Hemopexin-mediated Heme Transport to the Liver

EVIDENCE FOR A HEME-BINDING PROTEIN IN LIVER PLASMA MEMBRANES*

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Isolated liver plasma membranes interact with heme-hemopexin and effect the removal of heme from the complex. This heme is rapidly accumulated by a previously undescribed heme-binding membrane component (HBC). This intrinsic membrane component can be solubilized from the membrane with Triton X-100 in a form that retains the ability to bind heme. Solubilized HBC was shown to be distinct from hemopexin itself, free heme, ligandin, globin, heme oxygenase, cytochrome P-450, and albumin. Since formation of the heme-HBC complex is effected by the interaction of heme-hemopexin with its receptor, HBC may either be a subunit of the heme-hemopexin receptor or a separate protein that interacts with the receptor. HBC can also bind heme (Kd apparent 200 nM) that is presented to it in a nonprotein bound form, showing true heme-binding activity. HBC is proteinaceous since treatment with proteases, heat, and disulfide bond reducing agents diminishes its ability to bind heme. HBC and any associated detergent elutes from Sephacryl S-200 with an apparent molecular weight of 115,000 and Stokes radius of 7.5 nm. This component, which may comprise 0.5% of liver plasma membrane protein, appears to have an acidic pI since it adsorbs to DEAE-cellulose at pH 7.4 but not to CM-cellulose at pH 6.4. In sucrose gradients, HBC migrates with S values of 1.69 and 4.02, suggesting that it has subunits or that it forms multimers under these conditions.

Hemopexin functions to conserve body iron stores (1-3) and may also have a bacteriostatic action as proposed for transferrin (4) and recently for haptoglobin (5). The transport of heme1 to the parenchymal cells of the liver by hemopexin has recently been shown to be a receptor-mediated process (2, 6, 7). In this transport system, heme-hemopexin interacts via the protein moiety of the complex (2, 6) with a receptor on the hepatocyte. The hemopexin returns intact to the circulation (2, 8) while the heme is taken into the cell and catabolized (3). This protein-conserving transport mechanism has some features resembling the iron-transferrin-reticulocyte system but is distinct from protein-destroying transport systems like hemoglobin-haptoglobin (9) and cobalamin-trascobalamin II (10).

The details of the interaction between heme-hemopexin and its specific receptor on the hepatocyte plasma membrane are under investigation in this laboratory. Recently we have prepared membranes from rabbit livers which are capable of interacting rapidly and specifically with heme-hemopexin (11). As a result of this interaction, heme is transferred from hemopexin to the membranes (11). In this report we present evidence that the heme from heme-hemopexin is bound by a specific, integral membrane component. This heme-binding component (HBC) is a protein, possibly a subunit of the heme-hemopexin membrane receptor, and may be an intermediate between the receptor and the site of heme degradation in the endoplasmic reticulum by heme oxygenase.

MATERIALS AND METHODS

Hemopexin was isolated from nonhemolyzed rabbit serum (Pel-Freeze Biologicals) and its purity and heme-binding activity assessed as previously described (2). The preparation of [55Fe]heme and [65Fe]mesoheme using carrier-free Fe and the characterization of equimolar, saturated [55Fe]heme-hemopexin complexes have been described (2). Mesoheme is used in most experiments since it is more stable than protoheme and is equivalent to protoheme in hemopexin complexes in vivo and in vitro. Heme was labeled with 125I as previously described (11) and suffered no detectable loss of ability to bind heme or interact with its receptor (2, 11). 125I was measured using a Beckman 8000 γ-spectrometer.

