Competition: Physical Interactions between CYP2D6, NADPH-Cytochrome P450 Reductase, and Heme Oxygenase-1

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Introduction

Cytochrome P450

- The human cytochromes P450 (CYPs or P450s) are a superfamily of 57 hemeproteins that play an essential role in the metabolism of numerous endogenous and exogenous compounds.
- P450 families 1, 2, and 3 are responsible for metabolizing xenobiotics and account for the phase I biotransformation of approximately 75% of commonly prescribed drugs, including antidepressants, opioids, beta-blockers, and antipsychotics.

NADPH-cytochrome P450 Reductase (POR)

- P450s obtain electrons from their redox partner NADPH-cytochrome P450 reductase (POR) for their catalytic reaction, requiring P450s to form physical complexes with POR.

Heme-oxygenase 1 (HO-1)

- HO-1 is expressed in most tissues and is induced by stress.
- Heme-oxygenase 1 (HO-1) also interacts with POR to obtain electrons for the metabolism of free heme.

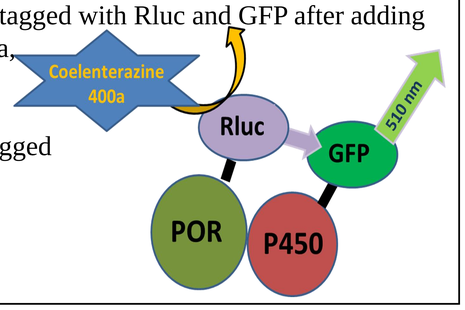
Interactions among CYP2D6, POR, and HO-1

- Since both P450s and HO-1 interact with POR, a complex network of protein-protein interactions is possible.
- Induction of HO-1 as a result of the stress response has the ability to interfere with the P450-POR interactions by forming complexes with either POR or P450.
- The goal of this study was to gain further insight into the network of interactions between CYP2D6, POR, and HO-1.

Methods

Bioluminescence Resonance Energy Transfer (BRET)

- Bioluminescence resonance energy transfer (BRET) was used to observe the physical interactions between CYP2D6, POR, and HO-1.
- Proteins were tagged with *Renilla* luciferase (Rluc) and green fluorescent protein (GFP) within the ER membrane of transfected 293T/17 cells.
- Cells were incubated for 48 hours to allow for protein expression.
- The BRET signal was used to measure the formation of protein complexes tagged with Rluc and GFP after adding coelenterazine 400a, and the potential disruption of these complexes by untagged proteins.



