HAPTOGLOBIN-MEDIATED TRANSFER OF HAEMOGLOBIN FROM SERUM INTO BILE

Richard H. HINTON, Miloslav DOBROTA and Barbara M. MULLOCK
Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford GU2 5XH, Surrey, England

Received 19 February 1980

1. Introduction

The majority of the proteins present in rat bile are also present in rat serum [1]. These proteins common to serum and bile fall into two distinct groups. In the first group are major serum proteins which are present in bile at ~0.1% of their concentration in serum. The relative concentrations of these proteins appear inversely related to their molecular weight (R.H.H., B.M.M. unpublished) and it would seem likely that, as has been suggested in man and dog [2,3], the presence of these proteins in bile is due to diffusion across the tight junctions between hepatocytes. However two serum proteins, identified in [1] as IgA and 'bile protein 8' are relatively concentrated in bile. IgA is known to be actively transported from blood to bile [4]. The mechanism of transport involves the binding of IgA to secretory component on the sinusoidal surface of hepatocytes [5,6] and transport across the hepatocytes in endocytic vesicles [7,8]. These vesicles are not, however, solely concerned with transporting IgA and its receptor secretory component, to bile. Examination of the distribution of a bile protein 8 amongst liver cell fractions showed that it was also associated with vesicles similar in size to the vesicles which contain IgA [9]. Moreover newly made bile protein 8 and newly made secretory component appeared in bile after a similar time lag [10]. We now show that the role of bile protein 8 is to transfer haemoglobin from serum to bile and that bile protein 8 is haptoglobin partially or totally saturated with haemoglobin.

2. Material and methods

Hooded rats of the University of Surrey strain were used in all experiments. Dako antiserum against human haptoglobin was obtained through Mercia Brocades Ltd. (Weybridge, Surrey). Rat erythrocytes were prepared from blood using EDTA as anticoagulant. The erythrocytes were collected by centrifugation and washed repeatedly by resuspension in 0.15 M NaCl/5 mM EDTA (pH 7.4). The washed cells were lysed by osmotic shock and the resulting haemolysate dialysed against 0.15 M NaCl/0.01 M phosphate (pH 7.4) and centrifuged for 1 h at 130 000 × g. As >94% of the non-sedimentable protein of erythrocytes is haemoglobin [11] this centrifuged haemolysate is referred to as a haemoglobin preparation. This haemoglobin preparation, after suitable dilution, was labelled using $Na^{125}I$ (Radiochemical Centre, Amersham) using either the procedure in [12] or a modification (B. Morris, unpublished) of the method in [13]. The iodinated protein was injected into the femoral vein of rats maintained under pentobarbital (Sagatal, May and Baker, Dagenham) anaesthesia soon after cannulation of the bile duct. Blood and bile samples were collected as in earlier experiments [10] and analysed by immunoelectrophoresis against Dako anti-(rat serum) (obtained through Mercia Brocades Ltd., Weybridge, Surrey) or an anti-(rat bile) antiserum prepared in our laboratory [10]. The molecular weight of bile protein 8 was estimated by gel exclusion chromatography of rat bile on Sephadex G-200 (Pharmacia, Uppsala). The column was eluted with 0.15 M NaCl/0.01 M Tris–HCl (pH 7.4). The distribution of the different bile proteins among the fractions was determined by crossed immunoelectrophoresis against anti-(rat bile) antiserum [10].

3. Results

Sephadex chromatography indicated that bile protein 8 has mol. wt 100 000–150 000 and SDS–poly-

