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Identification of identical binding polypeptides for cephalosporins and dipeptides in intestinal brush-border membrane vesicles by photoaffinity labeling

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The uptake of a photolabile derivative of the orally effective cephalosporin cephalexin, *N*-(4-azidobenzoyl)cephalexin, was investigated in brush-border membrane vesicles. The compound was taken up into the intravesicular space and inhibited the active uptake of cephalexin in a concentration-dependent manner. Therefore, this probe interacts with the transport system shared by α-aminocephalosporins and dipeptides. Photoaffinity labeling of brush-border membrane vesicles from rat small intestine with *N*-(4-azido[3,5-^{3}H]benzoyl) derivatives of the cephalosporin cephalexin and the dipeptide glycyl-L-proline resulted in the covalent incorporation of radioactivity into membrane polypeptides with apparent molecular weights of 127000, 100000, 94000 and 86000, the polypeptide of molecular weight 127000 being predominantly labeled. The specificity of labeling was demonstrated by a decrease in the labeling of the polypeptide of apparent molecular weight 127000 in the presence of β-lactam antibiotics and dipeptides, whereas glucose, taurocholate or amino acids had no effect on the labeling pattern. These data demonstrate an interaction of cephalosporins and dipeptides with a common membrane protein of molecular weight 127000, which could be a component of the intestinal transport system(s) responsible for the uptake of orally effective cephalosporins and dipeptides.

Introduction

Small peptides are taken up across the intestinal brush-border membrane by carrier-mediated transport processes [1–4] and a common mechanism for the uptake of di- and tripeptides has been found [5]. Aminocephalosporins – β-lactam antibiotics of low lipid solubility – are also efficiently absorbed from the small intestine by carrier-mediated uptake systems [6–16]. The uptake of dipeptides as well as of aminocephalosporins has been extensively studied using intestinal brush-border membrane vesicles [2,4,14–27]. The uptake of both classes of compounds was found to be Na^+-independent [14–16,19,22,25,27], in contrast to the Na^+-dependent transport systems for amino acids [28–30], and it was suggested that the uptake both of dipeptides and of aminocephalosporins is driven by an inwardly directed H^+-gradient [14,15,26,27,31,32]. Furthermore, evidence has been presented that dipeptides and aminocephalosporins share the same transport system(s) [9,13–15].

However, the actual molecular mechanisms of intestinal absorption of dipeptides and cephalosporins are still far from clear. The membrane

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proteins interacting with these substrates have not been identified. A promising approach to the molecular characterization of binding proteins is photoaffinity labeling [33–35]. Therefore, photoaffinity labeling studies have been performed with small-intestinal brush-border membrane vesicles using photolabile derivatives of an orally effective cephalosporin and a dipeptide.

Experimental procedures

Materials

N-Hydroxysuccinimidyl-4-azido[3,5-^{3}H]benzoate (48.9 Ci/mmol) was purchased from New England Nuclear (Dreieich, F.R.G.) and unlabeled *N*-hydroxysuccinimidyl-4-azidobenzoate was from Pierce (Rockford, IL, U.S.A.). Amino acids, taurocholate and dipeptides were from Serva (Heidelberg, F.R.G.). β-Lactam antibiotics were obtained from Sigma (München, F.R.G.). All other substances were from commercial sources and of highest purity available.

Animals

Male Wistar rats (Tierzuchthaltung Hoechst, Frankfurt/Main, F.R.G.) weighing 200–250 g and maintained on a standard diet and tap water ad libitum were used for all studies.

Synthesis of N-(4-azido[3,5-^{3}H]benzoyl)cephalexin

90 μl (90 μCi) of *N*-hydroxysuccinimidyl-4-azido[3,5-^{3}H]benzoate in isopropanol were transferred to a 1.5 ml polyethylene tube and dried in the vacuum. After evaporation of the solvent, 10 μl of dry dioxane was added and subsequently 20 μl of a 50 mM solution of cephalexin in 100 mM sodium phosphate buffer (pH 7.4) was vigorously mixed with the dioxane solution. The reaction mixture was kept at 20 °C in the dark for 12 h and subsequently put on an HPTLC-plate (20 × 5 cm, Merck, Darmstadt, F.R.G.). The chromatogram was developed using *n*-butanol/acetic acid/water (9/2/1, v/v) as solvent [36]. The plate was dried under vacuum and the distribution of radioactivity was determined with a radiochromatogram scanner LB 2842 (Berthold, Wildbad, F.R.G.). The plate was sprayed with water, the radioactive band with the desired band was scraped off and the substance was eluted with 700 μl of methanol. Overall yield was 70 μCi (77%). R_F values: (*n*-butanol/acetic acid/water = 9 : 2 : 1), 0.49; (chloroform/methanol/acetic acid = 100 : 10 : 1), 0.16.

