperature, reconstituted systems were diluted into buffer A (50 mM Hepes (pH 7.5), 15 mM MgCl₂, 0.1 mM EDTA). 7-ER was added to a final concentration of 4 μM. Onset of reductase activity was determined using a SpectraMax M5 plate reader to monitor resorufin fluorescence (excitation: 535 nm, emission 585 nm). Initial rates were used for calculation of activities using a standard curve generated from known quantities of resorufin.

**Data analysis**

Unless otherwise noted, POR titration data were fit to the Morrison equation for tight binding in GraphPad Prism 5 using nonlinear regression (49). Statistical comparison of each estimated parameter was done using an extra-sum-of-squares F test between a simple model in which all conditions share one value for the given parameter and another model, which allows each condition a separate value for the parameter (50). Parameters were considered significantly different if the model with separate values was preferred at p < 0.05. Data points are represented as the mean ± SD

**Data availability**

All data are contained within the manuscript.

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**Author contributions**—J. P. C. and G. F. C. conducted the experiments. J. P. C. wrote the initial draft of the manuscript. J. R. R. and W. L. B. conceptualized the study, supervised its progress, and read and edited the manuscript. W. L. B. obtained the funding.

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**Conflict of interest**—The authors declare that they have no conflicts of interest with the contents of this article.

**Abbreviations**—The abbreviations used are: 7-ER, 7-ethoxyresoruvin; BRET, bioluminescence resonance energy transfer; DLPC, 1,2-dilauroyl-sn-glycero-3-phosphocholine; EROD, 7-ethoxyresoruvin deethylation; GFP, green fluorescent protein; HO-1, Heme oxygenase 1; POR, NADPH-cytochrome P450 reductase.

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