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Azidobenzamido-008, a new photosensitive substrate for the 'multispecific bile acid transporter' of hepatocytes: evidence for a common transport system for bile acids and cyclosomatostatins in basolateral membranes

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Cyclo(-Phe(*p*-NH[1-^{14}C]Ac)-Thr-Lys-(CO(*p*-N_3)C_6H_4)-Trp-Phe-DPro), in the following named azidobenzamido-008, was synthesized in order to identify binding sites for c(Phe-Thr-Lys-Trp-Phe-DPro), named 008, (a cyclosomatostatin with retro sequence) in liver cell plasma membranes. In the dark the above photolabel was taken up into isolated hepatocytes, inhibiting the sodium dependent uptake of cholate and taurocholate in a competitive manner (K_i for cholate uptake inhibition = 1 μM; K_i for taurocholate uptake inhibition = 5 μM). When activated by flashed light the inhibition became irreversible (IC_{50} for cholate uptake inhibition = 2 μM; IC_{50} for taurocholate uptake inhibition = 9 μM) and the activated cyclopeptide bound chiefly to hepatocellular membrane proteins of 67, 54, 50, 37 kDa. Excess of the initial 008, or of cholate or phalloidin partially protected the above membrane components against labeling with ^{14}C-labeled azidobenzamido-008. In contrast AS 30 D ascites hepatoma cells, known to be deficient in bile acid and cyclosomatostatin transport, could not be specifically labeled by azidobenzamido-008. The membrane proteins preferentially labeled in hepatocytes (50 and 54 kDa) are integral glycoproteins. The 67 kDa protein is a hydrophilic nonglycosylated membrane component. Independent of labeling with ^{14}C-labeled azidobenzamido-008 or with ^{14}C-labeled azidobenzamido-taurocholate, the main radioactive peaks in the pH region of 7, 5.5, 5.25 were identical after solubilization with Nonidet P-40 and subsequent isoelectric focusing. Proteins of 67, 54, 50 and 37 kDa could be enriched by use of 008-containing gels in affinity electrophoresis. Binding sites for 008 were not destroyed by SDS or Nonidet P-40 treatment of plasma membranes.

Introduction

Various cyclic modifications of somatostatins, in particular such with retro sequences, protect liver cells against phalloidin [1]. They inhibit the inward transport of both bile acids and phalloidins in isolated hepatocytes in a competitive manner [1]. We suspected therefore that those cyclopeptides are transported by the sodium dependent bile salt carrier [2–5]. We proposed the term 'multispecific transporter' in order to describe the broad specificity of one of the bile acid carriers located at the basolateral membrane of liver cells [1].

Protein components of the latter could be identified by (photo)affinity labeling with reactive bile acid derivatives [6–11] and also with photolysable

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compounds derived from phalloidin and antamanide [12]. In this paper a new photolabel namely cyclo(Phe(*p*-NH[1-^{14}C]Ac)-Thr-Lys(CO(*p*-N_3) C_6H_4)-Trp-Phe-DPro) [13] is described, which binds to the same membrane proteins as do reactive derivatives of bile acids, phalloidin and antamanide. For the above photoaffinity label the term azidobenzamido-008 is used in the following because the corresponding cyclopeptide was introduced into earlier papers under the code number 008.

Materials

008, benzamido-008, acrylamidobenzamido-008, azidobenzamido-008, ^{14}C-labeled azidobenzamido-008 were synthesized as recently described [13]. [^{14}C]Cholate (spec. act. 1.85–2.2 GBq/mmol) and Amplify were purchased from Amersham Buchler, Braunschweig, F.R.G.; [^{3}H]taurocholate (spec. act. 74–185 GBq/mmol) from NEN, Dreieich, F.R.G.; mercaptoethanol, iodoacetamide and glycin from Merck, Darmstadt, F.R.G.; acrylamide, SDS and Tris from Serva, Heidelberg, F.R.G.; leupeptin from Sigma, Deisenhofen, F.R.G.; and benzamidine from Fluka AG, Buchs, Switzerland.

All other reagents used were of the highest quality commercially available.

Methods

Isolation of hepatocytes

Hepatocytes were isolated as described in Ref. 14. Livers were perfused for 15 min with 0.05% collagenase in 6.92 g/l NaCl, 0.82 g/l KCl, 0.29 g/l $MgSO_4$, 0.16 g/l KH_2PO_4, 2.1 g/l $NaHCO_3$ (pH 7.4). 85–90% of the cells were viable as determined by Trypan blue exclusion.

Isolation of AS 30 D ascites hepatoma cells

AS 30 D ascites hepatoma cells were isolated one week after intraperitoneal injection in Sprague-Dawley rats.

