

# Intracellular distribution of haem after uptake by different receptors

## Haem–haemopexin and haem–asialo-haemopexin

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How the interaction of haemopexin with two different receptors affects its subsequent metabolism and 'intracellular' haem transport was examined by using mesohaem–haemopexin and mesohaem–asialo-haemopexin. The physical properties of the two haem proteins, including their absorption and c.d. spectra, are similar. Binding studies *in vitro* showed that haem–asialo-haemopexin interacts with both the haemopexin-specific and galactose-specific receptors on liver plasma membranes, but that haem–haemopexin interacts only with the haemopexin receptor. *In vivo* haem–asialo-haemopexin rapidly interacts with the liver via the galactose-specific receptor, since the protein is extensively catabolized and uptake is blocked by asialofetuin. Haem iron from haem–asialo-haemopexin is not accumulated in the liver to the same extent as from intact haem–haemopexin, and the native sialylated protein is not proteolysed. Moreover, after fractionation of homogenized liver by using colloidal-silica gradients, liver-associated haem–haemopexin and haem–asialo-haemopexin produced distinctly different patterns for both protein and ligand, consistent with their uptake by two distinct receptors. These results demonstrate that the interaction of haemopexin with different receptors influences its subsequent metabolic fate and that haem iron from haem–haemopexin is efficiently conserved only if it enters the liver cell via the specific haemopexin receptor.

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## INTRODUCTION

The serum glycoprotein haemopexin binds its ligand haem with high affinity ( $K_D < 10^{-12}$  M; Hrkal *et al.*, 1974), yet intracellular accumulation of haem occurs rapidly via a specific receptor for haemopexin (Smith & Morgan, 1978, 1979), and haemopexin is not degraded during this process (Smith & Morgan, 1979). The haemopexin system is separate and distinct from that for haemoglobin–haptoglobin (Kino *et al.*, 1980) and iron–transferrin (Tsunoo & Sussman, 1983); nonetheless, haemopexin is part of the iron-conservation mechanisms of the body (Hershko, 1975; Davies *et al.*, 1979), and the iron from haem delivered to the hepatocyte by haemopexin is rapidly stored on ferritin (Davies *et al.*, 1979).

The intracellular route by which haem passes from the plasma membrane to its site of catabolism by haem oxygenase on the endoplasmic reticulum after haemopexin-mediated transport of haem is under study in this laboratory. The lysosomes have been implicated as sites of catabolism of haemoglobin–haptoglobin complexes in the liver (Higa *et al.*, 1981). Since haemoglobin is also a source of haem, an intracellular transport route for haem might exist to move haem from the lysosomes to haem oxygenase, and this route might join that for haem transported by haemopexin.

Asialo-haemopexin has been shown to be rapidly transported to the liver and catabolized both *in vivo* (Conway *et al.*, 1975) and by isolated hepatocytes (Bernard *et al.*, 1982). Isolated rabbit liver plasma membranes bind haem–haemopexin complexes and, moreover, readily accumulate haem from haem–haemo-

pexin (Smith & Morgan, 1984). Therefore the interactions of haem–haemopexin and haem–asialo-haemopexin with the asialo-glycoprotein receptor and haemopexin receptors on isolated liver plasma membranes were compared. The interaction of [ $^{55}\text{Fe}$ ]mesohaem– $^{125}\text{I}$ -labelled rabbit asialo-haemopexin complexes with the liver *in vivo* was examined to compare the transport of haem by asialo-haemopexin with that of intact haemopexin.

A previous study on the interaction of  $^3\text{H}$ -asialo-haemopexin and haem– $^3\text{H}$ -asialo-haemopexin complexes in isolated rat hepatocytes concluded that haem–asialo-haemopexin complexes are recognized by the hepatic asialo-glycoprotein receptor but not by the specific receptor for haem–haemopexin complexes (Bernard *et al.*, 1982). In contrast, evidence is now presented that haem–asialo-haemopexin complexes interact with both the asialo-glycoprotein and the haem–haemopexin receptors. Further, hepatic haem uptake *in vivo* mediated by intact haemopexin is far more efficient than that mediated by asialo-haemopexin, demonstrating that binding to different receptors not only affects the fate of this protein but also influences the intracellular distribution of its ligand as well.