For transport studies, unlabeled *N*-(4-azidobenzoyl)cephalexin was synthesized as follows. 500 μl of a freshly prepared 100 mM solution of *N*-hydroxysuccinimidyl-4-azidobenzoate (13 mg) in dry dioxane was added in 100 μl portions during 8 h to 2 ml of a freshly prepared 50 mM solution of cephalexin in 50 mM sodium phosphate buffer/dioxane (1 : 1, v/v). After further 20 h of stirring the solvent was evaporated and *N*-(4-azidobenzoyl)cephalexin was purified by flash chromatography (37) on a 15 × 1 cm column with chloroform/methanol/acetic acid (100 : 10 : 1, v/v) as solvent. The fractions containing the pure product were combined and evaporated to dryness. Yield: 50–60%. Ultraviolet (ethanol): λ_{max} = 210 nm ($\varepsilon = 2.4 \cdot 10^4$), 270 nm ($\varepsilon = 1.6 \cdot 10^6$). ^{1}H-NMR (CDCl$_3$/TMS): δ = 2.66 (C(3)CH$_3$, 3H, s); 3.02, 3.38 (C(2)CH$_2$-, 2H, dd, J = 18 Hz); 5.68 (C(6)H, 1H); 5.97 (C(7)H, 1H); 7.01 (CH-arom-N$_3$, 2H, d, J = 8 Hz); 7.32 (C$_6$H$_5$-, 5H, s); 7.83 (CH-arom-CONH, 2H, d, J = 8 Hz). Infrared spectrum: 2120 cm^{-1} (-N$_3$), 1775 cm^{-1} (gb-lactam), 1710 cm^{-1} (CO-NH).

Synthesis of N-(4-azido[3,5-^{3}H]benzoyl)glycyl-L-proline

The synthetic procedure is identical to that for the cephalexin derivative, with the exception that a 100 mM solution of glycyl-L-proline in sodium phosphate buffer was used. Overall yield: 73 μCi (81%). R_F values: (*n*-butanol/acetic acid/water = 9 : 2 : 1, v/v), 0.39; (chloroform/methanol/acetic acid = 100 : 10 : 1, v/v), 0.21.

Both compounds were stored in methanolic solution at −20 °C in the dark at a concentration of 0.1 μCi/μl. Under these storage conditions no change of the compounds occurred within 4 weeks.

Isolation of small-intestinal brush-border membrane vesicles

Brush-border membrane vesicles from rat small intestine were prepared by the Mg^{2+}-precipitation method as described [38] and further purified by KSCN treatment [39]. The enrichment of the specific activities of the brush-border membrane

marker enzymes aminopeptidase M (EC 3.4.11.2), γ-glutamyltransferase (EC 2.3.2.2) and sucrase (EC 3.2.1.48) was 25 ± 6, 23 ± 5 and 22 ± 5, respectively. Protein was determined according to Bradford [40] using the Bio-Rad kit (München, F.R.G.). Aminopeptidase M and γ-glutamyltransferase were tested using the Merckotest kits 3359 and 3394 (Merck, Darmstadt, F.R.G.), whereas sucrase was tested according to Dahlqvist [41].

Uptake measurements

Uptake in brush-border membrane vesicles was measured by the membrane-filtration technique [42,43]. The composition of the media is given in the legends to figures. Transport studies were carried out at 30 °C and individual tubes were used for each time point. Usually, 20 μl of the vesicle suspension (100 μg) were pipetted as a drop on the wall of disposable polystyrene tubes (11 × 70 mm) containing 180 μl of medium with the respective ligands. Transport was initiated by stirring on a Vortex mixer for 2 s. At the desired time, transport was stopped by addition of 1 ml of ice-cold stop solution (150 mM KCl/10 mM Tris-Hepes (pH 7.4)). This mixture was immediately pipetted onto the middle of a pre-wetted cellulose nitrate filter (HAWP 0.45 μm, Millipore, Eschborn, F.R.G.) kept under suction (25–35 mbar) and the filter was washed with 5 ml of ice-cold stop solution. For uptake measurements with radioactively labeled compounds the filters were dissolved with 4 ml of Quickszint 361 (Zinsser Analytic, Frankfurt, F.R.G.) and radioactivity was determined by liquid scintillation counting. For uptake measurements with unlabeled substrates a disc of 1 cm diameter was cut from each filter and the substrates were extracted with 300 μl of water [44]. 200 μl of each probe were analyzed by high-pressure liquid chromatography using a Waters chromatography system (M 740 data module, M 490 detector, M 680 automated gradient controller, M 501/510 HPLC pumps, M 712 automated sample processor, Waters Instruments, Eschborn, F.R.G.) on a Bischoff 250 × 4 mm column (Bischoff, Leonberg, F.R.G.) filled with LiChrosorb RP 18, 7 μm (Merck). Cephalexin was analyzed with 16.4% acetonitrile/83.6% 30 mM sodium phosphate buffer (pH 7.0) as eluent and ultraviolet-detection at 262 nm, whereas L-carnosine was eluted with 30 mM sodium phosphate buffer (pH 7.0) and detection at 210 nm. The flow rate in all experiments was 1 ml/min.