Bile acid and ^{14}C-labeled azidobenzamido-008 uptake measurements

Hepatocytes were suspended in Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 12 mM $NaHCO_3$, 1 mM glucose, 0.42 mM NaH_2PO_4) at a concentration of $2 \cdot 10^6$ cells/ml. Transport of ^{14}C-labeled azidobenzamido-008 was analyzed in the dark at 37°C in presence or absence of sodium. Energy dependence of transport was determined after 30 min incubation of hepatocytes in N_2/CO_2 atmosphere (Trypan blue exclusion: for O_2/CO_2, 90 ± 5%; for N_2/CO_2, 85 ± 5% viable cells).

Transport of cholate and taurocholate was measured in the dark at 37°C after a 30 s preincubation with varying concentrations of azidobenzamido-008 by adding a mixture of 1 μM [^{14}C]cholate or 1 μM [^{3}H]taurocholate plus increasing concentrations of the non-radioactive substrates. At the appropriate time, an aliquot (100 μl) of the cell suspension was rapidly centrifuged through a silicone oil layer [15]. The radioactivity associated with the cell pellet was measured in Lipoluma/ Lumasolve/ water in a Packard-Tricarb 2660 scintillation counter. Sodium-dependent uptake was determined by carrying out the transport assay in the presence and absence of sodium, where NaCl was replaced with choline chloride. The type of uptake inhibition was determined according to Lineweaver and Burk and the K_i value according to Dixon. Irreversible uptake inhibition was measured after 3 min preincubation of hepatocytes with increasing concentrations of azidobenzamido-008 at 37°C. The azido moiety was photolysed by a single UV flash. These irradiation conditions did not impair the structure and functions of hepatocytes (no change in the polypeptide pattern in SDS-gel electrophoresis; no reduction of cholate transport capacity: before UV flash 160 ± 34 pmol/mg per min; after UV flash 152 ± 69 pmol/mg per min). Unbound azidobenzamido-008 was removed by washing of the cells in Tyrode buffer. Thereafter the cells were analyzed for bile acid transport capacity as described. IC_{50} values were determined (concentration needed for 50% inhibition of substrate transport).

Isolation of rat liver basolateral plasma membranes

Plasma membranes were isolated according to Touster et al. [16] with some modifications as previously described [10]. Na^+/K^+-ATPase the marker enzyme for basolateral membranes was

enriched 28-fold, Mg^{2+}-ATPase the marker for canalicular membranes 2-fold compared with the crude homogenate. Minor contaminations of the plasma membrane fraction with intracellular membranes were obvious by a 2-fold enrichment of glucose-6-phosphatase. These determinations suggest that the membrane fraction arose primarily from the basolateral portion of the surface membrane.

Photoaffinity labeling with ^{14}C-labeled azidobenzamido-008

Photoaffinity labeling with ^{14}C-labeled azidobenzamido-008 (spec. act. 23 mCi/mmol) was done as described [17]. Protein concentration was

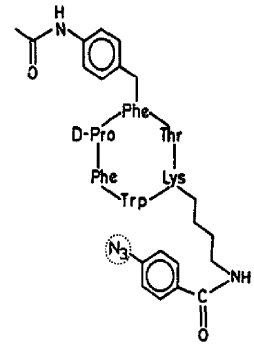

Fig. 2. Photosensitive 008 derivative. Shown is the structure of the photolabile analog.

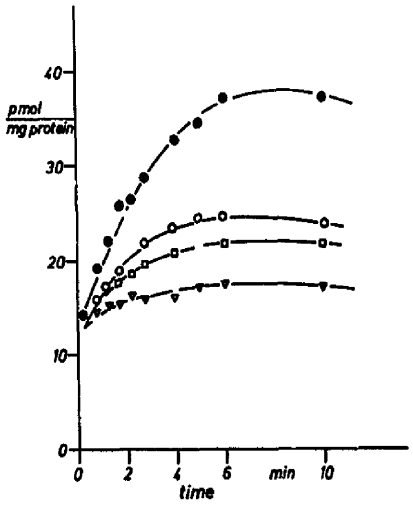

Fig. 1. Uptake of ^{14}C-labeled azidobenzamido-008. Isolated hepatocytes ($2 \cdot 10^6$/ml Tyrode buffer) were incubated in the dark at 37 °C with ^{14}C-labeled azidobenzamido-008 (400 nM). At the times indicated 100-µl aliquots were withdrawn and centrifuged through a silicon oil layer. The uptake (pmol/mg protein) was measured as described in Methods (●——●). The energy dependency of uptake was evaluated after preincubation of cells for 30 min in N_2/CO_2 atmosphere. Thereafter uptake of 400 nM of ^{14}C-labeled azidobenzamido-008 was measured (▽——▽). Sodium dependency was analyzed in a sodium free buffer, where sodium chloride was replaced by choline chloride (□——□). Transport inhibition by taurocholate (○——○) was determined by addition of 100 µM of taurocholate to $2 \cdot 10^6$ cells/ml 30 s before addition of 400 nM of ^{14}C-labeled azidobenzamido-008. Shown is the result of a typical experiment. The same experiments were done with three different cell preparations and comparable results.

