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Evidence for glycoprotein components of the hepatocellular bile acid transporter

K. Ziegler

Institut für Pharmakologie und Toxikologie, Fachbereich Veterinärmedizin, Justus Liebig Universität, Giessen (F.R.G.)

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The hepatocellular transporter, responsible for the uptake of bile acids and some foreign substances, can be shown to contain carbohydrate moieties. The hepatocellular uptake of cholate and phallotoxin is immediately inhibited by addition of wheat-germ agglutinin. Concanavalin A and lentil lectin reduce the uptake in a time-dependent manner. Apparently sialic acids or *N*-acetylglucosamine residues are involved in the translocation process. Polypeptides (M_r 50000, 54000) of the above transport system, identified by affinity labeling with [^{3}H]isothiocyanatobenzamido cholate and [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid, are heterogenously glycosylated. Binding of 80–90% of the 54, 50 kDa polypeptides to all immobilized lectins tested suggests that both high-mannose and complex type oligosaccharides with fucose and terminal sialic acid residues occur as carbohydrate chains. A 67 kDa labeled polypeptide is not glycosylated. Pilot experiments for purification of the above glycosylated membrane proteins on concanavalin A, lentil lectin and wheat-germ lectin columns are described. However, lectin affinity chromatography is not suitable as a one-step purification procedure for the labeled polypeptides.

Introduction

In a series of previous papers, a transport system in hepatocellular plasma membranes has been described which physiologically transports bile acids and also some foreign substrates [1–4]: e.g., fusidic acid [5], various organic anions, e.g., 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid, cholecystographic agents [3,7] some cyclopeptides, e.g., somatostatin [8], phalloidin [1,2,4] and antamanide [2].

Because of the heterogeneity of the compounds transported, the term 'multispecific transporter' was proposed in 1982 [9] and has been apparently accepted by other investigators [10]. Multispecificity was proved not only by kinetic data [1–8] but also by affinity labeling studies [11–17]. (Photo) affinity labels derived either from bile acids [11–16] or from various cyclopeptides (phalloidin, antamanide [17], or somatostatin [18]) bound preferentially to hepatocellular plasma membrane proteins of 67, 54, 50 and 37 kDa.

Little is known about chemical properties of the involved transport protein(s). Accatino et al. [19] suggested that the cholate binding and transporting protein in isolated rat liver plasma membranes is a glycoprotein. Isoelectric focusing of labeled transport proteins (M_r 50000, 54000) showed multiple spots. This was interpreted by

Correspondence: K. Ziegler, Institut für Pharmakologie und Toxikologie, Fachbereich Veterinärmedizin, Justus Liebig Universität, Frankfurter Strasse 107, D-6300 Giessen, F.R.G.

charge differences due to heterogeneous glycosylation [13].

In order to test the role of carbohydrates in cholate and phalloidin uptake, the effect of various lectins on the uptake of both substrates by isolated hepatocytes was investigated. Furthermore, immobilized lectins were used to evaluate the occurrence of carbohydrate residues in the labeled polypeptides. Finally, preliminary efforts to isolate the labeled polypeptides by lectin affinity chromatography will be described.

Materials

Concanavalin A, wheat-germ agglutinin, Serva blue G and phenylmethylsulfonyl fluoride (PMSF) were purchased from Serva, Heidelberg, F.R.G.; concanavalin A-Sepharose, lentil lectin-Sepharose, wheat-germ lectin-Sepharose from Pharmacia, Freiburg, F.R.G.; *N*-acetyl-D-glucosamine from Fluka AG, Buchs, Switzerland, and methyl α-D-mannoside from Sigma, München, F.R.G. [^{14}C]-Cholic acid (specific activity 1.85–2.2 GBq/mmol) was purchased from Amersham Buchler, Braunschweig, F.R.G.

[$^{3}H_2$]Diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid (spec. act. 37 GBq/mmol), and 3′-isothiocyanatobenzamido [^{3}H]cholate (spec. act. 22.59–29.6 GBq/mmol) were synthesized as described earlier [11,12]. All other reagents were of analytical grade.