Photoaffinity labeling

Prior to photoaffinity labeling the desired amount of the photolabile derivatives was dried in the vacuum and redissolved in 10 mM Tris/Hepes buffer (pH 7.4)/150 mM NaCl. For photoaffinity labeling, 200 μg of freshly prepared brush-border membrane vesicles were suspended in a total volume of 200 μl in 10 mM sodium phosphate buffer (pH 7.4)/150 mM NaCl and incubated with 1.5–2 μCi of the photolabile derivatives for 5 min in the dark at 20 °C. This incubation mixture was transferred to cell culture dishes (30 mm diameter) and photolyzed for 20 s at 254 nm in a Rayonet RPR 100 photochemical reactor (The Southern Ultraviolet Company, Hamden, CT, U.S.A.) equipped with 16 RPR 2530 Å lamps at a distance of 15 cm from the lamps. After photoaffinity labeling, the vesicles were washed with 1.5 ml of 10 mM sodium phosphate buffer (pH 7.4)/4 mM PMSF/4 mM iodoacetamide/4 mM EDTA [45] and centrifuged at 48000 × *g* for 30 min.

SDS-polyacrylamide gel electrophoresis

The pellets containing the photolabeled brush-border membranes were resuspended in 200 μl of water and delipidated as described [46]. The dried protein precipitates were dissolved in 40 μl of 62.5 mM Tris-HCl buffer (pH 6.8)/2% SDS/10% glycerol/ 5% 2-mercaptoethanol/ 0.001% Bromophenol blue. After boiling for 5 min, the probes were submitted to discontinuous SDS-gel electrophoresis on 0.7 mm × 20 cm × 15 cm gels as described [47]. The Coomassie blue stained gels were scanned at 595 nm with a Densitometer CD-50 (DESAGA, Heidelberg, F.R.G.). Radioactivity was determined by liquid scintillation counting after cutting the gels into 2-mm slices and after digestion of proteins with Biolute (Zinsser).

Results and Discussion

Photolabile derivatives of cephalosporins and dipeptides

Aminocephalosporins and dipeptides are taken up carrier-mediated across the intestinal brush-

border membrane by a common transport system [9,13–15]. In order to identify cephalosporin- and dipeptide-binding proteins in the brush-border membrane by photoaffinity labeling, the photoreactive derivatives used must be resistant to hydrolysis by brush-border enzymes. Otherwise, labeling of other proteins than the cephalosporin and dipeptide binding proteins may occur. Since cephalexin – an orally effective cephalosporin – is taken up into intestinal brush-border membrane vesicles by a carrier-mediated transport process [14,15], the photoreactive 4-azido[3,5-^{3}H]benzoyl substituent was attached to the cephalexin molecule. To allow a comparison of the cephalosporin-binding proteins with the dipeptide-binding proteins of the intestinal brush-border membrane, the dipeptide glycyl-L-proline, which shows a remarkable resistance to hydrolysis by brush-border enzymes [19,22,24,25], was also modified to a photosensitive *N*-(4-azidobenzoyl) derivative.

The effect of intestinal brush-border enzymes on the photoreactive analogues of cephalexin and glycyl-L-proline was investigated by thin-layer chromatography after incubation of the respective compounds with brush-border membrane vesicles. Fig. 1 shows that both derivatives are resistant to hydrolysis by intestinal peptidases and remain unchanged. This is an important prerequisite for the identification of cephalosporin- and dipeptide-binding proteins of the brush-border membrane. Photolysis of both compounds in the presence of albumin resulted in a clear photogenerated incorporation of radioactivity into the albumin molecule (data not shown). The photolysis of the azido group in both derivatives was complete within 20 s under the conditions used, and a half-life time of about 3.5 s was determined (Fig. 2). Therefore,