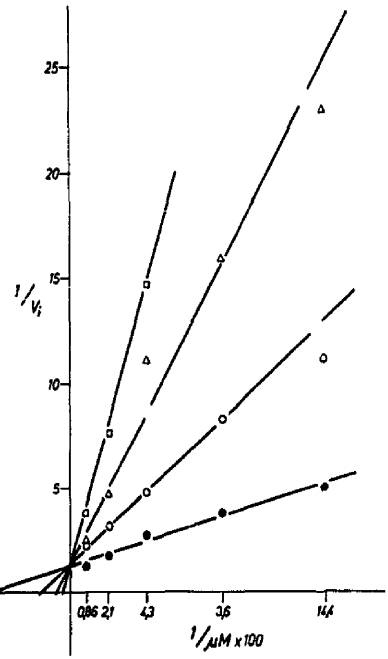

Fig. 3. Lineweaver-Burk plot of the sodium-dependent uptake of cholate in the presence of azidobenzamido-008. Isolated hepatocytes ($2 \cdot 10^6$/ml Tyrode or choline chloride buffer) were preincubated for 30 s in the dark with 0 (●——●); 1 (○——○); 5 (△——△); 10 µM (□——□) of azidobenzamido-008. Uptake measurements were started by addition of 1 µM [^{14}C]cholate plus 6.9, 11.5, 23, 46 and 115.2 µM of cholate. The initial uptake rate (v_i) was calculated for the sodium-dependent uptake and the results analysed on a double-reciprocal plot. K_m for cholate uptake was 27 ± 8 µM; V_{max} 1027 ± 209 pmol/mg per min ($n = 3$).

0.6 mg/ml, concentration of the label 2 μM. Hepatocytes or plasma membranes were preincubated for 3 min in the dark at 37°C before photolysis. Protein content was determined according to Lowry et al. [18]. Radioactive labeled proteins were identified after electrophoresis by fluorography using an intensifier fluorochrome, Amplify. Bound radioactivity was quantitated after slicing SDS rod gels.

Triton X-100, Nonidet P-40 and Triton X-114 solubilization and phase separation

Photoaffinity labeled plasma membrane proteins were solubilized in 1% Triton X-100 or Nonidet P-40 for 1 h at 4°C. By centrifugation at 100000 × g, solubilized proteins were separated from those which were insoluble. Triton X-114 phase separation was done as described [19]. Wheat germ lectin Sepharose affinity chromatography was performed as described earlier [20]. Isoelectric focusing in polyacrylamide rod gels was done as described previously [6].

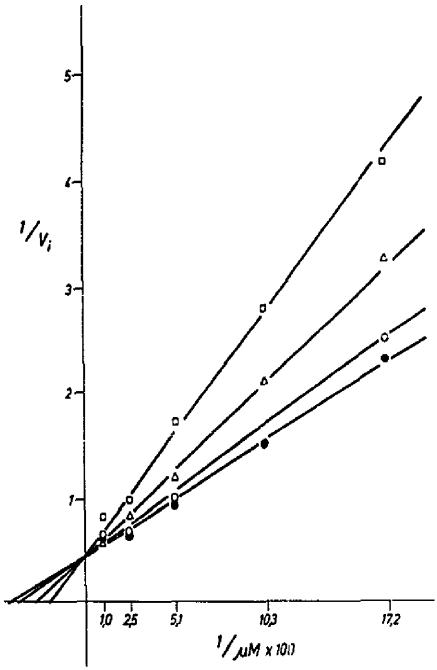

Fig. 4. Lineweaver-Burk diagram of the sodium-dependent taurocholate uptake in the presence of azidobenzamido-008. Isolated hepatocytes were incubated for 30 s with 0 (●——●); 1 (○——○); 5 (△——△) and 10 μM (□——□) of azidobenzamido-008 before addition of 1 μM of [^{3}H]taurocholate plus 5.8, 9.7, 19.4, 38.8 and 97 μM of taurocholate. The experiments were done either in Tyrode buffer (NaCl) or choline chloride buffer. The initial uptake rates were calculated within the period of linear uptake. The sodium dependency was determined and the data plotted according to Lineweaver and Burk. K_m for taurocholate uptake was 25 ± 2 μM; V_{max} 1929 ± 484 pmol/mg per min ($n = 3$).

