Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 338 (2005) 653–659
BBRC
www.elsevier.com/locate/ybbrc

# Dynamic changes in expression of heme oxygenases in mouse heart and liver during hypoxia

Feng Han [a,b], Kazuhisa Takeda [a], Satoru Yokoyama [a], Hideo Ueda [c], Yotaro Shinozawa [b], Kazumichi Furuyama [a], Shigeki Shibahara [a,*]

[a] Department of Molecular Biology and Applied Physiology, Tohoku University School of Medicine, Sendai 980-8575, Japan
[b] Department of Emergency and Critical Care Medicine, Tohoku University School of Medicine, Sendai 980-8575, Japan
[c] Taiyo Instruments Inc., Osaka 536-0025, Japan

Received 15 July 2005
Available online 24 August 2005

**Abstract**

Heme oxygenase cleaves heme to form biliverdin, carbon monoxide (CO), and iron, and consists of two structurally related isozymes, HO-1 and HO-2. HO-2 is also known as a potential oxygen sensor. Here we show that the relative CO content in arterial blood, which reflects the total amount of endogenous heme degradation, dynamically changes in mice during acclimatization to normobaric hypoxia (10% $O_2$), with the two peaks at 1 day and 21 days of hypoxia. The expression levels of HO-1 and HO-2 proteins were decreased by 20% and 40%, respectively, in the mouse liver at 7 days of hypoxia, which returned to the basal levels at 14 days. On the other hand, HO-1 and HO-2 proteins were increased 2-fold and 1.3-fold, respectively, in the heart at 28 days of hypoxia. Thus, hypoxia induces or represses the expression of HO-1 and HO-2 in vivo, depending on cellular microenvironments.


*Keywords:* Bilirubin; Carbon monoxide; Heme oxygenase; Heart; Hypoxia; Liver; Oxygen sensor

Heme oxygenase, the rate-limiting enzyme in heme catabolism, cleaves heme to form biliverdin IXα, carbon monoxide (CO), and iron [1,2]. Biliverdin IXα is reduced to bilirubin IXα during the last step of heme breakdown reaction. There are two structurally related isozymes of heme oxygenase, heme oxygenase-1 (HO-1) and heme oxygenase-2 (HO-2) [3,4]. Expression of HO-1 mRNA is induced by various environmental factors [5], including the substrate heme [6]. The induction of HO-1 has been considered as a protective response against oxidative stress, because bilirubin IXα, hereafter termed bilirubin, functions as a natural radical scavenger [7]. The physiological importance of HO-1 has been supported by the severe phenotypes of the HO-1 deficient mice [8] and a patient with HO-1 deficiency [9]. In contrast, the expression levels of HO-2 mRNA are maintained within narrow ranges in human cells [10–13].

HO-2 has been proposed to sequester heme to maintain the intracellular heme level [14], because HO-2, unlike HO-1 [15], contains two copies of the potential heme-binding site, a dipeptide of cysteine and proline (CP motif) [14,16]. Poss et al. [17] generated HO-2 deficient ($HO\text{-}2^{-/-}$) mice, showing that $HO\text{-}2^{-/-}$ mice are fertile and survive normally for at least 1 year. Recently, we have shown that $HO\text{-}2^{-/-}$ mice exhibit hypoxemia and attenuated hypoxic ventilatory responses with normal hypercapnic ventilatory responses [18], which led us to propose a novel function of HO-2 as an oxygen sensor. Subsequently, it has been reported that HO-2 interacts with α-subunit of a large conductance, calcium-sensitive potassium channel (the BK channel), and functions as an oxygen sensor for the BK channel [19].

Hypoxemia is frequently associated with sleep apnea syndrome and a variety of disease processes that affect the airways or the pulmonary parenchyma, such as chronic

* Corresponding author. Fax: +81 22 717 8118.
*E-mail address:* shibahar@mail.tains.tohoku.ac.jp (S. Shibahara).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.08.100

obstructive pulmonary disease, asthma, and pneumonia [20]. Sleep apnea syndrome is a newly recognized risk factor for the development of systemic hypertension, ischemic heart disease, and congestive heart failure. Hypoxemia is a hemodynamic stress and generates pressure overload to the right ventricle eventually leading to pulmonary hypertension and right heart failure. It is therefore of particular significance to explore whether normobaric hypoxia, a common cause of hypoxemia at the sea level, influences the expression levels of HO-1 and HO-2, a potential oxygen sensor, in various organs.

The present study was designed to examine the expression profiles of HO-1 and HO-2 in the heart, lung, and liver during acclimatization to normobaric hypoxia (10% oxygen). Accordingly, C57BL/6 mice were maintained for up to 28 days under normobaric hypoxia, in which the barometric pressure is kept at the sea level value in Sendai but with the $O_2$ concentration at an altitude of about 5000 m. We also assessed the changes in the total amount of heme breakdown during acclimatization to normobaric hypoxia by measuring arterial blood CO contents.

