Intestinal Absorption of Peptides by Coupling to Bile Acids*

(Received for publication, July 23, 1993, and in revised form, December 21, 1993)

Werner Kramer†, Günther Wess, Georg Neckermann, Gerrit Schubert, Jürgen Fink, Frank Girbig, Ulrike Gutjahr, Simone Kowalewski, Karl-Heinz Baringhaus, Georg Böger, Alfons Enhsen, Eugen Falk, Michael Friedrich, Heiner Glombik, Axel Hoffmann, Christoph Pittius, and Matthias Urmann

From the Hoechst Aktiengesellschaft, D-65926 Frankfurt am Main, Germany

Poor intestinal absorption of peptides greatly limits their use as drugs for the treatment of chronic diseases. Since bile acids are efficiently absorbed by an active, Na*-dependent transport system in the ileum of mammals, model peptides of different chain length were attached to the 3-position of modified 3β-(ω-aminoalkoxy)-7α,12α-dihydroxy-5β-cholan-24-oic acid. These peptide-bile acid conjugates inhibited Na*-dependent [3H]taurocholate uptake into brush-border membrane vesicles isolated from rabbit ileum in a concentration-dependent manner. Furthermore, photoaffinity labeling of the bile acid-binding proteins of M, 93,000 and 14,000, identified as the protein components of the ileal Na*-dependent bile acid transport system in rabbit ileum (Kramer, W., Girbig, F., Gutjahr, U., Kowalewski, S., Jovenal, K., Mülle, G., Tripier, D., and Wess, G. (1993) J. Biol. Chem. 268, 18035–18046) by the photoactive taurocholate analogue, (3,3-azo-7α,12α-dihydroxy-5β[7β,12β]-3H-cholan-24-oyl)-2-aminoethanesulfonic acid, was inhibited by the peptide-bile acid conjugates. In contrast, the parent peptides and amino acids neither had a significant effect on [3H]taurocholate uptake by ileal brush-border membrane vesicles nor on photoaffinity labeling of the ileal bile acid-binding membrane proteins. The inhibitory effect of peptide-bile acid conjugates on [3H]taurocholate transport and photoaffinity labeling of the bile acid-binding proteins in rabbit ileal vesicles decreased with increasing chain length of the attached peptide radical. By in vivo ileum perfusion in anesthetized rats an intestinal absorption of the bile acid conjugate S3744 of the fluorescent oxaproylpeptide 4-nitrobenzo-2-oxa-1,3-diazol-β-Ala-Phe-5-Opr-Gly (S1037) and secretion of the intact compound into bile could be demonstrated, whereas the parent peptide S1037 or its β-butyrel S4404 were not absorbed. The intestinal absorption of S3744 showed a similar temperature dependence as [3H]taurocholate absorption and was inhibited by the presence of taurocholate indicating a carrier-mediated uptake of S3744 via the ileal bile acid transporter. In conclusion, these results indicate that oligopeptides can be made enterally absorbable by coupling to modified bile acid molecules making use of the specific intestinal absorption pathway for bile acids. This finding may be of great importance for the design and development of orally active peptide drugs.

* Part of this work has been published in preliminary form (55). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

‡ To whom correspondence should be addressed: SBU Metabolism, Hoechst Aktiengesellschaft, D-65926 Frankfurt am Main, Federal Republic of Germany. Tel.: 069-69-305-3357; Fax: 069-69-305-13333.

