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FUNCTIONAL EXPRESSION OF INTESTINAL DIPEPTIDE/β-LACTAM ANTIBIOTIC TRANSPORTER IN *XENOPUS LAEVIS* OOCYTES

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Abstract—An intestinal active transport system specific to small peptides and peptide-like drugs such as β-lactam antibiotics was functionally expressed in *Xenopus laevis* oocytes after microinjection of messenger RNA (mRNA) derived from rat intestinal mucosal cells. The transport activity was evaluated by measuring the uptake of a tripeptide-like cephalosporin antibiotic, ceftibuten, which has high affinity for the intestinal peptide/H^+ co-transporter and is resistant to peptidases. Ceftibuten transport in mRNA-injected oocytes was pH dependent (a proton gradient is the driving force), stereo selective (uptake of the *cis*-isomer of ceftibuten was about 4-fold higher than that of the *trans*-isomer), saturable and temperature dependent. Furthermore, various dipeptides showed *cis*-inhibitory and *trans*-stimulatory effects on the uptake of ceftibuten by mRNA-injected oocytes, suggesting that ceftibuten and dipeptides are transported by a common carrier protein. These results are in accordance with the functional properties of native proton-coupled peptide transporter previously clarified by studies with isolated intestinal brush-border membrane vesicles and other experimental systems. A protein with a molecular mass of about 130 kDa expressed in the membrane of mRNA-injected oocytes was identified as the transport protein by specific labeling with a photoreactive β-lactam antibiotic, [^{3}H]benzylpenicillin, followed by SDS–PAGE analysis of the radiolabeled protein. Furthermore, an experiment with mRNA size-fractionated by sucrose density gradient centrifugation indicated that the peptide transporter is encoded by mRNA of between 1.8 and 3.6 kb. These results, obtained using a heterologous gene expression technique, confirm that intestinal absorption of β-lactam antibiotics occurs through a carrier-mediated mechanism and show that biologically stable β-lactam antibiotics can be useful probes for molecular analysis of intestinal peptide transporter.

Key words: intestinal absorption; proton-coupled transport; intestinal uptake; gene expression; cephalosporin; ceftibuten

The significance of an intestinal peptide transporter in the ingestion of digestive products of dietary proteins has been shown by membrane physiological studies with intestinal tissues and isolated brush-border membrane vesicles. It has been established that the peptide transporter accepts only dipeptides and tripeptides as substrates [1, 2], is energized by a proton gradient [3, 4] and is electrogenic [5]. A similar transporter was suggested to function in the brush-border membrane of the renal proximal tubules [6, 7]. Interestingly, the intestinal H^+ peptide co-transporter has a relatively broad substrate specificity [2, 4] and accepts certain peptide-like drugs such as β-lactam antibiotics [8–13], angiotensin converting enzyme inhibitors [14] and renin inhibitors [15], as well as endogenous dipeptides, and tripeptides.

Although a substantial amount of information has been accumulated about the functional properties of the peptide transporter, the molecular features of the transporter remain to be clarified. Functional heterologous expression of the transport activities in *Xenopus laevis* oocytes is useful, since it allows study of the transport proteins at the molecular level and of the genes encoding the transport proteins [16–19]. Recently, Miyamoto *et al.* [20] detected the expression of small peptide transporter in oocytes after microinjection of mRNA¶ from rabbit intestinal mucosa by measuring the uptake of a dipeptide, [^{3}H]glycylsarcosine. Although they observed an enhanced transport activity of the peptide, the increment of the activity was relatively small, and the proton-gradient dependence was not marked. In contrast to isolated membrane vesicles, oocytes are expected to be metabolically more active, and so it

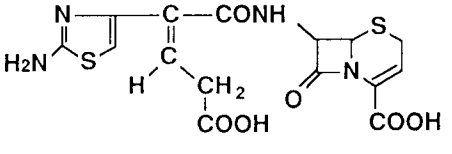

Fig. 1. The chemical structure of ceftibuten.