Plasma membranes were prepared from frozen rabbit livers (Pel-Freeze) by the method developed by Morell and Scheinberg (12) to obtain membranes containing the hepatic asialoglycoprotein receptor. We used 15 mM Hepes, 0.5 mM CaCl2, pH 7.4, throughout in place of bicarbonate buffer. The final membrane pellet was suspended in the Hepes buffer and aliquots (10-15 mg of protein/ml) were snap frozen in liquid N2, stored at −70 °C until use. Protein was determined by a modification of the method of Lowry et al. (33) after trichloroacetic acid precipitation (13). Membranes (1-4 mg of membrane protein/ml) were incubated with [55Fe]mesoheme-hemopexin (at 0.1-1.0 μM) in 10 mM Hepes, 2 mM CaCl2, pH 6.5, containing 1 mg/ml bovine serum albumin at 30 °C with shaking (1.7 Hz) in flat-bottomed glass vials. At various times during the incubation, duplicate aliquots (200 μl) of the membrane suspension were applied to individual bovine serum albumin-precoated, cellulose acetate filters (0.45 μm, Celotape, Millipore), shown in control experiments to bind negligible amounts of heme-hemopexin. The membranes retained by the filters, after aspiration of the medium through the filters using gentle vacuum, were washed with 2 × 5 ml of buffer and then dissolved in 10 ml of Cyto-Scint (West-Chem Co., San Diego, CA). The [55Fe]heme content of the membranes was determined by liquid scintillation radiometry using a Beckman 7500 or 9800 liquid scintillation counter. To assess specific binding and transfer of heme, parallel incubations were carried out. To one vial, 16 μl unlabeled heme-hemopexin was added 15 min before 0.4 μl labeled heme-hemopexin was added to both vials. The difference in binding between the two is evidence for a saturable process and a defined as specific uptake.

To prepare [55Fe]mesoheme-labeled detergent extracts, membranes at 4 mg of membrane protein/ml were incubated with 1 μM [55Fe]mesoheme-hemopexin at 4 °C for 30 min. At the end of the incubation the membranes were pelleted by centrifugation at 4,000 g for 3 min. The supernatant was removed and the membranes washed three times.
times (equivalent to a 1,000-fold dilution) by resuspension in fresh buffer (10 mM Hepes, 2 mM CaCl₂, pH 6.5) and centrifuged. The pelleted membranes were treated with 0.5% Triton X-100 in 50 mM Hepes, pH 7.7, for 30 min at 4 °C, then centrifuged to remove unsolubilized material. The supernatant, after centrifugation at 4,000 x g for 5 min, was subjected to ultracentrifugation at 110,000 x g for 1 h at 3 °C. The membranes were then solubilized in a suspension containing 15% (w/v) polyethylene glycol 3500 and 0.5% Triton X-100 in 50 mM Hepes, pH 7.7. After incubation of Triton X-100, 0.1% Triton X-100, pH 7.7, at a flow rate of 10 ml/ h. Fractions (1.0 ml) were collected without disturbing the gradient, and the radioactive heme determined by counting in 4 ml of Betaphase (West-Chem). Recovery of chromatographed [55Fe]meso-hemopexin-rabbit hemopexin and of radiolabeled HBC was greater than 75% and 80%, respectively. Elution constant (Kₘ) values were calculated using (Vₑ/Vₛ) [Vₑ + Vₛ where Vₑ, is the elution volume of the standard; Vₛ, the elution volume of the sample. Each sample was chromatographed at least three times. Other proteins employed as standards were γ-globulin, transferrin, catalase, carbonic anhydrase, and myoglobin.

Ion-exchange chromatography was carried out using 1 x 5 cm columns containing 2-2.5 ml of resin. Phosphate buffers (15 mM, at the appropriate pH) and linear salt gradients (0-0.5 M NaCl) over 30 ml at 80 ml/h were used throughout. Absorption at 280 nm was monitored and fractions (1.0 ml) were collected and their radioactive heme determined by counting in 4 ml of Betaphase (West-Chem) which forms a gel with buffered salt solutions.

Isopycnic gradient centrifugation in colloidal silica of plasma membranes after incubation with [55Fe]meso-hemopexin produces a profile with a single peak of radioactivity (Fig. 2). The extensive washing of the membranes both

RESULTS AND DISCUSSION

The binding of heme-hemopexin with its specific receptor is rapid and saturable both in vivo (2) and with isolated hepatocytes (6). Detailed studies of the binding of [55Fe]meso-hemopexin to isolated rabbit liver plasma membranes have recently been published (11). These studies show that when [55Fe]meso-hemopexin is incubated with liver plasma membranes, there is a specific interaction between the heme-hemopexin and a membrane receptor which leads to the transfer of heme from hemopexin to the membrane.

