Molecular Characterization of a Newly Identified Heme-binding Protein Induced during Differentiation of urine Erythroleukemia Cells*

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A heme-binding protein with a molecular mass of 22 kDa, termed p22 HBP, was purified from mouse liver cytosol, using blue Sepharose CL-6B. We identified a cDNA encoding p22 HBP, and sequence analysis revealed that p22 HBP comprises 190 amino acid residues (M, 21,063) and has no homology to any other known heme-binding protein. The p22 HBP mRNA (~1.0 kilobases) is ubiquitously expressed in various tissues and is extremely abundant in the liver. cDNA allows for expression of active p22 HBP, with a high affinity for $^{55}$Fe-hemin, with a $K_a$ of 26 ±1.8 nM. The $B_{max}$ of hemin binding to p22 HBP was 0.55 ± 0.021 mol/mol of protein, a value consistent with one heme molecule binding per molecule of protein. The order of potency of different ligands to compete against $^{55}$Fe-hemin binding to p22 HBP was hemin = protoporphyrin IX > coproporphyrin III > bilirubin > palmitic acid > all-trans-retinoic acid. Treatment of mouse erythroleukemia (MEL) cells with dimethyl sulfoxide or hemin resulted in an increase in p22 HBP mRNA. The immunoblot analysis showed that p22 HBP increased with time in dimethyl sulfoxide- and hemin-induced MEL cells. Conversely, transfer of antisense oligonucleotides to p22 HBP cDNA resulted in a decrease of p22 HBP in dimethyl sulfoxide-treated MEL cells, and the heme content in these cells decreased to 66–71% of sense oligonucleotides-transferred cells. Thus, this newly identified heme-binding protein, p22 HBP, may be involved in heme utilization for hemoprotein synthesis and even be coupled to hemoglobin synthesis during erythroid differentiation.

Heme regulates protein syntheses transcriptionally and translationally. The first enzyme of heme biosynthetic pathway, ALAS$^1$ consists of two gene products. The housekeeping-type ALAS (ALAS-1) was down-regulated at levels of transcription and translation into the mitochondrion by hemin (1, 2). Translocation of the erythroid-type ALAS (ALAS-2) into the mitochondrion is also inhibited by hemin (3). Heme as well as iron mediate the interaction of the iron-responsive element-binding protein with its target mRNAs, including those of ferritin, ALAS-2, and transferrin receptors (4–6). In addition, an increase in free heme in cells leads to transcriptional induction of heme oxygenase-1 (7, 8). Cells seem to maintain adequate heme levels by a combination of synthetic and degradative mechanisms. Cells are equipped with a sensing system to monitor changes in size of the uncommitted heme pool. Although such a system has not been identified, identification of uncommitted heme is one approach to elucidate cellular regulation by heme.

Conditions of uncommitted heme may be linked to cytosolic heme-binding proteins because heme is not so soluble in aqueous solutions at body pH and tends to form large aggregates at concentrations as low as 10$^{-7}$ (9). Several heme-binding and heme-transport proteins from the cytosol have been isolated. FABP constituting 3–5% of the total cytosolic proteins has an affinity for heme that is 10-fold higher than that for oleic acid (9, 10). GSTs constituting 3–5% of total cytosol proteins not only catalyze the conjugation of glutathione with xenobiotics but also bind organic anions, such as bilirubin and bile acid (11, 12), and heme (13). HBP23 has been purified from rat liver cytosol, using chromatography on hemin-agarose (14). cDNA cloning revealed that HBP23 was a homologue of MSP23, the mouse macrophage 23-kDa stress-induced protein that may function as an antioxidant (15, 16) and belongs to thioredoxin peroxidase family (17). However, as studies on functions of these proteins are few, their participation in cellular regulation by heme and the intracellular transport of heme has remained poorly understood.

We earlier reported that ferrochelatase, the terminal enzyme of heme biosynthesis, bound tightly to blue Sepharose (18, 19). Once the enzyme bound to the blue dye, it was not released from the dye even after washing with solution containing more than 1 M NaCl and nonionic detergents such as Triton X-100 and Nonidet P-40. There are reports that heme-binding proteins (20, 21) and enzymes of chlorophyll biosynthesis (22, 23) can bind to blue dye. Although mechanisms involved in such binding to proteins interacted with heme and porphyrins are unclear, it is considered that chromatography on blue Sepharose would be useful for purifying proteins interacting with heme and porphyrins. We purified three major proteins from the cytosol of mouse liver. Although two of them have been reported (24, 25), the third was a heretofore unrecognized heme-binding protein with a molecular mass of 22 kDa, termed...
as p22 HBP. Molecular cloning and functional expression showed that p22 HBP binds heme, protoporphyrin, and coproporphyrin, with relatively high affinities. Induction of the protein in MeS-O- or heme-treated MEL cells undergoing erythroid differentiation was also noteworthy.