## MATERIALS AND METHODS

Haemopexin was isolated from rabbit serum (Pel-Freeze Biologicals, Rogers, AK, U.S.A.) and its purity established as previously described (Hrkal & Muller-Eberhard, 1971). Asialo-haemopexin was prepared by nueraminidase digestion of the apo-protein for 16 h at

room temperature (Conway *et al.*, 1975). Released sialic acid was measured (Skoza & Mohos, 1976) and completion of digestion checked by comparison of the number of mol of sialic acid released enzymically with that released by hydrolysis in 0.05 M-H<sub>2</sub>SO<sub>4</sub> at 80 °C for 2 h. Rabbit haemopexin contains eight sialic acid residues per molecule of protein (Conway *et al.*, 1975). Asialofetuin was prepared from fetuin (Behring) by using the method described for haemopexin (Conway *et al.*, 1975). Rabbit haemopexin has been shown to be functionally equivalent to rat haemopexin in rats (Smith & Morgan, 1978, 1979).

Concentrations of apo- and asialo-haemopexin and the saturation of their equimolar complexes with haem were measured spectrophotometrically by using reported absorption coefficients (Seery *et al.*, 1972). Absorption and c.d. spectra were recorded as described previously (Morgan & Muller-Eberhard, 1972). [<sup>55</sup>Fe]Mesohaem was prepared (Adler *et al.*, 1970) and used to form equimolar haem-haemopexin complexes as previously described (Smith & Morgan, 1981). The same procedure was used to form and characterize [<sup>55</sup>Fe]mesohaem-asialo-haemopexin.

<sup>55</sup>Fe was measured in Beta-blend (West-Chem Scientific) after solubilization in NCS (Amersham) and neutralization with acetic acid for certain tissue samples in a Beckman LS-9800 liquid-scintillation spectrometer. Haemopexin and asialo-haemopexin were radiolabelled with <sup>125</sup>I with a mild chloramine-T method (McConahey & Dixon, 1966), and <sup>125</sup>I was measured by using a Beckman 8000  $\gamma$ -radiation counter. The initial specific radioactivity of the proteins was  $5 \times 10^{15}$ – $6 \times 10^{15}$  c.p.m./mol. This was diluted so that the specific radioactivity of the [<sup>55</sup>Fe]mesohaem-<sup>125</sup>I-protein complexes was  $5 \times 10^{13}$ – $6 \times 10^{13}$  and  $5 \times 10^{13}$ – $10 \times 10^{13}$  c.p.m./mol for the haem and protein moieties respectively in order to carry out dual-label liquid-scintillation counting. Mesohaem is routinely employed instead of protohaem because of its stability and proven physical and functional equivalence in haemopexin complexes *in vivo* (Smith & Morgan, 1979).

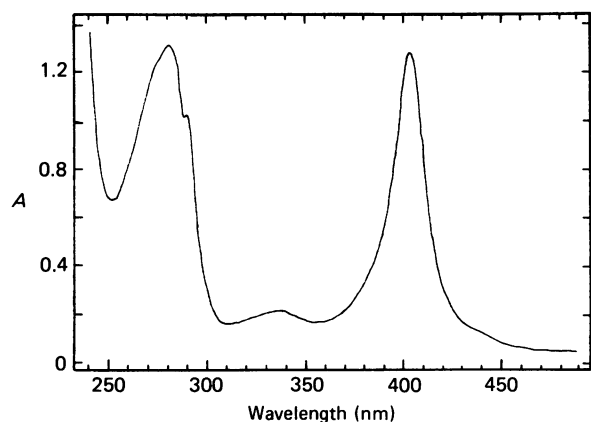
Methods to measure liver uptake and serum concentrations of asialo-fetuin, asialo-haemopexin, haem-haemopexin and haem-asialo-haemopexin in intact rats were as reported previously (Smith & Morgan, 1979). Briefly, after intravenous injection of radiolabelled material into the tail vein of anaesthetized rats, blood samples are taken from the tail at 2 min and then at 5, 10, 15 or 30 min. At the end of the experiment, livers are perfused with ice-cold 0.9% NaCl before excision and homogenization (1 g of liver in 9 ml of buffer) in 15 mM-Hepes/NaOH buffer, pH 7.4, containing 0.25 M-sucrose and 2 mM-CaCl<sub>2</sub> with a Thomas C homogenizer (0.15–0.23 mm clearance). The homogenate was centrifuged on isopycnic gradients to monitor the association of radiolabelled haem and/or protein with liver cellular organelles after being filtered through gauze or centrifuged at 750 g for 10 min. The concentrations of radiolabelled protein and its metabolites in serum were measured by using precipitation by trichloroacetic acid and analysed by using sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Smith & Morgan, 1979). The specific radioactivities of the radiolabelled proteins and complexes were used to calculate the amounts of haem or protein present in serum and tissue samples. In certain samples both radiolabels (<sup>55</sup>Fe and <sup>125</sup>I) may be

present in a form other than that injected, and this has been noted in the text where appropriate.

