Interaction between Heme Oxygenase-1 and -2 Proteins*

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The three isoforms of heme oxygenase (HO), the rate-limiting enzyme in heme degradation, are the products of different genes that show marked differences in regulation and expression. Why is there redundancy in the heme degradation pathway, and why are there differences in tissue expression of HO isoenzymes that are unanswered questions? An interaction between HO-1 and HO-2 is suspected by the co-localization of these enzymes in the lung and regions of the brain. Using multiple models and assays, we demonstrated an interaction between HO-1 and HO-2 at amino acids 0–45 of HO-2 and amino acids 58–80 of HO-1. The latter corresponds to a highly conserved, hydrophilic, and exposed region of the protein. Furthermore, the observed activity of the HO-1-HO-2 complex was lower than that expected from the sum of HO-1- and HO-2-derived activities, suggesting that this interaction serves to limit HO enzymatic activity. We speculate that this HO-1-HO-2 protein interaction may promote non-enzymatic functions of HO.

The enzymatic degradation of heme by a microsomal enzyme, heme oxygenase (HO), was first characterized by Tenhunen et al. (1). The isoenzymes HO-1 (the inducible form) and HO-2 (the constitutive form) have been attributed antioxidiant, cytoprotective, neurotransmitter, and anti-inflammatory functions (2–5) among others. A third isofrom HO-3 is less well understood. In the reaction catalyzed by HO, heme is degraded to generate equimolar quantities of iron, biliverdin, and carbon monoxide (CO) (see Fig. 1). All of these reaction substrates are unique in that they are pro-oxidants (heme and iron) or antioxidants (biliverdin) or signaling molecules (CO and biliverdin). Both isoenzymes of HO degrade heme at the α-meso bridge. Why there is redundancy of the heme-degradative function is not well understood, but as with nitric-oxide synthase (6, 7), there are differences in the function of the HO isoenzymes? The HO-1 form is inducible by a wide variety of stresses including oxidative, heavy metal, and inflammation (2–4). The HO-2 isozyme is largely constitutive and also serves as a heme-binding protein (8, 9). The HO-3 form is thought to primarily serve as a heme-binding protein (10).

In most tissues, there is variable abundance of each HO isozyme. The spleen has increased abundance of HO-1 protein (11), whereas the brain expresses mostly HO-2 protein (12). Although there is differential distribution of HO-1 and HO-2 within tissues, the two isoenzymes can also co-localize. In the brain, expression of HO-1 protein was observed in the neurons from the hypothalamus, cerebellum, and brain stem (13). The distribution of HO-2 was more widespread and included pyramidal, granule, and endothelial cells (13). In null mutant mice for the ataxia-telangiectasia mutated (ATM) gene product, HO-1 and HO-2 immunoreactive proteins showed increased expression and were co-localized to the Purkinje and endothelial cells of the cerebellum of 2-month-old animals as compared with wild type controls (14). In rat lung tissue slices, HO-1 and HO-2 immunoreactive signals colocalize in the bronchoalveolar epithelium and vascular smooth muscle cells throughout development (15). HO-1 predominates in alveolar macrophages, whereas HO-2 is expressed in the vascular smooth muscle but is also found in alveolar macrophages (16). Further documentation of co-localization of HO-1 and HO-2 to the endothelium has been published previously (17).

Co-localization of proteins does not necessarily imply that the proteins interact functionally but this occurs in many cases. For example, the proximity of two proteins may allow for transfer of energy as in the case of phosphofructokinase-1 and the “a” subunit of the proton pump. This serves to link the proton pump and glycolysis (18). In another example, carbonic anhydrase II binds to the mammalian Na+/H+ exchanger, enhancing its efficiency (19).