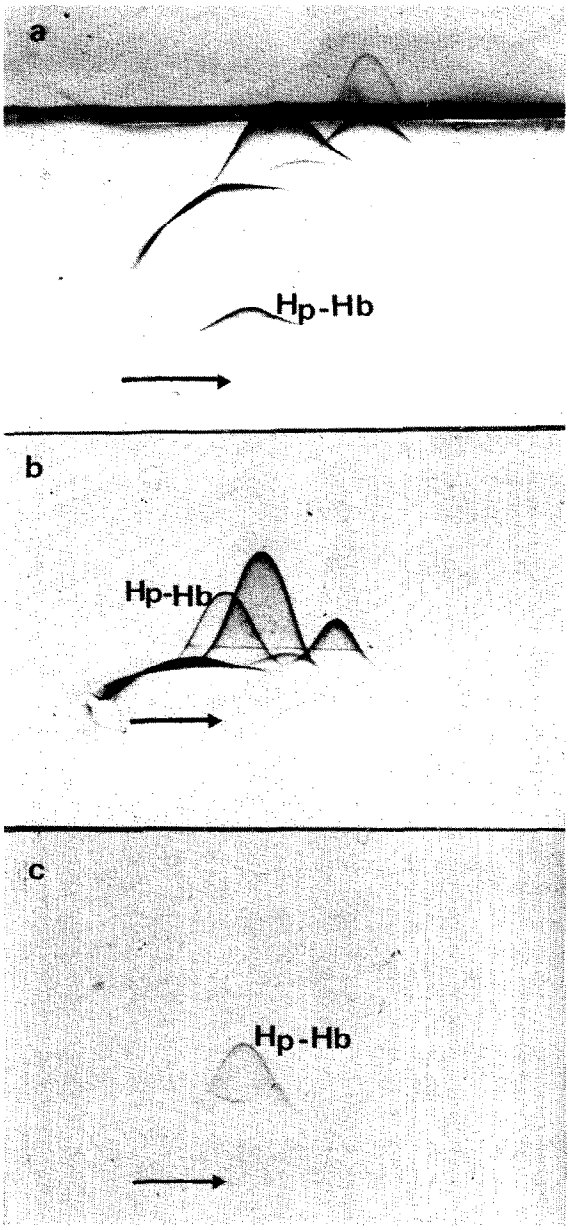

Fig.1. Patterns obtained after crossed immunoelectrophoresis of: (a) rat bile against an anti-(human haptoglobin) antiserum in the intermediate gel and an anti-(rat bile) antiserum in the upper gel; (b,c) rat bile, collected 45–52.5 min after intravenous injection of ^{125}I-labelled haemolysate proteins, against an anti-(rat bile) antiserum. In (a,b) the plates were stained for protein using Coomassie brilliant blue R; (c) is an autoradiograph of the plate shown in (b). Arrows show the direction of first dimension electrophoresis. Hp–Hb, haptoglobin–haemoglobin complex.

acrylamide gel electrophoresis showed that fractions enriched in bile protein 8 were also enriched in a peptide with mol. wt 35 000. These observations, together with the electrophoretic mobility (slow α_2 or fast β) suggested that bile protein 8 could be haptoglobin. Rat and human haptoglobin crossreact immunologically (J. Fowler, personal communication); crossed immunoelectrophoresis showed clearly that bile protein 8 was, indeed haptoglobin (fig.1a).

Since plasma haptoglobin binds haemoglobin we examined the possibility that haptoglobin carries haemoglobin into bile. Scanning rats with a radioactive monitor after intravenous injection of a ^{125}I-labelled haemoglobin preparation showed that label was concentrated in the liver. Measurement of plasma and bile radioactivity showed rapid clearance of material from plasma with considerable amounts of label appearing in bile after a short lag phase (fig.2). Immunoelectrophoresis showed that label in both plasma and bile was only associated with haptoglobin (illustrated for bile in fig.1b,c). About 8% of the injected label was excreted into bile in the 5 h following injection. As this is more than the total estimated amount of contaminants in our haemoglobin preparation and as haptoglobin specifically binds haemoglobin, it is cer-