The self-generating sigmoidal gradients employed were obtained by centrifuging homogenized liver samples with colloidal silica (Percoll from Pharmacia) in an iso-osmotic solution (15 mM-Hepes/1 mM-EDTA/0.25 M-sucrose; 38% Percoll). The initial density was 1.08 g/ml. After centrifugation at 60000 g for 60 min, 1.0 ml fractions were collected without disturbing the gradient, and distributions of radioactive haem and protein together with marker enzyme activities were determined. The density (g/ml) of each fraction was measured gravimetrically. In accordance with published work, 5'-nucleotidase (Aronson & Touster, 1974a), glucose-6-phosphatase (Aronson & Touster, 1974b), uricase (de Duve *et al.*, 1955), cytochrome *c* oxidase (Dianzani & Viti, 1955) and  $\beta$ -galactosidase (Barrett & Heath, 1977) were used as markers for plasma membranes, endoplasmic reticulum, peroxisomes, mitochondria and lysosomes respectively. Filtered homogenate was examined to determine whether plasma-membrane sheets were lost by centrifugation. The distribution of marker enzymes was similar whether filtered homogenate or its 750 g supernatant was employed, and the results obtained with the 750 g supernatant are presented here. More than 80% of the protein banded at densities between 1.10 and 1.05 g/ml.

Membranes were prepared from frozen rabbit livers (obtained from Pel-Freeze Biologicals) by the method of Morell & Scheinberg (1972), and the preparations were shown to be enriched in plasma membranes by their content of 5'-nucleotidase compared with glucose-6-phosphatase. Total protein was measured by a modification of the Lowry procedure (Bensadoun & Weinstein, 1976), with bovine serum albumin as standard. Membrane preparations (at 10–15 mg of protein/ml in 10 mM-Hepes, pH 7.0) were separated into small portions, snap-frozen in liquid N<sub>2</sub> and stored at –20 °C. These preparations showed minimal loss of haem-haemopexin-binding ability, but repeated freezing and thawing did result in loss of binding.

Membrane binding was measured in 10 mM-Hepes/NaOH buffer containing 0.15 M-NaCl, 2 mM-CaCl<sub>2</sub> and 1 mg of bovine serum albumin (Behring)/ml at pH 6.5 and 30 °C, with 4 mg of membrane protein/ml in glass vials in a shaking water bath at 80 cycles/min. As in previous work with hepatocytes (Smith & Morgan, 1981) and membranes (Smith & Morgan, 1984), specific binding is defined as the difference in uptake of radiolabel between duplicate sets of vials incubated in parallel, one with and one without excess unlabelled haem-haemopexin. Preliminary experiments showed that incubation for 15 min in the presence of 8–16  $\mu$ M unlabelled complex produced nearly maximal competition of binding of radioactive complex, usually added at 0.1–0.4  $\mu$ M final concentration. Duplicate or triplicate samples (0.2 ml) of each reaction mixture were taken at the indicated time after addition of tracer complex and applied to filters (Millipore type EH, 0.5  $\mu$ m pore size) on a 12-position vacuum-filtration apparatus (Hofer Scientific). After the sample was applied with vacuum off, the tap was opened and the filter quickly washed twice with 3 ml of buffer. The time required to take the sample, apply to filter and rinse was of the order of 30–60 s, with a filtration time of 5 s. Results presented in the Figures are expressed as the mean of these values (which varied by no more than 15%). To measure [<sup>55</sup>Fe]haem the washed filters were



**Fig. 1. Characteristic u.v.-absorption spectrum of equimolar  $[^{55}\text{Fe}]$ mesohaem-rabbit asialo-haemopexin complexes**

Equimolar amounts of  $[^{55}\text{Fe}]$ mesohaem and rabbit asialo-haemopexin ( $11.6 \mu\text{M}$ ) were incubated for 30 min at  $22^\circ\text{C}$  in 10 mM-sodium phosphate buffer, pH 7.3, containing 0.15 M-NaCl. The absorption spectra were recorded from 550 to 250 nm with the use of 1.0 mm slits and a scan rate of 0.5 nm/s.