## Experimental procedures

*Animal treatment*. Male C57BL/6 mice (5 weeks old) were obtained from the Animal Experimental Center of Tohoku University School of Medicine (Sendai, Japan) and were housed for 1 week before the beginning of the study. All animal experiments were performed based on the institutionally approved protocols of Tohoku University School of Medicine. Mice were maintained under 12-h light/12-h dark cycle at 20 °C and allowed free access to standard mice food and water. Mice were randomly selected into two groups. One group of mice was maintained in a normobaric hypoxic chamber with 10% oxygen for up to 28 days. Normobaric hypoxia was established by diluting ambient air with nitrogen in a special ventilated chamber, in which the $N_2$-enriched air supply is controlled with an $O_2$ sensor-driven inlet valve, as detailed previously [21,22]. Mice were maintained in the chamber and exposed to normobaric hypoxia (10% $O_2$) for 1, 2, 3, 5, 7, 14, 21, or 28 days ($n = 3$–9 for each time point). An oxygen analyzer was used to monitor the concentration of oxygen in the hypoxic chamber. The age-matched control mice were kept under normoxia (room air) in the same room, in which the hypoxic chamber was placed. The chamber was opened twice a week for 15 min for feeding and cleaning up cages. At the end of the exposure, the animals were anesthetized with diethyl ether, and the blood was taken from abdominal aorta for counting red blood cells (RBCs) and measuring hematocrit with a heparinized capillary tube (Drummond Scientific, Broomall, PA).

*Measurement of relative CO contents*. CO contents were measured as an indicator for endogenous heme degradation. Arterial blood (50 μl) was taken from abdominal aorta with a non-heparinized capillary tube (Drummond Scientific), transferred in a 7-ml glass vial, and stored at −70 °C. To this vial, 0.5 ml of saturation potassium ferricyanide and 1 ml tetraborate pH standard solution (pH 9.18) were added. The solution in the tightly capped vial was mixed vigorously for 30 s on a vortex mixer to release CO from carboxyhemoglobin (COHb) and was left standing for 10 min. Gas phase (1 ml) was taken with a 1-ml syringe from the vial and injected into a TRIlyzer mBA-3000 machine (TAIYO instruments, Osaka, Japan) for measurement of a CO concentration. CO contents are expressed as the ratio of the values in the hypoxia-exposed mice to those of the age-matched control mice, kept under normoxia.

*Northern blot analysis*. Total RNA was extracted from the liver, lung, and heart with TRIzol reagents (Invitrogen) and subjected to Northern blot analysis, as detailed previously [12,23]. HO-1 and HO-2 cDNAs used as Northern probes were synthesized by reverse transcriptase and polymerase chain reaction (RT-PCR) from mouse spleen RNA and testis RNA, respectively. The primers used were a forward primer, 5′-ACCAG CCTGAACTAGCCCAGTCCGGTGATG-3′ (102–131) and a reverse primer, 5′-TGCATTTACATGGCATAAATTCCCACTGCC-3′ (974–1003) for HO-1 cDNA, and a forward primer, 5′-ACTACTCAGCCAC AATGTCTTCAGAGGTGG-3′ (151–180) and a reverse primer, 5′-C AAACAACATGACAGGTCCTTCACATGTAG-3′ (1103–1132) for HO-2 cDNA. The primer set was designed from the murine genome database (GenBank Accession No. NM_010442.1 for HO-1 and No. NM_010443.1 for HO-2). The amplified fragment was cloned into pGEM-Teasy vector (Promega, Madison, WI). The nucleotide sequences of the cDNAs were confirmed by sequencing. The expression of β-actin mRNA was examined as an internal control. The probe for β-actin mRNA was the *Sma*I/*Sca*I fragment (nucleotides 124–1050) of a human β-actin cDNA provided by Dr. T. Yamamoto (Tohoku University). These DNA fragments were labeled with [α-$^{32}$P]dCTP (Amersham Biosciences) by the random priming method and were used as hybridization probes. Total RNA (20 μg per lane) was electrophoresed on 1.0% agarose gels containing 2 M formaldehyde, transferred to nylon membrane filters (Zeta-probe membrane; Bio-Rad), and fixed with a UV-linker (Stratalinker 1800; Stratagene). The RNA blot was hybridized with each $^{32}$P-labeled probe, as described previously [24]. Radioactive signals were detected by exposing the filters to X-ray films (X-AR5; Kodak) or with a Bioimage Analyzer (BAS1500; Fuji Film). The exposure time to X-ray films varied depending on the experiments. The intensity of hybridization signals was determined by photo-stimulated luminescence with a Bioimage Analyzer.

*Western blot analysis*. Each tissue sample was homogenized on ice in triple detergent lysis buffer, containing 50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 100 μg/ml phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml Nonidet P-40, and 0.5% sodium deoxycholate [10]. The homogenates were centrifuged at 15,000*g* for 10 min, and the supernatant (20 μg of protein) was analyzed on a SDS–polyacrylamide gel (10%). The proteins in the gel were treated with 20% methanol buffer containing 48 mM Tris, 39 mM glycine, and 0.037% SDS, and electrophoretically transferred to a polyvinylidene difluoride membrane (Immobilon-P, Millipore), which was pretreated with the same buffer. The membranes were treated overnight at 4 °C in Tris-buffered saline (TBS) with 0.1% Tween 20 (TBS-T), containing 5% non-fat dried milk, and were washed three times each for 5 min in TBS-T at room temperature. The membranes (Western blots) were then incubated with a rabbit polyclonal antibody against rat HO-1 [25] or mouse HO-2 (SPA-897, StressGen Biotechnologies, Canada) in TBS-T for 1 h at room temperature or overnight at 4 °C. The reaction mixture contained 5% non-fat milk for HO-1 antibody (a dilution of 1:1000) and 1% non-fat milk for HO-2 antibody (a dilution of 1:2000). The specific immunocomplexes were detected with a Western blot kit (ECL Plus, Amersham Biosciences). Expression of α-tubulin was determined as an internal control with α-tubulin antibody (Neo Markers, CA, USA).