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¶ Abbreviations: mRNA, messenger RNA; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

may be difficult to detect clearly the transport characteristics by using endogenous peptides, which may be liable to undergo enzymatic degradation. Furthermore, such dipeptides are more likely to be taken up by the native transporter of the oocytes, resulting in lower sensitivity in detecting the newly expressed activity. As is the case for orally active β-lactam antibiotics [8–13], ceftibuten (see structure in Fig. 1), a biologically stable β-lactam antibiotic, has been shown to be transported by the small peptide transport system, to have relatively high affinity for the carrier and to show clear stereoselective and proton-gradient-dependent transport characteristics in rat intestinal brush-border membrane vesicles [21, 22]. Accordingly, this tripeptide-like drug should be a suitable substrate for the detection of the expressed peptide transport activity in mRNA-injected oocytes.

The purpose of the present study was to achieve functional expression of the small-intestinal transporter of dipeptides and tripeptides in oocytes as the first step toward molecular characterization of the transporter through expression cloning. Here we describe the expression of ceftibuten transport in oocytes injected with mRNA from rat small intestinal epithelial cells. The expressed transport features are consistent with those of intestinal brush-border membrane vesicles as regards pH and temperature dependences, saturation kinetics, stereo specificity and substrate specificity [21, 22]. Furthermore, we estimated the molecular size of the transporter by observing the expression of increased transport activity after injection of size-fractionated mRNA and by identification of the peptide-binding protein in membranes of mRNA-injected oocytes.

MATERIALS AND METHODS

Materials. Ceftibuten was kindly supplied by Shionogi & Co. (Osaka, Japan). mRNA purification kit and collagenase (type I) were from Pharmacia LKB (Piscataway, NJ, U.S.A.), and Sigma Chemical Co. (St Louis, MO, U.S.A.), respectively. [Phenyl-4(*n*)-^{3}H]benzylpenicillin (699 GBq/mmol) was obtained from Amersham International (Buckinghamshire, U.K.). All other chemicals were of reagent grade and were used without further purification.

Isolation and fractionation of poly(A)$^+$RNA. Mucosal scrapings from the rat small intestine were used to isolate poly(A)$^+$RNA as described previously [23]. Briefly, total RNA was extracted using a guanidinium thiocyanate extraction method followed by cesium chloride gradient centrifugation. Poly(A)$^+$RNA was separated with an mRNA purification kit on an oligo(dT)-cellulose column and stored as an ethanol precipitate at −20°. For size fractionation of poly(A)$^+$RNA, linear 5–25% (w/w) sucrose gradient centrifugation (2 mM EDTA, 0.5% SDS and 10 mM Tris adjusted to pH 8.0 with HCl) was used. Poly(A)$^+$RNA (500 μg) was heated at 65° for 5 min and rapidly chilled on ice. The samples were then loaded on the top of the gradient and centrifuged for 24 hr at 26,000 rpm at 4° (Beckman, SW-28 rotor). Each sample was collected manually in 11 fractions, each of which was precipitated in ethanol and dissolved in RNase-free water. The size of the mRNA in each fraction was determined by formaldehyde gel electrophoresis.

Expression of mRNA in Xenopus laevis *oocytes.* The methods of isolation of oocytes and microinjection have been described in detail previously [23]. Briefly, a frog, *Xenopus laevis* (Sankyo Laboratory Co., Toyama, Japan), was anesthetized by hypothermia and a small portion of the ovarian lobe was surgically removed, placed in medium A (96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$ and 5 mM HEPES adjusted to pH 7.6 with NaOH) and treated with collagenase (0.5 mg/mL) for 4 hr in modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 50 units/mL penicillin G, 50 μg/mL streptomycin and 10 mM HEPES adjusted to pH 7.5 with NaOH) at 18°. Follicular cells were then removed by using forceps under a dissecting microscope. Only healthy-looking stage V–VI oocytes were used for microinjection. Approximately 50 nL of total mRNA (1 μg/μL), fractionated mRNA (0.5 μg/μL) or water was injected into oocytes. mRNA-injected or water-injected oocytes were incubated in modified Barth's solution at 18° for 5–7 days until used for transport studies.