Earlier evidence to suggest that HO-1 and HO-2 protein could interact is indicated in the first description of HO-1 purification where a 68-kDa protein was identified (20). It is now known that the molecular mass of HO-1 is 32 and HO-2 36 kDa. It could be that a complex of the two proteins was initially isolated under partially denaturing conditions. Therefore, we hypothesized that there is a physical interaction between HO-1 and HO-2 proteins. In other systems, such interactions may modify protein function. If HO-1 binding to HO-2 alters HO activity, the relative abundance of HO-1 to HO-2 may modulate the effects of HO in the various tissues. Using several methods, an interaction between HO-2 and HO-2 was demonstrated. We also show the effect of the HO-1-HO-2 protein complex on HO activity.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Interaction of HO-1 and HO-2—The yeast strain Y187 (Matchmaker GAL4, Clontech, Palo Alto, CA) was grown in YPD (yeast extract/peptone/dextrose) adjusted to pH 5.8 with 2% glucose. The HO-1 and HO-2 fusions were formed by ligation of HO-1 and HO-2 (yeast extract/peptone/dextrose) adjusted to pH 5.8 with 2% glucose. The HO-1 and HO-2 fusions were formed by ligation of HO-1 and HO-2 rat cDNA with pAS2–1 and pACT2 vectors, respectively. More specifically, 100 ng of HO cDNA was incubated for 30 min with 0.1 mg of carrier DNA (herring testis DNA). Yeast cells were heat-shocked at 42 °C and then chilled and centrifuged at 10,000 × g. The cell pellet was resuspended in buffer containing 0.1 mM Tris, pH 8, and 0.1 mM EDTA and plated on selection medium (synthetic dropout agar) at 30 °C for 2–4 days. In some experiments, HO-1 was used as the bait protein.
**Table I** Primers used for the generation of PGEXT-1/HO-2 deletion constructs

| Construct     | 5’ Sense | 3’ Antisense |
|---------------|----------|--------------|
| HO-2/FL       | GGTGATCATGTGTCCTGAGGTGAGAC | ACACCTGAGTCACATGTAAGTTACGGGCA |
| HO-2/235      | GGTGATCATGTGTCCTGAGGTGAGAC | GCGCTGAGTCACATGTAAGTTACGGGCA |
| HO-2/148      | GGTGATCATGTGTCCTGAGGTGAGAC | GCCCTGAGTCACATGTAAGTTACGGGCA |
| HO-2/44       | GGTGATCATGTGTCCTGAGGTGAGAC | CGCTCAGACTGCAGCCTGCTGCTGCTG |
| HO-2/45–319   | TAGCTGCATGTGGCAGCAAGAAATACCCAG | ACACCTGAGTCACATGTAAGTTACGGGCA |

**Table II** 3’ Antisense primers for the generation of PGEXT-1/HO-1 deletion constructs

| Construct     | 3’ Antisense |
|---------------|--------------|
| HO-1/FL       | CGCTCAGATTACGCTGGCATAAATTCCCA |
| HO-1/230      | CGGTACATATCGTCTGGGCCTTCTGTCGACG |
| HO-1/80–289   | CGCTCAGATTACGCTGGCATAAATTCCCA |
| HO-1/117      | CGGTACATATCGTCTGGGCCTTCTGTCGACG |
| HO-1/67       | CTGTGATATCGTCTCTATCTCCTTTCCAG |
| HO-1/58       | CGGTACATATCGTCTGGCATAAATTCCCA |

**Table III** Verification of an HO-1 and HO-2 protein interaction with the yeast two-hybrid system

| Yeast colonies | β-Galactosidase activity |
|---------------|--------------------------|
| pY3A + pTD (positive control) | ++ |
| pACT2/HO-2 + pAS2-1/HO-1 | + |
| pACT2 + pAS2-1/HO-1 | – |
| pACT2/HO-2 + pAS2-1 | – |

**Fig. 1. Pathway of heme degradation.** Heme is metabolized by heme oxygenase to form CO and iron (Fe²⁺). This reaction is energy-requiring as the reduced form of NADPH and molecular oxygen (O₂) are metabolized by cytochrome c P450 reductase to the oxidized form of NADPH and water (H₂O₂). Biliverdin is further reduced to bilirubin by biliverdin reductase, and NADPH is also oxidized to NADP in this step. HO-2 protein at room temperature overnight. The purified GST-HO-1 fusion protein on the column was then washed five times with phosphate-buffered saline, eluted with 100 μl of glutathione elution buffer containing 10 mM reduced glutathione, and run on a 12% SDS-PAGE as described above. The gel was dried and developed on Kodak X-Omat AR film. The presence of a radioactive signal was determined by autoradiography. In other experiments, a GST-HO-2 fusion protein was incubated with the glutathione-Sepharose column and incubated with 35S-labeled HO-1. The column was washed and eluted as above.