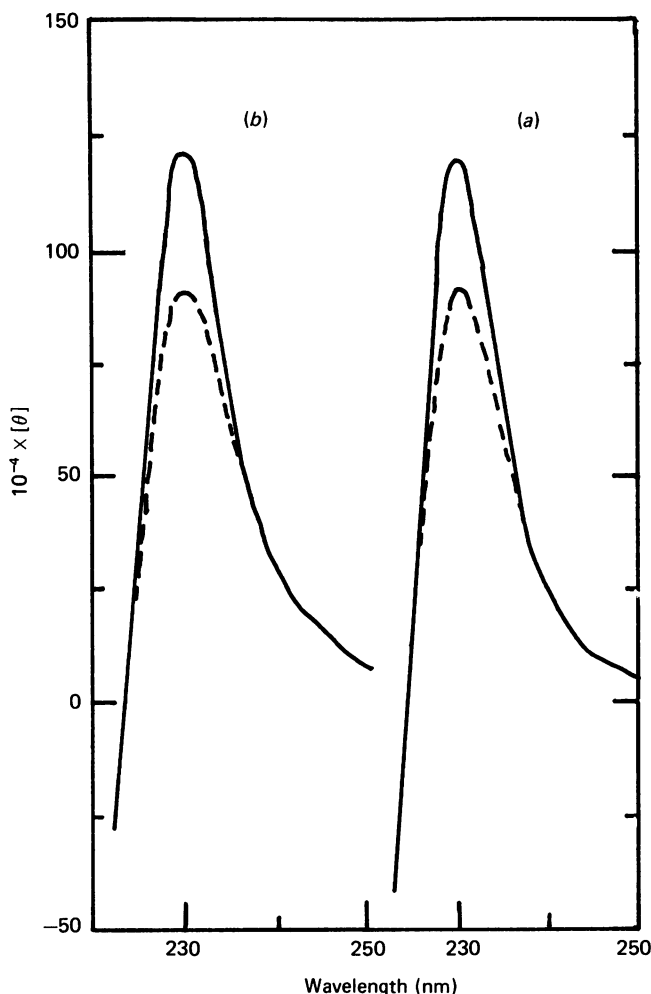
transferred to plastic scintillation vials, sample side up. Cyto-Scint (West-Chem Scientific) (15 ml) was added to each vial with vigorous shaking to dissolve the membrane protein. For  $^{125}\text{I}$ -asialo-haemopexin, the washed filters were placed in suitable containers and their radioactivities measured in a  $\gamma$ -radiation counter. Control experiments showed less than 0.1% of the added radioactivity (either  $^{55}\text{Fe}$  or  $^{125}\text{I}$ ) to be retained by the filters in the absence of membrane.

## RESULTS AND DISCUSSION

The physical properties of asialo-haemopexin and its haem-binding activity are similar to those of haemopexin. The absorption spectra of an equimolar complex of rabbit mesohaem-asialo-haemopexin (Fig. 1) closely resembles that characteristic of rabbit haem-haemopexin complexes in both the u.v. and Soret regions of the spectrum (Morgan & Muller-Eberhard, 1972). The affinity of asialo-haemopexin for haem was also assessed by using human serum albumin as a competitive binder. Haemopexin readily removes haem from the haem-human serum albumin complex ( $K_D$  approx. 10 nM), but human serum albumin cannot remove haem from haem-haemopexin ( $K_D < 1 \text{ pM}$ ) (Morgan *et al.*, 1976). Similar experiments with haem-asialo-haemopexin and human serum albumin show that haem-asialo-haemopexin behaves the same as haem-haemopexin.

Importantly, addition of haem to asialo-haemopexin produces an increase in the protein's molar ellipticity at 231 nm (Fig. 2), as described for native haemopexin (Morgan & Muller-Eberhard, 1972). This change in circular dichroism has been attributed to a conformational change in the protein required for recognition by the haem-haemopexin receptor (Morgan, 1976). Therefore haem-asialo-haemopexin complexes appear to be suitable for interaction with the haem-haemopexin receptor as well as with the hepatic asialo-glycoprotein receptor.

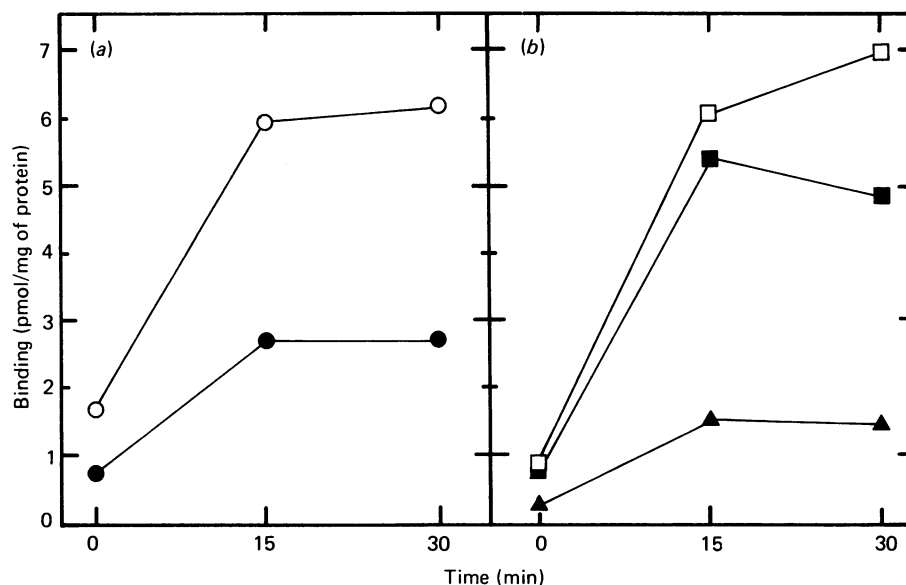
The ability of haem-asialo-haemopexin to bind to the haemopexin receptor was demonstrated *in vitro* by