As shown in Fig. 1, net transfer of heme to the membrane ceases by about 15 min with the bulk of the transfer occurring by 10 min. The heme bound by the membrane represents up to 10% of the original heme-hemopexin. If the accumulated heme was derived from heme spontaneously dissociating from hemopexin or released due to breakdown of hemopexin rather than via the interaction of heme-hemopexin with its receptor, a linear accumulation of heme would be expected, rather than a rapid uptake reaching a plateau (Fig. 1). However, hemopexin is unaltered during incubation with membranes (shown by profiles on sodium dodecyl sulfate-polyacrylamide gel electrophoretograms), and the amount of heme which accumulates is at least 500-fold greater than expected from spontaneous dissociation of heme-hemopexin, Kₛ <1 × 10⁻¹⁵ M (23).

Interestingly, 10-fold more heme than protein is bound to the membranes within a few minutes (Fig. 1). This suggests that a heme-binding component or components must exist in the membranes. The work presented here demonstrates the existence of such a membrane component and describes its preliminary characterization.

Isopycnic gradient centrifugation in colloidal silica of plasma membranes after incubation with [55Fe]meso-hemopexin was determined by the difference in [55Fe]meso-hemopexin or [55Fe]meso-hemopexin as described above) can be precipitated a pH 7.7 by 15% (w/v) polyethylene glycol 3500 (Sigma) at 0-5 °C. This pH and PEG concentration precipitation of [55Fe]meso-hemopexin (0.05-1 μM) is negligible. PEG precipitation was routinely carried out overnight at 4 °C. After centrifugation at 5,000 x g for 20 min, the supernatant was aspirated and the pellet washed with 1.0 ml of buffer containing 15% PEG. After centrifugation, the resulting pellet was dissolved in 400 μl of NCS solubilizer (Amersham Corp.) and aliquots counted, after neutralization with glacial acetic acid, in Betabond (West-Chem) to determine the amount of [55Fe]meso-hemopexin precipitated. After incubation of Triton X-100-solubilized membrane protein (1000 μg) with [55Fe]meso-hemopexin (0.05 μM) at 4 °C for 30 min, more than 57% of the radioactive heme precipitated by this treatment. In the absence of membranes only 3% of the radiolabeled heme and only 0.6% of 0.05 μM [55Fe]meso-hemopexin were precipitated.

The sedimentation of [55Fe]meso-hemopexin was analyzed in linear sucrose gradients (4.8 ml) of 5-20% (w/v) sucrose in 0.1 M Tris-HCl, pH 7.4. Gradients were prepared using a gradient maker, and the concentration of the sucrose was checked using the refractive index of the solutions. Solubilized [55Fe]meso-hemopexin samples were centrifuged in gradients containing 0.1% Triton X-100. Samples or standards, with known sedimentation coefficients (s₂₀,w) were applied to the gradients. The standards used were: ribonuclease, 1.9 S (16); carbonic anhydrase, 2.75 S (17); ovalbumin, 3.5 S (18); bovine serum albumin 4.4 S (19); aldolase, 7.7 S (20), and catalase, 11.3 S (21). These standards were iodinated using lodo-Beads (Pierce) and carrier-free ¹²⁵I (ICN) (22). Specific activities ranged from 1.3 × 10⁶ to 3.1 × 10⁶ dpm/μg of protein, and proteins

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Heme-binding Membrane Protein