Methods

Isolation of rat liver cells. Rat hepatocytes were isolated according to Berry and Friend [20]. Livers of male Wistar rats (220–250 g) were perfused with 0.05% collagenase in Krebs-Henseleit buffer: (composition, in g/l: NaCl, 6.92; KCl, 0.32; $MgSO_4$, 0.29; KH_2PO_4, 0.16; $NaHCO_3$, 2.10) at 37 °C (pH 7.4) for 15 min in an O_2/CO_2 (95 : 5%) atmosphere. After isolation, the liver cell suspension was equilibrated for 30 min in Tyrode buffer (composition, in mmol/l: NaCl, 137.0; KCl, 2.7; $MgCl_2$, 1.05; $CaCl_2$, 1.8; $NaHCO_3$, 12.0; glucose, 1.0; NaH_2PO_4, 0.42) (pH 7.4, 37 °C, carbogen 95% O_2/5% CO_2). Cell viability was evaluated by Trypan blue exclusion and 85–90% of the cells remained viable after the isolation procedure. Uptake studies were performed within 2 h of cell isolation.

Uptake studies. The uptake of cholic acid and of phalloidin was measured by rapid centrifugation of hepatocytes through a silicon oil layer [21]. Briefly: $2 \cdot 10^6$ hepatocytes/ml Tyrode buffer, at 37 °C, in O_2/CO_2 atmosphere, were incubated with or without lectins 30 s prior to the addition of 1 μM [^{14}C]cholate plus 6 μM cholate, or 0.1 μM [^{3}H]demethylphalloin plus 5 μM phalloidin (referred to as phallotoxin). At 15, 45, 75, 105, 135 s, 5 and 10 min, 100-μl aliquots were withdrawn and centrifuged through silicon oil [21]. The radioactivity associated with the cell pellet was measured, after addition of a mixture of Lipoluma/Lumasolve/water, in a Packard Tricarb 2660 scintillation counter. In studies of the time-dependency of lectin inhibition, hepatocytes were preincubated with lectins for 10–30 min. The unbound lectin was then removed by washing. Uptake measurements were then carried out as described above.

Preincubation of isolated hepatocytes with lectins or with the various monosaccharides had a negligible effect on cell viability (90–95% of control cells excluded Trypan blue, 83–88% of lectin preincubated cells excluded the dye).

Isolation of rat liver plasma membranes. Rat liver surface membranes were prepared according to Touster [22] with some modifications [12]. The plasma membrane preparation was enriched in marker enzymes of the basolateral membrane fraction: ($Na^+ + K^+$)-ATPase was enriched 28-fold.

Affinity labeling of rat liver plasma membranes. Isolated plasma membranes were labeled with [$^{3}H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid, or [^{3}H]isothiocyanatobenzamidocholate as previously described [11–13]. Unbound label was removed by repeated washing in 20 mM Tris-HCl/0.1 mM NaCl/0.5 mM PMSF/1 mM $CaCl_2$/1 mM $MgCl_2$ (pH 7.4).

Solubilization of labeled plasma membranes. Affinity-labeled plasma membranes were solubilized in 2% Nonidet P-40 or 1% Triton X-100. The solubilized proteins were separated from the insoluble fraction by centrifugation at 100 000 × *g* for 1 h.

Lectin affinity chromatography. Nonidet P-40- or Triton X-100-solubilized proteins were sep-

arated on lectin affinity columns as follows. Concanavalin A-Sepharose or lentil lectin (*Lens culinaris*)-Sepharose were washed as described by Zanetta [23]. Columns (2.4 × 6 cm) were filled with concanavalin A- or lentil lectin-Sepharose in 20 mM Tris-HCl/0.5 M NaCl/0.05% Nonidet P-40 or Triton X-100/1 mM $CaCl_2$/1 mM $MgCl_2$/0.5 mM PMSF (pH 7.4) at room temperature. 5 mg Nonidet P-40- or Triton X-100-solubilized labeled proteins were applied to the columns. After a binding period of 30 min, the unbound proteins were eluted with the above buffer at a flow rate of 10 ml/h. Fractions of 2 ml volume were collected. Thereafter, the columns were washed with nonspecific sugars (*N*-acetylglucosamine in the case of concanavalin A- and lentil lectin-Sepharose) to remove any protein nonspecifically adsorbed to the column. Glycoproteins were then eluted with methyl α-D-mannoside or methyl α-D-glucoside (0.5 M). For removal of strongly bound proteins the columns were eluted with buffers at pH 5.2. In control experiments, washing and elution of the columns led to some leakage of coupled lectin as measured by the presence of protein in the eluate.