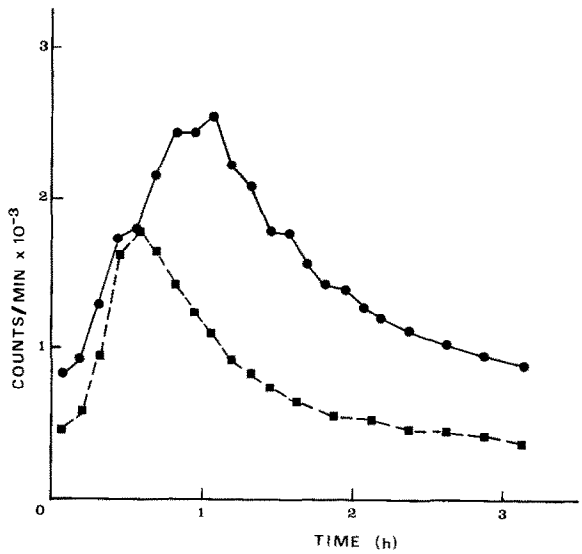

Fig.2. Appearance of radioactivity in bile following intravenous injection of ^{125}I-labelled haemolysate proteins into rats. Results from 2 different animals are presented separately. Of the injected radioactivity ~70% had cleared from the plasma after 1 h. The radioactivity level of the bile was still falling gradually 5 h after injection.

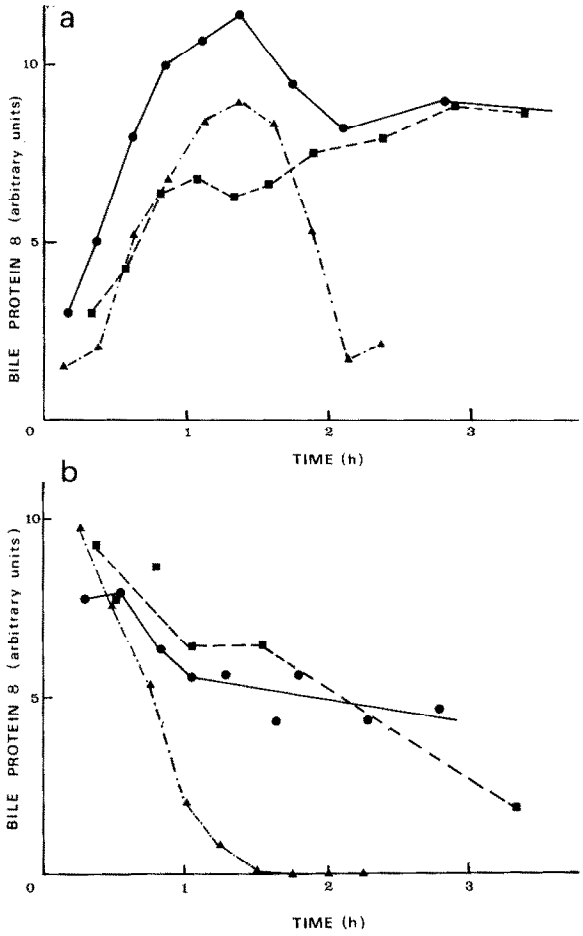

Fig.3. Changes in amounts of 'bile protein 8' in (a) bile and (b) plasma with time after bile duct cannulation. Results from 3 different animals are presented separately. Bile protein 8 was measured from the areas under the peak on immunoelectrophoresis plates; antisera were standardised for all the points within each time course, but absolute values for the different animals are not comparable. Rat 1 (•), rat 2 (■), rat 3 (▲).

tain that the excretion which we observe is of haemoglobin.

Although our experiments show that haptoglobin is involved in the transport of haemoglobin from blood to bile, they do not prove that preformed haemoglobin–haptoglobin complexes are transferred. Haptoglobin is made in the liver and it is conceivable that haemoglobin is transported only if it binds to haptoglobin molecules associated with the sinusoidal membrane of hepatocytes. Examination of the overall changes in the level of haptoglobin in plasma and bile indicated that this was not the case. Following bile duct cannulation, the levels of haptoglobin in plasma fell while those in bile rose (fig.3). When, as in rat 3, there was severe haemolysis (in this case due to cutting the femoral vein while attempting an intravenous injection), the plasma haptoglobin levels fell close to zero and there was an equally rapid, although slightly delayed fall in bile haptoglobin. This would indicate that haptoglobin–haemoglobin complexes in plasma are, indeed, precursors of the haptoglobin found in bile.