Previous Findings

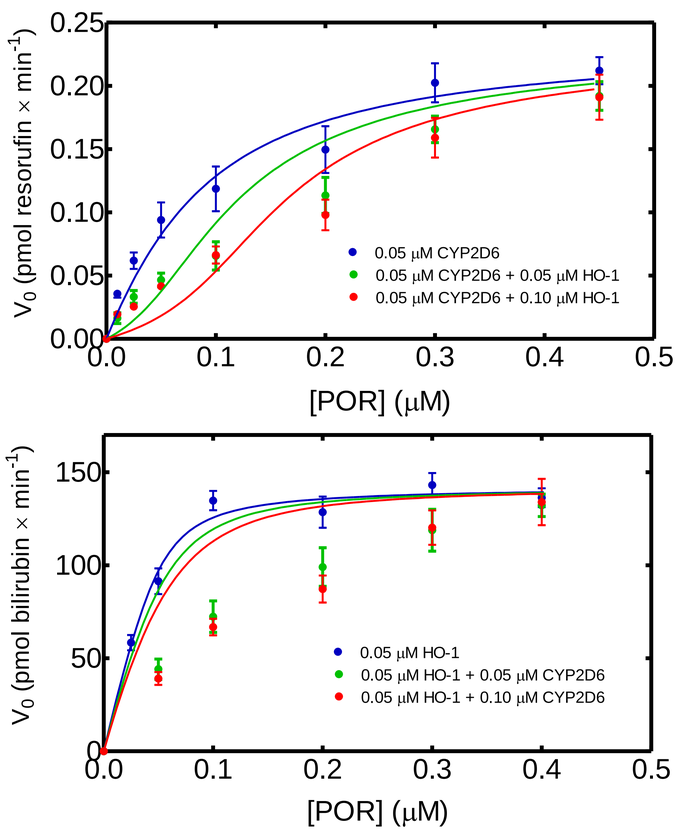


Figure 1. Kinetic assays for CYP2D6 (top) and HO-1(bottom). Measurement of activity as a function of [POR] in the absence of competitor (blue curves) allowed the determination of the affinity of each enzyme for POR. The affinity of HO-1 for POR was 7 times higher than that of CYP2D6. The expected effect HO-1 and CYP2D6 on each other's activity was modeled assuming simple competition for POR binding (green and red curves). CYP2D6 caused significantly more inhibition of HO-1 than expected while HO-1 did not inhibit CYP2D6 as much as predicted at subsaturating POR levels.

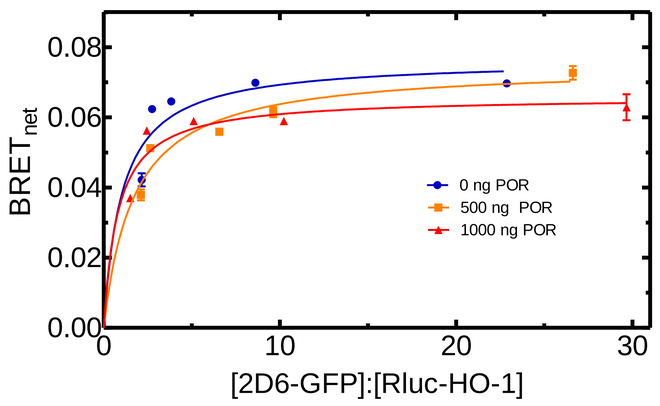


Figure 2. Effect of POR on CYP2D6:HO-1 complex. BRET assay demonstrates a physical interaction between CYP2D6, labeled with GFP, and HO-1, labeled with Rluc. The CYP2D6:HO-1 complex was not significantly impacted by the addition of POR.

Does the HO-1:CYP2D6 complex affect POR binding for either enzyme?

Can CYP2D6 form homomeric complexes and, if so, are they stable in the presence of POR?

Results

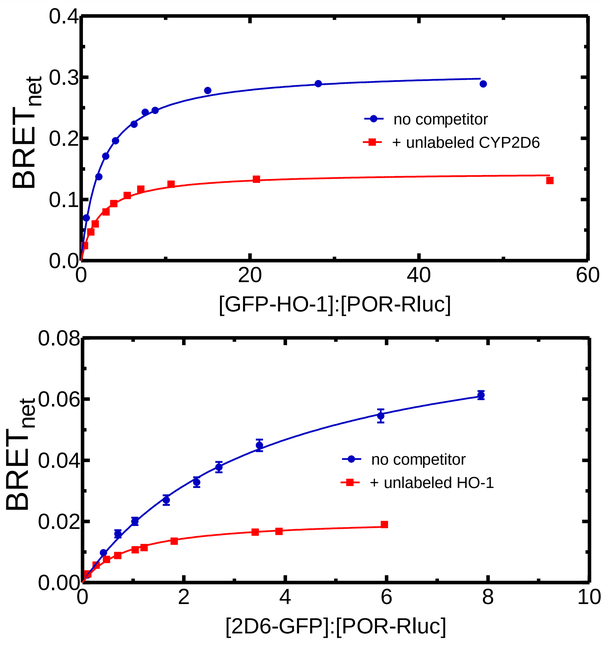
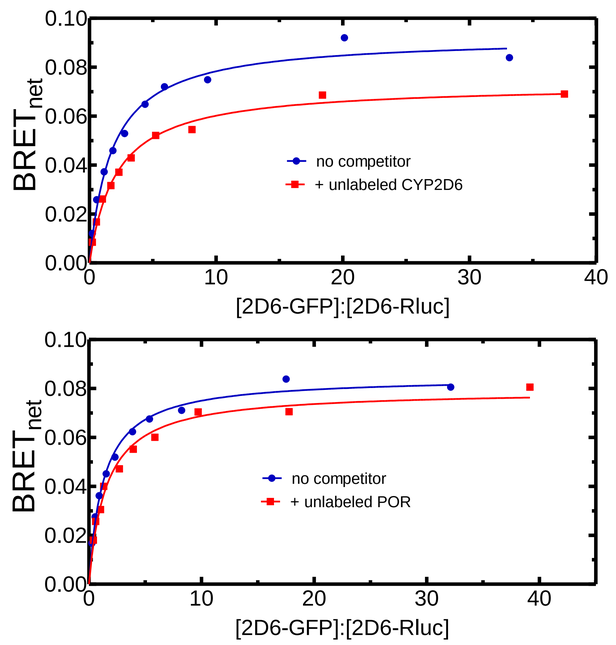