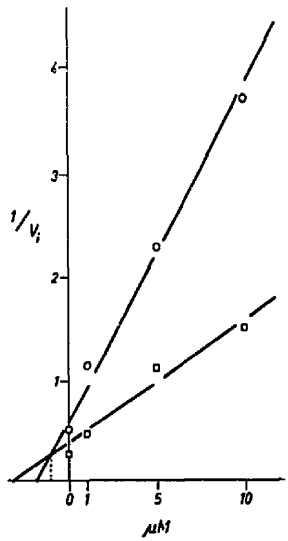

Fig. 5. Dixon plot of the inhibition of the sodium-dependent cholate uptake in the presence of azidobenzamido-008. The sodium-dependent uptake of cholate (6.9 (○——○) and 23 (□——□) μM) was measured in the dark (uptake in the presence minus uptake in the absence of sodium) in the presence of 1, 5 and 10 μM of azidobenzamido-008 at 37°C. The initial uptake rates were calculated and plotted according to Dixon ($n = 3$).

Affinity electrophoresis

Acrylamidobenzamido-008 was copolymerized in acrylamide-bisacrylamide gels. The copolymer content was 0.6%. A 10% SDS-acrylamide gel was cast (16.5 cm × 7 × 0.15 cm). On top the copolymerized affinity gel was poured (10% acrylamide with 0.6% 008 (2 cm high)) and then a 5% stacking gel, all containing 0.1% SDS. Plasma membranes were solubilized in SDS and 6 mg of solubilized membrane proteins were put on top of the gel. Electrophoresis was started at 50 V. After the bromophenol-blue band entered the separation gel electrophoresis was continued at 100 V.

After electrophoresis the affinity gel was cut into small pieces and the proteins were obtained by electroelution. After dialysis and lyophilisation proteins were separated again by SDS gel electrophoresis and visualized by silver staining. In other experiments proteins were solubilized with Nonidet P-40, 5% acrylamide separation gels and affinity gels contained 1% Nonidet P-40.

Results

Transport of ^{14}C-labeled azidobenzamido-008 into isolated rat hepatocytes

In order to demonstrate the usefulness of azidobenzamido-008 for labeling of transport proteins, it must be shown whether the label itself is taken up by hepatocytes. The uptake studies must be performed in the dark (dim light). As expected isolated hepatocytes took up ^{14}C-labeled azidobenzamido-008 in a time- and concentration-dependent manner ($K_m = 6 \pm 3$ μM; V_{max} 165 pmol/mg per min). Omission of sodium (replacement by choline chloride) reduced the transport capacity as well as preincubation of the cells in N_2 atmosphere (Fig. 1). The transport was inhibited in the presence of taurocholate (Fig. 1).

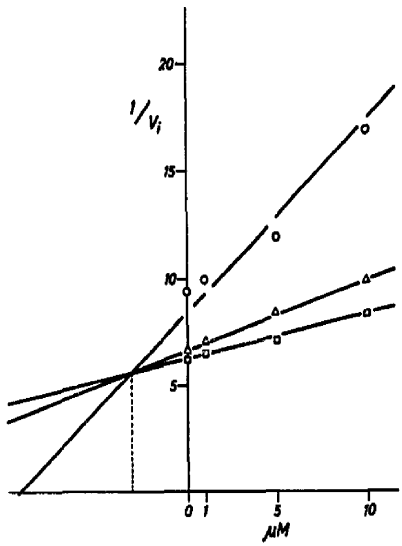

Fig. 6. Dixon plot of the inhibition of the sodium-dependent taurocholate uptake in the presence of azidobenzamido-008. The sodium-dependent taurocholate uptake (18.4 (○——○), 38.8 (△——△), 97 (□——□) μM) was measured in the dark in presence of 1, 5 and 10 μM of azidobenzamido-008. Initial uptake rates were determined and plotted according to Dixon ($n = 3$).

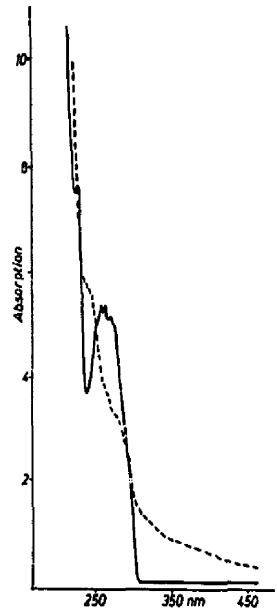

Fig. 7. The effect of a single UV flash on the absorption spectrum of azidobenzamido-008. Azidobenzamido-008 (20 μM in ethanol) was photolysed at room temperature with one UV flash. The absorption spectrum was recorded before (——) and after (- - -) photolysis. Under our experimental conditions, 80% of the azido derivative was converted to nitrene.