**EXPERIMENTAL PROCEDURES**

**Materials**—[β-32P]dCTP (3000 Ci/mol), [35S]FeSO₄ (60 mcg/ml) and nylon membranes were obtained from Amersham. [35S]Fe-heme was prepared, as described previously and dissolved in MeS-O (26). The specific radioactivity of [35S]Fe-heme was 1.06 × 10⁶ dpm/nmol heme. Restriction enzymes and other nucleic acid-modifying enzymes were from Toyobo Co. and Takara Shuzo Co. A 5'-RACE cDNA library from mouse liver mRNA was from CLONTECH. Blue Sepharose CL-6B, EAH-Sepharose CL-6B, and pGEX-4T-1 were from Amersham Pharmacia Biotech. Protoporphyrin IX and coproporphyrin III were from Porphyrin Products Co. Hemin-bound Sepharose were prepared by incubation of hemin with EAH-Sepharose CL-6B by the method of Olsen (27). Amino acid sequencing was carried out using a Hewlett Packard Protein Sequencer GI000A. All other chemicals used were of analytical grade.

**Purification of p22 HBP and Amino Acid Sequencing**—All procedures for the purification of p22 HBP were done at 4 °C. Solution containing 10 mg of Triton X-100 buffer, pH 8.0, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 150 mM NaCl was designated as Solution A. Fresh mouse livers (20 g) were minced and homogenized in 6 volumes of Solution A with 6 strokes of a Teflon pestle in a glass homogenizer. After crude lysates were centrifuged at 10,000 × g for 10 min to remove nuclei and mitochondria, cytosol was separated from the postmitochondrial supernatant by ultracentrifugation at 105,000 × g for 1 h. Then, to the obtained cytosolic fraction, 10% Triton X-100 solution was added at the final concentration of 1%. The resulting samples (865 mg of protein) were applied to a column of blue Sepharose CL-6B (1.5 × 5.0 cm) that was equilibrated with Solution A containing 1% Triton X-100. The column was washed with 500 ml of Solution A containing 1% Triton X-100 and 1 mM NaCl at a flow rate of 30 ml/h. The proteins were eluted with Solution A containing 1% sodium cholate and 1 mM NaCl. Fractions containing proteins were collected, concentrated using an Amicon YM-10 membrane, and dialyzed with Solution A for 16 h. Amounts of protein in the final preparation were 0.23 mg. For chromatography on hemin-Sepharose CL-6B, eluates of blue Sepharose were applied to a small column of hemin-Sepharose (1.0 × 2.0 cm) equilibrated with Solution A containing 0.5% Tween 20, with which the column was washed with 100 ml of Solution A containing 1 mM NaCl. The proteins were eluted from the column with 50 mM sodium acetate, pH 5.0. Fractions containing proteins were collected and dialyzed against Solution A for 16 h. Protein concentration was determined by the method of Lowry et al. (28).

To identify the N-terminal amino acid sequence of the obtained proteins, the proteins were analyzed by SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Bio-Rad), as described (29). After staining the proteins, the parts of the filter corresponding to molecular masses of 26, 24, and 22 kDa (see Fig. 1) were cut out and directly sequenced.

**Isolation and Sequencing of p22 HBP cDNA**—A primer (5'-GTTGCCCAGCAATGACCCACG-3') complementary to amino acid sequence (FGSVET) was prepared, and PCR was carried out using as a template 5'-RACE mouse liver cDNA library. The PCR products were separated by agarose gel electrophoresis, purified, and ligated to pGEM-T vector (Promega), and then the insert was examined by DNA sequencing (30). To isolate full-length cDNA, the MEI cell cDNA library in Agi11 (29) was screened with the radiolabeled partial fragment of p22 HBP cDNA. Five positive plaques were isolated from 1.5 × 10⁶ plaques, and the longest insert (1.0 kbp) of clone 5 was cut out and subcloned into a pBluescript vector for sequencing.