For the treatment of chronic diseases oral route is the most desirable method of drug administration. The medicinal importance of peptides as drugs will tremendously increase in the future due to the discovery of intelligent synthesis and screening strategies for biologically active peptides (1). A major limiting factor of using peptides as drugs is their poor oral availability and susceptibility to enzymatic hydrolysis (2, 3). A variety of membrane-bound peptidases in the brush-border membrane of the intestinal absorptive epithelial cells hydrolyze proteins and peptides to di- and tripeptides as well as amino acids, which are taken up by the enterocytes via specialized transport systems (4). Thus, the intestinal mucosa is a highly selective filter for the absorption of peptides and many strategies to improve their intestinal absorption including coating with polymers (5), usage of penetration enhancers (6), or co-administration with protease inhibitors (7, 8) have been applied. A more specific way to increase the intestinal permeability of peptides would be the “smuggling in” by coupling to a natural ligand which is absorbed by a specific transport pathway, either by receptor-mediated endocytosis as with vitamin B12 (9) or by a carrier mechanism. Our approach makes use of the enterohepatic circulation of bile acids involving the small intestine and the liver under physiological conditions (10). This ontogenousism of bile acids is expanded by the existence of a highly selective, Na*-dependent bile acid transport system in the plasma membrane of enterocytes and hepatocytes (11–14). The protein components of the membrane transporters for the uptake of bile acids by enterocytes (15–18) and hepatocytes (19, 20) have been identified with photolable bile acid derivatives (21–23). The high capacity of these transport systems recommends bile acids as a natural shuttling system to improve the intestinal absorption of poorly absorbable drugs and to deliver drugs selectively to the liver (24), the latter being demonstrated recently (25–27).

EXPERIMENTAL PROCEDURES

Materials

Photoaffinity labeling was carried out with the sodium salt of (3,3-azo-7α,12α-dihydroxy-5β[7β,12β]-3H-cholan-24-oyl)-2-aminoethanesulfonic acid (specific radioactivity 5.9 Ci/mmol) synthesized as described (21, 22). [3H]Taurocholic acid (specific radioactivity 2.1 Ci/mmol) was purchased from Du Pont-NEr (NEN Division, Dreieich, Germany). Acetylamide, N,N-bis(methylene) acrylamide, and marker proteins for the determination of molecular weights were from Sigma. Cellulose nitrate filters for transport measurements (25-mm diameter, 0.45-μm pore size, ME 25) were from Schleicher & Schuell (Dassel, Germany) and scintillators Quickszint 501, 361 and Unisolve I from Zinsser Analytic (Frankfurt, Germany). The kits (Mercokests) for the determination of the activity of the marker enzymes aminopeptidase N and γ-glutamyltranspeptidase were from Merck (Darmstadt, Germany). Serva Blue R-250 and all other materials for electrophoresis were from Serva (Heidelberg, Germany). All other substances were obtained from the usual commercial sources and were of analytical grade.
Chemical Synthesis

The fluorescent oxaprolylpeptides S4404, S1037, and their bile acid conjugates S3744 and S2831 were synthesized as described elsewhere (28-30).

α-Alanine Amino Acid and Peptide Derivatives—N-Protected α-alanine and (α-alanyl) peptides (n = 2-4) were synthesized by standard methods of peptide chemistry (31-33). N-Protection was either by the Boc (tert-butyloxycarbonyl) group or the Z (carbobenzyloxy) group. N-Protected (α-alanyl) (α-prolyl) peptides (n = 3, 6, 8; m = 1, 2) were synthesized by stepwise solid phase peptide synthesis (34) following an orthogonal protecting scheme throughout most of the chain assembly. The α-coupled Sarsin resin (35) was chosen as polymeric support and the β-coupled Fmoc (9-fluorenylmethoxycarbonyl) group was used as a temporary N-protection (36) except for the coupling of the N-terminal amino acid. In this case the Boc group was chosen for N-protection in order to enhance the solubility of the cleaved peptide at the subsequent reaction with bile acid. Coupling of the amino acids was done with diisopropycarbodiimide in the presence of 1-hydroxybenzotriazole in dimethylformamide. The completeness of the coupling reactions was monitored by the bromphenol blue method (37). Final cleavage of the N-protected peptide from the resin was by 1-2% trifluoroacetic acid in dichloromethane. The N-protected peptides were characterized by thin-layer chromatography, high performance liquid chromatography, and fast atom bombardment-mass spectrometry.