Reversible and irreversible inhibition of bile acid transport by azidobenzamido-008

To demonstrate that bile acids and cyclosomatostatins share a common transport system, the effect of azidobenzamido-008 on cholate and taurocholate transport into isolated hepatocytes was investigated.

In the dark azidobenzamido-008 (Fig. 2) inhibits both the sodium-dependent uptake of cholate (Fig. 3) and taurocholate (Fig. 4) in a competitive manner. The K_i value for cholate transport inhibition was calculated to be 1 μM (Fig. 5) and for taurocholate uptake inhibition 5 μM, respectively (Fig. 6). Azidobenzamido-008 was also a competitive inhibitor of the sodium-inde-

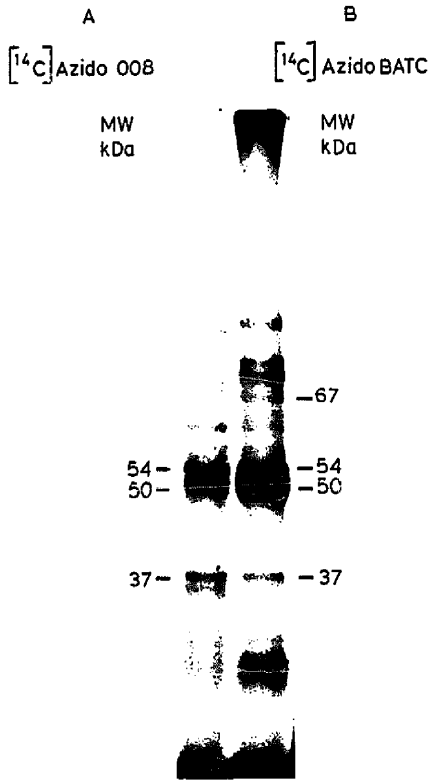

Fig. 8. Comparison of labeled proteins in isolated rat liver basolateral plasma membranes after photoaffinity labeling with ^{14}C-labeled azidobenzamido-008 (A) and with ^{14}C-labeled azidobenzamidotaurocholate (B). Isolated membranes (0.6 mg/ml) were flash photolabeled after 3 min preincubation in the dark with 2 μM of the photosensitive derivatives. Thereafter unbound label was removed by washing and proteins (100 μg) were separated by SDS slab gel electrophoresis. Labeled proteins were visualized by fluorography using Amplify as fluorochrome. Shown is the result of a typical experiment.

pendent uptake part of bile acid transport (data not shown).

After photolysis of the azido moiety of azidobenzamido-008 (Fig. 7) inhibition of bile acid transport became irreversible (IC_{50} for taurocholate transport inhibition 9 ± 2 μM and IC_{50} for cholate transport inhibition 3 ± 1 μM).

Photoaffinity labeling of isolated plasma membranes by ^{14}C-labeled azidobenzamido-008

Freshly prepared basolateral plasma membranes were photoaffinity labeled with ^{14}C-labeled azidobenzamido-008. The label preferentially binds to membrane proteins of M_r 54 and 50 kDa (highly reproducible in more than 50 labeling experiments) and to a lesser extent to a 67, 43 and 37 kDa protein. Labeling of proteins with molecular weight of 60 and 34 kDa is not reproducible and probably represents contaminations with intracellular membranes. A comparison of proteins labeled with bile acids or 008 shows binding of both substrates with slightly different affinity to proteins in the same molecular weight range, especially 50 and 54 kDa (Fig. 8). In the experiment shown, the bile acid label bound to ad-