*Statistical analysis*. All data were derived from 3 to 9 animals per each time point and are expressed as means ± SEM. Two-tailed Student's *t* test was used for comparison between the two groups. Differences between mean values were considered significant when $p < 0.05$.

## Results

### *Erythrocytosis as an adaptive response to normobaric hypoxia*

We initially assessed the effects of normobaric hypoxia on erythropoiesis in C57BL/6 mice, as the inter-strain difference in hypoxic response has been known in mice [26,27]. Male C57BL/6 mice were maintained in a normobaric hypoxic chamber with 10% oxygen for up to 28 days. RBC number and hematocrit were measured by arterial blood analysis at each time point (Fig. 1). The number of

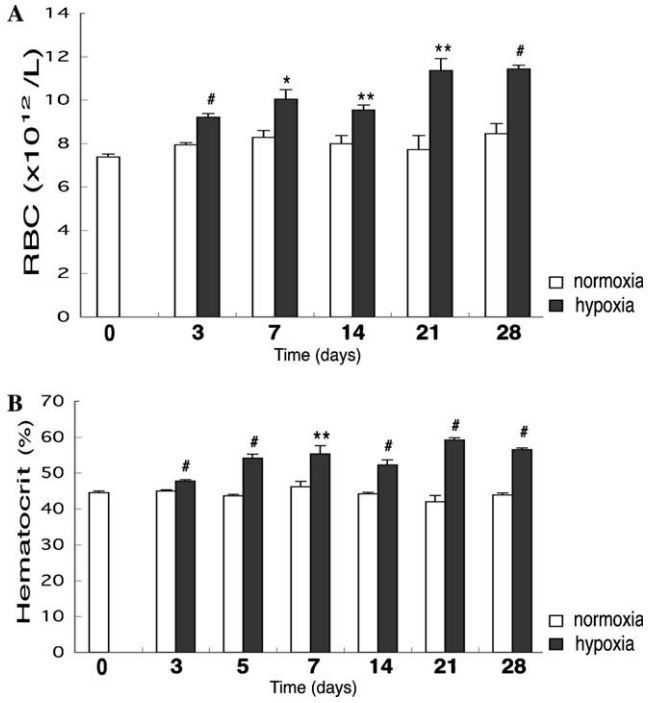


Fig. 1. Erythrocytosis in mice during acclimatization to hypoxia. RBC numbers (A) and hematocrit (B) values were measured in arterial blood taken from mice kept under normoxia or hypoxia (10% oxygen) for the indicated time. Symbols represent statistically significant differences compared to the respective control ($^{\#}p < 0.01$; $^{**}p < 0.03$; $^{*}p < 0.05$).

RBCs increased to reach the highest values at 3 days of hypoxia and appeared to increase further between 14 and 21 days of hypoxia (Fig. 1A). In parallel, the hematocrit values started to increase from 44% after 3 days of hypoxia, reaching a peak of 59% at 21 days of hypoxia (Fig. 1B). In contrast, the number of RBCs and the hematocrit values remained constant in the age-matched control mice, maintained under normoxia. Thus, C57BL/6 mice are able to respond to normobaric hypoxia by enhancing erythropoiesis.

### *Biphasic increases in arterial blood CO contents during hypoxia*

We also measured the CO contents in arterial blood to assess the amount of heme degradation, as heme catabolism accounts for about 86% of endogenously produced CO [28]. The CO gas analyzer employed enabled us to detect the low levels of CO released from carboxyhemoglobin. Relative CO contents dynamically changed in a time-dependent manner, compared with the age-matched control mice maintained under normoxia (Fig. 2). The CO contents increased within 1 day of hypoxia and returned to the basal levels at 2 days. The CO levels increased again after 7 days and remained at the highest level at 21 days of hypoxia ($p < 0.05$). Notably, at 28 days of hypoxia, CO contents returned to the basal levels. These dynamic changes in the arterial CO contents reflect the similar changes in the total amount of endogenous heme degradation during acclimatization to hypoxia.

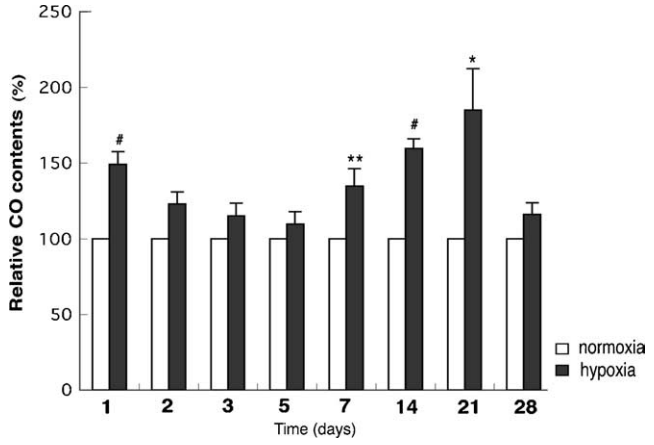


Fig. 2. Biphasic increases in the relative CO contents in arterial blood during hypoxia. Relative CO content is a ratio to the value of each age-matched control and shown as percentage. Symbols represent statistically significant differences compared to the respective control ($^{\#}p < 0.01$; $^{**}p < 0.03$; $^{*}p < 0.05$).