Synthesis of α-Alanyl Peptide-Bile Acid Conjugates—Compounds I-XV were synthesized by coupling of the N-protected α-alanine or α-alanyl-derivative peptide to the amino function of β-(2-aminoethoxy)phenylalanine-7α,12α-dihydroxy-5β-cholane-24-acid methyl ester (28-30). In the case of I-VIII, coupling was done via a linker strategy, utilizing either the N-hydroxysuccinimide or 1-hydroxybenzotriazole ester of N-protected α-alanine and of the N-protected α-alanyl peptide, respectively. Compounds I-XV were synthesized using diicyclohexylcarbodiimide or 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (98) in the presence of N-hydroxysuccinimide or 1-hydroxybenzotriazole as N-protecting ester of N-protected α-alanine and of the N-protected α-alanyl peptide, respectively. Deprotection of the N-protecting group was either done by acidolysis (in the case of Boc) or hydrogenation (in the case of Z), whereas the methylester group of the bile acid was cleaved under basic conditions. The products were purified by column chromatography and characterized by TLC and 'H NMR spectroscopy (100 MHz or 400 MHz).

Animals

Male Wistar rats (Tierzucht Hoechst AG, Kastengrund, Frankfurt am Main, Germany) weighing 300-400 g were maintained on a standard diet (Altromin®) with free access to water. Food was withdrawn 18 h prior to the ileal perfusion studies.

Preparation of Brush-border Membrane Vesicles

Brush-border membrane vesicles from the ileum of male white New Zealand rabbits (weighing 4-5 kg) were prepared by the Mg2+ precipitation method (39) as described previously (13, 18, 40, 41). The entire small intestine was removed, cut into 10 segments of equal length, numbered 1-10, proximal to distal. Segments 8-10 were used for the preparation of ileal brush-border membrane vesicles. The brush-border membranes were enriched (19 ± 4-fold) with regard to aminopeptidase N (EC 3.4.11.2) and (16 ± 7-fold) for γ-glutamyltransferase (EC 2.3.2.2) and free of contamination by other cell contributions as shown by enzymatic and immunological methods (18). Immediately after preparation, the vesicles were stored in liquid nitrogen without loss of transport and enzymatic activity for at least 4 weeks. The intactness of the vesicles was determined by measuring Na+-dependent α-glucose uptake after 15 s of incubation; usually the overshoot uptake at 15 s was greater than 20-fold. The enzymatic activities of aminopeptidase N and γ-glutamyltransferase were determined with Morcktest kits and protein was determined according to Bradford (42) using the Bio-Rad assay (Bio-Rad, München, Germany).

Transport Measurements

Uptake of radioiodinated substrates by brush-border membrane vesicles was determined by the membrane filtration method (39) as described previously (13, 17, 18, 40, 41). Typically, the transport reaction was initiated by adding 10 μl of the vesicle suspension (50-100 μg of protein) equilibrated with 10 μm Tris-Hepes buffer (pH 7.4), 300 mM mannitol and 90 μl of incubation medium containing the radioactively labeled substrate kept at 30°C. The composition of the incubation medium for measurements in the presence of a Na+ gradient usually was 10 μM Tris-HCl (pH 7.4), 100 mM NaCl, 100 mM mannitol and in the absence of a Na+ gradient, 10 μM Tris-HCl (pH 7.4), 100 mM KCl, 100 mM mannitol. For measurement of procholic uptake, these media contained 50 μM (0.75 μCi) [3H]taurocholate and for glucose uptake 19 μl (1 μCi) [U-14C]glucose. At desired time points, the transport reaction was terminated by the addition of 1 ml of ice-cold stop solution (10 μM Tris-Hepes buffer (pH 7.4), 150 mM KCl). The entire content was pipetted onto the middle of a prewashed, prechilled filter kept under suction with the aid of a vacuum controller. The filter was rinsed immediately with 5 ml of ice-cold stop solution and then solubilized in scintillator Quickzint 361. The radioactivity remaining on the filter was counted with standard liquid scintillation techniques. After correction of medium radioactivity bound to the filter in the absence of membrane vesicles and eventual chemiluminescence, absolute solute uptake was calculated and expressed as nanomole/mg protein. All experiments were performed in triplicate and uptake values are given as mean ± S.D.