Construction of Deletion Mutants of HO-2 to Determine the Region of Binding to HO-1—Deletion analysis was used to identify the area of binding of HO-1 on HO-2. 35S-Labeled in vitro translated full-length HO-1 was incubated with GST-HO-2 fusion products of various lengths and passed onto a GST column. The primers used to generate the PGEXT-1/HO-2 constructs are listed in Table I.

**Site-directed Mutagenesis of HO-2**—Plasmid DNA from the rat HO-2 expression clone, pRC/CMV-HO2, served as the substrate to carry out site-directed mutagenesis using the mutagenic primers 5’-GGACCAGGGTAAGGCCTCCCGAGGCTTGGGCC-3’. Complementary to nucleotides 495–524 with a GC mismatch for CA to convert Cys-126 into alanine. The primer 5’-CCGAGAAGGCGTTGCGCTCC TGCC-3’, complementary to nucleotides 442–464 with mismatches to convert His-151 to alanine, was also used as described previously (9). The mutations were produced with the QuickChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). The mutated cDNA products were sequenced to verify that the appropriate mutation had been produced. Thereafter, the cDNA clones were ligated into the PGEX4T-1 vector at the BamHI and XhoI sites.

Construction of Deletion Mutants of HO-2 to Determine the Region of Binding to HO-2—In other experiments, various HO-1 constructs of differing lengths were PCR-amplified using the sense primer 5’-TATGGATCATGGAGGCGCCACAGCAGCTGTCGACG-3’ and the antisense primers described in Table II. The PCR products were in vitro translated using the TNT T7 Quick coupled transcription/translation system (Promega).

To generate the 80–289 fragment, a Bsu361 restriction enzyme allowing for a single cleavage at bp 239 of HO-1 cDNA was used and the resultant fragment was then in vitro translated after blunt ligation. The 35S-labeled products were then incubated with GST-labeled full-length HO-2 and eluted onto a GST column as described above.

(FL, full-length HO-2 yielding a 319-amino acid protein fragment. Numbers denote amino acids in the fragment starting from the N terminus. 44–319 indicates truncation at the N-terminal amino acid 44.)

To generate the 80–289 fragment, a Bsu361 restriction enzyme allowing for a single cleavage at bp 239 of HO-1 cDNA was used and the resultant fragment was then in vitro translated after blunt ligation. The 35S-labeled products were then incubated with GST-labeled full-length HO-2 and eluted onto a GST column as described above.
The binding of HO-1 fragments to HO-2 was evaluated by gel electrophoresis and autoradiography as described above. Evaluation of an HO-1/HO-2 Complex in Native Gels—5 μg each of GST-purified, thrombin-cleaved HO-1 and HO-2 proteins in 0.1 M phosphate-buffered saline were co-incubated at room temperature for 8 h. Samples were mixed with non-denaturing loading buffer (lacking reducing agents sodium dodecyl sulfate or mercaptoethanol) and electrophoresed onto a 12.5% polyacrylamide gel at pH 8.8. Gels were electrophoresed under non-denaturing conditions, transferred onto polyvinylidene difluoride membranes, and probed for HO-1 and HO-2 immunoreactive proteins by Western analysis as described previously (21). The gel was then stained with Coomassie Blue.

Determining the Effects of HO-1-HO-2 Protein Complex on HO Activity—In vitro translated HO-1 and HO-2 (1 μg each in 0.1 M phosphate buffer pH 7.4) were combined at differing ratios (1:1, 1:2, 1:3, 2:1, and 3:1 of HO-1 to HO-2) in the presence of excess hemin (150 μM, Sigma), NADPH (10 μM, Sigma), and cytochrome c P450 reductase (1 μM,

**Fig. 2. In vitro interaction between HO-1 and HO-2 proteins.** Radiolabeled [35S]HO-1 was incubated with GST-HO-2 and vice versa (i.e. [35S]HO-2 with GST-HO-1). A, [35S]-labeled HO-1 protein signal before (lane 1) or after (lane 2) purification with a glutathione-Sepharose 4B MicroSpin column. B, [35S]-labeled HO-2 protein signal before (lane 1) or after (lane 2) purification with a glutathione-Sepharose 4B MicroSpin column. Solid arrows, HO-1 (A) and HO-2 (B) proteins; empty arrow, nonspecific products. *+, molecular weight markers.