**Expression of GST-p22 HBP Fusion Protein and Binding Studies**—The cDNA corresponding to mouse p22 HBP was subcloned into BamHI-NolI site of an expression vector pGEX-4T-1 in the correct reading frame. p22 HBP was expressed as GST fusion protein in E. coli BL21 (DE3) strain. The cDNA for p22 HBP was subcloned into NdeI-RKI site of an expression vector pAR-HF, as described (32). p22 HBP was purified using blue Sepharose, as described above. For binding of heme to p22 HBP, the purified p22 HBP (100 μg) in 10 mM Tris-HCl buffer, pH 8.0, was incubated with 20 μM heme at 4 °C for 20 min. The sample was loaded onto a column of DEAE-cellulose (1.0 × 2.0 cm) equilibrated with 10 mM Tris-HCl buffer, pH 8.0. The column was washed with 10 ml of an above solution and the protein was eluted with 10 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl.

**Cell Culture**—MEL cells (clone 745A) were grown in DMEM supplemented with 7% fetal calf serum and antibiotics. To induce erythroid differentiation, cells at an initial density of 1 × 10⁶/ml were grown for 3 days in medium containing 2% MeSO or 100 μM heme. Mouse macrophage RAW 264.7 cells were grown in RPMI 1640 medium containing 10% fetal calf serum (39).

**Northern Blot Analysis**—A mouse multiple tissue Northern blot membrane was purchased from CLONTECH Co. Total RNA was isolated from MEL cells and RAW264.7 cells, as described previously (29). 10 μg of RNA were applied to a 1% agarose-formaldehyde gel, electrophoresed, and transferred onto a nylon membrane (Amersham Pharmacia Biotech Hybrid N°) for hybridization with DNA probes. The filters were then hybridized and washed as described (29). The mRNA concentration was quantified by densitometry using an Advanced DMC-33 densitometer.

**Antibodies and Immunoblot Analysis**—Antibodies against p22 HBP were prepared by injecting a rabbit with 0.5 mg of the purified GST-p22 HBP fusion protein in Freund's complete adjuvant. After three subsequent injections at 2-week intervals, the resulting antisera were collected and antibodies were purified as described (29). Antibodies for ferrochelatase and MSP23 were prepared, as described (16, 29). For immunoblotting, cells were lysed with Laemmli's sample buffer (33). The lysate was then sonicated and boiled for 1 min. After the proteins had been resolved by SDS-PAGE on 12% gel, the protein in the gel were electrophoblated onto a polivinyldene difluoride membrane. Conditions of immunoblotting and detection of the cross-reacted antigen were as described (29).

**Transfection of Oligonucleotides and Determination of Heme in MEL Cells**—Phosphorothioate sense (5′-ATGTTGGGCGATCATGAA-3′) oligonucleotides, which were identical to nucleotide positions 58–75 of p22 HBP cDNA, were transcribed and purified. MEL cells (5 × 10⁶) treated with 2% MeSO for 16 h were collected and rinsed twice with serum-free DMEM. Phosphorothiate sense or antisense oligonucleotides (5 μM) were transfected into the cells, using a DOTAP Liposomal Transfection Reagent (Boehringer Mannheim GmbH) (34). After a 6-h transfection, the medium was changed to DMEM containing 7% fetal calf serum, 2% MeSO, and antibiotics. The cells were further cultured for 48 or 48 h and then collected. The heme content in the cells were estimated as described previously (29).