Uptake measurements. To measure the uptake of ceftibuten, five oocytes were used for each uptake and experiments were repeated at least three times using the same batch of mRNA- or water-injected oocytes. mRNA- or water-injected oocytes were preincubated for 30 min in medium B (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM HEPES adjusted to pH 7.5 with Tris) at 25° before the initiation of the uptake measurement. Then the oocytes were placed in 2 mL of the uptake medium, which has the same constituents as medium B, plus ceftibuten, at 25°. In most experiments the concentration of ceftibuten used was 2 mM. The pH of the uptake medium was adjusted with Tris or Mes to the desired pH value. To terminate the uptake, oocytes were washed three times with ice-cold medium B, homogenized in 300 μL of phosphate buffer (pH 7.4), and then deproteinized with 0.5% perchloric acid for HPLC analysis of ceftibuten. The HPLC system (model BIP-I, Japan Spectroscopic Co., Tokyo, Japan) was equipped with a UV detector (model UVIDEC-100V, Japan Spectroscopic Co.) and recorder-integrator (model Chromatopac CR-4A, Shimadzu Co., Kyoto, Japan). The analytical column used was a reversed-phase TSK-gel ODS-80 TM (4.6 mm × 15 cm, Tosoh Co., Tokyo, Japan). A Guard-Pak™ (μ-Bondapak C_{18}, Waters, Milford, MA, U.S.A.) was used between the analytical column and injector. The mobile phase was composed of 4% acetonitrile–96% water containing 10 mM ammonium acetate (pH 4.0). The flow rate of mobile phase was 1.0 mL/min and the effluent was monitored at 260 nm.

Photoaffinity labeling. Membranes of oocytes were purified by differential centrifugation [24]. Photoaffinity labeling of membrane proteins with [^{3}H]benzylpenicillin was performed according to the method of Kramer *et al.* [25]. The isolated membranes (100 μg of protein) were suspended in medium C (300 mM mannitol and 10 mM Tris adjusted to

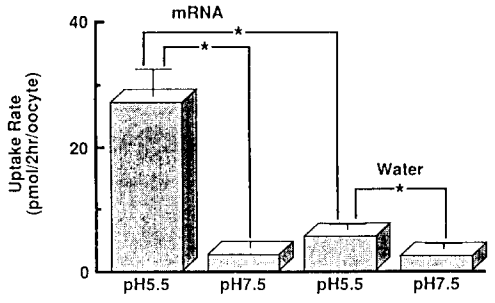

Fig. 2. Effect of extracellular pH on ceftibuten uptake. Uptake of ceftibuten (2 mM) was measured at 25° for 2 hr. Each datum represents the mean ± SE of four to six experiments. * Significantly different ($P < 0.05$) between the indicated pairs.

pH 7.4 with HEPES) and incubated with 3 μCi of [^{3}H]benzylpenicillin in 100 μL of medium D (100 mM citrate and 140 mM KCl to pH 6.0 with Tris) in either the absence or the presence of inhibitors. After 15 min incubation in the dark at 20°, samples were irradiated at 254 nm for 150 sec at a distance of 15 cm from the UV lamps (Model XX-15S, UVP Inc., San Gabriel, CA, U.S.A.). After irradiation, the samples were diluted with ice-cold medium E (300 mM mannitol, 4 mM phenylmethylsulfonyl fluoride, 4 mM EDTA, 4 mM iodoacetamide and 10 mM Tris adjusted to pH 7.4 with HEPES) and centrifuged at 48,000 g for 30 min. The resultant pellet was resuspended in 100 μL of water and the proteins precipitated by addition of methanol were analysed by 7.5% SDS–PAGE. Radioactivity in gels was measured by liquid scintillation counting after slicing the gels into 2 mm pieces.

RESULTS

Effect of extracellular pH on ceftibuten uptake

The effects of extracellular pH on the uptake of ceftibuten in water-injected or mRNA-injected oocytes are shown in Fig. 2. At an external pH of 5.5, the uptake of ceftibuten in mRNA-injected oocytes was about five times higher than that in water-injected oocytes. Furthermore, oocytes injected with mRNA showed a 10-fold greater uptake at pH 5.5 than that at pH 7.5. In contrast, water-injected oocytes exhibited only a modest uptake rate of ceftibuten at an external pH of 5.5 or 7.5 with a slight pH dependence. To confirm that the expressed pH dependence of ceftibuten transport in mRNA-injected oocytes is attributable to a proton gradient as the driving force, the effects of FCCP, a protonophore, were examined at external pH of 5.5 and 7.5. As shown in Fig. 3, FCCP significantly decreased the uptake of ceftibuten in the presence of the proton gradient (pH 5.5), whereas it did not alter ceftibuten uptake in the absence of the proton gradient (pH 7.5).