**Fig. 3. Co-immunoprecipitation of HO-1 and HO-2 proteins.** Rabbit anti-rat HO-1 antibody and in vitro translated [35S]-labeled HO-2 were incubated with or without extracts of cells transfected with pRC-CMV/HO-1 to express HO-1 protein. The samples were run on a 12% SDS gel and viewed by autoradiography. Lane 1, samples without cell extract; lane 2, samples with the cell extracts. *+, the input is 5% in vitro translated HO-2 protein. The solid arrow indicates the HO-2 protein signal.

**Fig. 4. Visualization of HO-1-HO-2 complex and determination of HO activity.** A, visualization of the HO-1-HO-2 protein complex with native gels. In vitro translated HO-1 and HO-2 were run on a 12% non-denaturing gel alone or after co-incubation. The gels were then stained with Coomassie Blue. B, verification of the HO-1-HO-2 complex with Western analysis using anti-HO-1 and anti-HO-2 antibodies. Incubation of HO-2 with either GST-purified full-length HO-1 (fl) or rat HO-1 from E. coli (tr) where the membrane binding domain has been truncated resulted in an HO-1/2 immunoreactive complex (arrows). Note the increased intensity of the complex when HO-1(tr) is added to HO-2 protein (HO-1 tr/HO-2). Two immunoreactive bands were detected for HO-2. C, HO activity of solutions where HO-1 and HO-2 proteins are co-incubated at various concentrations. 1 μmol of GST-purified, thrombin-cleaved HO-1 was used for the experiments. Purified HO-2 was added at 1-, 2-, and 3-μmol concentrations to the HO-1 solution (lanes 1:1, 1:2, and 1:3, respectively). Total activity was determined in the presence of non-limiting concentrations of hemin, NADPH, and cytochrome c P450 reductase. Values are the mean ± S.E. of four measurements. *+, p < 0.05 versus expected value based on the sum total of HO-1 + HO-2.
Stressgen, Vancouver, British Columbia, Canada), an enzyme that participates in the HO reaction by providing reducing equivalents. Total HO activity was determined by gas chromatography and expressed as nmol CO/mg protein/h (22).

In some experiments, 20 μl of spleen homogenate (predominantly composed of HO-1) from C57BL/6 mice were incubated for 1 h at room temperature with 0–25 μg in vitro translated HO-2 to determine the effect of HO protein binding on total HO activity in tissues.

In other experiments, 20 μl of brain homogenates (predominantly HO-2) from C57BL/6 or HO-2 knock-out mice were incubated for 1 h at room temperature with 0–25 μg of pure rat HO-1 protein. Total heme oxygenase activity of the mixture was measured.

Lastly, 20 μl of brain homogenates from C57BL/6 were incubated for 1 h at room temperature with 12.5 μg of HO-1 fragment, which binds to HO-2. Total HO activity was then measured.

RESULTS

Defining an Interaction between HO-1 and HO-2 Proteins—Several approaches were used to demonstrate that HO-1 binds to HO-2. 1) The specific interaction of HO-1 and HO-2 proteins was confirmed by co-transformation of both pAS2-1/HO-1 and pACT2/HO-2 into Y187 cells to reconstruct the GAL4 transcription activity. Additionally, GAL4 transcription activity was confirmed with the reverse reaction, namely pAS2-1/HO2 and pACT2/HO2 in the Y187 cells (Table III). 2) Recovery of [35S]HO-2 was demonstrated after incubation with GST-HO-1 fusion protein, elution on a glutathione column, and separation by SDS-PAGE (Fig. 1). The reverse process, namely the recovery of [35S]-labeled HO-1 with HO-2-GST fusion protein, was also successfully observed (Fig. 2). 3) In vitro translated [35S]-labeled HO-2 was recovered after incubation with extracts from NIH 3T3 cells transfected with pRC-CMV/HO-1 and immunoprecipitation with HO-1 antibodies (Fig. 3). 4) The appearance of an additional band on a non-denaturing gel after incubation of purified HO-1 and HO-2 proteins was documented. Incubation with anti-HO-1 and anti-HO-2 antibodies verified that the new band had both HO-1 and HO-2 immunoreactivity. This finding demonstrates the formation of an HO-1/HO-2 protein complex (Fig. 4A). Incubation of HO-2 with either GST-purified full-length HO-1 (fl) or rat HO-1 from E. coli where the membrane binding domain has been truncated (tr) resulted in an HO-1/2 immunoreactive complex (Fig. 4B). However, the signal intensity of the complex was enhanced when the soluble rat HO-1 protein was added to HO-2 (Fig. 4B). This suggests that the soluble form of HO-1 is more likely to bind to HO-2. 5) When spleen was incubated with purified HO-2, a new HO-1 and HO-2 immunoreactive band was observed. This band increased in intensity with increasing amounts of HO-2 (Fig. 5, A and B). 6) When brain tissue was incubated with purified GST-HO-1 and electrophoresed on a native gel, a new HO-1 and HO-2 immunoreactive band was observed. This band increased in HO-1 signal intensity with...
the addition of increasing amounts of HO-1 protein (Fig. 6, A and B). The signal intensity of HO-2 diminished with the addition of purified HO-1, suggesting that the HO-2 antigenic site may have been obscured by complexation of HO-1 with HO-2 (Fig. 6B).