**RESULTS**

**Purification, Characterization, and cDNA Cloning of Mouse p22 HBP**—After blue Sepharose CL-6B chromatography of mouse hepatic cytosolic protein, three major bands, 22, 24, and 26 kDa, appeared on SDS-PAGE (Fig. 1). To determine these proteins would bind to heme, eluates of blue Sepharose were loaded to hemin-bound Sepharose, the column was washed with Solution A containing 1 mM NaCl, and proteins were eluted with the solution at pH 5.0. All proteins were bound to heme-Sepharose. Thus, three proteins with relatively low molecular weights that were tightly bound to blue dye could bind to heme. We then analyzed the N-terminal amino acid sequences of three proteins. The protein with a molecular mass of 22 kDa had the following amino acid sequence: (NH₂)-N-S-L-F-G-S-V-
E-T-W. When we searched for the above sequence in the GenBank Data Bank, we found no homology with any known proteins. The 26 and 24 kDa proteins showed the sequence of (NH2)-V-L-E-L-Y-L-D-L-L-S- and (NH2)-P-P-Y-T-I-V-Y-F-P-V-, respectively. The latter sequences perfectly matched with mouse GST-T1 (25). We then prepared a primer complementary to amino acid sequence positions 4–9 (FGSVET) and obtained a 112-bp partial cDNA fragment (clone 20) corresponding to a 22-kDa protein by the 5’-RACE PCR amplification method, using a mouse liver 5’-RACE cDNA library. The nucleotide sequence of clone 20 showed no homology to known sequences. We termed this 22-kDa heme-binding protein, p22 HBP. When we isolated the full-length cDNA, p22 HBP cDNA proved to be a 1033-bp insert of 41 kDa is induced with concomitant increases in heme biosynthesis, total RNA was obtained from MEL cells at various periods after Me2SO treatment, and RNA blots were carried out using p22 HBP cDNA as a probe. As shown in Fig. 7A, p22 HBP mRNA corresponding to about 1.0 kbp increased gradually with time up to 72 h. Hemin is an inducer of erythroid differentiation of MEL cells (38). We next examined the induction of p22 HBP mRNA in MEL cells treated with 100 μM hemin. Treatment of cells with hemin resulted in an increase in hemoglobin synthesis, similar to findings in MEL cells treated with Me2SO. p22 HBP mRNA increased with time, similar to the case of Me2SO-treated cells, but at 72 h the level of the RNA slightly decreased (Fig. 7B). Thus, the p22 HBP mRNA was induced during MEL cell differentiation.

Density of p22 HBP in MEL Cells—MEL cells were induced to differentiate with 2% Me2SO, heme biosynthesis increased with time, and then over 90% of the cells synthesized large quantities of hemoglobin at 4 days (29). To examine involvement of p22 HBP in heme biosynthesis, total RNA was obtained from MEL cells at various periods after Me2SO treatment, and RNA blots were carried out using p22 HBP cDNA as a probe. As shown in Fig. 7A, p22 HBP mRNA corresponding to about 1.0 kbp increased gradually with time up to 72 h. Hemin is an inducer of erythroid differentiation of MEL cells (38). We next examined the induction of p22 HBP mRNA in MEL cells treated with 100 μM hemin. Treatment of cells with hemin resulted in an increase in hemoglobin synthesis, similar to findings in MEL cells treated with Me2SO. p22 HBP mRNA increased with time, similar to the case of Me2SO-treated cells, but at 72 h the level of the RNA slightly decreased (Fig. 7B). Thus, the p22 HBP mRNA was induced during MEL cell differentiation.

Decrease of Heme Content in Me2SO-treated MEL Cells by Antisense Oligonucleotides to p22 HBP—Expression of p22 HBP in MEL cells with oligonucleotides was estimated by immunoblotting. As shown in Fig. 9A, p22 HBP in cells transfected with sense-oligonucleotides was similar to that in control cells, whereas the protein in cells transfected with antisense oligonucleotides was markedly reduced compared with control cells. We finally examined effects of sense or antisense oligonucleotides on heme content during Me2SO-induced differentiation of MEL cells. The heme contents in cells transfected with antisense oligonucleotides were lower than those in control cells (Fig. 9B). Thus, heme biosynthesis is dependent on the expression of p22 HBP during Me2SO induced differentiation.
We have described herein the molecular cloning and functional characterization of p22 HBP, a novel heme-binding protein expressed as a cytosolic protein that is particularly abundant in the liver. Three major proteins of the cytosolic fraction of mouse liver which bound tightly to blue Sepharose were eluted with solution containing 1% sodium cholate and 1 M NaCl, methods similar to those used for purification of ferrochelatase from mitochondria (18, 19). The three proteins eluted have affinities for heme because all bound to hemin-coupled Sepharose. The use of blue Sepharose is appropriate to purify proteins interacting with heme and porphyrins. The amino acid sequences of N termini revealed that two proteins corresponded to isoforms of GST (GST-T1 and GST-P1). GSTs not only catalyze the conjugation of glutathione with xenobiotics but also organic anions such as bilirubin and bile acids (11, 12, 36). Binding of GST-T1 and P1 for heme has not been reported, however, our present study shows the binding of these enzymes to hemin-Sepharose. Our newly identified p22 HBP also belongs to heme-binding protein family.