Figure 3A. Effect of CYP2D6 on POR:HO-1 complex (top). Physical interactions between HO-1, labeled with GFP, and POR, labeled with Rluc were visualized using a BRET assay. The interaction is significantly lowered in the presence of the competitor, unlabeled CYP2D6. Unlabeled CYP2D6 DNA comprised 60% of total transfected DNA.
B. Effect of HO-1 on POR:CYP2D6 complex (bottom). Physical interactions between CYP2D6, labeled with GFP, and POR, labeled with Rluc were visualized using a BRET assay. The interaction is significantly decreased in the presence of the competitor, unlabeled HO-1. Unlabeled HO-1 DNA comprised 33% of total transfected DNA.

HO-1 and CYP2D6 can disrupt each other's ability to bind POR.

Figure 4A. Effect of CYP2D6 on CYP2D6:CYP2D6 complex (top). BRET assay demonstrates physical interactions between CYP2D6 molecules, labeled with GFP and Rluc. In the presence of the competitor, unlabeled CYP2D6, there are significantly fewer homomeric interactions between CYP2D6 molecules. Unlabeled CYP2D6 DNA comprised 33% of total transfected DNA.
B. Effect of POR on CYP2D6:CYP2D6 complex (bottom). BRET assay demonstrates physical interactions between CYP2D6 molecules, labeled with GFP and Rluc. However, in the presence of the competitor, unlabeled POR, there are slightly fewer interactions between CYP2D6 molecules, but the interactions are not as significantly decreased, as with unlabeled CYP2D6. Unlabeled POR DNA comprised 33% of total transfected DNA.



CYP2D6 forms a homomeric complex that is stable in the presence of excess POR.

Conclusions

- Interactions between HO-1 and POR are significantly impacted in the presence of CYP2D6.
- Interactions between CYP2D6 and POR are significantly disrupted in the presence of HO-1.
- CYP2D6 can form homomeric complexes that are stable in the presence of POR.
- These results point to a network of interactions that likely play a role in balancing CYP2D6 and HO-1 activity when both proteins are expressed in excess of POR.
- The potential of a stress response to affect drug metabolism via HO-1 induction and protein-protein interactions merits further study.

References

Marohnic CC, Huber WJ, Connick JP, et al. Mutations of Human Cytochrome P450 Reductase Differentially Modulate Heme Oxygenase-I Activity and Oligomerization. *Archives of biochemistry and biophysics*. 2011;513(1):42-50.

Connick JP, Reed JR, and Backes WL (2012). Characterization of Interactions Among CYP1A2, CYP2B4, and NADPH-cytochrome P450 Reductase: Identification of Specific Protein Complexes. *Drug Metab Dispos.* 2018 Mar;46(3):197-203.