Concentration and temperature dependence of ceftibuten uptake

Figure 4 shows the concentration dependence of ceftibuten uptake, examined to determine the kinetic parameters for ceftibuten transport. The uptake rate was measured at concentrations of ceftibuten between 0.1 and 5 mM in both water-injected and mRNA-injected oocytes. The expressed transport of ceftibuten due to mRNA injection was calculated as the difference between the uptake in mRNA-injected oocytes and that in water-injected ones. By nonlinear least-squares analysis using the MULTI program [26], the Michaelis constant (K_t) and the maximum uptake rate (J_{max}) of the expressed ceftibuten transport were estimated to be 0.11 ± 0.03 mM and 7.30 ± 0.40 pmol/2 hr/oocyte,

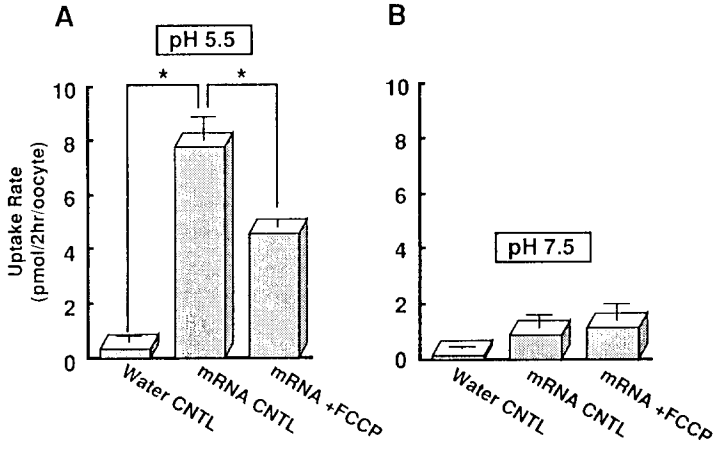

Fig. 3. Effect of the protonophore FCCP on ceftibuten uptake. After incubation of oocytes at 25° for 30 min with 50 μM FCCP in medium B, uptake of ceftibuten (2 mM) was measured at pH 5.5 (A) or pH 7.5 (B) at 25° for 2 hr. Each datum represents the mean ± SE of four to nine experiments. * Significantly different ($P < 0.05$) between the indicated pairs.

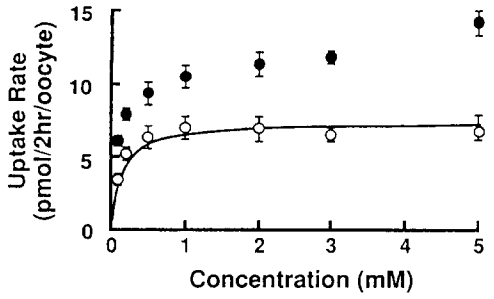

Fig. 4. Concentration dependence of ceftibuten uptake. Uptake of ceftibuten was measured in uptake medium (pH 5.5) containing various concentrations (0.1–5 mM) of ceftibuten at 25° for 2 hr. mRNA-injected oocytes (closed circles); and increment due to mRNA calculated as the difference between the uptakes in mRNA-injected oocytes and in water-injected ones (open circles). Each point represents the mean ± SE of four to eleven experiments. The line represents the carrier-mediated uptake estimated from the kinetic parameters as described in the text.

Table 1. Effect of various compounds on ceftibuten uptake by mRNA-injected oocytes

Inhibitor	Concentration (mM)	% of control
Glycyl-L-proline	20	18.5 ± 3.2*
Glycylglycine	20	13.7 ± 9.4*
Cyclacillin	20	29.9 ± 3.1*
Cephradine	20	2.7 ± 1.1*
Cefixime	5	42.6 ± 3.9*
Benzylpenicillin	20	20.4 ± 29.6*
Proline	20	102.6 ± 7.4
Glycine	20	79.8 ± 13.9

Uptake of ceftibuten (2 mM) was measured at 25° for 2 hr in uptake medium (pH 5.5) containing each inhibitor at the concentration shown in the table. Each bit of data represents the uptake % of control study (100%) with mean ± SE of three to nine experiments.