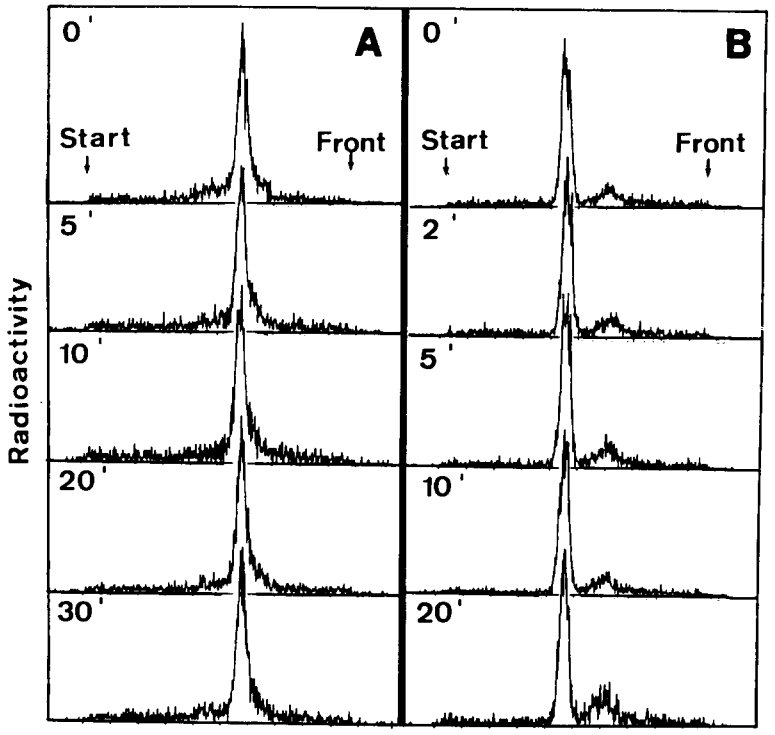


Fig. 1. Effect of intestinal brush-border membrane vesicles on photolabile derivatives of cephalexin and glycyl-L-proline. 1 μCi of *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin (A) or *N*-(4-azido[3,5-^{3}H]benzoyl)glycyl-L-proline (B) in 60 μl of 10 mM Tris-Hepes buffer (pH 7.4)/150 mM NaCl were incubated at 20 °C with 100 μg of small-intestinal brush-border membrane vesicles. At definite time points (in minutes) 10-μl aliquots were removed and protein was precipitated with 20 μl of methanol. After centrifugation the clear supernatant was put on HPTLC plates. The chromatograms were developed in *n*-butanol/acetic acid/water (9:2:1, v/v) as solvent. Radioactivity was detected with a radiochromatogram scanner.

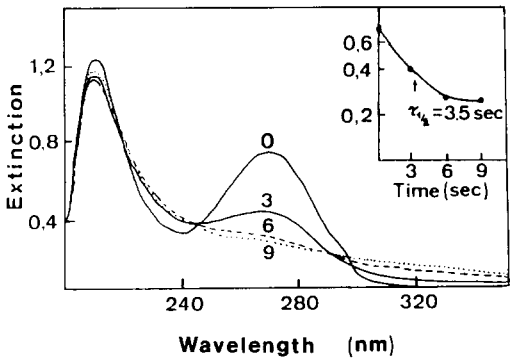


Fig. 2. Photolysis of *N*-(4-azidobenzoyl)cephalexin. A 50 μM solution of *N*-(4-azidobenzoyl)cephalexin in ethanol was photolyzed at 254 nm for 0, 3, 6, 9 and 12 s in a Rayonet photochemical reactor equipped with 16 RPR 2530 A lamps with recording of ultraviolet spectra after photolysis.

photoaffinity labeling experiments were performed at 254 nm for 20 s. These irradiation conditions did not impair the brush-border membranes, as was shown by two experiments: (a) the polypeptide pattern in SDS-gel electrophoresis did not change, and (b) the enzyme activities of the brush-border marker enzymes were unchanged.

Interaction of N-(4-azido[3,5-^{3}H]benzoyl)cephalexin with the intestinal transport system for α-aminocephalosporins and dipeptides

Prior to the detection of putative transport proteins by photoaffinity labeling it must be demonstrated that the photolabile derivative interacts with the transport system for the 'natural' substrate. To determine whether the photolabile cephalosporin derivative was taken up into small-intestinal brush-border membrane vesicles by the transport system shared by α-aminocephalosporins and dipeptides [9,13–15], transport experiments were performed under red lighting. As shown in Fig. 3, *N*-(4-azidobenzoyl)cephalexin showed a rapid uptake by brush-border membrane vesicles. The uptake of both *N*-(4-azidobenzoyl) cephalexin (Fig. 3B) and cephalexin (Fig. 3A) was stimulated by an inward H^+ gradient ($[pH]_o$ = 6.0, $[pH]_i$ = 7.4). To ascertain that the uptake of cephalexin and its photolabile derivative represents transport rather than membrane binding the osmotic sensitivity of the uptake of cephalexin and *N*-(4-azidobenzoyl)cephalexin into brush-border membrane vesicles was determined. Fig. 4

