MECHANISMS BY WHICH NEWLY MADE GLYCOPROTEINS ARE TRANSFERRED FROM HEPATOCYTES INTO BILE

Barbara M. MULLOCK and Richard H. HINTON

Institute for Industrial and Environmental Health and Safety, University of Surrey, Guildford GU2 5XH, Surrey, England

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1. Introduction

Hepatocytes differ from other secretory cells in that they secrete materials into two separate fluids, the blood and bile. Serum proteins and glycoproteins are obviously discharged at the sinusoidal surface of hepatocytes [1], but the route by which proteins enter bile is less evident. Although many of the proteins present in rat bile are also to be found in rat serum, the relative concentrations of the different proteins are markedly different in the two fluids [2]. In addition to 'serum' proteins, bile contains the secretory component of IgA which is not found in blood [2,3] together with small amounts of hepatocyte plasma membrane proteins. There must, it appears, be some mechanism which sorts proteins destined for bile from proteins destined for blood.

Recent experiments have suggested a simple mechanism for routing at least some of the major proteins into bile without an elaborate system of sorting secretory granules. IgA is carried rapidly from serum to bile [4,5]. The IgA binds to a receptor on the sinusoidal surface of hepatocytes [6] and is taken up into specialised endocytic vesicles [7] which discharge their contents into bile. The receptor is probably secretory component ([8], E. Orlans, J. Peppard, J. R. Fry, R. H. H. and B. M. M., unpublished experiments). This system immediately accounts for the presence of IgA in bile and, if excess secretory component were present in the vesicles, would also account for the presence of the free secretory component. If, as seems likely, these or similar vesicles carry other proteins across hepatocytes, one can postulate that all 'export' proteins made in hepatocytes are transferred to the sinusoidal face of the cell but that some proteins are then transferred across to the bile canalicular face in association with endocytic vesicles. At the bile–canalicular face such vesicle-associated proteins are released, together with small amounts of typical plasma membrane proteins, probably by the detergent action of bile salts [9].

If bile proteins travel initially to the sinusoidal surface of the hepatocyte and then have to travel back across the cell, one would expect that newly synthesised proteins would appear in serum first and would appear in bile only after a lag phase representing the time taken to recross the cell. We have, therefore, studied the release of newly synthesised glycoproteins, labelled by L-[1-^{14}C]fucose and monitored their appearance in serum and bile. All the major proteins of rat bile except albumin have bound carbohydrate groups [2]. Fucose is incorporated into glycoproteins immediately before their discharge from liver [10] so that labelling with fucose, rather than an amino acid, avoids uncertainties over the rates of processing of different proteins in the endoplasmic reticulum and Golgi apparatus. We find the expected lag phase in the appearance of newly synthesized glycoproteins in bile.

2. Material and methods

Hooded rats of the University of Surrey strain were used in all experiments. The rats were maintained under pentobarbital (Sagatal, May and Baker, Dagenham, Kent) anaesthesia throughout the experiment. The common bile duct was cannulated and the

body wall and skin ligatured to avoid dehydration. L-[1-^{14}C]fucose (0.2–3 ml) (50 μCi/ml, purchased from the Radiochemical centre, Amersham) was injected into the femoral vein. Bile was collected for 15 min periods. Blood samples (~20 μl) were collected from the tip of the tail. The samples were diluted with ~80 μl of 0.15 M NaCl/5 mM EDTA pH 7.4 and the red blood cells removed by centrifugation.

Acid-precipitable proteins in serum and bile were assayed by the method in [11]. The method was modified in that the initial precipitate was collected by centrifugation to permit sampling of the supernatant. The pellet was resuspended, transferred to a GF-B glass fibre filter (Whatman Lab. Sales Ltd, Maidstone, Kent) and thereafter processed according to the original scheme. The distribution of label among the different glycoproteins present in serum and bile was determined by crossed immunoelectrophoresis [12] against anti-(rat serum) (Mercia Brocades Ltd, West Byfleet, Surrey) or an antiserum prepared against rat bile using the dosage schedule given in [13].