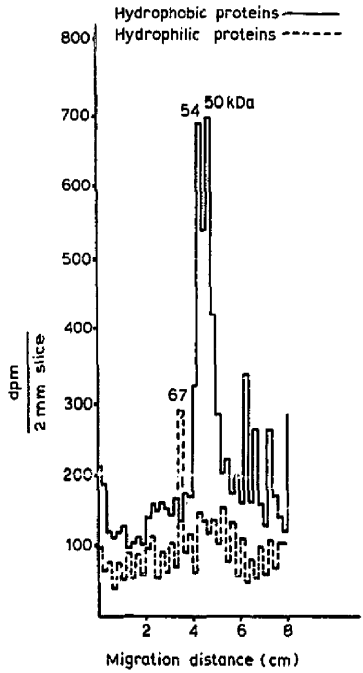

Fig. 9. Separation of hydrophilic and hydrophobic proteins by Triton X-114 phase separation. ^{14}C-labeled azidobenzamido-008 labeled plasma membranes were solubilized with Triton X-114 (1 h, 4°C). Insoluble proteins were removed by centrifugation at 100000×g for 30 min. The fraction of soluble proteins was phase separated as described [19]. Hydrophilic (water phase) and hydrophobic proteins (detergent phase) were subjected to SDS rod gel electrophoresis. The protein-associated radioactivity was quantitated by slicing the rod gels and counting the radioactivity in a Packard scintillation counter after incubation in Lipoluma/Lumasolve/water for 4 h at 40°C.

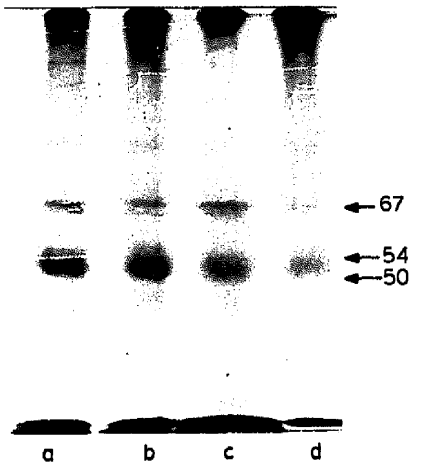

Fig. 10. Wheat germ Lectin Sepharose chromatography. Labeled proteins were solubilized in 1% Triton X-100 for 1 h at 4°C. The soluble protein fraction was obtained by 100000 × g centrifugation (60 min). This fraction was poured on a wheat germ Lectin Sepharose column. The chromatographic procedure was as described [20]. Shown is a fluorogram of an SDS-slab gel. Protein content is a–c: 100 μg; d: 50 μg. a, plasma membranes; b, Triton X-100 supernatant; c, flow through; d, elution.

ditional proteins of 70, 85 and 95 kDa. These proteins are not always identified [7–11]. The broad band in the molecular weight region of 20 kDa is a proteolytic degradation product. The radioactivity on top of the separation gel represents aggregated protein. This phenomenon has also been reported by others [7–11].

The labeled proteins of 50, 54 and 37 kDa are hydrophobic integral ones. They are only soluble in detergents like Triton X-100 or Nonidet P-40. This is a further parallelism to the behavior of bile acid labeled proteins [6,9–11]. The hydrophobic character of the 50, 54 and 37 kDa proteins was also shown by Triton X-114 phase separation. Those proteins were concentrated in the detergent phase (Fig. 9), whereas the labeled 67 kDa protein was enriched in the aqueous phase after separation. Bile acid labeled proteins behave in this manner [6,9–11].

The labeled proteins of 54 and 50 kDa are heterogeneously glycosylated as is also true for bile acid labeled proteins [20]. Part of these proteins bind to Wheat Germ Lectin-Sepharose (Fig. 10) and can be eluted with 500 mM *N*-acetylglucosamine. In the experiment shown small amounts of the labeled 67 kDa proteins are also eluted by

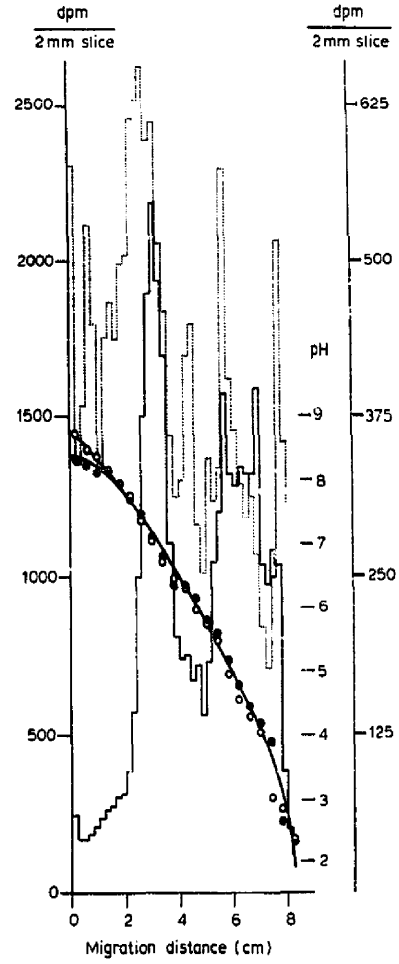

Fig. 11. Isoelectric focusing of ^{14}C-labeled azidobenzamido-008 and ^{14}C-labeled azidobenzamido-taurocholate labeled proteins. Isolated plasma membranes were photolabeled with the above analogs. After solubilization with Nonidet P-40, soluble proteins were subjected to isoelectric focusing in polyacrylamide rod gels. The proteins were focused 1 h at 200 V and further 15 h at 300 V. The distribution of radioactivity was estimated by slicing the gels and counting the radioactivity. Shown is the distribution of radioactivity and the pH gradient, measured in a gel without protein. dpm scala on the left side for 008 labeled proteins: dpm scala on the right side for bile acid labeled proteins.