The wheat-germ agglutinin Sepharose was equilibrated with 50 mM Tris-HCl, 500 mM NaCl, 0.5 mM PMSF/0.05% Nonidet P-40. Nonspecific elution of proteins was done by addition of 500 mM rhamnose in the above buffer. Thereafter, glycoproteins were eluted with buffer containing 500 mM *N*-acetylglucosamine (pH 7.4). Of the solubilized proteins applied, 70–75% did not bind to the affinity columns.

Preparation of samples for SDS-polyacrylamide gel electrophoresis. The peak fractions of unbound or specifically bound and subsequently eluted proteins were dialysed against double-distilled water to remove monosaccharides and detergents. The samples were frozen and lyophilized. The lyophilized proteins were redissolved in sample buffer containing 2% SDS and 5% mercaptoethanol (pH 6.7). After boiling for 3 min, polyacrylamide gel electrophoresis was performed either on slab gels (10% acrylamide) or on rod gels. Proteins were visualized by either Coomassie blue or silver staining.

Radioactive proteins separated in slab gels were analysed by fluorography [24]. Radioactivity bound to the protein was quantified by slicing rod gels and counting the radioactivity in a Packard scintillation counter.

Staining of concanavalin A and wheat-germ agglutinin reactive glycoproteins was done according to Clegg [25] after transfer of polypeptides separated on SDS slab gels to nitrocellulose [26].

Results

Functional changes of hepatocellular uptake of cholate and of phallotoxins in presence of concanavalin A, lentil and wheat-germ lectin

Uptake studies were carried out with two representative substrates of the multispecific carrier, namely cholate as the physiological type and phallotoxin as the nonphysiological one. To evaluate the role of glycoproteins in the above system, the effect of lectins (concanavalin A, lentil lectin, and wheat-germ lectin) on the uptake were studied.

(A) Addition of concanavalin A (400 μg/2 · 10^6 hepatocytes per ml) to isolated hepatocytes 30 s before starting the uptake measurements did not influence the initial uptake velocity for both substrates. In contrast, a 30–50% reduction of the initial uptake velocity was observed after preincubation with the lectin for 10–30 min (Fig. 1).

There was no agglutination of hepatocytes during the preincubation period and inclusion of methyl α-D-mannoside during the preincubation abolished the inhibitory effect of concanavalin A. In contrast, *N*-acetylglucosamine did not prevent the inhibition of cholate and phallotoxin uptake by concanavalin A. Methyl α-D-mannoside or methyl α-D-glucoside (25–100 mM) had no effect on the uptake of either substrate (Table I).

(B) Wheat-germ agglutinin (200 or 400 μg/ml) inhibited the initial uptake of both substrates by 30% when added 30 s prior to starting the uptake. This inhibition increased to 70% after a 30 min preincubation with lectin (Fig. 2). *N*-Acetylglucosamine (100 mM) also inhibited the uptake of cholate by isolated hepatocytes, whereas 25 mM of the monosaccharide had no effect on cholate transport. Preincubation of wheat-germ agglutinin with *N*-acetylglucosamine for 10 min before the addition to hepatocytes prevented the inhibition of cholate by the lectin/carbohydrate mixture. Methyl α-D-mannoside or methyl α-D-glucoside

