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TRANSPORT OF HEME BY HEMOPEXIN TO THE LIVER: EVIDENCE FOR RECEPTOR-MEDIATED UPTAKE

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SUMMARY

We used carefully defined heme-hemopexin complexes to investigate the role of hemopexin in the catabolism of heme *in vivo*. Uptake of rabbit [^{59}Fe]heme-[^{125}I]hemopexin by rat liver was rapid. The liver-associated ^{125}I reached a maximum 5 minutes after injection, nearly 7-fold higher than apo-hemopexin, whereas liver-associated ^{59}Fe increased with time. This together with an inverse relationship of [^{125}I]hemopexin in the liver and serum during the course of heme transport suggests that hemopexin was released from the liver back to the circulation. Saturation of uptake with heme-hemopexin, reaching about 170 pmol [^{125}I]hemopexin (gm liver)$^{-1}$ 5 minutes after injection of 11 nmol, indicates a receptor-mediated process.

We conclude that hemopexin delivers heme to the liver via interaction with a finite number of receptors and returns to the circulation.

A role for hemopexin in the catabolism of the heme* moiety of hemoglobin has been supported by physicochemical evidence (1,2), by autoradiographic studies indicating association of hemopexin and heme with liver parenchymal cells (3-5) and by decreased circulating levels of hemopexin in hemolytic states after depletion of haptoglobin (6,7). When hemopexin binds heme, a conformational change occurs in the protein which was proposed to be necessary for recognition of the complex by the hepatocyte (8).

Most selective uptake processes of ligands bound by serum transport proteins, e.g. iron-transferrin (9) and cobalamin-transcobalamin II (10),

*Heme is defined as iron-protoporphyrin IX.

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are rapid, usually on the order of minutes. Although injection of heme significantly decreased the plasma half-clearance time of native hemopexin in rabbits (11,12) and humans (13), the observed time was several hours. Furthermore, circulating hemopexin, which binds heme on an equimolar basis, did not decrease in proportion to the amount of heme administered. Our current research is intended to assess these observations and to obtain information on the mechanisms of the interaction of heme-hemopexin with the liver. In our initial experiments, we employed a heterologous system, i.e. rabbit hemopexin uptake in rats, because the rabbit protein is the most readily available and has been extensively characterized (4,8,14-16).

This paper provides evidence for a rapid and specific interaction of carefully defined heme-hemopexin complexes with the liver *in vivo*. We propose that hemopexin delivers heme to the liver cell via a receptor-mediated process and then returns to the circulation.

MATERIALS AND METHODS

Protein purification, iodination and characterization. Hemopexin was purified from rabbit serum as previously described (17) and its purity assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (18) and immunological analysis with antiserum to whole rabbit serum and monospecific antisera to rabbit hemopexin. Concentrations of apo- and heme-hemopexin solutions were determined spectrophotometrically using published extinction coefficients (19). For storage, protein solutions were quickly frozen with liquid nitrogen and kept at -20°.

Proteins were labeled with ^{125}I using 0.5-1.0 mg/mL protein solutions at 20 µg chloramine T and 500 µCi ^{125}I per mg protein (20) and dialyzed. At least 95% of the ^{125}I was precipitable with 12.5% trichloroacetic acid (v/v, final concentration) and 1 mg/mL bovine serum albumin used as a co-precipitant. No evidence for altered immunological, biological or heme-binding properties of [^{125}I]hemopexin was found. In some experiments [^{125}I]hemopexin was mixed with unlabelled hemopexin. Specific activity (SpA, cpm/mol) of apo- and heme-[^{125}I]hemopexin was calculated from the absorbance and radioactivity of each solution. ^{125}I and ^{59}Fe (see below) were measured using a Nuclear-Chicago Model 1185B well-type γ-counter. Quantitative immunoprecipitation of [^{125}I]hemopexin was carried out as previously described (21).

Preparation of [^{59}Fe]heme and [^{59}Fe]heme-[^{125}I]hemopexin. ^{59}Fe as solid ferrous sulfate (Amersham, SpA 20.6 mCi/g) was inserted into protoporphyrin IX (Porphyrin Products, Logan, Utah) under refluxing in dimethylformamide (22). The concentration (23) and SpA of the [^{59}Fe]heme was measured in dimethylsulfoxide.