Ileal Perfusion

Male Wistar rats were anesthetized with urethane (1.2 g/kg) and the biliary duct was cannulated with polyethylene tubing. Eight cm proximal to the ileocecal valve an incision was made into the ileum and a silicone tubing inserted. A second incision into the cecum was made and silicone tubing was passed through the ileocecal valve, fixed in place, and connected to a peristaltic pump. The loop was filled with buffer I (137 mM NaCl, 0.9 mM CaCl2, 0.51 mM MgCl2, 8.1 mM Na2HPO4, 2.7 mM KCl, 1.47 mM KH2PO4 (pH 7.4), 8% (v/v) ethanol, containing 1 μM concentrations of the respective test compounds usually at 37°C (12 or 4°C in some experiments). The volume of the whole system was 2 ml and the flow was adjusted to 250 μl/min. For determination of substance absorption, bile was collected over a period of 90 min and samples were taken from the perfusion medium and analyzed as the bile samples by thin layer chromatography.

Analysis of Bile Samples by Thin layer Chromatography

From the bile samples collected during the ileum perfusion experiments, 10-μl aliquots were applied onto high performance TLC plates (20 x 10 cm) together with the respective reference standards. Subsequently the chromatograms were developed using the following solvent systems: I, 1-butanol/water/acetic acid, 5/3/2 (v/v/v); II, 1-butanol/water/acetic acid, 9/2/1 (v/v/v); III, 1-butanol/water/acetic acid, 10/1 (v/v/v); IV, chloroform/methanol, 3/1 (v/v/v). The respective fluorescent compounds were visualized under UV light and densitometry at characteristic ultraviolet absorption at 510 nm using a densitometer CD 50 (DESAGA, Heidelberg, Germany). Quantification was achieved by calibration with definite amounts of the respective reference standards using either the linear or the zig-zag scan mode of the densitometer.

Photoaffinity Labeling

Photoaffinity labeling with photoreactive bile acids was performed as described previously (15, 21, 22, 43). Typically, 15 μl of brush-border membrane vesicles (150 μg of protein) equilibrated with 10 μM Tris-Hepes buffer (pH 7.4), 300 mM mannitol, were added in the dark to 185 μl of 10 mM Tris-Hepes buffer (pH 7.4), 100 mM NaCl, 100 mM mannitol containing the radiolabeled photoactive 3,3,5-azido-derivative of taurocholic acid and nonradioactively labeled putative inhibitors. After 5 min of preincubation, the suspensions were irradiated for 10 min at 350 nm in a Rayonet Photochemical Reactor RPR-100 (The Southern Ultraviolet Co., Hamden, CT) equipped with 16 RPR 3500-Å lamps. Afterwards, the suspensions were diluted with 1 ml of ice-cold buffer (10 μM Tris-Hepes buffer (pH 7.4), 300 mM mannitol) and centrifuged for 30 min at 48,000 x g. The supernatant was carefully removed and membrane proteins were precipitated (44). The dried membrane proteins were solubilized in 50 μl of 62.5 mM Tris-HCl buffer (pH 6.8), 2% SDS (w/v), 10% glycerol (v/v), 5% 2-mercaptoethanol (v/v), 0.001 bromphenol blue (w/v), and submitted to SDS-PAGE.

GeI Electrophoresis

SDS-PAGE was carried out in vertical slab gels (20 x 17 x 0.15 cm) using an electrophoresis System LE 2/4 (LKB Pharmacia Biotechnologie, Freiburg, Germany) as described (15, 18, 45). After staining with Serva Blue R 250, the gels were scanned with a densitometer CD 60 (Deutsche Analytische Laborgeräte) and the individual lanes were cut into slices of 1-1.5 cm thickness. Each slice was solubilized with 250 μl of tissue solubilizer Biolute S overnight and after addition of 4 ml of scintillator Quickzint 501 the

1 The abbreviations used are: Boc, tert-butyloxycarbonyl; NBD, 4-nitrobenzo-2-oxa-1,3-dioxol; Opr, 5-oxoproline, PAGE, polyacrylamide gel electrophoresis.
samples were counted for radioactivity. Additionally, radioactivity was detected by fluorography as described elsewhere (18, 45).