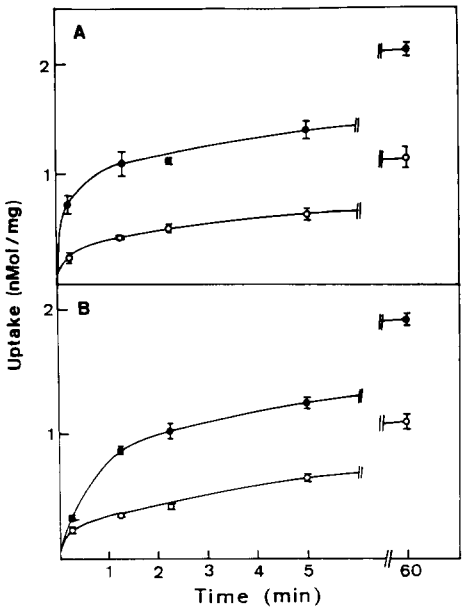


Fig. 3. Uptake of cephalexin (A) and *N*-(4-azidobenzoyl)-cephalexin (B) by small-intestinal brush-border membrane vesicles. Intestinal brush-border membrane vesicles (100 μg/20 μl) loaded with 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol were incubated at 30 °C with 180 μl of the following media with a concentration of 1 mM cephalexin (A) or 100 μM (0.22 μCi) *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin: (a) 10 mM Tris-Hepes buffer (pH 7.4)/140 mM NaCl (○); (b) 10 mM citrate-Tris buffer (pH 6.0)/140 mM KCl (●).

shows that the equilibrium uptake of both compounds was inversely proportional to medium osmolarity, demonstrating that both cephalexin and *N*-(4-azidobenzoyl)cephalexin were transported through the brush-border membrane into the intravesicular space. Extrapolation to infinite osmolarity yielded a positive *y*-intercept representing membrane binding. Under the conditions usually used (300 mosM), the binding of cephalexin was 73% and that of *N*-(4-azidobenzoyl)cephalexin 72%.

To demonstrate that *N*-(4-azidobenzoyl)cephalexin and cephalexin share a common transport system, the effect of *N*-(4-azidobenzoyl)cephalexin on the uptake of the orally effective cephalosporin cephalexin and the dipeptide L-carnosine was investigated. Fig. 5A and B show that the uptake of both compounds was significantly inhibited by the photolabile cephalosporin derivative. Increasing concentrations of *N*-(4-azidobenzoyl)cephalexin in the incubation media led to an increasing inhibi-

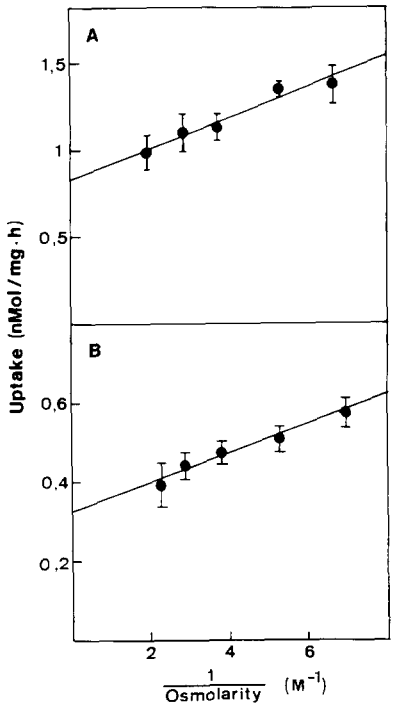
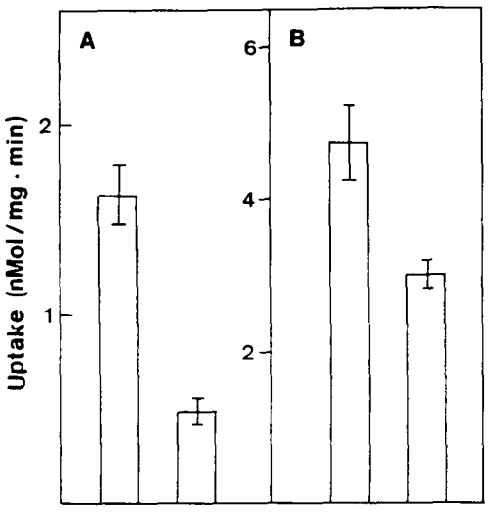


Fig. 4. Effect of medium osmolarity on the uptake of cephalexin (A) and *N*-(4-azidobenzoyl)cephalexin (B) by small-intestinal brush-border membrane vesicles. Small-intestinal brush-border membrane vesicles (100 μg, 20 μl) loaded with 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol were incubated at 30 °C for 60 min with 180 μl of medium containing either 1 mM cephalexin (A) or 100 μM (0.2 μCi) *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin (B) in 10 mM sodium phosphate buffer (pH 7.4) and varying amounts of cellobiose to attain the desired medium osmolarity.