**Defining the Binding Region of HO-2 on HO-1**—Once we had demonstrated the HO-1-HO-2 complex in several model systems, we wanted to identify the specific region of binding on each protein. Using deletion analysis and site-directed mutagenesis, we identified the area of binding of HO-2 on HO-1 and vice versa. Truncating the HO-1 protein from the C-terminal did not alter the binding to HO-2. A loss of the first 80 amino acids resulted in a loss of binding, but a truncated protein of 80–289 amino acids from the N terminus retained the HO-2 binding. In contrast, a protein of 0–67 amino acids from the N terminus did not retain the binding ability. Based on these observations, we deduce that the binding region of HO-1 to HO-2 resides in amino acids 58–80. (Table IV.) These residues correspond to a portion of the C-helix and loop of HO-1 protein (see Table VI).

With HO-2, modifying Cys-126 or His-151 did not alter HO-1/HO-2 binding. This suggests that the binding does not involve sulphydryl groups or the putative heme regulatory motif suggested by McCoubrey et al. (9). Truncating the HO-2 protein from the C terminus did not result in the loss of binding. However, when amino acids 0–45 were deleted from the HO-2 protein, binding to HO-1 was eliminated (Table V). This region is immediately adjacent to His-45, which is also thought to be a heme regulatory motif (Table VI).

**Determination of the Enzymatic Activity of the HO-1-HO-2 Protein Complex**—Several strategies were employed to evaluate the effect of HO-1-HO-2 on total HO activity as follows: 1) The addition of increasing concentrations of *in vitro* translated HO-2 to *in vitro* translated HO-1 protein in the presence of excess substrate and cofactors did not increase the total HO activity of the solution. In fact, at the highest concentrations, a decrease in activity was noted suggesting inhibition of HO-1 activity with HO-2 (Fig. 4C). 2) When mouse spleen homogenate (predominantly HO-1) was combined with *in vitro* translated HO-2, total HO activity increased at the lowest concentration but decreased as more HO-2 was added, suggesting competitive inhibition (Fig. 5C). 3) When mouse brain homogenate (predominantly HO-2) was combined with *in vitro* translated HO-1, HO activity increased initially but then decreased with the addition of increasing concentrations of HO-1 (Fig. 6C). Furthermore, the levels of HO activity achieved with the addition of HO-1 to HO-2 knock-out mice brain were significantly higher than those observed in the WT control (Fig. 6D) and the addition of higher concentrations of HO-1 protein to these samples resulted in decreased HO activity (Fig. 6E).

**DISCUSSION**

Using multiple methods, we have described a novel interaction between HO-1 and HO-2 proteins in cell-free systems and in tissues. Also, with GST fusion assays, we were able to determine that the binding involves the first 44 amino acids of HO-2 and involves amino acids 58–80 of HO-1.

In the case of HO-2, the loss of binding to HO-1 occurred if residues 0–44 were removed. This location is proximal to His-45. This residue is involved in heme binding and HO catalytic activity as described by Ishikawa et al. (23). Others had implicated His-152 of HO-2 as the heme catalytic site and showed that mutation of His-152 to Ala results in total HO activity of the complex (decreased to 0.23 ± 0.03 *versus* 0.36 ± 0.04 nmol CO/mg protein/h in samples incubated with 0–117 fragment *versus* brain homogenates alone). Values are the mean ± S.E. of four separate experiments (*p* < 0.05).