RESULTS AND DISCUSSION

Design of Peptide-Bile Acid Conjugates—For optimal recognition of a bile acid molecule by the active Na⁺-dependent bile acid transport systems in the liver and in the small intestine, a negative charge in the side chain of the bile acid molecule and at least one hydroxyl group in positions 3, 7, or 12 of the steroid moiety is a prerequisite (12, 18, 46–49). Photoaffinity labeling with a bile acid derivative lacking the 12α-oriented hydroxyl group, 7,7-azo-3α,12α-dihydroxy-5β[12α-3H]-cholane-24-oic acid revealed the significance of the 12α-hydroxy group for optimal recognition of a bile acid by the ileal bile acid transporter (18) explaining the low ileal absorption of 12β-hydroxy bile acids such as lagodeoxycholic acid (50). In previous studies we could show that drug-bile acid conjugates with attachment of the respective drug to the 3-position of the steroid nucleus are recognized like natural bile acids by the bile acid transport systems of the liver (25, 27). Consequently, peptides of different chain length were covalently attached to the 3-position of linker-modified bile acids (Fig. 1) thoroughly considering the structural requirements of bile acids for optimal molecular recognition by the intestinal transporter (12, 18, 46–49). Peptides containing t-amino acids were chosen as model substrates to avoid enzymatic hydrolysis of the peptide backbone by brush-border enzymes. In order to investigate mainly the influence of the peptide chain length on the molecular interaction of the peptide-bile acid conjugates with the ileal bile acid carrier system(s) and to avoid other effects, e.g., effects of functional side chains or ordered conformations, no trifunctional amino acid, but D-alanine as the simplest optically active amino acid was chosen. When synthesizing longer homo-alanyl peptides, it is necessary to introduce a different amino acid into the peptide backbone to avoid ordered conformations as they are known for homo-oligopeptides. In the case of polyalanyl peptides, a transition from random-coil to β-structure occurs for chain lengths of n = 6 in most organic solvents and in water (51). Furthermore, homo-alanyl peptides containing 10–20 alanyl residues
are highly helical (52, 53). The helical structure leads to poor coupling yields at the stepwise formation of homo-alanyl peptides using solid phase peptide synthesis (52). To avoid these difficulties D-proline was introduced at position 2 (XI), 4 and 8 (XII-XV) of the peptides for α-proline is known to disrupt peptide-a-helices (54).

Interaction of Peptide-Bile Acid Conjugates with the Ileal Na*-dependent Bile Acid Transport System—Molecular recognition of the peptide-bile acid conjugates with the ileal Na*/bile acid co-transport system was investigated by measuring the effect of these compounds and of the corresponding free oligopeptides on the uptake of [3H]taurocholate by brush-border membrane vesicles prepared from rabbit ileum. Fig. 2 shows that peptide-bile acid conjugates led to a concentration-dependent inhibition of [3H]taurocholate uptake, whereas the peptides alone had no significant inhibitory effect on bile acid uptake. Attachment of peptide radicals greater than 4 amino acid residues resulted in a significant drop in the affinity of the peptide-bile acid conjugates to the ileal bile acid transport system as indicated by an increase of the IC50, IC50, or IC50 values (Table I).

To evaluate whether the synthesized peptide-bile acid conjugates are recognized by the ileal Na*-dependent bile acid transport system, their interaction with the protein components of this transport system was investigated by photoaffinity labeling using (3,3-azo-7a,12α-dihydroxy-5β,7β,12β-[3H]cholan-24-oyl)-2-aminoethanesulfonic acid, a photolabile taurocholate analogue (21) being modified by the photoreactive diazirino (azi) group in the 3-position as in the peptide-bile acid conjugates and behaving as taurocholate during enterohepatic circulation (21). Photoaffinity labeling of the bile acid-binding proteins of M, 93,000 and 14,000 which have been identified as the essential protein components of the Na*/bile acid co-transporter from rabbit ileum (17, 18), was inhibited by the peptide-bile acid conjugates, whereas the free peptides did not show a significant inhibitory effect on photoaffinity labeling of these bile-acid-binding proteins (Fig. 3). The labeling of the integral bile-acid-binding membrane protein of M, 87,000 thought to be involved in membrane bile acid transport by a facilitated transport system (18) was also inhibited by peptide-bile acid conjugates. These transport and photoaffinity labeling studies demonstrate a specific interaction of the peptide-bile acid conjugates with the ileal bile acid carrier system(s).