![Fig. 1. Time course of the specific accumulation of [55Fe]meso-hemopexin from heme-hemopexin by isolated liver plasma membranes. The specific binding of heme (■) and hemopexin (○) was determined by the difference in [55Fe]meso-hemopexin or [55Fe]hemopexin bound to the membranes in the presence and absence of excess 16 μM unlabeled heme-hemopexin using a filter assay as described under "Materials and Methods." Binding was measured in 10 mM Hepes, 2 mM CaCl₂, pH 6.5, with 1 mg/ml bovine serum albumin at 30 °C. The initial concentration of [55Fe]meso-hemopexin was 0.4 μM and the membranes were at 4 mg of membrane protein/ml.](image-url)
physical-chemical characteristics (described below) exhibited by heme or by addition of [\textsuperscript{55}Fe]mesoheme to detergent extracts with a macromolecular component. The membrane complex was subjected to isopycnic centrifugation, and the distribution of radioactivity bilizes over 50% of the membrane-associated [\textsuperscript{55}Fe]mesoheme. The membrane component was solubilized using the nonionic detergent Triton X-100. After preparation of [\textsuperscript{55}Fe]mesoheme-labeled membranes, the membranes were then subjected to isopycnic centrifugation, and the distribution of radioactivity (○) in the isolated fractions determined. Parallel samples of washed membranes were set up for the determination of 5'-nucleotidase activity (■, left scale) and glucose-6-phosphatase activity (▲, right scale). The recovery of [\textsuperscript{55}Fe]mesoheme was greater than 80%.

during their isolation and after incubation with heme-hemopexin (a 1000-fold dilution) ensures that the component which becomes radiolabeled with [\textsuperscript{55}Fe]mesoheme is located in the membranes and is not a contaminant. The marker enzyme activity profiles indicate that the bound heme is preferentially localized in the plasma membrane fraction enriched in 5'-nucleotidase rather than in the small amount of endoplasmic reticulum present in this preparation (indicated by the residual glucose-6-phosphatase activity).

Desferrioxamine (100 \textmu M), an avid iron-chelator, has no effect on the accumulation of radioactivity in the membranes whether incubated with the membranes or presented simultaneously with [\textsuperscript{55}Fe]mesoheme-hemopexin (at 0.4 \textmu M). This indicates that intact heme and not free iron is accumulated by the membrane and distinguishes HBC from the iron-binding reticuloocyte plasma membrane protein recently reported (24).

Both heme-hemopexin (Fig. 1) and mesoheme (Table I) are good sources for heme accumulation by membranes. This shows that the membranes have intrinsic heme-binding activity and do not rely exclusively on the hemopexin receptor. However, the hemopexin receptor is involved in the accumulation by the membrane of mesoheme from mesoheme-hemopexin since the amount of mesoheme taken up is at least 50 times higher than that can be attributed to the spontaneous dissociation of heme from the very tight heme-hemopexin complex (\textsl{K}_b below 1 \textmu M).

As a first step towards characterizing the HBC, membrane components were solubilized using the nonionic detergent Triton X-100. After preparation of [\textsuperscript{55}Fe]heme-loaded membranes by incubation with either [\textsuperscript{55}Fe]mesoheme-hemopexin or [\textsuperscript{55}Fe]mesoheme, treatment with 0.5% Triton X-100 solubilizes over 50% of the membrane-associated [\textsuperscript{55}Fe]mesoheme. The product is not free [\textsuperscript{55}Fe]mesoheme but heme associated with a macromolecular component. The membrane component which becomes radiolabeled by [\textsuperscript{55}Fe]mesoheme or [\textsuperscript{55}Fe]mesoheme-hemopexin is currently considered to be the same membrane component. This inference is based on the similar physical-chemical characteristics (described below) exhibited by this membrane component prepared by the incubation of membranes with [\textsuperscript{55}Fe]mesoheme-hemopexin or [\textsuperscript{55}Fe]mesoheme or by addition of [\textsuperscript{55}Fe]mesoheme to detergent extracts of membrane.