**Fig. 2. U.v. c.d. spectra of rabbit apo-haemopexin (a) and asialo-haemopexin (b) and their mesohaem complexes**

C.d. spectra were recorded from 250 to 220 nm in 0.1 M-sodium phosphate buffer, pH 7.3, at  $25^\circ\text{C}$ . To form the haem complex an equimolar amount of mesohaem was added to the protein 30 min before the spectrum was recorded. The broken lines indicate the spectra recorded before, and the continuous lines those after, haem addition.

examining the interaction of  $[^{55}\text{Fe}]$ mesohaem-haemopexin,  $[^{55}\text{Fe}]$ mesohaem-asialo-haemopexin and  $^{125}\text{I}$ -asialo-haemopexin with isolated rabbit liver plasma membranes. Specific binding of  $^{125}\text{I}$ -asialo-haemopexin (initial concn.  $0.4 \mu\text{M}$ ) to the galactose-specific receptor is about 1.6 pmol/mg of membrane protein after 30 min at  $37^\circ\text{C}$ , as determined by competitive inhibition with  $10 \mu\text{M}$ -asialo-fetuin. Asialo-haemopexin complexed with haem also binds to the galactose-specific receptor (Bernard *et al.*, 1982); however, haem-haemopexin complexes do not (Smith & Morgan, 1979). Evidence for a specific interaction between haem-asialo-haemopexin and the haemopexin receptor is presented by the competition experiments shown in Fig. 3. Membranes were incubated with either haem-protein complex in the presence and in the absence of unlabelled mesohaem-haemopexin as competitive inhibitor. The rate and amount of membrane-associated  $[^{55}\text{Fe}]$ haem is similar for both  $[^{55}\text{Fe}]$ mesohaem-haemopexin and  $[^{55}\text{Fe}]$ -



**Fig. 3. Specific interaction of both mesohaem-haemopexin and mesohaem-asialo-haemopexin with the haemopexin receptor on isolated liver plasma membranes**

(a) Interaction at 30 °C of rabbit [ $^{55}\text{Fe}$ ]mesohaem-haemopexin (0.4  $\mu\text{M}$ ) with rabbit liver plasma membranes in the absence ( $\circ$ ) or in the presence ( $\bullet$ ) of excess unlabelled haem-haemopexin (16  $\mu\text{M}$ ). (b) Interaction of [ $^{55}\text{Fe}$ ]mesohaem-asialo-haemopexin (0.4  $\mu\text{M}$ ) with membranes in the absence ( $\square$ ) or in the presence of excess unlabelled haem-haemopexin ( $\blacksquare$ , 16  $\mu\text{M}$ ) or asialo-fetuin ( $\blacktriangle$ , 10  $\mu\text{M}$ ).