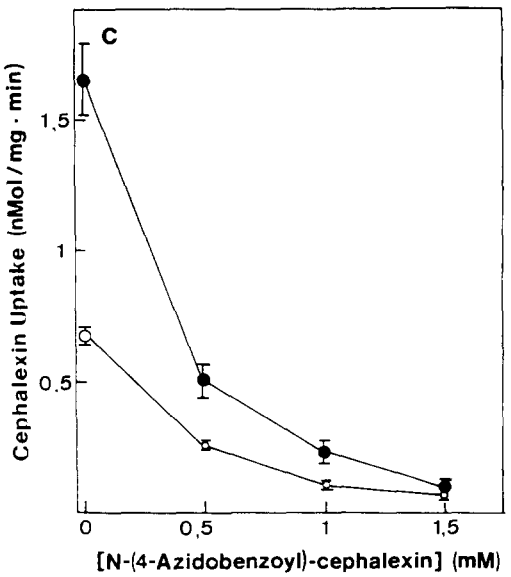


Fig. 5. Effect of *N*-(4-azidobenzoyl)cephalexin on the uptake of cephalexin and L-carnosine. Small-intestinal brush-border membrane vesicles (100 μg, 20 μl) loaded with 10 mM Tris-Hepes buffer (pH 7.4)/300 mM mannitol were incubated at 30 °C for 1 min with 180 μl of 10 mM citrate-Tris buffer (pH 6.0)/140 mM KCl containing: (A) 0.5 mM cephalexin either in the absence (left bar) or in the presence of 0.5 mM *N*-(4-azidobenzoyl)cephalexin (right bar); (B) 1.5 mM L-carnosine either in the absence (left bar) or in the presence of 0.5 mM *N*-(4-azidobenzoyl)cephalexin (right bar); (C) 0.5 mM (○) or 1.5 mM (●) mM cephalexin in the presence of 0, 0.5, 1 and 1.5 mM *N*-(4-azidobenzoyl)cephalexin.

tion of cephalexin uptake (Fig. 5B). 400 μM of *N*-(4-azidobenzoyl)cephalexin produced half-maximal inhibition (I_{50}) of the H^+-dependent cephalexin uptake.

These transport studies indicate that *N*-(4-azidobenzoyl)cephalexin interacts with the same transport system in the small-intestinal brush-border membrane as orally effective α-aminocephalosporins which are taken up by the dipeptide transport system [9,13–15]. *N*-(4-Azidobenzoyl)cephalexin is therefore a suitable probe for the identification of the putative intestinal transport system(s) for α-aminocephalosporins and dipeptides.

Identification of binding proteins for cephalosporins and dipeptides by photoaffinity labeling

In order to identify binding polypeptides for cephalosporins in the intestinal brush-border

membrane, freshly prepared membrane vesicles were incubated with the photolabile *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin and submitted to photoaffinity labeling. Subsequently, the brush-border membrane vesicles were washed and proteins were separated by discontinuous SDS-gel electrophoresis. Fig. 6 shows that photoaffinity labeling with *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin led to a clear incorporation of radioactivity into membrane polypeptides with apparent molecular weights of 127 000, 100 000, 94 000 and 86 000, the polypeptide of molecular weight 127 000 being predominantly labeled. With the photolabile dipeptide analogue, *N*-(4-azido[3,5-^{3}H]benzoyl)glycyl-L-proline, a very similar distribution of radioactivity was obtained (Fig. 7). With both photolabile derivatives, the distribution of radioactivity along the gel is not identical with the distribution of polypeptides, demonstrating a specific labeling of membrane proteins. Repeated experiments revealed that these labeling patterns were highly reproducible. Control experiments with hydroxysuccinimidyl-4-azido[3,5-^{3}H]benzoate as photo-