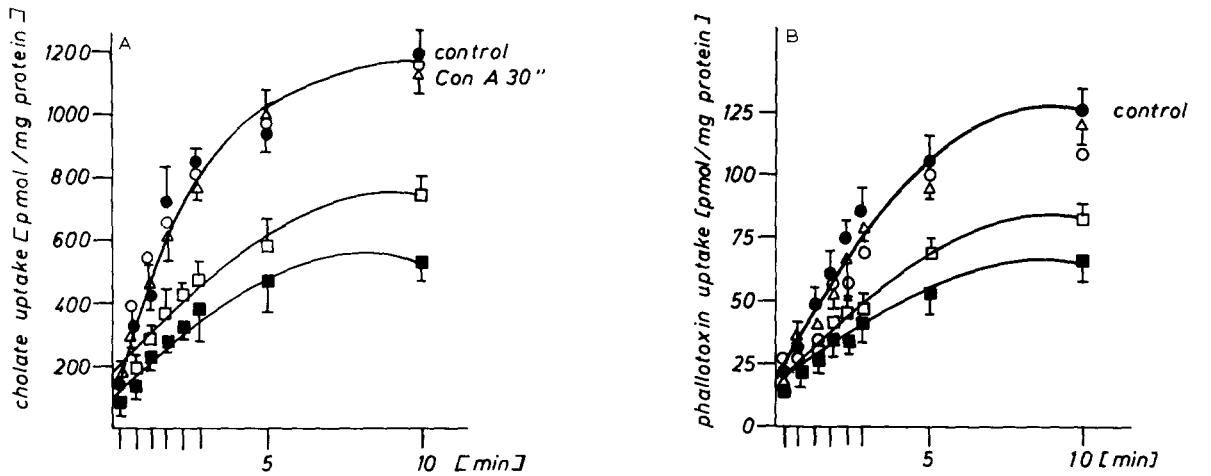

Fig. 1. (A) Influence of concanavalin A on cholate uptake in isolated hepatocytes. Isolated hepatocytes were incubated with or without concanavalin A 30 s or 30 min prior to the addition of 1 μM [^{14}C]cholate plus 6 μM cholate to 1 ml of cells ($2 \cdot 10^6$ hepatocytes/ml). After 15, 45, 75, 105, 135 s, and 5 and 10 min, 100-μl aliquots were withdrawn and centrifuged through silicon oil. ●, Control; △, concanavalin A, 30 s; □, 200 μg/ml concanavalin A, 30 min preincubation and removing unbound lectin by washing; ■, 400 μg/ml concanavalin A, 30 min preincubation and removing unbound lectin by washing; ○, 200 μg/ml concanavalin A plus 50 mM methyl α-D-glucoside. ($N = 4$; $\bar{x} \pm$S.D.). (B) Influence of concanavalin A on phallotoxin uptake. The experimental procedure is as described in (a). ●, control; △, concanavalin A plus methyl α-D-mannoside; □, 200 μg/ml concanavalin A, 30 min preincubation and removing unbound lectin by washing; ■, 400 μg/ml concanavalin A, 30 min preincubation and removing unbound lectin by washing.

did not abolish the inhibition of cholate or phallotoxin uptake by wheat-germ agglutinin.

Lectins do not prevent binding of [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid or [^{3}H]-isothiocyanatobenzamido cholate to protein components of the hepatocellular plasma membrane

Protein components of the multispecific transporter were previously identified using various affinity and photoaffinity labels [11–18]. It was of interest whether affinity labeling of proteins involved in the function of the multispecific transporter could be inhibited in the presence of certain lectins.

Simultaneous incubation of isolated plasma

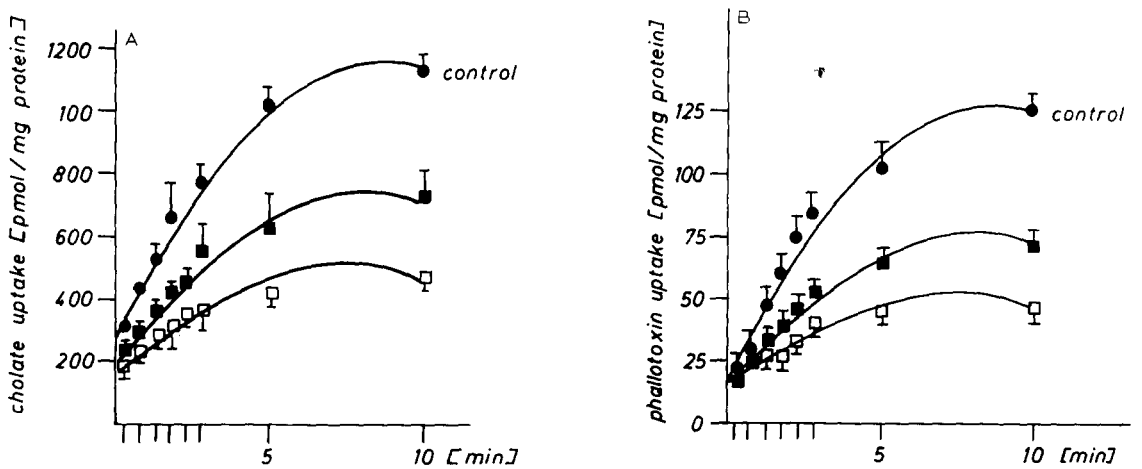

Fig. 2. Influence of wheat-germ agglutinin on (A) cholate uptake and (B) phallotoxin uptake in isolated hepatocytes. For details, see legends to Fig. 1a and b, respectively. ●, Control; ■, 200 μg/ml wheat-germ agglutinin, 30 s; □, 200 μg/ml wheat-germ agglutinin, 30 min preincubation and removing unbound lectin by washing; ($n = 4$; $\bar{x} \pm$S.D.).