3. Results

In these experiments we examined the appearance of acid-precipitable ^{14}C-labelled glycoproteins in plasma and in bile following the intravenous injection of [^{14}C]fucose into rats. In plasma, acid-soluble fucose disappeared very rapidly and ^{14}C-labelled glycoproteins accumulated progessively over the 2 h period of study (fig.1a) with no apparent lag phase. The rate of accumulation of ^{14}C-labelled glycoproteins in plasma does, however, slowly decrease, due, probably, to the dilution of the pool of [^{14}C]fucose in liver. These results are consistent with those obtained in [10].

The kinetics of appearance of ^{14}C-labelled glycoproteins in bile (fig.1b) were markedly different from those of ^{14}C-labelled glycoproteins in plasma. There was a marked lag phase; only small amounts of labelled glycoproteins appeared in bile during the first 30 min after injection of [^{14}C]fucose. The amount of ^{14}C-labelled glycoproteins in bile then increased rapidly up to ~70 min following injection of the fucose and then slowly declined.

In order to determine which proteins are labelled by [^{14}C]gucose, we fractionated plasma and bile proteins by crossed immunoelectrophoresis using autoradiography to determine which proteins had incorporated the [^{14}C]fucose. The results (fig.2) showed that all but one of the major bile proteins were labelled by [^{14}C]fucose. The non-radioactive protein

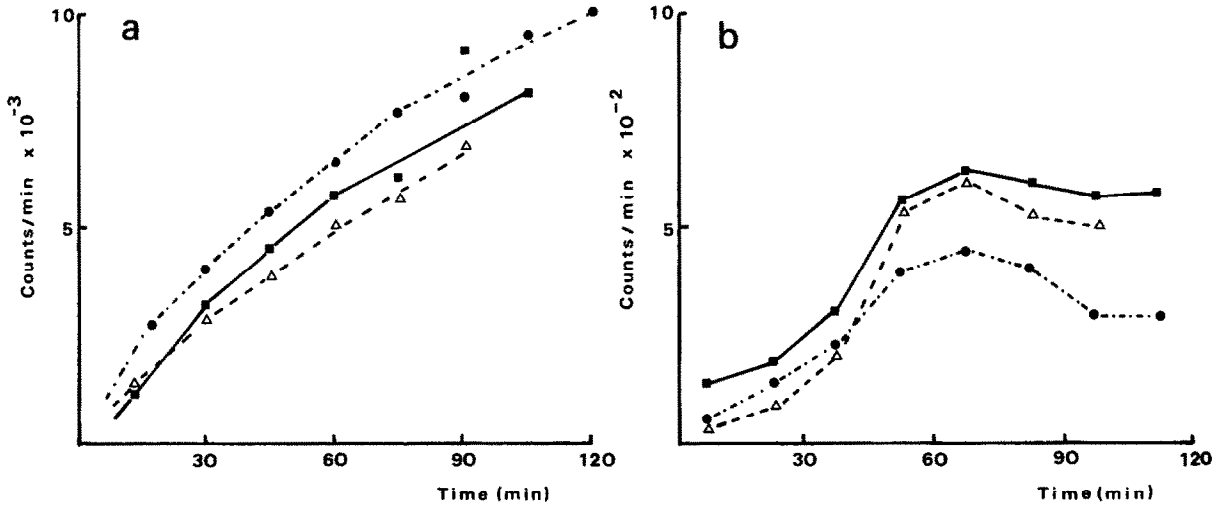

Fig.1. Appearance of acid-precipitable ^{14}C-labelled glycoproteins in (a) plasma and (b) bile following intravenous injection of L-[1-^{14}C]fucose into rats. The results from 3 different animals are presented separately. The results are presented as cpm/ml fluid in the case of rats 1 and 3 (●,△) and cpm/0.5 ml fluid in the case of rat 2 (■) which received a larger dose of fucose.