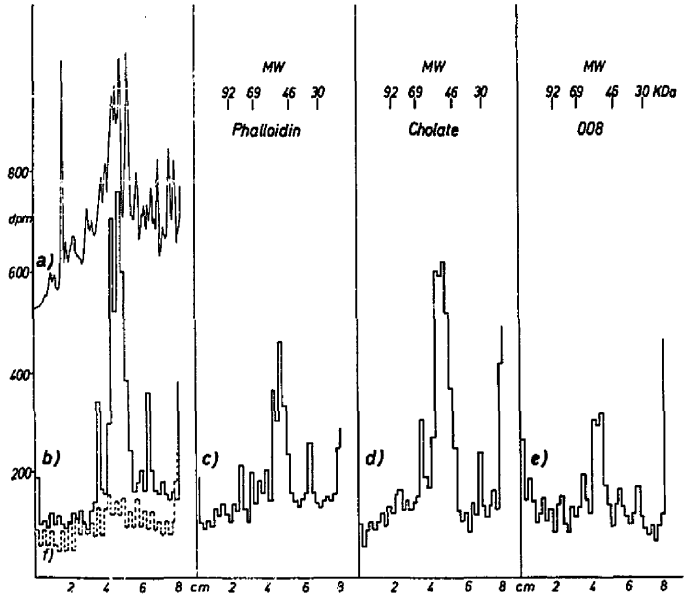

Fig. 12. Protection of ^{14}C-labeled azidobenzamido-008 photoaffinity labeling of isolated basolateral rat liver plasma membranes by substrates of the multispecific transporter. Labeling of AS 30 D ascites hepatoma cells. Isolated plasma membranes (0.6 mg/ml) or AS 30 D ascites hepatoma cells ($10 \cdot 10^6$/ml) were preincubated in the dark at room temperature for 3 min without or with a 100-fold molar excess of phalloidin (200 μM), 008 or cholate prior to photoaffinity labeling with 2 μM of ^{14}C-labeled azidobenzamido-008. Unbound label was removed by washing the membranes and cells in 20 mM Tris-HCl plus 100 μM phenylmethylsulfonyl fluoride, 5 mM EDTA and 5 μg/ml leupeptin. Proteins (100 μg) were separated on SDS rod gels (10% acrylamide). Quantitation of protein bound radioactivity was done by slicing the rod gels (2-mm slices) and counting the radioactivity. The protein pattern of basolateral membranes was analyzed after Coomassie blue staining. (a) Protein pattern of Coomassie blue-stained basolateral membranes. (b) Distribution of radioactivity in basolateral membranes photolabeled with 2 μM of ^{14}C-labeled azidobenzamido-008. (c) Protection of photoaffinity labeling was by 200 μM phalloidin. (d) 200 μM 008. (e) 200 μM cholate. (f) Photoaffinity labeling of AS 30 D ascites hepatoma cells.

the specific sugar as well as a 95–100 kDa protein, which was also found in bile acid labeled membranes [20].

To compare proteins labeled with bile acids or 008 further, those proteins were solubilized with Nonidet P-40 and were subjected to isoelectric focusing in polyacrylamide rod gels (Fig. 11). Main radioactive peaks of ^{14}C-labeled azidobenzamido-008 labeled proteins were detected in the pH region of 7, 5.5 and 5.25. Additional peaks at pH 4.5 and 3 represent the unbound label (no protein bands; peaks in these regions are also found after focusing the label in the absence of protein). ^{14}C-labeled azidobenzamido-taurocholate labeled proteins focused at pH values of 7.5, 7, 7.5, 5.5 and 5.2. The peaks at 8.2, 8 and 3.0 represent the free label only. This experiment reveals that both labels have affinity to proteins which focused to pH values of 7, 5.5 and 5.25.

Specificity of photoaffinity labeling

Cholate, phalloidin and 008 in 100-fold molar excess partially protect plasma membranes against photoaffinity labeling (Fig. 12). In accordance with the different affinity (K_m values) of cholate, phalloidin and 008 to the transport system, 008 exerts the strongest protective activity. AS 30 D ascites hepatoma cells, which neither transport bile acids nor cyclosomatostatins, are not specifically labeled (Fig. 12). Binding of ^{14}C-labeled azidobenzamido-008 to hepatocellular membrane proteins seems therefore to be specific.