### *Increased expression of HO-1 and HO-2 proteins in the heart during hypoxia*

We then analyzed the expression of HO-1 and HO-2 in the heart and lung, as hypoxia generates hemodynamic stress [22]. Northern blot analysis showed that the expression of HO-1 mRNA was increased in the heart at 5 days of hypoxia, compared with the age-matched control. The HO-1 mRNA increased again to reach the highest levels at 28 days of hypoxia (Fig. 3A). In contrast, there were no noticeable changes in the expression levels of HO-2 mRNA in the heart under hypoxia. In parallel with the changes in HO-1 mRNA, HO-1 protein tended to increase in the heart at 7 days of hypoxia, but the changes were not statistically significant (Figs. 3B and C). HO-1 protein reached the highest levels at 28 days, when HO-2 protein also increased by 30%, although HO-2 mRNA level remained at the basal levels (see Fig. 3A). The increased expression of HO-1 and HO-2 may reflect the adaptation processes to hemodynamic stress [18], as normobaric hypoxia (10% oxygen) for 3 weeks increases pulmonary vascular resistance and induces right ventricular hypertrophy in C57BL/6 mice [29]. On the other hand, we were unable to detect the significant changes in the expression levels of HO-1 and HO-2 mRNAs and proteins in the lung during hypoxia (data not shown).

### *Decreased expression of HO-1 and HO-2 proteins in the liver*

We next analyzed the expression profiles in the liver, which is actively involved in degradation of a large amount of endogenous heme. HO-1 mRNA levels tended to increase after 1 day of hypoxia and returned to the basal levels at 5 days (Figs. 4A and B), although the increase was not statistically significant. After a lag period, HO-1 mRNA levels increased 2.4-fold at 21 days. In contrast, HO-2 mRNA remained unchanged during hypoxia. In par-

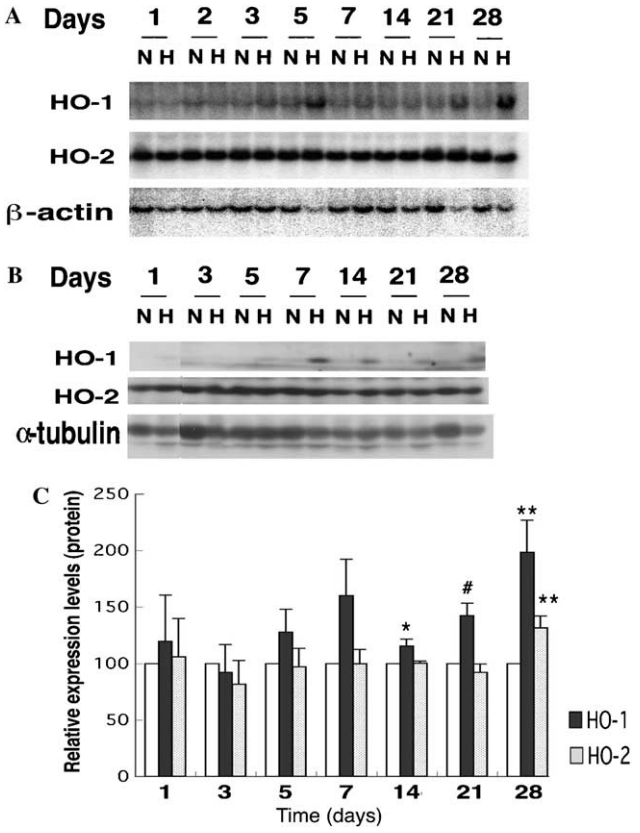


Fig. 3. Increased expression of HO-1 and HO-2 proteins in the heart. (A) Northern blot analysis of HO-1 and HO-2 mRNAs. The hearts were isolated from mice after exposure to hypoxia (H) for the indicated time or age-matched control kept under normoxia (N). Total RNAs were extracted from each heart tissue and subjected to Northern blot analysis. Each lane contains 20 μg of total RNA. The bottom panel shows the expression of β-actin mRNA as an internal control. Shown is one of the two independent experiments. (B) Western blot analysis of HO-1 and HO-2 proteins. The tissue extracts were prepared from the hearts and subjected to Western blot analysis. Each lane contains 20 μg protein. The data shown are one of four independent experiments with similar results. To normalize the expression levels of HO-1 and HO-2, the same Western blot was reused for α-tubulin. (C) Relative expression levels of HO-1 and HO-2 proteins. The intensities of the signals in the Western blots were quantified, and the intensity of HO-1 or HO-2 protein was normalized with respect to the intensity for α-tubulin. The relative expression level of HO-1 or HO-2 protein indicates the ratio of each normalized value to that of the respective age-matched control kept under normoxia and is shown as percentage. The data shown are derived from four independent experiments. For easy comparison, the level of HO-1 or HO-2 under normoxia at each time point is shown as open column. Symbols represent statistically significant differences compared to the respective control ($^{\#}p < 0.01$; $^{**}p < 0.03$; $^{*}p < 0.05$).

allel with the early changes in HO-1 mRNA levels, HO-1 protein transiently increased by 27% at 3 days of hypoxia (Figs. 4C and D). Subsequently, HO-1 protein decreased below the basal levels (20% reduction) at 7 days and then returned to the basal levels at 14 days. HO-1 protein remained at the basal levels for up to 28 days, despite the induction of HO-1 mRNA at 21 days of hypoxia. Unexpectedly, HO-2 protein decreased by about 40% at 7 days and returned to the basal levels at 14 days of hypoxia. To confirm these surprising findings, we repeated this series of experiments five times. We therefore conclude that HO-1 and HO-2 proteins are transiently reduced in the liver during hypoxia.