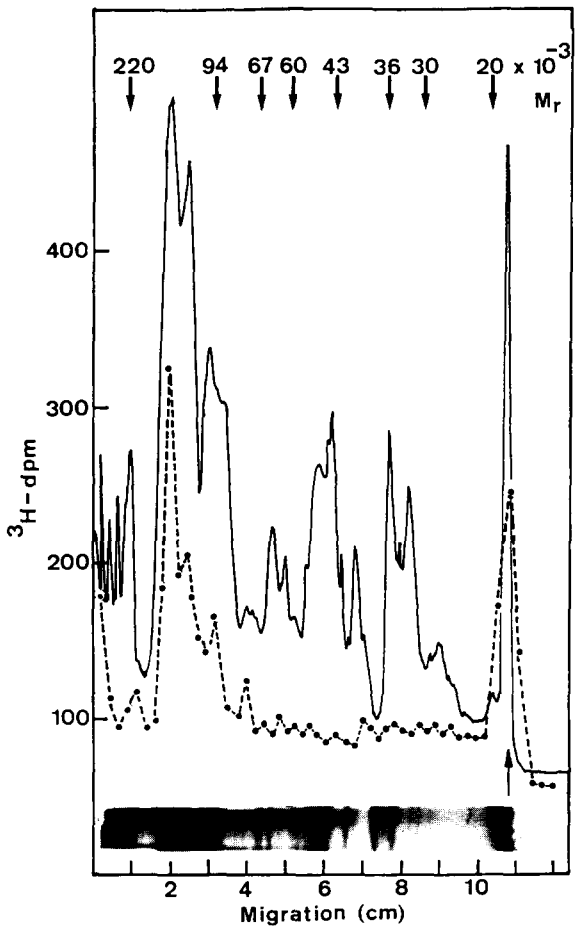


Fig. 6. Distribution of radioactivity after SDS-gel electrophoresis of small-intestinal brush-border membrane vesicles after photoaffinity labeling. 200 μg of brush-border membrane vesicles were photolabeled with 0.16 μM (1.72 μCi) of *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin. Total acrylamide concentration was 9%. The solid line shows the densitometer tracing of the Coomassie-blue-stained polypeptides, whereas the dotted line indicates the distribution of radioactivity. The position of the molecular weight marker proteins (M_r) and of the tracking dye, Bromophenol blue, are indicated by arrows.

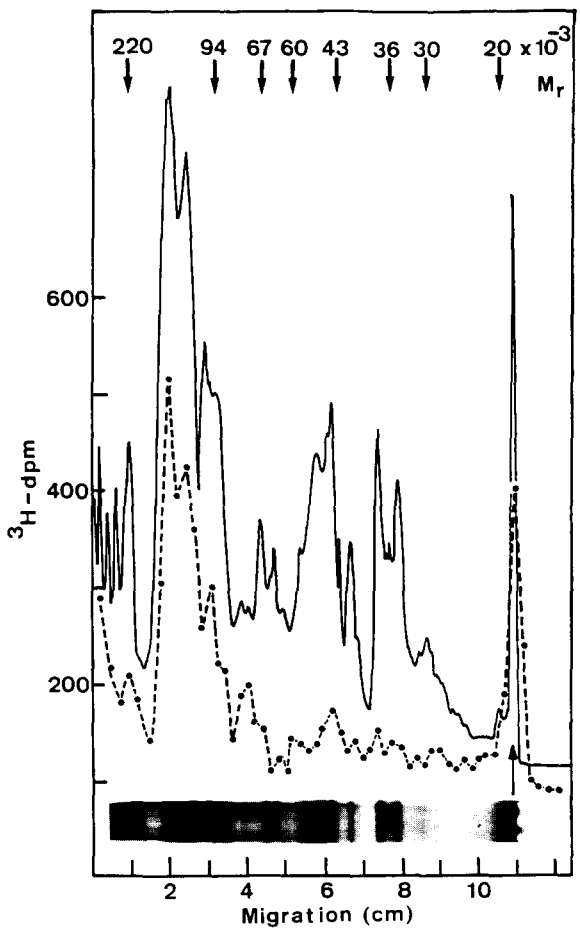


Fig. 7. Distribution of radioactivity after SDS-gel electrophoresis of small-intestinal brush-border membrane vesicles after photoaffinity labeling. 200 μg of brush-border membrane vesicles were photolabeled with 0.18 μM (1.8 μCi) of *N*-(4-azido[3,5-^{3}H]benzoyl)glycyl-L-proline. For all other conditions see legend to Fig. 6.

label resulted in an unspecific labeling of all membrane proteins and incubation of the membrane vesicles with the *N*-(4-azido[3,5-^{3}H]benzoyl) derivatives of cephalexin and glycyl-L-proline in the dark did not result in a covalent labeling of membrane proteins. Photoaffinity labeling at 4°C had no effect on the labeling pattern.

The specificity of labeling was further investigated by labeling experiments in the presence of various intestinally absorbed substances. Amino acids do not share the intestinal transport system for dipeptides [23,24] or α-aminocephalosporins [15]. The presence of the amino acids glycine or alanine had no effect on the labeling pattern, as would be expected (Fig. 8). The intestinal uptake systems for D-glucose and for bile acids have been identified by photoaffinity labeling, and membrane polypeptides of molecular weights 72000 (for glucose) and 99000 (for bile acids) have been shown to be components of these Na^+-dependent transport systems [47,48]. The presence of these substrates during photoaffinity labeling with photoreactive cephalosporin and dipeptide analogues had, as expected, no effect on the labeling of membrane polypeptides (Fig. 8). However, the presence of cefadroxil or cephalexin – α-aminocephalosporins taken up carrier-mediated into brush-border membrane vesicles [14–16] – resulted in a clear concentration-dependent decrease in the extent of labeling, especially on the polypeptide of molecular weight 127000, as is shown in Fig. 9. This demonstrates the specificity of this protein for the binding of cephalosporins and dipeptides. Additional experiments revealed that various β-lactam antibiotics (cephalexin, cefadroxil, ampicillin, amoxicillin, penicillin G) and dipeptides (L-carnosine, glycyclglycine, glycyl-L-proline) decreased the labeling of the polypeptide of apparent molecular weight 127000 in a concentration-dependent manner. All these substances are absorbed in the small intestine. Substrates which are also absorbed, but not accepted by the dipeptide transport system, e.g., sugars, amino acids and bile salts, had no influence on the extent of labeling. It is evident that the binding protein for cephalosporins and dipeptides with apparent molecular weight 127000 is different from the known binding proteins of the small-intestinal brush-border membrane for D-glucose (M_r 72000–75000), bile salts (M_r 99000) and the imino acid, L-proline (M_r 100000), which have been identified by labeling techniques [47–50].