![Fig. 2. Percoll gradient centrifugation of [\textsuperscript{55}Fe]mesoheme-labeled membranes. After incubating membranes with 1.0 \textmu M [\textsuperscript{55}Fe]mesoheme-hemopexin for 30 min at 37 °C, the membranes were sedimented by centrifugation and washed extensively to remove loosely associated material. The washed membranes were then subjected to isopycnic centrifugation, and the distribution of radioactivity (○) in the isolated fractions determined. Parallel samples of washed membranes were set up for the determination of 5'-nucleotidase activity (■, left scale) and glucose-6-phosphatase activity (▲, right scale). The recovery of [\textsuperscript{55}Fe]mesoheme was greater than 80%.

Effects of temperature, unlabeled mesoheme, and proteases on the formation of [\textsuperscript{55}Fe]mesoheme-HBC

In these experiments, 1 mg of solubilized membrane protein was incubated with 0.05 \textmu M [\textsuperscript{55}Fe]mesoheme at 4 °C for 30 min in a total volume of 1 ml and the [\textsuperscript{55}Fe]mesoheme-HBC formed was measured by precipitation in 15% (w/v) polyethylene glycol at pH 7.7 as described under "Materials and Methods." Protease treatments were carried out at 37 °C for 30 min using 50 \textmu g of protease/ml. Treatments of apo-HBC were carried out after solubilization but before addition of [\textsuperscript{55}Fe]mesoheme. Dithioerythritol was added (final concentration 20 mM) to parallel samples after the indicated treatment but before addition of the PEG. In the standard assay more than 57% of [\textsuperscript{55}Fe]mesoheme (0.05 \textmu M) was precipitated while in the absence of solubilized membrane protein only 3% precipitated. Under these conditions, only 0.6% [\textsuperscript{55}Fe]mesoheme-hemopexin (0.05 \textmu M) was precipitated.

Gel-permeation and ion-exchange chromatography of solubilized [\textsuperscript{55}Fe]mesoheme-labeled membranes were employed to further characterize HBC and to exclude involvement of free mesoheme, mesoheme-hemopexin, or known heme- or iron-binding proteins (like globin, ligandin, or transferrin) present in liver or serum. Gel permeation resolved two peaks of radioactivity in the Triton-solubilized membrane extract (Fig. 3). The minor first peak eluted just after the void volume and probably represents a small amount of heme and/or HBC in Triton X-100 micelles (see below). The majority of the [\textsuperscript{55}Fe]mesoheme is in the second peak, 
\[ K_b = 0.11 \pm 0.01 \] which we define as HBC. Heme-hemopexin elutes with a 
\[ K_b = 0.16 \pm 0.02 \] clearly separated from HBC (Fig. 3). The molecular weight of HBC is in the second peak, 
\[ K_b = 0.4 \pm 0.05 \]
and corresponds to the minor shoulder of radioactivity observed in some of the HBC samples (Fig. 3). In other experiments, [\textsuperscript{55}Fe]mesoheme-hemopexin was mixed with solubilized [\textsuperscript{55}Fe]mesoheme-labeled membranes and passed through the Sephacryl column. Three radioactive components were clearly resolved: the smallest near the void volume, one at the HBC elution position, and one at the position of authentic mesoheme-hemopexin. The Stokes radius of the [\textsuperscript{55}Fe]mesoheme-HBC (and possibly associated detergent) complex was determined to be 5.9 nm by the method of protein versus its Stokes radii (26). The molecular weight of HBC, calculated from a plot of 
\[ K_b = 0.13 \] versus (molecular weight)\(^{1/2}\) (27), is 115,000.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% control</th>
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<tbody>
<tr>
<td>None</td>
<td>100</td>
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<tr>
<td>100 °C, 15 min</td>
<td>37</td>
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<tr>
<td>Unlabeled mesoheme</td>
<td>15</td>
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<td>5 \textmu M</td>
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<td>50 \textmu M</td>
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<td>Trypsin</td>
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<td>Proteinase K</td>
<td>46</td>
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<tr>
<td>Before heme</td>
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<tr>
<td>After heme(^b)</td>
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\(^{a}\) ND, not determined.