## Discussion

We have shown the dynamic changes in the expression levels of HO-1 and HO-2 in mice during acclimatization to normobaric hypoxia. The expression levels of HO-1 and HO-2 proteins are transiently reduced in the liver at 7 days of hypoxia, whereas HO-1 and HO-2 proteins increased in the heart at 28 days. This is the first report, showing that the expression levels of HO-1 and HO-2 proteins are decreased or increased during acclimatization to hypoxia. Consistent in part with the changes in HO-1 and HO-2 proteins, the relative CO contents in arterial blood exhibit the two peaks under hypoxia: the first peak at 1 day and the second peak at 21 days of hypoxia. Notably, the CO contents in arterial blood return to the basal levels at 28 days of hypoxia, despite the fact that the HO-1 and HO-2 proteins are expressed at the highest levels in the heart (Fig. 3). Thus, the heart may contribute to only a small portion of the in vivo heme degradation. On the other hand, the changes in the arterial CO contents appear to parallel with the expression levels of HO-1 and HO-2 proteins in the liver, suggesting that the CO contents in arterial blood may reflect the amount of heme degradation in the liver. In fact, the liver is responsible for the degradation of a large amount of endogenous heme [5,30].

The biphasic changes in arterial CO contents indicate the time-dependent adaptation processes to normobaric hypoxia (Fig. 2). The CO contents increased within 1 day, returned to the basal levels after 2–5 days, and then increased again after 7 days of hypoxia. During acute phase (within 1 day), mice have to cope with hypoxia by enhancing ventilation and increasing heart rate. The rapid increase in heme breakdown may be due to the induction of HO-1 in certain organs, including the liver (Fig. 4), resulting in the sufficient supply of bilirubin as a potent antioxidant [7]. The second peak seen at 21 days of hypoxia may reflect the late-onset adaptation in the vascular system, such as the remodeling of the pulmonary artery [29] and the hypertrophy of the pulmonary venous myocardium [18]. In this context, it has been reported that CO may dilate blood vessels, cause smooth muscle cell relaxation, and inhibit smooth muscle cell proliferation by activating soluble guanylate cyclase and elevating intracellular cGMP levels [31,32]. It is therefore conceivable that CO may play beneficial roles during adaptation to hypoxia. Likewise, iron, which is also released during heme breakdown, may be efficiently reused for heme synthesis in the bone marrow [5], as erythropoiesis is enhanced under hypoxia (Fig. 1).

Other investigators have reported that normobaric hypoxia (8–10% $O_2$) causes the remodeling of the pulmonary artery, which generates pressure overload to right ventricle and eventually pulmonary hypertension and right ventricular hypertrophy in mice [29,33]. However, in the