In Vivo Ileal Absorption of Peptide-Bile Acid Conjugates—In order to investigate the intestinal absorption of peptide-bile acid conjugates in a living animal, peptide-bile acid analogues containing additionally a fluorescent reporter group (NBD, Fig. 1) were studied in an in vivo ileum perfusion model. After cannulation of the common bile duct, the respective compounds were instilled as a 1 mM solution into a closed loop ileal segment and recirculated at a flow rate of 0.25 ml/min. Bile was collected over a period of 90 min and samples were taken from the collected bile and the perfusion medium for analysis by thin layer chromatography. Since the intestinal absorption is the rate-limiting step during enterohepatic circulation of bile acids, the appearance of bile acids in bile can be used to determine the intestinal absorption of bile acid derivatives.

As a model compound for these investigations we have chosen S3744, where the NBD-modified tetrapeptide β-Ala-Phe-Opr-Gly was linked to the 6-atom spacer modified cholic acid derivative 3β-(5-aminopentoxy)-7α,12α-dihydroxy-5β-cholan-24-oic acid. To detect an intestinal absorption of NBD-β-Ala-Phe-Opr-Gly in S3744 by making use of the ileal bile acid
transport system distinct from an absorption by other mechanisms, the intestinal absorption of the parent peptide S1037 and a lipophilic prodrug thereof, the t-butylerster S4404, was also investigated in an identical experimental setup. Neither the parent peptide S1037 (Fig. 4A) nor its t-butylerster S4404 (Fig. 4B) appeared in bile and furthermore, also no fluorescent fragments of these compounds possibly generated by luminal, membrane, or intracellular hydrolysis could be detected in bile. These findings clearly indicate that peptides S1037 and S4404 are not absorbed by the rabbit ileum. In contrast, after instillation of the bile acid conjugate S3744 into an ileal segment, the intact peptide-bile acid conjugate was secreted into bile with a secretion profile similar to a natural bile acid (Fig. 4C) with a secretion maximum after 12–16 min compared with 8–12 min for taurocholate. In addition, a metabolite M₁ was secreted into bile with a secretion profile parallel to the parent compound S3744. Thin layer chromatographic analysis of bile and perfusion medium probes in different solvent systems proved the identity of M₁ detected in bile after ileal perfusion with S3744 with M₁ formed intraluminally from S3744, S1037, and S4404. The exact structure of M₁ is not known but presumably is a derivative of NBD-β-Ala-Phe-Opr. Control experiments with M₁ isolated from the perfusion medium revealed that M₁ did not inhibit [3H]taurocholate uptake into ileal brush-border membrane vesicles. Furthermore, since the metabolite M₁ is formed intraluminally from compounds S3744, S1037, and S4404 and since no M₁ appears in bile after ileal perfusion with peptides S1037 and S4404, it can be excluded that the amounts of M₁ detected in bile after ileal perfusion with S3744 arise from an intestinal absorption of M₁. The M₁ detected in bile after ileal perfusion from S3744 is formed from S3744 during liver passage as could be shown by liver perfusion experiments after bolus injection of S3744 into a peripheral mesenteric vein (25). Within a collection period of 80 min, around 1.1% of the applied amount of S3744 was secreted into bile in intact form and a further 1.15% as metabolite M₁ giving a resorption rate of >2.25% for S3744 within the collection period. Analysis of the perfusion medium revealed that S3744 was intraluminally rapidly degraded to metabolite M₂; after 20 min around 30% of S3744 remained as intact bile acid conjugate S3744 within the intestinal lumen. Since the metabolite M₂ is not transferred from the intestinal lumen into bile to a significant extent, an ent transport process, uptake of ligands by this transport system, had to be considered a prerequisite for bile acid absorption. However, bile acids cannot be absorbed by the rabbit ileum, and therefore, the transport system investigated is not responsible for bile acid absorption.