**Table 1. Liver uptake of asialo-fetuin, asialo-haemopexin, haem-haemopexin and haem-asialo-haemopexin**

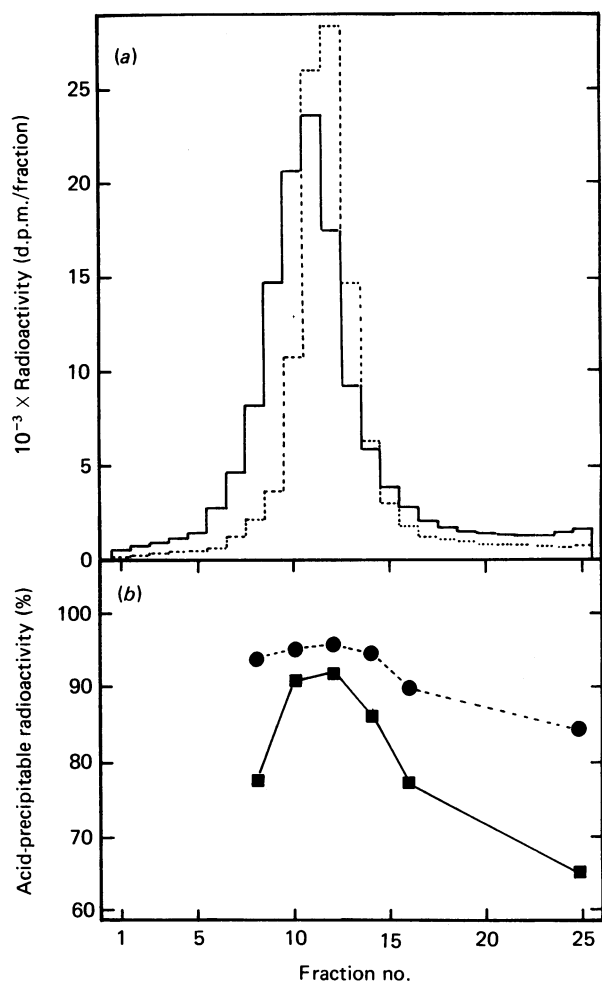
Anesthetized rats were injected intravenously with  $^{125}\text{I}$ -asialo-fetuin (0.2 mg),  $^{125}\text{I}$ -asialo-haemopexin (0.4 nmol), mesohaem- $^{125}\text{I}$ -haemopexin (4 nmol) and mesohaem- $^{125}\text{I}$ -asialo-haemopexin (4 nmol) to assess liver uptake and metabolism. The  $^{125}\text{I}$  in serum (shown as percentage of the total radioactivity in serum at that time) and liver (shown as percentage of the injected dose) samples were measured at the time indicated as described in the Materials and methods section. Abbreviations: TCA, trichloroacetic acid; N.D., not determined.

Protein	Time (min)	Serum radioactivity (% TCA-precipitable)	Liver radioactivity (% of dose)
Asialo-fetuin	2	98	N.D.
	5	97	57
	15	86	68
Asialo-haemopexin	2	94	N.D.
	30	48	68
Mesohaem-haemopexin	2	99	N.D.
	10	98	17
	30	97	15
Mesohaem-asialo-haemopexin	2	98	N.D.
	10	96	63
	30	15	89

mesohaem-asialo-haemopexin. In addition, both are inhibited by unlabelled haem-haemopexin, albeit to different extents, but only haem-asialo-haemopexin is inhibited by asialofetuin. This suggests that some of the [ $^{55}\text{Fe}$ ]mesohaem-asialo-haemopexin complexes are bound to the haemopexin receptor. Because the extent of competition of [ $^{55}\text{Fe}$ ]haem-asialo-haemopexin is less than that of [ $^{55}\text{Fe}$ ]haem-haemopexin, the data suggest that the asialo-haemopexin complexes are also binding at another site on the membranes, namely the galactose-specific receptor, as shown by the competitive inhibition with asialo-fetuin. Taken together, these observations

clearly demonstrate that haem-asialo-haemopexin interacts both with the haemopexin receptor and with a functional hepatic asialo-glycoprotein (galactose-specific) receptor on these isolated membranes *in vitro*.

To determine whether the interaction of haem-asialo-haemopexin with both the haemopexin receptor and the asialo-glycoprotein receptor also occurs *in vivo*, liver uptake in rats was studied. The results summarized in Table 1 show that asialo-haemopexin, like asialo-fetuin but unlike intact haemopexin, is rapidly taken up and extensively catabolized by the liver. More than half of the injected asialo-fetuin, asialo-haemopexin or haem-asialo-