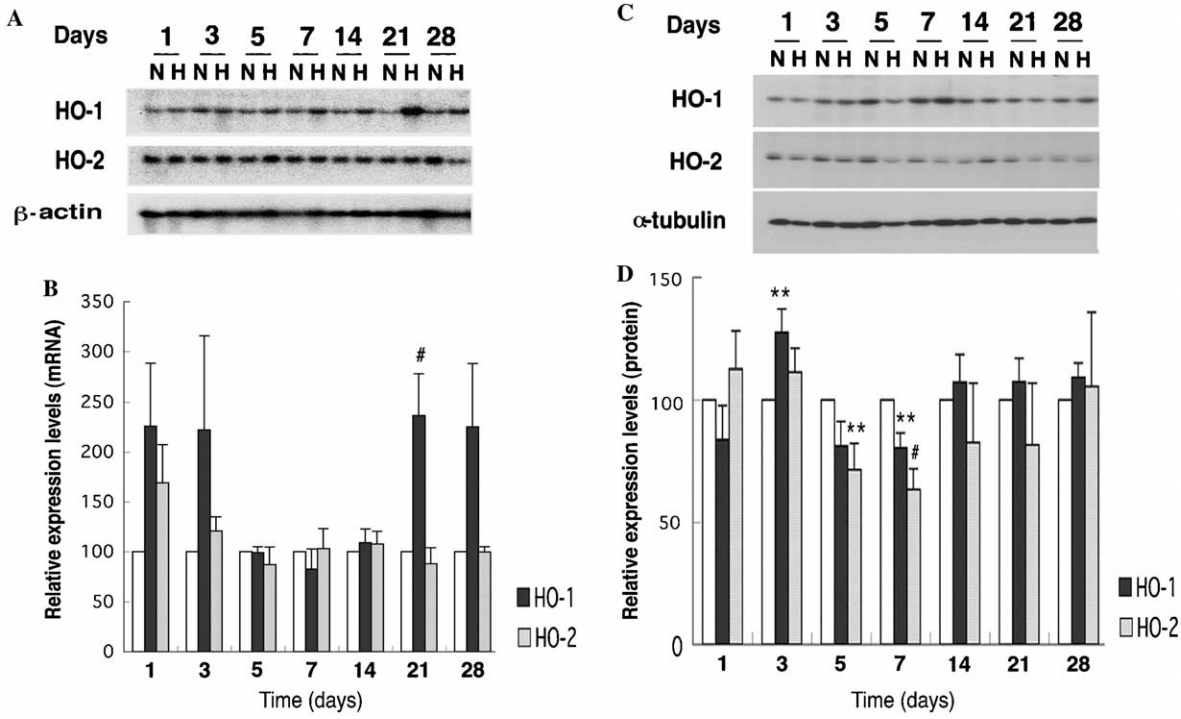


Fig. 4. Decreased expression of HO-1 and HO-2 proteins in the liver. (A) Northern blot analysis of HO-1 and HO-2 mRNAs. Each lane contains 20 μg of total RNA prepared from the liver. Other conditions are described in Fig. 3A. The data shown are from one of three independent experiments with similar results. (B) Relative expression levels of HO-1 and HO-2 mRNAs. The intensities of the signals in (A) were quantified, and the intensity of representing HO-1 or HO-2 mRNA was normalized with respect to the intensity for β-actin mRNA. The relative expression level of HO-1 or HO-2 mRNA indicates the ratio of each normalized value to the respective age-matched control and is shown as percentage. A symbol represents statistically significant difference compared to the control ($^{\#}p < 0.01$). (C) Western blot analysis. Total proteins were extracted from the livers, which were isolated from mice kept under hypoxia (H) or normoxia (N) for the indicated time. The data shown are one of five independent experiments with similar results. Other conditions are described in Figs. 3B and C. (D) Relative expression levels of HO-1 and HO-2 proteins. The data shown are derived from five independent experiments. Symbols represent statistically significant differences compared to the respective control ($^{\#}p < 0.01$; $^{**}p < 0.03$).

present study, normobaric hypoxia did not cause statistically significant changes in the expression of HO-1 and HO-2 at mRNA and protein levels in the lung, as judged by Northern and Western blot analyses (data not shown). These results suggest that the changes in the expression levels of HO-1 and/or HO-2 may be localized to a small population of certain cell types in the lung, such as pulmonary arterial smooth muscle cells. In contrast, the liver and the heart consist of a predominant cell population, hepatocytes, and myocardium, respectively.

In a rat model of mountain sickness, where rats were exposed for up to 3 weeks to hypobaric hypoxia, which is equivalent to the condition at an altitude of about 5000 m, the COHb level (%) increased within 1 day, then decreased to the basal levels at 3 days, and again increased to the highest level at 3 weeks [34]. These changes in rats under hypobaric hypoxia are similar to the changes observed in mice under normobaric hypoxia. Thus, the blood CO content is a good indicator to assess the acclimatization to normobaric and hypobaric hypoxia. Likewise, the HO activity was increased by 20% in the liver after exposure of rats to hypobaric hypoxia for 28–30 days [35]. In the present study, the expression levels of HO-1 and HO-2 proteins tend to increase in the liver at 28 days of normobaric hypoxia, although the changes are not statistically significant (Fig. 4). Thus, normobaric and hypobaric hypoxia may cause similar changes in heme degradation in mice and rats, despite the inter-species difference in the regulation of HO-1 expression [5,27].

Here we have shown that the expression of HO-2 protein is increased and decreased in the heart and liver, respectively, during acclimatization to normobaric hypoxia, despite the constant levels of HO-2 mRNA (Figs. 3 and 4). Thus, hypoxia may regulate the expression of HO-2 protein at the post-transcriptional levels, such as protein stability. Moreover, recent reports have shown the decreased expression of HO-2 protein in the placental tissues of abnormal pregnancies [36,37]. In cultured human trophoblast cells, HO-2 protein is decreased by hypoxia, whereas HO-1 protein and HO enzymatic activity were unaffected by hypoxia [38]. It is therefore conceivable that reduced expression of HO-2, a potential oxygen sensor, may represent an important response under certain conditions.

Hypoxia reduces the expression of HO-1 in several types of cultured human cells [39,40]. The present study has also shown the distinct hypoxic responses of HO-1 in the heart and liver; namely, normobaric hypoxia

decreases or increases the expression of HO-1, depending on the organs or the exposure time. Likewise, expression of HO-2 protein is decreased or increased during acclimatization to hypoxia. We therefore propose that both HO-1 and HO-2 are involved in the adaptive responses to hypoxia, thereby contributing to the maintenance of the host homeostasis.

## Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research (B), for Scientific Research on Priority Areas, and by the 21st Century COE Program Special Research Grant "the Center for Innovative Therapeutic Development for Common Diseases" from the Ministry of Education, Science, Sports and Culture of Japan.