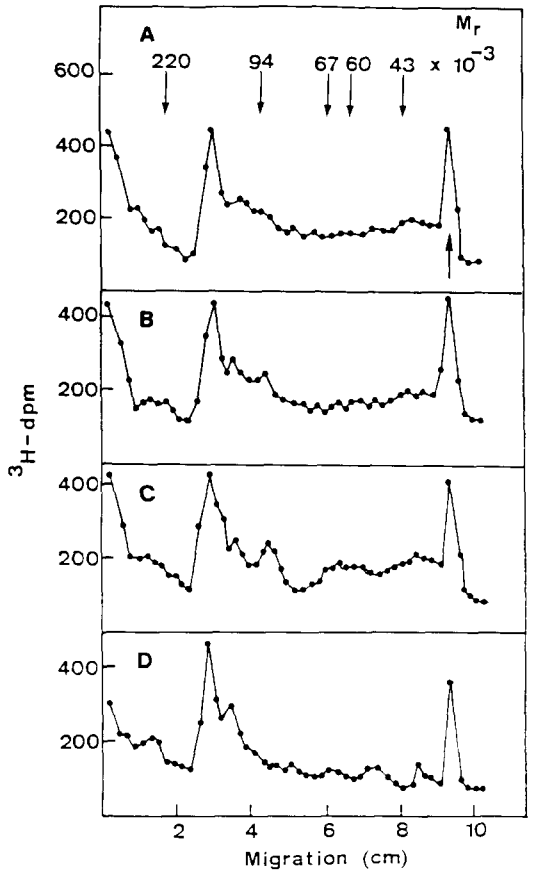
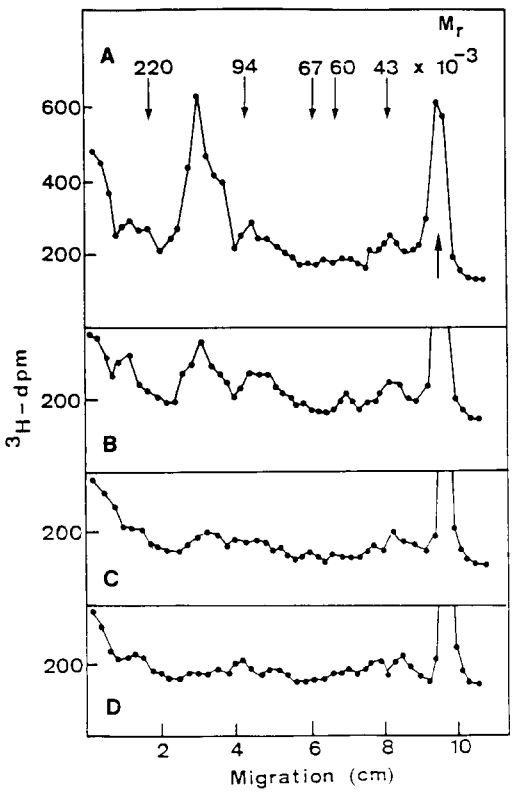


Fig. 8. Photoaffinity labeling of brush-border membrane vesicles with *N*-(4-azidobenzoyl)cephalexin in the presence of various intestinally absorbed substances. 200 μg of brush-border membrane vesicles were photolabeled with 0.16 μM (1.72 μCi) of *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin in the absence of inhibitors (A) and in the presence of 625 μM of L-alanine (B), D-glucose (C) or taurocholate (D). Total acrylamide concentration was 7%.

In conclusion, the experimental results presented here demonstrate for the first time that orally effective cephalosporins and dipeptides bind to an identical membrane polypeptide with a molecular weight of 127000 in the brush-border membrane of the small intestine. This polypeptide may be involved in the intestinal uptake of cephalosporins and dipeptides. Further work is in progress to elucidate the precise role of this polypeptide for the intestinal absorption of β-lactam antibiotics and dipeptides.



Flg. 9. Photoaffinity labeling of brush-border membrane vesicles with *N*-(4-azidobenzoyl)cephalexin in the presence of orally effective cephalosporins. 200 μg of brush-border membrane vesicles were photolabeled with 0.16 μM (1.72 μCi) of *N*-(4-azido[3,5-^{3}H]benzoyl)cephalexin in the absence (A) and in the presence of 25 μM (B), 100 μM (C) and 250 μM (D) of cefadroxil. Total acrylamide concentration was 7%.

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