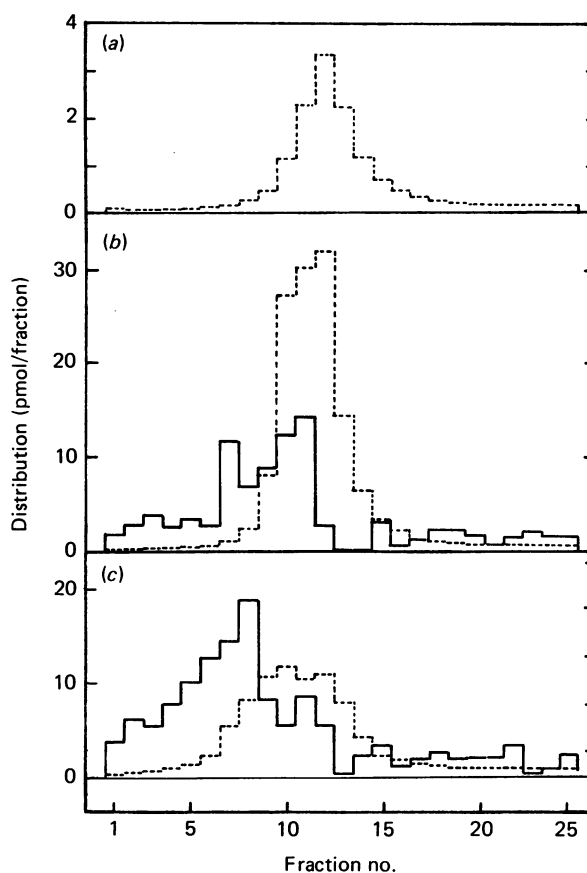


**Fig. 4.** Distribution of  $^{125}\text{I}$ -asialo-fetuin on isopycnic gradients

The distribution of  $^{125}\text{I}$ -asialo-fetuin (0.2 mg/rat) 5 min (-----) and 15 min (—) after intravenous injection is shown in (a) and the profile of trichloroacetic acid-precipitable radioactivity in (b). Liver homogenates were mixed with Percoll, and gradients were generated and analysed as described in the Materials and methods section.

haemopexin is located in the liver within 10–15 min after intravenous injection. By 30 min after injection of asialo-haemopexin or of haem-asialo-haemopexin, serum concentrations of intact protein, as represented by trichloroacetic acid-precipitable radioactivity, are significantly lowered. In contrast, only 13% of the protein from haem-haemopexin is liver-associated by 30 min and more than 97% of the radioactive haemopexin in the circulation remains trichloroacetic acid-precipitable. By this time 57% of the haem (part of which is likely to be radiolabelled iron derived from the haem) delivered by intact haemopexin is present in the liver. However, haem from haem-asialo-haemopexin is not accumulated as effectively by the liver (see below).

The uptake processes were further characterized by comparing the subcellular distributions of asialo-fetuin, asialo-haemopexin, haem-asialo-haemopexin and haem-haemopexin at two times after injection. The distribution of  $^{125}\text{I}$ -asialo-fetuin together with the trichloroacetic acid-precipitable radioactivity across the density gradient

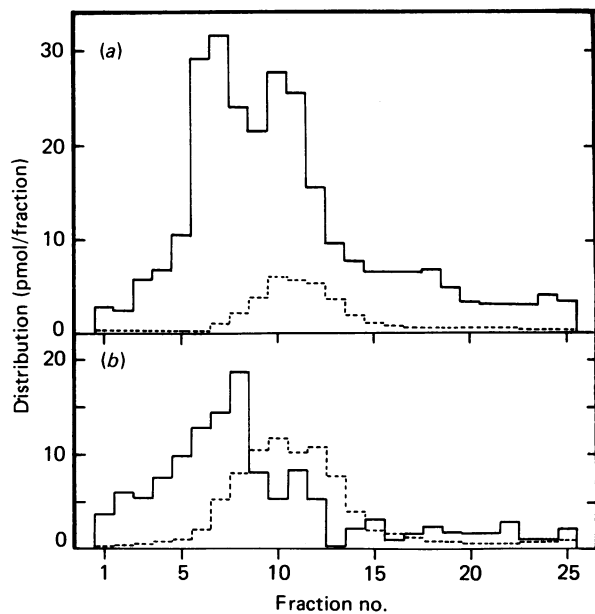


**Fig. 5.** Distribution of haem and protein on isopycnic gradients after injection of  $^{55}\text{Fe}$ mesohaem- $^{125}\text{I}$ -asialo-haemopexin

The distribution on Percoll gradients of  $^{55}\text{Fe}$ mesohaem- $^{125}\text{I}$ -asialo-haemopexin (4 nmol/rat) in liver homogenates at 10 and 30 min after intravenous injection is shown in (b) and (c) respectively. The distribution of 0.4 nmol of rabbit  $^{125}\text{I}$ -asialo-haemopexin 30 min after injection used as a 'marker' is included for comparison in (a). Continuous lines denote  $^{55}\text{Fe}$ haem and broken lines  $^{125}\text{I}$ -protein.

is shown in Fig. 4. Under these conditions asialo-fetuin has been shown to be transported via endocytosis and, within 15 min after injection, to have reached the lysosomes (LaBadie *et al.*, 1975). Since asialo-haemopexin (Fig. 5a) has a profile similar to asialo-fetuin (see Fig. 4), it appears that asialo-haemopexin and the protein moiety of haem-asialo-haemopexin (Fig. 5b) follow the same intracellular route as asialo-fetuin, resulting in catabolism of the protein in the lysosomes.