## References

[1] R. Tenhunen, H.S. Marver, R. Schmid, The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase, Proc. Natl. Acad. Sci. USA 61 (1968) 748–755.
[2] T. Yoshida, G. Kikuchi, Features of the reaction of heme degradation catalyzed by the reconstituted microsomal heme oxygenase system, J. Biol. Chem. 253 (1978) 4230–4236.
[3] S. Shibahara, R. Muller, H. Taguchi, T. Yoshida, Cloning and expression of cDNA for rat heme oxygenase, Proc. Natl. Acad. Sci. USA 82 (1985) 7865–7869.
[4] M.D. Maines, G.M. Trakshel, R.K. Kutty, Characterization of two constitutive forms of rat liver microsomal heme oxygenase, only one molecular species of the enzyme is inducible, J. Biol. Chem. 261 (1986) 411–419.
[5] S. Shibahara, The heme oxygenase dilemma in cellular homeostasis: new insights for the feedback regulation of heme catabolism, Tohoku J. Exp. Med. 200 (2003) 167–186.
[6] S. Shibahara, T. Yoshida, G. Kikuchi, Mechanism of increase of heme oxygenase activity induced by hemin in cultured pig alveolar macrophages, Arch. Biochem. Biophys. 197 (1979) 607–617.
[7] R. Stocker, Y. Yamamoto, A.F. McDonagh, A.N. Glazer, B.N. Ames, Bilirubin is an antioxidant of possible physiological importance, Science 235 (1987) 1043–1046.
[8] K.D. Poss, S. Tonegawa, Heme oxygenase 1 is required for mammalian iron reutilization, Proc. Natl. Acad. Sci. USA 94 (1997) 10919–10924.
[9] A. Yachie, Y. Niida, T. Wada, N. Igarashi, H. Kaneda, T. Toma, K. Ohta, Y. Kasahara, S. Koizumi, Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency, J. Clin. Invest. 103 (1999) 129–135.
[10] S. Shibahara, M. Yoshizawa, H. Suzuki, K. Takeda, K. Meguro, K. Endo, Functional analysis of cDNAs for two types of human heme oxygenase and evidence for their separate regulation, J. Biochem. (Tokyo) 113 (1993) 214–218.
[11] K. Takeda, S. Ishizawa, M. Sato, T. Yoshida, S. Shibahara, Identification of a *cis*-acting element that is responsible for cadmium-mediated induction of the human heme oxygenase gene, J. Biol. Chem. 269 (1994) 22858–22867.
[12] K. Takahashi, E. Hara, H. Suzuki, H. Sasano, S. Shibahara, Expression of heme oxygenase isozyme mRNAs in the human brain and induction of heme oxygenase-1 by nitric oxide donors, J. Neurochem. 67 (1996) 482–489.
[13] K. Takahashi, E. Hara, K. Ogawa, D. Kimura, H. Fujita, S. Shibahara, Possible implications of the induction of human heme oxygenase-1 by nitric oxide donors, J. Biochem. (Tokyo) 121 (1997) 1162–1168.
[14] W.K. McCoubrey Jr., T.J. Huang, M.D. Maines, Heme oxygenase-2 is a hemoprotein and binds heme through heme regulatory motifs that are not involved in heme catalysis, J. Biol. Chem. 272 (1997) 12568–12574.
[15] T. Yoshida, P. Biro, T. Cohen, R.M. Muller, S. Shibahara, Human heme oxygenase cDNA and induction of its mRNA by hemin, Eur. J. Biochem. 171 (1988) 457–461.
[16] K. Ishikawa, N. Takeuchi, S. Takahashi, K.M. Matera, M. Sato, S. Shibahara, D.L. Rousseau, M. Ikeda-Saito, T. Yoshida, Heme oxygenase-2. Properties of the heme complex of the purified tryptic fragment of recombinant human heme oxygenase-2, J. Biol. Chem. 270 (1995) 6345–6350.
[17] K.D. Poss, M.J. Thomas, A.K. Ebralidze, T.J. O'Dell, S. Tonegawa, Hippocampal long-term potentiation is normal in heme oxygenase-2 mutant mice, Neuron 15 (1995) 867–873.
[18] T. Adachi, K. Ishikawa, W. Hida, H. Matsumoto, T. Masuda, F. Date, K. Ogawa, K. Takeda, K. Furuyama, Y. Zhang, T. Kitamuro, H. Ogawa, Y. Maruyama, S. Shibahara, Hypoxemia and blunted hypoxic ventilatory responses in mice lacking heme oxygenase-2, Biochem. Biophys. Res. Commun. 320 (2004) 514–522.
[19] S.E. Williams, P. Wootton, H.S. Mason, J. Bould, D.E. Iles, D. Riccardi, C. Peers, P.J. Kemp, Hemoxygenase-2 is an oxygen sensor for a calcium-sensitive potassium channel, Science 306 (2004) 2093–2097.
[20] Y. Tun, W. Hida, S. Okabe, H. Ogawa, Y. Kikuchi, M. Oikawa, T. Kitamuro, K. Shirato, Can nasal continuous positive airway pressure decrease clinic blood pressure in patients with obstructive sleep apnea? Tohoku J. Exp. Med. 201 (2003) 181–190.
[21] D. Katayose, M. Ohe, K. Yamauchi, M. Ogata, K. Shirato, H. Fujita, S. Shibahara, T. Takishima, Increased expression of PDGF A- and B-chain genes in rat lungs with hypoxic pulmonary hypertension, Am. J. Physiol. 264 (1993) L100–L106, Lung Cell Mol. Physiol. 8.
[22] D. Katayose, S. Isoyama, H. Fujita, S. Shibahara, Separate regulation of heme oxygenase and heat shock protein 70 mRNA expression in the rat heart by hemodynamic stress, Biochem. Biophys. Res. Commun. 191 (1993) 587–594.
[23] E. Hara, K. Takahashi, T. Tominaga, T. Kumabe, T. Kayama, H. Suzuki, H. Fujita, T. Yoshimoto, K. Shirato, S. Shibahara, Expression of heme oxygenase and inducible nitric oxide synthase mRNA in human brain tumors, Biochem. Biophys. Res. Commun. 224 (1996) 153–158.
[24] R. Udono-Fujimori, K. Takahashi, K. Takeda, K. Furuyama, K. Kaneko, S. Takahashi, M. Tamai, S. Shibahara, Expression of heme oxygenase-1 is repressed by interferon-gamma and induced by hypoxia in human retinal pigment epithelial cells, Eur. J. Biochem. 271 (2004) 3076–3084.
[25] S. Taketani, H. Sato, T. Yoshinaga, R. Tokunaga, T. Ishii, S. Bannai, Induction in mouse peritoneal macrophages of 34 kDa stress protein and heme oxygenase by sulfhydryl-reactive agents, J. Biochem. (Tokyo) 108 (1990) 28–32.
[26] M.L. Nohr, Inefficacy of erythropoietic response of BALB/c mice to hypoxia, Am. J. Physiol. 213 (1967) 1285–1289.
[27] Y. Hoshikawa, P. Nana-Sinkam, M.D. Moore, S. Sotto-Santiago, T. Phang, R.L. Keith, K.G. Morris, T. Kondo, R.M. Tuder, N.F. Voelkel, M.W. Geraci, Hypoxia induces different genes in the lungs of rats compared with mice, Physiol. Genom. 12 (2003) 209–219.
[28] H.J. Vreman, R.J. Wong, K.D. Stevenson, Carbon monoxide in breath, blood, and other tissues, in: D.G. Penney (Ed.), Carbon Monoxide Toxicity, CRC Press, Boca Raton, FL, 2000, pp. 19–60.
[29] M. Ozaki, S. Kawashima, T. Yamashita, Y. Ohashi, Y. Rikitake, N. Inoue, K. Hirata, Y. Hayashi, H. Itoh, M. Yokoyama, Reduced hypoxic pulmonary vascular remodeling by nitric oxide from the endothelium, Hypertension 37 (2001) 322–327.
[30] N. Goda, K. Suzuki, M. Naito, S. Takeoka, E. Tsuchida, Y. Ishimura, T. Tamatani, M. Suematsu, Distribution of heme oxygenase isoforms in rat liver. Topographic basis for carbon monoxide-mediated microvascular relaxation, J. Clin. Invest. 101 (1998) 604–612.