* Significantly different ($P < 0.05$) from control study.

respectively. When the temperature was lowered to 4°, the uptake of ceftibuten was significantly decreased to 2.5% of the uptake measured at 25° (data not shown).

Substrate specificity of the transporter

It has been shown [21, 22] that ceftibuten is transported in the intestinal brush-border membrane *via* a carrier-mediated mechanism shared with small peptides and some β-lactam antibiotics. Therefore, the effects of β-lactam antibiotics, dipeptides and amino acids on ceftibuten uptake in mRNA-injected oocytes were examined. As shown in Table 1, all of the β-lactam antibiotics and dipeptides, glycylglycine and glycyl-L-proline, tested significantly inhibited ceftibuten uptake, whereas amino acids, glycine and L-proline, did not. Furthermore, in order to confirm that the transport system is shared with peptides, we examined the countertransport effect on ceftibuten uptake in mRNA-injected oocytes. As is clear from Fig. 5, the uptake rate of ceftibuten was increased in mRNA-injected oocytes preloaded with 10 mM glycyl-L-proline (Fig. 5A), whereas no change of ceftibuten uptake was observed in water-injected oocytes (Fig. 5B). These results suggest that ceftibuten is indeed transported by the dipeptide transporter.

Uptake of the geometrical isomers of ceftibuten

Ceftibuten has a *cis* double bond in its side-chain at the 7 position (Fig. 1). In contrast to the *cis*-isomer, the *trans*-isomer is not readily absorbed from the intestine, and low uptake activity of the *trans*-isomer was observed in intestinal brush-border membrane vesicles [21, 22]. In order to determine whether the expressed transporter retains this stereo specificity, the uptakes of these isomers in water-injected and mRNA-injected oocytes were compared (Fig. 6). In mRNA-injected oocytes, the uptake of *cis*-isomer was about 4-fold higher than that of *trans*-

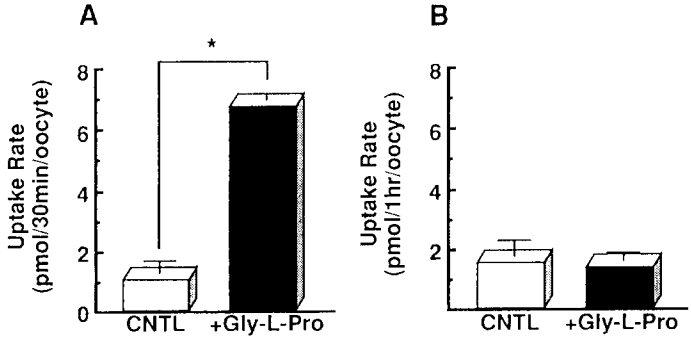

Fig. 5. Countertransport effect of glycyl-L-proline on ceftibuten uptake. Oocytes were injected with mRNA (A) or water (B) and were preincubated with or without 10 mM glycyl-L-proline. Then uptake of ceftibuten (2 mM) was measured for 30 min (A) or 1 hr (B) at 25°. Each datum represents the mean ± SE of four to five experiments. * Significantly different ($P < 0.05$) between the indicated pairs.

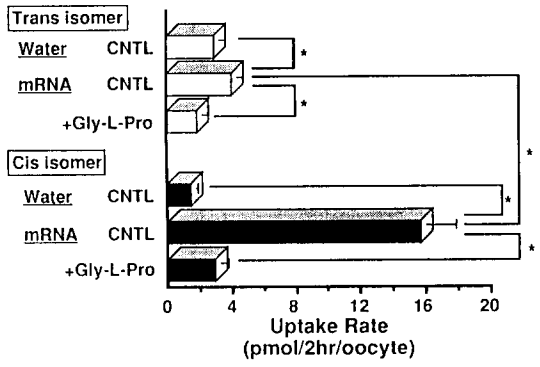

Fig. 6. Uptake of the geometrical isomers of ceftibuten. Uptake of *trans*-isomer (open columns) or *cis*-isomer (closed column) of ceftibuten (2 mM) was measured in the absence (CNTL) or presence of 25 mM glycyl-L-proline at 25° for 2 hr. Each datum represents the mean ± SE of four to eight experiments. * Significantly different ($P < 0.05$) between the indicated pairs.