TABLE I

INFLUENCE ON CHOLATE UPTAKE OF METHYL α-D-GLUCOSIDE

For details, see legend to Fig. 1.

Methyl α-D-glucoside	V_i (pmol/mg protein per min)
Control	314 ± 10
25 mM	300 ± 25
50 mM	295 ± 13
100 mM	295 ± 20

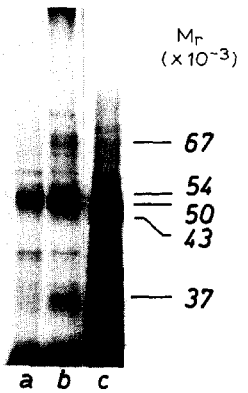

Fig. 3. Lectin affinity chromatography of [^{3}H]isothiocyanatobenzamidocholate-labeled plasma membranes. The amount of protein applied to the slab gel was: 100 μg, lanes a, b; 300 μg lane c. (a) Labeled plasma membranes: (b) Nonidet P-40 supernatant; (c) Nonidet P-40 pellet.

membranes with concanavalin A (0.4 mg/ml; 2 mg plasma membrane protein/ml) and with [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid or [^{3}H]isothiocyanatobenzamido cholate did not prevent binding of the above labels to plasma membrane proteins. Labeling after 40 min preincubation of plasma membranes with concanavalin A showed a radioactive pattern identical to that in control experiments. Identical results were obtained with lentil lectin and with wheat-germ agglutinin (data not shown).

Binding of [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid- or [^{3}H]isothiocyanatobenzamido cholate-labeled membrane proteins to immobilized lectins

Plasma membranes labeled with [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid or [^{3}H]isothiocyanatobenzamidocholate were solubilized either with 1% Triton X-100 or 2% Nonidet P-40. Of the protein and radioactivity, 80% were recovered in the supernatant after 100 000 × g centrifugation. Small amounts of labeled polypeptides remained in the Triton X-100- or Nonidet P-40-insoluble pellet, in particular a 43 kDa protein (Fig. 3). Proteins remaining in the supernatant were applied to columns of concanavalin A, lentil lectin or wheat-germ agglutinin immobilized on Sepharose.

Fig. 4A depicts representative elution profiles from lentil lectin and concanavalin A columns. It should be emphasized that ^{3}H (cpm × 10^{-2}/50 μl) represents radioactivity in the plasma membranes following labeling and solubilization. It does not necessarily represent specific and covalently labeled transport proteins. Since [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid and also [^{3}H]isothiocyanatobenzamido cholate are highly hydrophobic molecules [12,13], they penetrate into membrane lipids and, even after washing the membranes, a significant amount of the label remains trapped in lipid. The large initial peak of radioactivity thus represents a mixture of nonretained transport proteins and unincorporated or lipid-bound label. For elution of nonspecifically bound protein, the columns were washed with a buffer containing a noncompeting sugar. No radioactivity could be detected in the fractions. In contrast, by inclusion of the specific monosaccharide competitor in the buffer, the glycoproteins could be eluted from the columns, producing a radioactive peak.

Fig. 4B shows the fluorogram of the SDS-polyacrylamide gel electrophoresis of the indicated fractions from the lectin columns. In concanavalin A and lentil lectin affinity chromatography the 67 kDa protein was found almost quantitatively in the unbound fraction. In this fraction large amounts of lipid bound label can be detected. Of the 50 and 54 kDa proteins, 10–20% did not bind to the lectins. In the retained fraction, the proteins of 50 and 54 kDa were predominant. A protein of 95–100 kDa, which is usually not identified in labeled membranes, is concentrated by lentil lectin affinity chromatography. Affinity chromatography of [^{3}H]isothiocyanatobenzamidocholate-labeled