4. Discussion

Bile protein 8, which, like IgA, is present in bile at a high concentration relative to major serum proteins such as albumin [1] can now be identified as haptoglobin–haemoglobin complex since it reacts with an anti-(human haptoglobin) antiserum and is radiolabelled after injection of ^{125}I-labelled haemoglobin. On two-dimensional electrophoresis bile protein 8 splits to give two spots [1]; this might be expected if, like human haptoglobin [14], the rat protein can complex with either 1 or 2 $\alpha\beta$-globin dimers.

In haemolytic states in man, serum haptoglobin levels are known to fall rapidly as the haptoglobin–haemoglobin complex forms and is taken up by hepatocytes much faster than haptoglobin is synthesised [14]. This work shows a similar fall in plasma haptoglobin levels in rat after surgery and further indicates that at least part of the haptoglobin–haemoglobin complexes taken up by the hepatocytes are transferred to the bile, apparently by carriage in vesicles of a similar size to those transferring IgA [9,10]. Haemolytic anaemia in man and acetylphenylhydrazine-induced haemolytic anaemia in dog and rat have been shown to increase total biliary iron output 4–10-fold [15], suggesting that haptoglobin–haemoglobin complex output into bile is increased in these conditions. Further work on the mechanism and scope of this pathway for haemoglobin is clearly required.

Acknowledgements

We thank Miss L. Shaw for expert technical assistance, Professor J. Bridges for helpful discussion and Mr B. Morris of the Department of Biochemistry at this University and Dr J. Fowler of ICI for communication of unpublished results. Financial support was provided by the Medical Research Council.

References

[1] Mullock, B. M., Dobrota, M. and Hinton, R. H. (1978) Biochim. Biophys. Acta 543, 497–507.
[2] Dive, C. and Heremans, J. F. (1974) Eur. J. Clin. Invest. 4, 235–239.
[3] Dive, C., Nadalini, R. A., Vaerman, J.-P. and Heremans, J. F. (1974) Eur. J. Clin. Invest. 4, 241–246.
[4] Orlans, E., Peppard, J., Reynolds, J. and Hall, J. (1978) J. Exp. Med. 147, 588–592.
[5] Orlans, E., Peppard, J., Fry, J. R., Hinton, R. H. and Mullock, B. M. (1979) J. Exp. Med. 150, 1577–1581.
[6] Socken, D. J., Jeejeebhoy, K. N., Bazin, H. and Underdown, B. J. (1979) J. Exp. Med. 150, 1538–1548.
[7] Birbeck, M. S. C., Cartwright, P., Hall, J. G., Orlans, E. and Peppard, J. (1979) Immunology 37, 477–484.
[8] Mullock, B. M., Hinton, R. H., Dobrota, M., Peppard, J. and Orlans, E. (1979) Biochim. Biophys. Acta 587, 381–391.
[9] Hinton, R. H., Mullock, B. M., Peppard, J. and Orlans, E. (1980) Biochem. Soc. Trans. 8, 114.
[10] Mullock, B. M. and Hinton, R. H. (1979) FEBS Lett. 106, 121–124.
[11] Diem, K. and Lentner, C. (1970) Documenta Geigy, Scientific Tables, 7th edn., p. 579, Geigy, Basle.
[12] McConahey, P. J. and Dixon, F. J. (1966) Int. Arch. Allergy Appl. Immunol. 29, 185–189.
[13] Salacinski, P., Hope, J., MacClean, C., Clement-Jones, B., Sykes, J., Price, J. and Lowry, P. J. (1979) J. Endocrinol. 81, 131P.
[14] Giblett, E. (1974) in: Structure and function of plasma proteins (Allison, A. C. ed) vol. 1, pp. 55–72, Plenum Press, London.
[15] Figueroa, W. G. and Thompson, J. H. (1968) Am. J. Physiol. 215, 807–810.