[^{59}Fe]heme-[^{125}I]hemopexin complexes were prepared by mixing 1.3 equivalents of heme with [^{125}I]hemopexin and incubating at ambient temperature for 1 hour. Excess heme and iron were removed with DE-52 ion exchange resin (Whatman) equilibrated with 15 mM sodium phosphate buffer, pH 7.4. The absorbance and radioactivity of the eluted heme-protein was determined. The saturation of hemopexin (> 90%) with heme was assessed and the integrity of the complex was confirmed by the characteristic absorption spectrum of heme-hemopexin (1,2,8). Unlabelled-heme-[^{125}I]hemopexin was prepared similarly.

Liver uptake. The uptake *in vivo* of apo- and heme-hemopexin was studied, unless otherwise specified, using male inbred Lewis rats (120-140 gm) maintained at this institute. The rats were anesthetized with pentobarbital (0.15 grain per rat i.p.) and the rectal temperature maintained at 34-36^{o}C by warming with an infrared lamp. The protein in phosphate-buffered saline was injected into the lateral tail vein, as near the tip of the tail as possible. Blood samples (25 µL) were taken into calibrated heparinized capillary tubes after making a small cut across the tail artery and rinsed out into phosphate-buffered saline (500 µL) for direct counting of ^{125}I. Rats were killed by decapitation and the livers immediately perfused (within 10 sec) through the portal vein with 50 mL ice cold 0.15 M sodium chloride (at 15-20 mL/min). Weighed samples of liver were taken for direct counting of radioactivity. Within an individual experiment, two rats were used for each point and every experiment was repeated.

RESULTS AND DISCUSSION

Time course of liver uptake of apo- and heme-hemopexin. Uptake of rabbit apo-hemopexin by rat liver was 5 pmol (gm liver)$^{-1}$ five minutes after injection and thereafter increased only slightly (Fig. 1). In contrast, significant liver uptake and decreased circulating levels of hemopexin occurred within two minutes after injection of heme-hemopexin complexes (Fig. 1). Liver associated-^{125}I reached a maximum at five minutes (32.9 $\pm$ 4.7 pmol (gm liver)$^{-1}$, mean $\pm$ S.D.), and then declined over the next thirty minutes to values similar to those for the apo-protein. This peak of radioactivity in the liver is reflected by a dip in the blood of circulating TCA- and immuno-precipitable ^{125}I. The increase in blood levels of precipitable radioactivity after five minutes (Fig. 1) suggests that the protein returns to the circulation after interaction with the liver. This is a subject of current studies.

Normal circulating serum levels of hemopexin in rats are 2-3x10^{-5}M. Thus, our injected tracer doses of 500-700 pmol, giving a serum concentration of 1-2x10^{-7}M, were less than 1% of the endogenous circulating

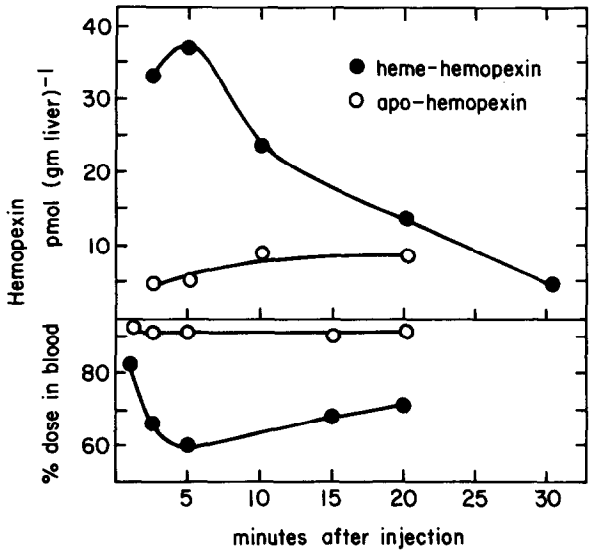

Figure 1. Time course of [^{125}I]hemopexin in blood and liver.