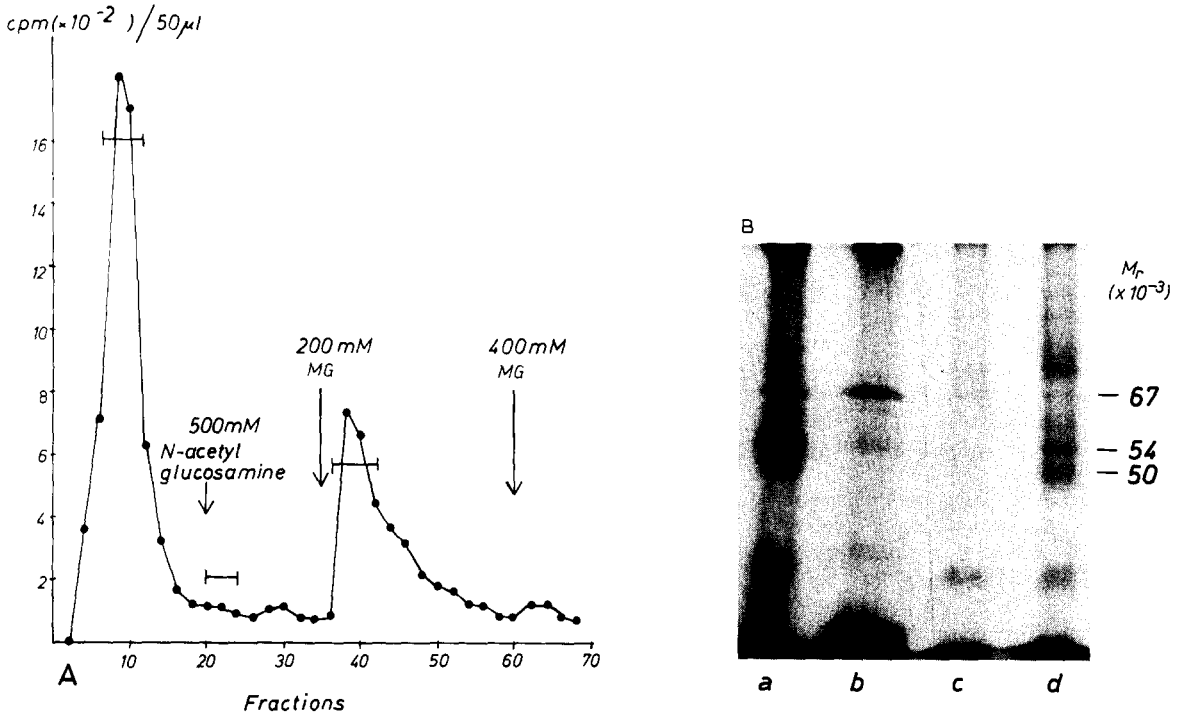

Fig. 4. Lentil lectin affinity chromatography of [^{3}H]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid labeled proteins in rat liver plasma membranes. (A) Elution profile of [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid labeled rat liver plasma membranes. Labeled rat liver plasma membranes were solubilized in 1% Triton X-100/20 mM Tris-HCl/500 mM NaCl (pH 7.4); chromatographed on lentil lectin columns; 2 ml fractions were collected, columns were initially washed with 20 mM Tris-HCl/0.5 M NaCl/0.05% Triton X-100/1 mM $CaCl_2$/1 mM $MgCl_2$/0.5 mM PMSF (pH 7.4) to elute unbound proteins. Then columns were eluted with the nonspecific monosaccharide *N*-acetylglucosamine (500 mM). Thereafter, the columns were eluted with 200 or 400 mM methyl α-D-glucoside (MG) or mannoside to desorb specifically bound glycoproteins. Shown is the distribution of radioactivity in the fractions collected. Of the applied radioactivity, 75% was found in the flow-through fraction. (B) Fluorogram of SDS-polyacrylamide gel electrophoresis of the indicated fractions from the lentil lectin column. The pooled fractions were prepared for electrophoresis as indicated under Methods. (a) Triton X-100 supernatant; (b) flow through; (c) unspecific elution; (d) specific elution.

proteins on concanavalin A-Sepharose gave the same results (Fig. 5). The recovery of the 50, 54 kDa proteins was poor after elution with competing monosaccharides. Therefore, tightly bound proteins were eluted by a buffer comprising 5 mM sodium acetate/200 mM NaCl/1 mM $CaCl_2$/1 mM $MgCl_2$/0.5 mM PMSF/0.05% Triton X-100 (pH 5.2). By this procedure, additional amounts of the 50 and 54 kDa proteins were found in the eluate. A 95–100 kDa protein was enriched in this fraction. As control for specific adsorption of the labeled proteins, affinity chromatography was performed in the presence of methyl α-D-mannoside. Lack of binding of labeled membrane components to any of the columns (concanavalin A or lentil lectin) demonstrated the absence of nonspecific adsorption.