Since the ileal uptake of bile acids is an active, Na⁺-dependent transport process, uptake of ligands by this transport system should greatly depend on temperature. Therefore, in vivo ileal perfusion experiments were carried out where the ileal segment was placed into an external bath kept at 12 or 4 °C and
CONCLUSIONS

In order to evaluate the active, Na+-dependent ileal bile acid uptake system as a means for the intestinal absorption of peptides, a series of small linear model peptides up to a chain length of 10 amino acids were covalently coupled to the 3β-position of modified bile acid yielding peptidyl-3β-(ω-aminoalkoxy)-7α,12α-dihydroxy-5β-cholan-24-oic acids. These compounds were able to interact with the ileal Na+/bile acid co-transport system as was shown by a concentration-dependent inhibition of Na+-dependent [3H]taurocholate uptake into brush-border membrane vesicles from rabbit ileum. Photoaffinity labeling of the protein components of M, 93,000 and 14,000 of the ileal bile acid transport system (17, 18) by (3,3-azo-7α,12α-dihydroxy-5β[7β,12β]3Hcholan-24-oyl)-2-aminoethanesulfonic acid was concentration-dependent inhibited by the peptide-bile acid conjugates. In contrast, the corresponding parent peptides neither inhibited taurocholate transport nor photoaffinity labeling of the transport proteins. The affinity of the peptide-bile acid conjugates to the ileal bile acid carrier system decreased with increasing chain length of the model peptide. By in vivo ileal perfusion experiments it could be shown that linear oligopeptides with modification of the N terminus by the fluorophore NBD (in size approximately corresponding to the molecular volume of the amino acids tyrosine or tryptophan) became intestinally absorbed after coupling to the 3β-position of a 5-atom linker-modified bile acid in contrast to the peptide itself which was not transported. A strong temperature dependence of uptake of the peptide-bile acid conjugate S3744 and its secretion into bile as well as an inhibition of uptake by taurocholate strongly argues for a carrier-mediated ileal uptake via the Na+/bile acid co-transport system. After replacement of the 4-atom spacer (NH2-CH2-CH2-CO) of β-alanine in the bile acid conjugate of NBD-β-Ala-Phe-0pr-Gly (S3744) by a 13-atom linker in 12-aminododecanoic acid (NH2-CH2-12CO) with conservation of all other structural elements, no significant transport of the corresponding bile acid conjugate S2831 from the intestinal lumen into bile could be detected. Since β-alanine was replaced by the lipophilic amino fatty acid 12-aminoendecanoic acid, it is possible that the increased lipophilicity of the peptide backbone in S2831 leads to a sticking of the molecule to biological membranes. In S3744, an 18-atom backbone made of a tetrapeptide and an aminopentyl-spacer group attached to the 3β-position of cholic acid was inserted between the NBD-reporter group and the bile acid molecule leading to a significant intestinal absorption of the peptide-bile acid conjugate. Since the space-filling size of the (NBD-β-Ala-Phe-0pr-Gly-NH2-(CH2)n)-ω-moity attached to cholic acid corresponds approximately to that of a linear heptapeptide, it seems probable that modified bile acid molecules can be used as a "hook-up" system to improve the intestinal absorption of therapeutically useful oligopeptides via the active Na+-dependent bile acid transport system in the ileum. These findings may be a first step to overcome the obstacle of nonabsorbable biologically active peptides and be of great importance for the development of orally active peptide drugs.

Acknowledgments—We thank Susanne Winkler and Silke Elsasser for excellent secretarial assistance.
REFERENCES


5. Saffran, M., Kumar, D., Deliuery Rev. 4, 171–207.