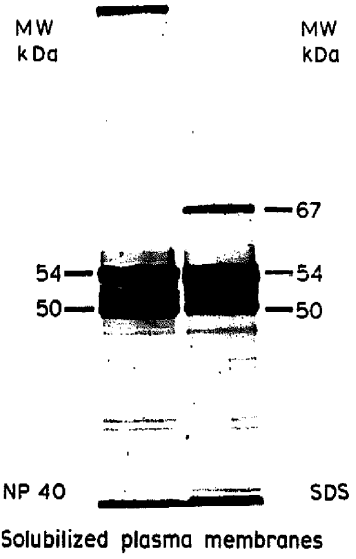

Fig. 13. Affinity electrophoresis. Copolymerised ligand, 008; reelectrophoresis in SDS. Isolated rat liver basolateral plasma membranes were solubilized for 1 h at room temperature either in 1% SDS or 1% Nonidet P-40. Soluble proteins were separated on a 008-affinity gel as described in Methods. After homogenization of the affinity gel, proteins were obtained by electroelution. Thereafter proteins were dialyzed and lyophilized and again separated on a SDS slab gel. Shown is a silver stained SDS slab gel.

Enrichment of the 67, 54, 50, 37 kDa proteins by affinity electrophoresis in gels containing copolymerized 008

To substantiate the results of photoaffinity labeling, SDS (or Nonidet P-40) solubilized proteins were separated by 008-affinity electrophoresis. In earlier studies with reactive bile acid derivatives, it was shown that solubilization by Triton X-100 did not destroy binding sites for bile acids [6]. Whether SDS destroys binding sites is unknown. Fig. 13 shows that proteins solubilized with SDS or Nonidet P-40 preserve their affinity to 008. Proteins in the above molecular weight range are enriched by the above method.

Discussion

Our earlier hypothesis that bile acids and somatostatins are both transported by a multispecific uptake system in isolated hepatocytes [1] is supported by the photoaffinity labeling studies described here. The photolabile derivative, azidobenzamido-008, is transported into hepatocytes by a sodium dependent transport system, which can be inhibited by taurocholate. Azidobenzamido-008 inhibits the sodium dependent (an independent) uptake of cholate and taurocholate in isolated hepatocytes in a competitive manner. The azido-derivative resembles its prototype 008, which competitively inhibited cholate and phalloidin uptake (the latter is a further substrate of the transporter [21,12]) and is also taken up by hepatocytes (detailed results to be published later).

^{14}C-labeled azidobenzamido-008 binds with slightly different affinity to membrane proteins in the same molecular weight range as photolabile bile acid analogs do. The ^{14}C-labeled azidobenzamido-008 labeled proteins posses identical physical properties as those labeled by bile acid derivatives. The 54 and 50 kDa polypeptides are integral hydrophobic membrane glycoproteins whereas the 67 kDa protein is hydrophilic and nonglycosylated. Membrane proteins either labeled with bile acids or with 008 have a p*I* of 7, 5.5 and 5.25 in isoelectric focusing experiments (in the case of bile acid labeled proteins two further peaks at 7.5 and 6.5 were found). Other possible substrates of the multispecific transporter in 100-fold molar excess partially blocked photoaffinity labeling with azidobenzamido-008. Phalloidin, a bicyclic heptapeptide which binds to the bile acid transporter [12] is a potent protector (55% inhibition).

Affinity electrophoresis in 008 containing gels demonstrated a specific interaction of membrane proteins with 008. Proteins of 67, 54, 50 and 37 kDa were enriched in 008 copolymerized gels. Apparently, during electrophoretic separation of solubilized proteins those with affinity to 008 were retained in the gel. Neither SDS nor Nonidet P-40 destroyed binding sites for 008. This was previously shown for other proteins [22,23].

The results presented are a further hint for a common transport system for bile acids and for certain cyclopeptides. Whether the transporter is composed of the labeled proteins only or contains additional unlabeled subunits can only be settled after isolation of the labeled proteins and recon-

stitution of the system. Recently, reconstitution of the taurocholic acid transport system was reported using proteoliposomes containing several membrane proteins in the range of 49–54 kDa [24]. To date reconstitution with single defined components has not been reported.

Whether the cyclosomatostatin and bile acid binding proteins possess identical amino acid sequences or represent a family of related proteins with nearly identical molecular weights (like the cytochrome *P*-450 system) remains unclear.

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