**Table IV**

*Deletion analysis to identify the binding region of GST-HO-1 fragments to *35S-HO-2*

| HO-1 fragment | HO-2 binding |
|---------------|--------------|
| N-terminus    | 289          |
|               | +            |
| 80            | 230          |
|               | +            |
| 80            | 117          |
|               | +            |
| 80            | 67           |
|               | +            |
| 80            | 58           |
|               | -            |

**Table V**

*Deletion analysis to identify the binding region of *35S-HO-2 fragments to GST-HO-1*

| HO-2 fragment | HO-1 binding |
|---------------|--------------|
| 45            | 319          |
|               | +            |
| 44            | 148          |
|               | +            |
| 319           | +            |
| Cys/Ala       | 319          |
|               | +            |
| 319           | +            |
less, it is possible that binding of a relatively large protein such as HO-2 in this location could modify HO-1 protein charge and configuration, resulting in deformation of the heme pocket. This could explain the relative loss of HO activity in the presence of the HO-1-HO-2 complex.

None of the distal residues of HO-1 or HO-2 appears to be involved in this HO-1-HO-2 binding. These have been implicated in proper HO-1 function, and they form the distal heme binding pocket (24, 25). For example, amino acids 134–136, 142, and 147 all directly contact heme but they do not appear to be important in the binding of HO-1 to HO-2 because truncated forms eliminating these amino acids did not result in modified binding to HO-2.

There is a positive electrostatic potential around the hydrophobic heme pocket of the human HO-1 protein, which could repel other proteins and prevent binding (26). Nonetheless, the amino acids in the initial portion of rat HO-2 (0–44 amino acids) are largely hydrophilic and thus could interact with the amino acids from the helix C in HO-1 that are equally hydrophilic. Because of the lack of an available crystal structure for HO-2, it is not yet possible to further define the region of binding of HO-1 to HO-2.

As stated, the proximity of HO-1 binding to the His-45 in HO-2 could limit accessibility of heme to the heme binding pocket and affect HO activity. In addition, one heme molecule could be shared or sequestered by HO-1 and HO-2, thereby limiting HO activity. In fact, HO activity was significantly decreased in the presence of the complex. This was demonstrated both with in vitro generated protein in the presence of excess cofactors as well as in tissues such as the spleen and the brain with relative abundance of HO-1 and HO-2, respectively. There are other examples of enzymes binding to proteins, thereby modifying the enzyme activity. For example, p53 protein binds to the ribonucleotide reductase subunit HRRM2, resulting in increased reductase activity as shown by increased nuclear translocation (27). In another example, carbonic hydrate binds to and enhances the activity of the Na+/H+ exchanger (19). This is done by binding at the N terminus of both proteins and occurs after phosphorylation (19). The HO-2 protein can also phosphorylated, rendering it more active (28). Whether phosphorylation or hypophosphorylation modifies the formation and activity of the HO-1-HO-2 binding complex is not known at this time.

In other examples, protein interactions allow for an approximation of proteins of a similar metabolic pathway leading to the formation of a “metabolon,” facilitating energy provision such as with H+-ATPase and phosphofructokinase 1 (18). In the case of HO-1 and HO-2, the interaction did not enhance HO activity but rather suppressed it. The regulation of HO enzyme activity by HO-1 binding to HO-2 could represent a useful negative feedback mechanism for limiting HO activity when there is excess HO protein. Overexpression of HO activity in the higher range (greater than 5-fold) has deleterious effects (29), whereas at low concentrations, HO activity is cytoprotective in most models (4, 30, 31). Maintaining HO activity within a narrow range could provide protection against acute changes in HO-1 expression with various inducers. We suspect that in tissues where HO-1 and HO-2 co-localize such as the lung and the brain, HO activity is modulated by the HO complex. The lung is particularly susceptible to oxidant stress because of its direct exposure to environmental toxins. Overexpression of HO beyond a certain threshold may be detrimental because of the release of redox active iron (29, 32). Allowing HO activity to remain in the cytoprotective range would be desired. This could be accomplished by the formation of the HO complex.

In summary, we demonstrate an interaction between HO-1 and HO-2 proteins using various methods and we suggest that this interaction serves to limit HO activity in certain tissues where the two co-enzymes co-localize. This negative regulation of HO activity may be important to ensure a cytoprotective range of HO expression.

Acknowledgment—We thank Dr. Paul Ortiz de Montellano for invaluable expertise and helpful suggestions.

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