Rats were injected with 500 pmol apo-hemopexin or heme-hemopexin and killed at the indicated times. The results are from a representative experiment with female rats and each point represents an average of two animals. Further details are given in the Materials and Methods.

hemopexin. Blood levels one minute after injection averaged 90% of the injected radioactivity. No differences in uptake between the various lobes of the liver were detected, and the radioactivity located in unperfused organs such as lung, spleen, kidney and bladder were routinely less than 1-2% of the dose. Since the presence of calcium (2 mM) is obligatory for other binding systems, e.g. of asialoglycoproteins *in vitro* to rat liver membranes (24), there was a possibility that some bound hemopexin might dissociate from the receptor during the perfusion. However, addition of calcium (2 mM) to the sodium chloride perfusate did not alter the results.

Influence of increasing the injected dose of apo- and heme-hemopexin on liver uptake. A comparison of liver uptake of rabbit apo- and heme-hemopexin was made over the dose range 0.3-11.4 nmol (Fig. 2). The proportion of apo-hemopexin in the liver was always 3-4% of the injected

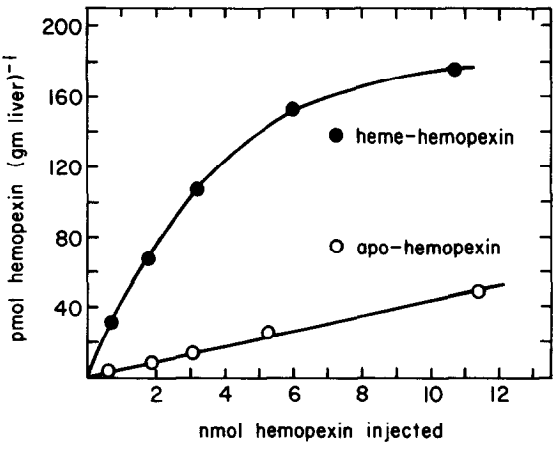

Figure 2. Effect of increasing the dose of apo-hemopexin or heme-hemopexin on liver uptake.

Rats were injected with the indicated amounts of apo-hemopexin or heme-hemopexin and killed 5 minutes later. Further details are given in the Materials and Methods.

dose whereas uptake of heme-hemopexin increased to a plateau of 160-180 pmol (gm liver)$^{-1}$ at a dose of 10-12 nmol (Fig. 2). This saturation of uptake suggests that a finite number of receptor molecules on the liver cell surface are involved in this recognition and uptake process.

Uptake of [^{59}Fe]heme-[^{125}I]hemopexin complexes by rat liver *in vivo*. Since hemopexin transports heme, it is necessary to determine the fate not only of the protein but also of the heme moiety. The Kd of 10^{-12}M for heme-hemopexin (2) precludes any significant dissociation of heme during the experimental period. The results of preliminary uptake experiments with doubly-labelled complexes of heme with rabbit hemopexin are summarized in Table 1. At 5 minutes, the ratio of ^{125}I:^{59}Fe in the liver is already 1:2.3 and by 15 minutes this has increased to 1:4.6 as the ^{59}Fe continues to accumulate in the liver while the protein content declines. These results support the concept of recycling of hemopexin.

In conclusion, our results show that heme-hemopexin complexes interact rapidly and specifically with the liver *in vivo* and that the

Table 1. Uptake of [^{59}Fe]heme and [^{125}I]hemopexin by rat liver *in vivo*.

Sample injected	Time (min)	[^{125}I]hemopexin (pmol/gm liver)	[^{59}Fe]heme (pmol/gm liver)
rabbit heme-hemopexin	5	26	61
	15	18	84

Rats were injected with 700 pmol of rabbit [^{59}Fe]heme-[^{125}I]hemopexin and killed at the times indicated. The results are the mean of two animals from a representative experiment. Further details are presented in the Materials and Methods.

interaction is saturable, thus indicating a receptor-mediated process. Moreover, hemopexin delivers heme to the liver and returns to the circulation. This resembles the action of transferrin (9) rather than that of transcobalamin II (10) in which the entire complex is taken into the target cell. We have recently purified rat hemopexin and are currently investigating this system in detail with homologous protein using rat liver membranes and hepatocytes.

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