**Fig. 2.** Nucleotide sequence of p22 HBP cDNA and the deduced amino acid sequences. N termini identical to that of purified p22 HBP are double underlined. The hydrophobic region possibly involved in heme-binding is underlined. The dotted line shows the putative polyadenylation signal.

**Fig. 3.** Tissue distribution of p22 HBP mRNA determined by Northern blot analysis. Multiple tissue blot membrane (CLONTECH) was probed with the fragment of p22 HBP cDNA. The membrane was washed and reprobed with β-actin cDNA. Lane 1, testis; lane 2, kidney; lane 3, skeletal muscle; lane 4, liver; lane 5, lung; lane 6, spleen; lane 7, brain; and lane 8, heart. The position of p22 HBP mRNA is shown by an arrow. Molecular size markers (1.35–4.4-kbp ladder) are indicated on the left.

**Fig. 4.** Absorption spectrum of the heme-p22 HBP complex. Purified p22 HBP (100 μg of protein) was incubated with 20 μM hemin, and unbound hemin was removed by DEAE-cellulose chromatography. Absorption spectra of 1 μM hemin-p22 HBP complex (emyemy) and 1 μM hemin itself (-----) in 10 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl were measured.

**DISCUSSION**

We have described herein the molecular cloning and functional characterization of p22 HBP, a novel heme-binding protein expressed as a cytosolic protein that is particularly abundant in the liver. Three major proteins of the cytosolic fraction of mouse liver which bound tightly to blue Sepharose were eluted with solution containing 1% sodium cholate and 1 mM NaCl, methods similar to those used for purification of ferrochelatase from mitochondria (18, 19). The three proteins eluted have affinities for heme because all bound to hemin-Sepharose. The use of blue Sepharose is appropriate to purify proteins interacting with heme and porphyrins. The amino acid sequences of N termini revealed that two proteins corresponded to isoforms of GST (GST-T1 and GST-P1). GSTs not only catalyze the conjugation of glutathione with xenobiotics but also organic anions such as bilirubin and bile acids (11, 12, 36). Binding of GST-T1 and P1 for heme has not been reported, however, our present study shows the binding of these enzymes to hemin-Sepharose. Our newly identified p22 HBP also belongs to heme-binding protein family. Comparison of amino
Acid sequences among p22 HBP, GST-T1, and GST-P1 showed no conserved region for heme-binding. MSP23 was rich in liver and one of the heme-binding proteins (14, 16), but the binding mechanism of MSP23 for heme has not been reported. MSP23 was not purified by blue Sepharose, as was the case for p22 HBP. Although the affinity of heme-binding with p22 HBP (K_d 526 nM) was similar to that seen with HBP23 (a rat homologue of MSP23) (K_d 588 nM) (14), mechanisms of binding of MSP23 for heme can be different from those of p22 HBP.

Analysis of nucleotides and deduced amino acid sequences revealed that p22 HBP has no homology to heme-binding proteins such as GSTs, FABP, and MSP23. There are various proteins and enzymes, including yeast HAP1 (40), mammalian ALAS-1 and ALAS-2 (3), and heme-regulated eIF-2a kinase (41), which have the potential to bind heme. They contain a heme-regulatory motif (-K/RCP-) in their sequences, but p22 HBP does not have this motif. Albumin binds heme with K_d 510^{-28} M and can bind several hemes per molecule (37). Only a hydrophobic region in albumin is responsible for heme-binding (35, 36).

Similarly p22 HBP contains the hydrophobic region spanning 73–82 amino acid residues, suggesting that this region contributes to heme-binding. p22 HBP expressed in E. coli presents as a monomer, as estimated by gel filtration, and B_max for hemin obtained by binding assay showed 0.55 mol/mol of protein, thereby suggesting that p22 HBP binds one heme. Thus, binding properties of a small heme-binding protein, p22 HBP, are unique as compared with those of other heme-binding proteins.