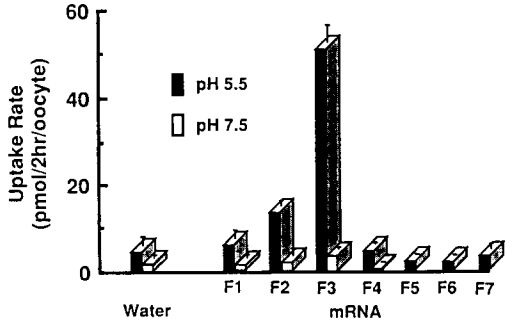

Fig. 7. Expression of ceftibuten uptake after injection of size-fractionated mRNA. Uptake of ceftibuten (2 mM) was measured at pH 5.5 (closed column) or pH 7.5 (open column) at 25° for 2 hr. Approximate size of mRNA of each fraction is as follows: F1, 3.0–6.6 kb; F2, 2.4–5.1; F3, 1.8–3.6; F4, 1.4–2.4; F5, 0.7–1.8; F6, 0.3–1.0; F7, –0.4. Each datum represents the mean ± SE of three to ten experiments.

isomer, and a greater inhibitory effect of glycyl-L-proline was observed in the uptake of *cis*-isomer, confirming that the expressed transporter is stereo selective.

Expression of ceftibuten uptake after injection of size-fractionated mRNA

In order to enrich the mRNA encoding the H^+-gradient-dependent dipeptide transporter and to estimate the approximate size of the mRNA, total mRNA was fractionated on a 5–25% sucrose density gradient centrifugation. As shown in Fig. 7, the highest expression of H^+-gradient-dependent ceftibuten uptake was observed in fraction 3, corresponding to the size of 1.8–3.6 kb as determined by agarose-gel electrophoresis under denaturing conditions (data not shown). Uptake of ceftibuten in oocytes injected with mRNA of fraction 3 was significantly higher than that of oocytes injected with total mRNA (Fig. 2). The difference of uptake between pH 5.5 and pH 7.5 in oocytes injected with mRNA of fraction 3 was approximately 14-fold, which was higher than that of total mRNA-injected oocytes (10-fold).

Photoaffinity labeling of dipeptide transport protein

Figure 8 shows the expression and the molecular size of the peptide transporter identified by photoaffinity labeling of membrane proteins from water-injected or mRNA-injected oocytes with a photoreactive specific binder, [^{3}H]benzylpenicillin [25]. Membranes of oocytes were incubated with [^{3}H]benzylpenicillin and subjected to photoaffinity labeling followed by SDS–PAGE. In membranes of mRNA-injected oocytes, two predominant binding proteins with apparent molecular masses of 130 kDa and 100 kDa were observed (Fig. 8A). As can be seen in Fig. 8(C and D), both of the specific labelings were significantly inhibited by ceftibuten and L-carnosine. Photoaffinity labeling of membranes of water-injected oocytes resulted in prominent labeling of a single binding protein with an apparent molecular mass of 100 kDa (Fig. 8B). These results suggest that the newly expressed binding protein with an approximate molecular mass of 130 kDa in mRNA-injected oocytes may be the peptide transporter originated from intestinal mucosal cells.

DISCUSSION

The most important finding in the present study is that the uptake of ceftibuten by oocytes was significantly increased by microinjection of mRNA derived from rat small intestinal mucosa (Fig. 2). Observed uptake by oocytes injected with mRNA was remarkably temperature dependent, saturable (Fig. 4) with K_t of 0.11 mM, which is very close to the value (0.17 mM) obtained in the study using intestinal brush-border membrane vesicles [21], and stereo selective (Fig. 6), showing that a specific carrier-mediated mechanism is involved. The accordance of the affinities for the transporter and the preferred stereo-isomer between the present study and the previous membrane vesicle study strongly indicates that the newly acquired transport activity in the oocytes originated from the gene which encodes the intestinal brush-border transporter, and was not a result of activation of the native oocyte's transporter.