\(^{b}\) Membranes were labeled with [\textsuperscript{55}Fe]mesoheme by incubation with [\textsuperscript{55}Fe]mesoheme-hemopexin and washed extensively before preparing the Triton X-100 extract used in the assay.

Table I
Heme-binding Membrane Protein

FIG. 3. Gel permeation chromatography of \(^{55}\text{Fe}\)meso-heme-labeled, solubilized liver plasma membranes. After incubating the membranes with \(^{55}\text{Fe}\)meso-heme-hemopexin complexes for 30 min at 30 °C at pH 6.5, the membranes were solubilized with 0.5% Triton X-100 buffered at pH 7.7. An aliquot was diluted to 0.1% Triton X-100, and chromatographed on a 1.6 × 25-cm column of Sephacryl S-200. Fractions were collected and the amount of \(^{55}\text{Fe}\)mesoheme determined in each. The absorbance at 280 nm and also in some cases at 406 nm was monitored. The columns were calibrated with blue dextran, mixtures of standard proteins, and L-tyrosine. Solubilized membranes (●) and \(^{55}\text{Fe}\)mesoheme-hemopexin (○) were chromatographed separately. Also shown is \(^{55}\text{Fe}\)mesoheme (△−△) run as a control without being incubated with membranes but subjected to the same homogenization treatment.

FIG. 4. Ion-exchange chromatography of \(^{55}\text{Fe}\)mesoheme-labeled, solubilized liver plasma membranes. Aliquots of membranes treated as described in the legend to Fig. 3 were applied to 1 × 5-cm columns of DE52 and eluted with 10 mM Na phosphate buffer, pH 7.4, containing 0.1% Triton X-100. Elution was carried out using a linear salt gradient (0–0.5 M NaCl) in the same buffer over 20 ml. \(^{55}\text{Fe}\)Mesoheme was measured in each fraction. Shown are: HBC (●−●) and \(^{55}\text{Fe}\)mesoheme-hemopexin (○−○) chromatographed separately under identical conditions.

28) nor hemoglobin (pI near 7.2, Ref. 29) which are retained. HBC adsorbs to diethylaminoethanecellulose at pH 7.4 and can be eluted by a gradient of NaCl (Fig. 4). "Free" (i.e. nonprotein-bound) \(^{55}\text{Fe}\)mesoheme is retained by DE52 and does not elute under these conditions; \(^{55}\text{Fe}\)mesoheme-hemopexin (pI 5.8, Ref. 30) like transferrin is only loosely bound (Fig. 4). Thus, HBC appears to be a component of plasma membranes that is distinguishable by its Stokes radius and ionic charge from several known heme- or iron-binding proteins as well as from free heme or heme-hemopexin.

FIG. 5. Effect of trypsin on the heme-binding activity of liver plasma membranes. Gel permeation chromatography on Sephacryl S-200 was carried out as described in the legend to Fig. 3 on isolated, Triton X-100-solubilized plasma membranes treated with trypsin (25 µg/mg of membrane protein) for 30 min at 37 °C before (C—○) and after (■—■) exposure of the membranes to 1 µM \(^{55}\text{Fe}\)mesoheme-hemopexin. Trypsin digestion was terminated by addition of a 10-fold excess (w/w) of soybean trypsin inhibitor, and the membranes solubilized as usual. \(^{55}\text{Fe}\)Mesoheme was measured in each fraction.

FIG. 6. Scatchard-type plot of the specific binding of \(^{55}\text{Fe}\)mesoheme to solubilized liver plasma membrane. Solubilized liver plasma membranes were incubated with increasing concentrations of \(^{55}\text{Fe}\)mesoheme (0.025–1 µM) at 4 °C overnight before polyethylene glycol precipitation. Specific binding was determined after subtracting nonspecifically bound ligand. Specifically bound ligand (picomoles in 1.0 ml) is plotted versus the ratio of bound over free ligand according to Scatchard (32). The incubation volume was 1.0 ml and contained 270 µg of solubilized membrane protein.