If haem-asialo-haemopexin remained as a complex after endocytosis, an equimolar distribution of both moieties on the gradient would be expected, particularly at early times after injection, and the profile of radioactivity should be similar to that of asialo-fetuin. However, even at 10 min, when 60% of the asialo-haemopexin is already located in the liver, the distributions of radiolabel from haem and protein on the gradient do not coincide, although the proportion of haem to asialo-protein across the whole of the gradients is 1:0.86 at 10 min and 1:1.5 at 30 min (Fig. 5). Haem is found at a position of greater density and in lower amounts than the asialo-haemopexin (Fig. 5), which migrates with the same mean density as asialo-fetuin (Fig. 4). Over 30 min



**Fig. 6. Distribution of haem and protein on isopycnic gradients after injection of [ $^{55}\text{Fe}$ ]mesohaem- $^{125}\text{I}$ -haemopexin**

The distribution of radioactive haem (—) and protein (-----) in homogenates of liver samples taken 30 min after intravenous injection of 4 nmol of doubly labelled rabbit mesohaem-haemopexin/rat is shown (a). For comparison the data for doubly labelled mesohaem-asialo-haemopexin at 30 min (from Fig. 5c) are reproduced below (b).

haem (or  $^{55}\text{Fe}$  derived from haem) hardly changes (e.g. from 28.8 to 31.6 pmol per gradient) but is now found in the more-dense regions of the gradient. The total amount of asialo-haemopexin decreases somewhat (from 33.5 to 20.9 pmol per gradient) and its mean density is only slightly increased (Fig. 5c), consistent with that of the asialo-fetuin profile.

The subcellular distribution of haem delivered to the liver by intact haemopexin at 30 min (Fig. 6) is in clear contrast with that delivered by asialo-haemopexin described above. More extensive hepatic accumulation of haem (or  $^{55}\text{Fe}$  derived from haem) with much lower accumulation of protein, as represented by  $^{125}\text{I}$  radioactivity, is observed with haem-haemopexin (Fig. 6a) than with haem-asialo-haemopexin [Fig. 6b; for clarity these data from Fig. 5(c) are reproduced again]. The ratio of total haem to haemopexin on the gradients at 30 min is 8:1 for haem-haemopexin compared with 1.5:1 for haem-asialo-haemopexin. Haem delivered by either form of haemopexin accumulates in the more-dense regions of the gradient and that from intact haemopexin shows a biphasic distribution. Haem from haemopexin is found coinciding with a region of density 1.05 g/ml containing high activity in marker enzymes for the endoplasmic reticulum (glucose-6-phosphatase) and also in a region of density 1.06–1.08 g/ml, containing mitochondria (cytochrome *c* oxidase). Haem originally injected complexed with asialo-haemopexin was found predominantly in these more-dense fractions but with a somewhat less-dense mean peak density (1.055 g/ml). This region is also high in  $\beta$ -galactosidase and 5'-nucleotidase activity, considered to be marker enzymes for lysosomes and plasma membranes respectively. However, the distribu-

tion of marker-enzyme activity on these gradients in the region 1.06–1.08 g/ml density cannot conclusively distinguish several organelles likely to contain haem, such as mitochondria (cytochrome *c* oxidase) or peroxisomes (uricase). Obviously, further investigation is required to identify which organelles are involved in the uptake of haem.

In conclusion, asialo-haemopexin binds haem in a manner similar to the native protein, as judged by absorption and c.d. spectra. The asialo-protein complexed with haem is also functional, since it is capable of interacting with the haemopexin receptor *in vitro*. Moreover, haem-asialo-haemopexin, like asialo-haemopexin, binds to the galactose-specific receptor on isolated membranes *in vitro*, and the protein undergoes rapid and extensive catabolism *in vivo*. Consequently, haem-asialo-haemopexin interacts *in vivo* and *in vitro* with both the haemopexin-specific and the galactose-specific receptors. However, *in vivo* asialo-haemopexin does not effect efficient accumulation of haem iron by this organ. The pattern of hepatic haem accumulation is consistent with transport via the haemopexin receptor of a small fraction of the injected haem-asialo-haemopexin. Extensive uptake and catabolism of the asialo-protein without concomitant recovery of haem suggests that after interaction of haem-asialo-haemopexin complexes with the galactose-specific receptor only the protein moiety is retained. This concept is supported by the observation that the plasma clearance of haem injected bound to asialo-haemopexin is slower than that of protein (G. Queen, personal communication). In contrast, intact haemopexin rapidly and efficiently transports haem to the liver; the haem accumulates in the liver, and the protein is not catabolized.

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