The transport activity of ceftibuten by oocytes injected with mRNA increased about five times more than that of oocytes injected with water. The increment is higher than the previous observations by using [^{3}H]glycylsarcosine or [^{3}H]bestatin, a dipeptide anticancer agent, as substrates: approximately 3- or 2-fold increment of uptake was observed after injection of mRNA derived from rabbit intestinal mucosa [20] or Caco-2 cells [27], respectively. Transport of peptides by brush-border membrane vesicles is characterized by its pH dependence [3, 4]. In the present study, ceftibuten uptake was increased about 10-fold at acidic pH

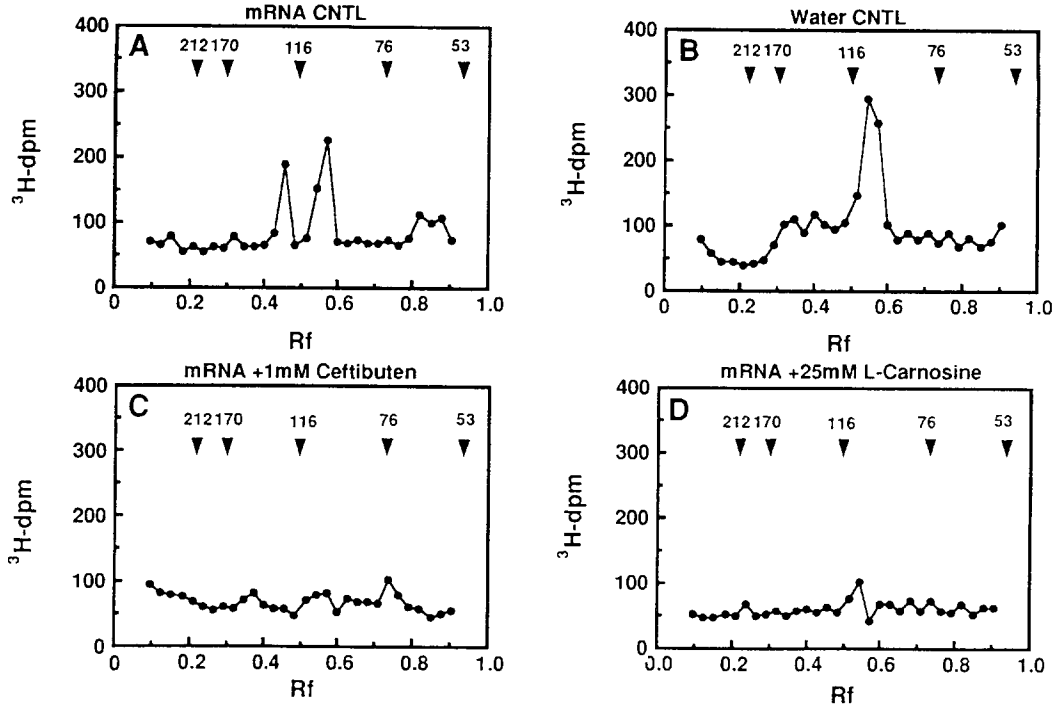

Fig. 8. Photoaffinity labeling of membrane proteins with [^{3}H]benzylpenicillin. Membranes (100 μg of protein) from oocytes injected with water (B) or mRNA (A, C, D) were photolabeled with 3 μCi of [^{3}H]benzylpenicillin in the absence (A, B) or presence of (C) ceftibuten (1 mM) or (D) L-carnosine (25 mM). The molecular mass is shown in the upper part of each figure with arrows in kDa.

compared to that at neutral pH, while the pH dependencies expressed in oocytes in the previous studies were much smaller [20, 27]. The efficient expression of ceftibuten transport may be due to the higher affinity of ceftibuten to peptide transporter, since it has a K_t value of 0.17 mM [21], whereas glycylsarcosine and bestatin have K_t values of 19 mM [28] and 0.5 mM [29], respectively. Further, ceftibuten may be less susceptible to biological degradation. Although increased uptake was also observed at acidic pH in oocytes injected with water, the degree of increment at acidic pH was much higher in oocytes injected with mRNA (about 10-fold increase) compared with oocytes injected with water (about 2-fold increase) (Fig. 2). The slight pH effect in water-injected oocytes may reflect the endogenous transport characteristics of oocytes or the increase of non-ionic passive diffusion, because the lipophilicity is increased 3-fold at pH 5.5 relative to that at pH 7.5 [21]. The pH dependence observed in the previous studies has been ascribed to the fact that the transport of small peptides, including ceftibuten, is energized by the proton gradient across the membrane [3, 4]. Our study of the effect of FCCP shown in Fig. 3 suggests the significance of a proton gradient in the transport of ceftibuten, since the transport was decreased by FCCP only in the presence of the proton gradient. Although a decreased viability of oocytes by FCCP cannot be excluded as a contributory factor, no change of ceftibuten uptake in the absence of the proton gradient indicates that the effect of FCCP specifically involves dissipation of the proton gradient.