In another series of experiments, labeled proteins were applied to a wheat-germ agglutinin-Sepharose column. The resulting elution profile resembled that of the concanavalin A and lentil lectin columns.

The 67 kDa protein was found quantitatively in the flow-through fraction of the column (Fig. 6). In the experiment shown in Fig. 6 some amounts of the 50 and 54 kDa proteins were not bound by the lectin; however, after rechromatography of the flow-through fraction on a second (wheat-germ lectin Sepharose) column, the flow-through fraction was almost free of 50 kDa protein. Only 15% of the 54 kDa protein was found in this fraction. After nonspecific elution, without any radioactivity in the fractions, specifically bound proteins were eluted by inclusion of *N*-acetylglucosamine

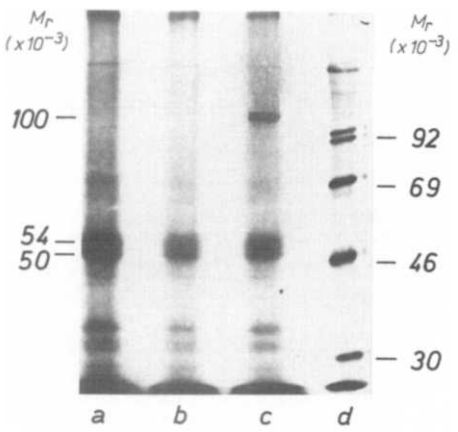

Fig. 5. Fluorogram of [^{3}H]isothiocyanatobenzamido cholate labeled proteins fractionated on concanavalin A-Sepharose. Proteins were separated on SDS slab gels after solubilization in 2% SDS and 5% mercaptoethanol. (a) Triton X-100 supernatant; (b) specific elution; (c) pH 5.2 elution; (d) marker proteins.

in the buffer. The 50 kDa protein was predominant, with small amounts of the 54 kDa protein also present (Fig. 6, lane d).

As a further control for the specificity of binding of labeled proteins to lectins we examined the binding of concanavalin A and wheat-germ lectin to unlabeled proteins in SDS-polyacrylamide slab

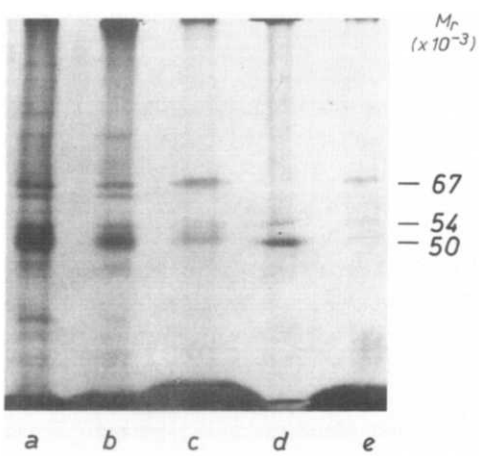

Fig. 6. Acrylamide electrophoresis fluorogram of [^{3}H]isothiocyanatobenzamido cholate labeled rat liver plasma membrane proteins fractionated on wheat-germ lectin Sepharose. (a) Labeled plasma membranes; (b) Triton X-100 supernatant; (c) flow through; (d) specific elution, second column; (e) flow through, second column.

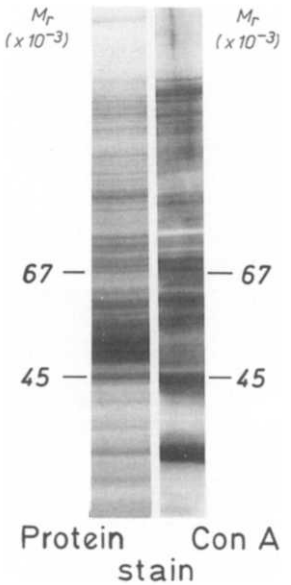

Fig. 7. Concanavalin-A-binding proteins in isolated rat liver plasma membranes. Isolated rat liver plasma membranes were separated on SDS acrylamide slab gels after solubilization in SDS and mercaptoethanol. One lane was stained with Coomassie blue (Protein stain) and one lane was blotted on nitrocellulose. The localization of glycoproteins was done by incubation of the nitrocellulose in a buffer containing concanavalin A, followed by horseradish peroxidase and a chromogenic peroxidase substrate (0.06% 4-chloro-1-naphthol/0.01% H_2O_2) (Con A stain).

gels. The separated proteins were transfered to nitrocellulose (blotting) and incubated with concanavalin A or wheat-germ lectin as described in Methods. These results demonstrate that concanavalin A and wheat-germ lectin binding glycoproteins of 54 and 50 kDa really do exist (apart from other binding proteins) in rat liver plasma membranes (Fig. 7).