Analysis of nucleotides and deduced amino acid sequences revealed that p22 HBP has not heretofore been identified. The overall amino acid composition indicated that the protein is soluble and contains a heme-binding site. The amino acid sequence of p22 HBP has no homology to heme-binding proteins such as GSTs, FABP, and MSP23. There are various proteins and enzymes, including yeast HAP1 (40), mammalian ALAS-1 and ALAS-2 (3), and heme-regulated eIF-2a kinase (41), which have the potential to bind heme. They contain a heme-regulatory motif (-K/RCP-) in their sequences, but p22 HBP does not have this motif. Albumin binds heme with K_d 510^{-28} M and can bind several hemes per molecule (37). Only a hydrophobic region in albumin is responsible for heme-binding (35, 36).

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N terminus of the deduced amino acid sequence of cloned p22 HBP differed from that of the purified p22 HBP because the...
shows that p22 HBP is also induced in MEL cells induced with Me₃SO and hemin. Based on observations that coproporphyrinogen oxidase markedly increased during MEL cell differentiation (44), p22 HBP may possibly function as a transporter of the coproporphyrinogen produced by cytosolic uroporphyrinogen decarboxylase to mitochondrial peripheral-type benzodiazepine receptors. The interaction of peripheral-type benzodiazepine receptors with p22 HBP is now under investigation.

Iwahara et al. (14) reported that a rat homologue of MSP23, HBP23 in rat liver, was induced after partial hepatectomy in vivo. During regeneration of the liver, the level of ALAS-1 decreases and heme oxygenase-1 increases (46). Moreover, treatment of rat primary hepatocyte culture with hemin resulted in an increase in HBP23 mRNA (39). Under these conditions, heme oxygenase-1 increases and ALAS-1 decreases (47). Ishii et al. (15, 16) also reported that MSP23 was markedly induced in mouse peritoneal macrophages by oxidative stress and may protect cells by binding heme which is a prooxidant molecule. Thus, changes in HBP23 mRNA levels are related to heme catabolism. In contrast, p22 HBP protein or its mRNA in macrophage RAW 264.7 cells was not induced by hemin, indicating that p22 HBP is not a stress-induced protein. Conversely, p22 HBP but not MSP23 was induced during Me₃SO-or hemin-induced differentiation of MEL cells. During erythroid differentiation, an increase in hemoglobin accumulation but a decrease of heme oxygenase-1 was observed (48). Heme metabolism in erythroid cells differs from that in nonerythroid cells. The present study demonstrated that heme content in Me₃SO-induced MEL cells transfected with antisense oligonucleotides. Therefore, induction of p22 HBP can be coupled to heme biosynthesis as well as formation of hemoglobin. Except for erythroid cells, heme biosynthesis is most active in the liver. Much of the synthesized heme in the liver is used for prosthetic groups of cytochromes, including cytochrome P-450 and b₅. Since p22 HBP mRNA is extremely abundant in liver, hepatic p22 HBP may have a role in heme metabolism (i.e., heme transport and heme binding).

Uncommitted heme plays a central role in the regulation of iron uptake in various species of cells (49) and hemoglobin synthesis in developing erythroid cells (50). Within the mitochondria, heme exerts negative feedback regulation on its own synthesis at ALAS-1 and ferrochelatase (51, 52). Once formed, heme must be translocated from mitochondria to sites of hemoprotein synthases in the cytoplasm and endoplasmic reticulum. Endogenously synthesized heme does not freely penetrate the inner membrane of the mitochondria, consequently, the transport of heme across this membrane seems to be a potential rate-limiting step in hemoprotein synthesis (52, 53). The efflux of heme from mitochondria depends on the presence of cytosolic protein (54). Other workers (10, 39, 55) found that GSTs, FABP, and HBP23 may be important for the transport of heme from mitochondria. The Kds of FABP, HBP23, and GSTs for hemin were 10⁻⁷ to 10⁻⁸ M. The affinity of p22 HBP for hemin approximates values as above, indicating that p22 HBP functions physiologically as heme-binding and/or heme-transport protein. Based on observations that p22 HBP is induced during erythroid differentiation and antisense oligonucleotides to p22 HBP decrease heme biosynthesis in Me₃SO-induced MEL cells, the induced p22 HBP can enhance efflux of synthesized heme in mitochondria, and p22 HBP-bound heme is utilized for hemoglobin maturation. An increase in p22 HBP unsaturated with heme with concomitant decrease of uncommitted heme leads to promotion of the biosynthesis of heme in the mitochondria. Considering that the production of heme can be dependent on the expression of p22 HBP, it is intriguing...
ing that p22 HBP acts as a positive regulator for heme biosynthesis.

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