[31] H.J. Duckers, M. Boehm, A.L. True, S. Yet, H. San, J. l. Park, R.C. Webb, M. Lee, G.J. Nabel, E.G. Nabel, Heme oxygenase-1 protects against vascular constriction and proliferation, Nat. Med. 7 (2001) 693–698.

[32] T. Morita, S.A. Mitsialis, H. Koike, Y. Liu, S. Kourembanas, Carbon monoxide controls the proliferation of hypoxia vascular smooth muscle cells, J. Biol. Chem. 272 (1997) 32804–32809.

[33] T. Minamino, H. Christou, C.M. Hsieh, Y. Liu, V. Dhawan, N.G. Abraham, M.A. Perrella, S.A. Mitsialis, S. Kourembanas, Targeted expression of heme oxygenase-1 prevents the pulmonary inflammatory and vascular responses to hypoxia, Proc. Natl. Acad. Sci. USA 98 (2001) 8798–8803.

[34] M.S. Carraway, A.J. Ghio, J.D. Carter, C.A. Piantadosi, Expression of heme oxygenase-1 in the lung in chronic hypoxia, Am. J. Physiol. Lung Cell. Mol. Physiol. 278 (2000) L806–L812.

[35] H.L. Bonkovsky, B. Lincoln, J.F. Healey, L.C. Ou, P.R. Sinclair, U. Muller-Eberhard, Hepatic heme and drug metabolism in rats with chronic mountain sickness, Am. J. Physiol. 251 (1986) G467–G474.

[36] A.C. Zenclussen, E. Lim, S. Knoeller, M. Knackstedt, K. Hertwig, E. Hagen, B.F. Klapp, P.C. Arck, Heme oxygenases in pregnancy II: HO-2 is downregulated in human pathologic pregnancies, Am. J. Reprod. Immunol. 50 (2003) 66–76.

[37] G.E. Lash, C.M. Taylor, A.J. Trew, S. Cooper, F.W. Anthony, T. Wheeler, P.N. Baker, Vascular endothelial growth factor and placental growth factor release in cultured trophoblast cells under different oxygen tensions, Growth Factors 20 (2002) 189–196.

[38] S.D. Appleton, G.S. Marks, K. Nakatsu, J.F. Brien, G.N. Smith, C.H. Graham, G.E. Lash, Effects of hypoxia on heme oxygenase expression in human chorionic villi explants and immortalized trophoblast cells, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H853–H858.

[39] M. Nakayama, K. Takahashi, T. Kitamuro, K. Yasumoto, D. Katayose, K. Shirato, Y. Fujii-Kuriyama, S. Shibahara, Repression of heme oxygenase-1 by hypoxia in vascular endothelial cells, Biochem. Biophys. Res. Commun. 271 (2000) 665–671.

[40] T. Kitamuro, K. Takahashi, K. Ogawa, R. Udono-Fujimori, K. Takeda, K. Furuyama, M. Nakayama, J. Sun, H. Fujita, W. Hida, T. Hattori, K. Shirato, K. Igarashi, S. Shibahara, Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells, J. Biol. Chem. 278 (2003) 9125–9133.