TABLE II

BINDING SPECIFICITY OF IMMOBILIZED LECTINS

		Ref.
Concanavalin A	mannose	27,28
Lentil lectin	mannose	27,28
	fucose	30–32
Wheat-germ lectin	*N*-acetylglucosamine	33
	sialic acids	34

Discussion

Polypeptides of 54 and 50 kDa which can be labeled with [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid or [^{3}H]isothiocyanatobenzamidocholate are heterogeneous populations with respect to their carbohydrate content. Of the labeled polypeptides 10–20% were not retained by the immobilized lectins, even after repeated application. On the other hand, binding of 80–90% of these polypeptides to all lectins tested suggests that both types of N-linked oligosaccharides occur as carbohydrate residues [27–29]. Clearly, conclusions based on lectin binding must be interpreted with caution, since the specificities are complex and not strictly defined. Table II gives the binding specificities of the immobilized lectins used in our studies. Apparently, both high-mannose and complex type oligosaccharides with fucose and terminal sialic acid residues occur as carbohydrate moieties. It is well known that these types of oligosaccharide often occur simultaneously on the same glycoprotein [35,36].

According to Accatino [19], binding of cholate to plasma membranes is prevented by neuraminidase treatment. Transport inhibition using wheat-germ lectin provides further evidence for the requirement of sialic acids in cholate uptake. Addition of this agglutinin to isolated hepatocytes immediately stopped the uptake of cholate and also of phalloidin. The inhibition was prevented in the presence of the monosaccharide competitor, *N*-acetylglucosamine (25 mM). On the other hand, high concentrations (100 mM) of the competitor also blocked the uptake of cholate. The reason for this phenomenon remains unclear.

Concanavalin A failed to exert a rapid effect on the uptake of both substrates, even when added at very high concentrations. On the other hand, preincubation for 10–30 min reduced the uptake of bile acids and phalloidin. During the preincubation period concanavalin A binds to liver-cell surface membranes [37]. Receptors for concanavalin A have been found evenly distributed over the surface of liver cells [38,39]. Binding of concanavalin A to its own receptors restricts the mobility of other proteins [40]. The activity of the multispecific transporter might also be reduced. On the other hand, binding of concanavalin A to its own receptor (also a glycoprotein [41]) may cause steric hindrance [37] or changes in membrane fluidity [42]. The latter mechanism has been shown to reduce bile acid and phallotoxin transport [43].

The lectins used in the above uptake studies did not protect the transport proteins against affinity labeling. Even wheat-germ agglutinin, which immediately inhibits transport in isolated hepatocytes, could not prevent affinity labeling. This suggests that binding sites for the affinity label and for wheat-germ lectin are not identical. In intact liver cells, wheat-germ lectin inhibits transport by interaction with sialic acids or *N*-acetylglucosamine residues on the transporter protein or nearby, e.g., due to steric hindrance.

Affinity chromatography of [$^3H_2$]diisothiocyano-1,2-diphenylethane-2,2′-disulfonic acid- or [^{3}H]isothiocyanatobenzamido cholate-labeled proteins on lectin columns can not be used as a one-step purification procedure for the labeled proteins. The 67 kDa protein, presumably identical to albumin [15], was not retained. This protein was found almost completely in the flow through-fraction. Although most of the labeled proteins of 50 and 54 kDa were bound specifically to the concanavalin A column, only 40% were recovered by specific elution with methyl glucoside or methyl mannoside. Poor recovery with saccharide elution has also been reported for other glycoproteins [44].

Proteins in the range of 49–54 kDa have been recently isolated by affinity chromatography and have been incorporated into liposomes [45]. Na^+-dependent taurocholic acid uptake could be measured, demonstrating that the labeled proteins of 50 and 54 kDa are parts of the bile acid carrier.

By affinity chromatography of labeled proteins on lentil lectin- or concanavalin A-Sepharose, a labeled protein of 95–100 kDa was enriched either in the specific elution (lentin lectin) or in the pH 5.2 elution (concanavalin A). This protein might be identical to a bile acid transport protein, a glycoprotein, recently identified in canalicular plasma membranes [46]. Obviously, basolateral membranes prepared according to Touster are cross-contaminated by canalicular membranes.

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