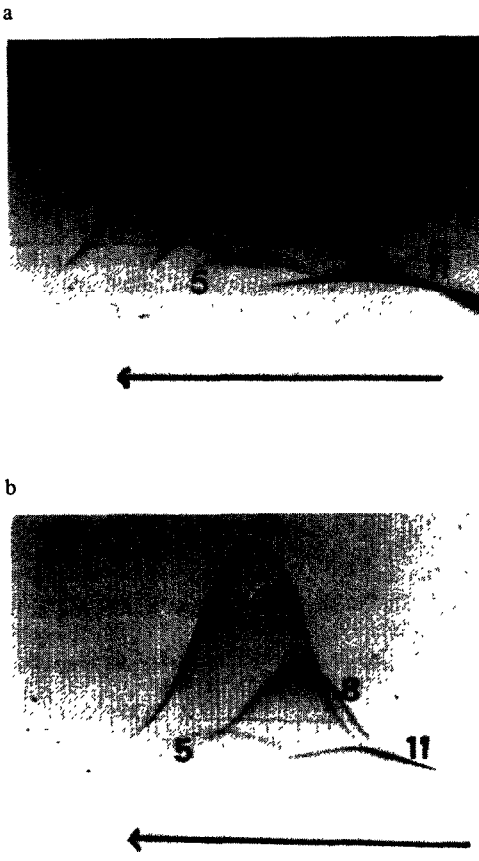

Fig.2. Pattern obtained after crossed immunoelectrophoresis of rat bile collected between 45 min and 60 min after intravenous injection of L-[1-^{14}C]fucose. Electrophoresis was against an anti-(rat bile) antiserum. The plate was stained for protein using Coomassie brilliant blue R (a) and glycoproteins containing [^{14}C]fucose were located by autoradiography (b). The proteins are numbered as in [2]. The arrows at the base of the figures show the direction of electrophoresis in the first dimension.

'1' is undoubtedly albumin. The autoradiographic studies make clear that the [^{14}C]fucose is principally incorporated into the three major bile proteins labelled 'D', '8' and '11' in fig.2. From [2] we can identify these as free IgA, secretory component, a glycoprotein of unknown function, and secretory IgA, respectively. The fucose label associated with the secretory IgA is most probably due to the bound secretory component. There is also some fucose label associated with the minor bile protein '5' which our earlier results [2] suggest is identical to a major serum glycoprotein. Electrophoresis of bile against anti-(rat serum) showed that the specific activity of this protein in bile was at least as high as the specific activity of the homologous protein in serum. In plasma the label seemed uniformly spread among the different glycoproteins.

4. Discussion

Our results show that there is, indeed, the lag phase in the appearance of newly made glycoproteins in bile which, as mentioned in section 1, would occur if newly made biliary glycoproteins are carried first to the sinusoidal surface of the cell and then back across the cell into bile. The time course is very similar to the time course of IgA transport; intravenously injected IgA binds almost instantaneously to the sinusoidal surface of hepatocytes [6] but there is a lag of ~30 min before IgA appears in bile [4]. The similarity in the time for newly made glycoproteins to appear in bile and the time taken for IgA to be transported across the hepatocyte, together with the known association of secretory component and bile protein 8 with the 'shuttle' vesicles [7,14] strongly suggests that the 'shuttle' vesicles not only transport IgA from blood to bile, but also convey newly made free secretory component and at least one other major bile protein.

The formation of secretory component and bile protein 8 represents a significant metabolic effort for the hepatocyte. The amount of glycoprotein appearing in bile at 30–60 min averages 515 cpm/min (the bile flow in our rats is ~1 ml/h). The blood volume of our rats is ~12 ml, hence we would calculate that the rate of discharge of newly made glycoproteins in the first 30 min of labelling as ~67 000 cpm/min. It must, however, be remembered that the label in serum is divided among many glycoproteins while the label in bile is essentially confined to two. The reasons for this investment of metabolic energy are not yet clear. Secretory component is, apparently, the receptor for IgA [8]. The function of bile protein 8 is not clear but some circumstantial evidence suggests that it may also have a transport function, possibly carrying small molecules. It would seem, then, that although leakage

of major serum proteins across tight junctions [15] may supply some minor bile proteins whilst others may be dissolved from the canalicular plasma membrane [9], those major bile proteins which have a high bile/blood concentration ratio are supplied via the endocytic vesicle shuttle.

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