The amount of \(^{55}\text{Fe}\)mesoheme bound by liver plasma membranes is significantly decreased when the membranes are treated with trypsin before exposure to radiolabeled heme-hemopexin (Fig. 5), suggesting that HBC is a protein. However, effects on formation of heme-HBC due to proteolysis of the hemopexin receptor in these membranes cannot yet be excluded. Trypsin treatment of membranes solubilized after incubation with radioabeled heme-hemopexin produced a slight decrease in amount of radioactivity in the HBC peak and a small amount of lower molecular size material analyzed by gel permeation chromatography under nonreducing conditions (Fig. 5). Untreated controls consistently produced the radioactivity profile shown for the component defined as HBC in Fig. 3. Heat treatment of membranes or solubilized mem-
branes (60 °C for 30 min) before incubation with heme-he- mopexin had little effect on heme-HBC formation, suggesting that neither HBC nor the receptor when membrane-associated is particularly heat-labile. This apparent stability of HBC is probably due to the presence of disulfide bonds which help maintain the integrity of the protein (as discussed next) and to areas of HBC necessary for heme-binding being less accessible when HBC is membrane-associated.

More conclusive evidence that HBC is a protein is provided by the results summarized in Table I. Heating the Triton X-100-extracted membranes at 100 °C for 15 min before addition of [55Fe]mesoheme reduced the formation of heme-HBC to 37% of control levels. Heme binding by solubilized membranes was also significantly decreased by mild treatment with several proteases including trypsin, papain, Pronase, and proteinase K. A further decrease in the heme-binding activity is caused by dithioerythritol whether alone or after treatment with protease, suggesting that disulfide bonds of HBC help maintain its native conformation. This observation and the plasma membrane location of HBC distinguish HBC from heme oxygenase, an endoplasmic reticulum protein containing no disulfide bonds (31). Heme bound to HBC did not protect detergent-solubilized HBC from proteolytic inactivation.

[55Fe]Mesoheme-HBC prepared by incubation of membranes with [55Fe]mesoheme-hemopexin before solubilization and exposure to proteinase K was affected to the same extent as "apo-HBC" (Table I).

Solubilized liver plasma membranes bind mesoheme in a saturable, and therefore specific, manner. This specificity is also shown by the competitive inhibition of unlabeled mesoheme exerted on [55Fe]mesoheme binding (Table I). A Scatchard-type analysis of the binding data (Fig. 6) yields an apparent dissociation constant for heme-HBC of 2 × 10−7 M and a binding capacity of 178 pmol/mg of solubilized membrane protein, suggesting that HBC of molecular weight 100,000 may comprise about 0.5% of the total membrane protein.

Sucrose gradient ultracentrifugation of Triton X-100-extracted [55Fe]mesoheme-labeled membranes revealed two peaks of radioactive heme with S values of 1.7 and 4.0, quite distinct from the free heme which remained at the least dense region of the gradient (Fig. 7). The biphasic distribution suggests that HBC has subunits or forms multimers under these conditions. Since solubilized membrane proteins often have lipid or detergent bound to hydrophobic areas on their surfaces, the S values derived for the HBC most likely represent forms of a [55Fe]mesoheme-HBC-detergent or lipid complex.

In conclusion, liver plasma membranes contain a previously undescribed, specific heme-binding component involved in hemopexin-mediated heme transport. This component, termed HBC, appears to be a protein and is not heme trapped in micelles of detergent or a known heme-binding protein. HBC could be a subunit of the heme-hemopexin receptor but only if it was present in more than 10-fold excess over the receptor subunit that interacts with hemopexin. We are currently extending our characterization of HBC with emphasis on its purification, molecular properties, and relationship to the hemopexin receptor. We are particularly interested in determining how HBC interacts with the hemopexin receptor; whether HBC serves as an intracellular transport protein by acting as an intermediary between the hemopexin-receptor or plasma membrane and the site of heme catabolism by heme oxygenase on the endoplasmic reticulum, and whether HBC also acts in the intracellular transport of heme derived from the breakdown of hemoglobin in lysosomes.

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