Expression of the peptide transporter and its substrate specificity were confirmed by studies on *cis*-inhibition and *trans*-stimulation of ceftibuten uptake. Dipeptides and β-lactam antibiotics tested significantly reduced ceftibuten uptake, whereas amino acids had no effect (Table 1). Furthermore, preloading of a relatively stable dipeptide, glycyl-L-proline, into oocytes significantly increased the uptake of ceftibuten only in the mRNA-injected oocytes (Fig. 5). These *cis*-inhibitory and *trans*-stimulatory effects show that the newly expressed transporter is specific to small peptides.

Specific labeling of membrane protein(s) by using a photoreactive substrate of the peptide transporter, [^{3}H]benzylpenicillin, confirmed expression of the peptide-specific transporter. Two different binding proteins with molecular masses of approximately 130 kDa and 100 kDa were found in mRNA-injected oocytes, whereas a single protein with a molecular mass of about 100 kDa was labeled in water-injected oocytes (Fig. 8). These labelings were inhibited in the presence of ceftibuten or L-carnosine. The molecular mass of the newly expressed binding protein (130 kDa) is in good accordance with the value of 127 kDa reported by Kramer *et al.* [25, 30] for the specific binding protein of small peptides and β-lactam antibiotics observed in rat or rabbit intestinal brush-border membranes. Therefore, it is suggested that the binding protein of 130 kDa

identified only in mRNA-injected oocytes is the peptide transporter. The 100 kDa binding protein is unlikely to be a newly expressed transporter, because it was also present in water-injected oocytes, which showed negligible transport activity.

Microinjection of mRNA with a size of 1.8–3.6 kb yielded the highest transport activity (Fig. 7). The significant induction of transport activity by a single mRNA fraction suggests that the transport protein is composed of a single polypeptide, and is not composed of different-sized subunits. Although it is difficult to estimate the molecular mass of the gene product from the size of the mRNA, owing to the probable presence of untranslated regions and the possible occurrence of modifications of the polypeptide, such as glycosylation, this size of mRNA appears to be consistent with the molecular mass of the protein identified by photoaffinity labeling, 130 kDa. The presence of a basolateral peptide transporter has been suggested [31], but the transporter identified in the present study is likely to be a brush-border peptide transporter, because we found that a single polypeptide functions in the transport of ceftibuten, and the expressed protein possesses functional characteristics similar to those of the native brush-border transporter with regard to driving force, stereo selectivity, substrate specificity, Michaelis constant and molecular mass.

In conclusion, using a β-lactam antibiotic, ceftibuten, which has a high affinity for the intestinal brush-border peptide transporter and is resistant to biological degradation, we were able to confirm the participation of a carrier-mediated mechanism in the transports of peptides and β-lactam antibiotics. Recent studies by ourselves and others, using brush-border membrane vesicles, suggested that specific transporters other than the peptide transporter function in the transports of certain β-lactam antibiotics such as cefdinir [32] and cefixime [33]. Furthermore, there is another study suggesting that passive diffusion is probably a major pathway in the intestinal absorption of orally active β-lactam antibiotics [34]. The precise molecular mechanisms of the transport and the structural requirements of β-lactam antibiotics for transport are not clear. Further studies utilizing the technique described in the present study should provide much information about the mechanisms of the intestinal brush-border transport of β-lactam antibiotics, as well as native dipeptides and tripeptides, leading to the clarification of the physiological functions of intestinal peptide transporter and aiding the rational molecular design of orally active peptide-like drugs.

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