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Rapid report

Preferential recognition of zwitterionic dipeptides as transportable substrates by the high-affinity peptide transporter PEPT2

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Abstract

We investigated the interaction of rat PEPT2, a high-affinity peptide transporter, with neutral, anionic, and cationic dipeptides using electrophysiological approaches as well as tracer uptake methods. D-Phe-L-Gln (neutral), D-Phe-L-Glu (anionic), and D-Phe-L-Lys (cationic) were used as representative, non-hydrolyzable, dipeptides. All three dipeptides induced H^+-dependent inward currents in *Xenopus laevis* oocytes heterologously expressing rat PEPT2. The H^+:peptide stoichiometry was 1:1 in each case. A simultaneous measurement of radiolabeled dipeptide influx and charge transfer in the same oocyte indicated a transfer of one net positive charge into the oocyte per transfer of one peptide molecule irrespective of the charged nature of the peptide. We conclude that the zwitterionic peptides are preferentially recognized by PEPT2 as transportable substrates and that the proton/peptide stoichiometry is 1 for the transport process.

Keywords: High-affinity peptide transporter; Proton/peptide stoichiometry; Charge transfer/peptide transfer ratio; Zwitterionic peptide; (Rat)

Proton-coupled peptide transporters are widely distributed in both prokaryotes and eukaryotes [1–6]. In animals and in humans, the presence of the peptide transport process has been established in the intestine and kidney. The intestine expresses a low-affinity peptide transport system whereas the kidney expresses predominantly a high-affinity peptide transport system. However, both systems are H^+-dependent and electrogenic [7–9]. The transporters responsible for the low-affinity and high-affinity peptide transport processes have been cloned (for a review, see [10–13]). PEPT1, the low-affinity transporter, is expressed principally in the intestine, and to a much smaller extent in the kidney. PEPT2, the high-affinity transporter, is expressed principally in the kidney, and to an appreciable extent also in the brain [14,15]. Both transporters have been expressed heterologously in mammalian cells as well as in *Xenopus laevis* oocytes. Both are H^+-dependent and electrogenic.

The functional characteristics of PEPT1 and PEPT2 have been investigated primarily using zwitterionic neutral dipeptides such as glycylsarcosine or glycylglutamine. However, the physiologically occurring substrates include neutral, anionic, and cationic dipeptides. Competition studies have indicated that

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PEPT1 and PEPT2 interact with peptides of varying ionic nature. The H^+/peptide stoichiometry and the electrogenicity of the transport process are expected to be closely related to the ionic nature of the transported peptide. This relationship has been investigated independently by at least three groups of investigators in the case of PEPT1. Amasheh et al. [16] studied the transport of charged dipeptides by rabbit PEPT1 in *X. laevis* oocytes and concluded that the uptake of the zwitterionic neutral form of the peptide contributes significantly to overall transport irrespective of the ionic nature of the peptide in the uptake medium. What was measured in these studies was the inward current induced by PEPT1 in the presence of peptide substrates. The direct relationship between the amount of charge transferred and the amount of peptide transported was not investigated. We have reported similar findings for human PEPT1 [17]. hPEPT1-mediated transport process is electrogenic irrespective of the ionic nature of the peptide in the medium and the H^+ activation of the induced current remains hyperbolic for all three groups of peptide substrates (neutral, anionic, and cationic). This suggests, but does not prove, that only the zwitterionic neutral form of the peptides is transported coupled with one H^+, because the exact quantities of the charge and the peptide substrate transported via the carrier was not measured for any of these differently charged peptides. Steel et al. [18] have analyzed the intracellular acidification rate (as a measure of H^+ transfer) and the initial uptake rate of radiolabeled peptides in association with the charge fluxes for rabbit PEPT1. This analysis has led to the conclusion that the H^+/peptide coupling ratio is 1, 2, and 1 for neutral, anionic, and cationic peptides, respectively. This suggests that the charge/peptide ratio may be different depending upon the ionic nature of the peptide.

Similar detailed studies have not been conducted with the high-affinity H^+/peptide cotransporter PEPT2. However, H^+ activation kinetics of rabbit PEPT2 with differently charged peptides have indicated that the electrogenic properties may be similar for PEPT1 and PEPT2 [19]. But the charge transfer and the peptide transfer have not been correlated for PEPT2 as has been done for PEPT [18]. Therefore, we undertook the present investigation in which we analyzed the H^+-coupled peptide transport mediated by rat PEPT2 in *X. laevis* oocytes with respect to peptide-evoked current, H^+ activation, charge transfer and peptide transfer. We carried out this analysis for neutral, anionic, and cationic peptides. Quantitation of charge transfer and peptide transfer was accomplished by a procedure using the 'Fetchex' algorithm to simultaneously measure the transfer of radiolabeled peptide and the transfer of charge associated with the transport process in the same oocyte. The results of these studies show that the ratio of charge transfer/peptide transfer for rat PEPT2 is 1 irrespective of whether the peptide is neutral, anionic or cationic.

The hydrolysis-resistant radiolabeled dipeptides D-[^{3}H]Phe-L-Gln (FQ), D-[^{3}H]Phe-L-Glu (FE), and D-[^{3}H]Phe-L-Lys (FK) were custom-synthesized commercially (Zeneca, Northwich, Cheshire UK; specific activity, 12 Ci/mmol) and the corresponding unlabeled dipeptides D-Phe-L-Gln, D-Phe-L-Glu, and D-Phe-L-Lys were synthesized as described previously [20].

The rPEPT2 cDNA was cloned from a rat brain cDNA library as previously reported [14]. In vitro transcription of the plasmid DNA was performed after linearization of the template DNA by restriction endonuclease (*Bam*HI) digestion at a downstream position from the 3′-end of the poly(A) tail. The transcription was carried out using bacteriophage T7 RNA polymerase. RNase inhibitor and mRNA cap analog (7-methyldiguanosine triphosphate [mG(5′)ppp(5′)G] (Ambion Inc., Austin, TX, USA) were present during transcription. The DNA template in the reaction mixture was removed by digestion with an RNase-free DNase following the in vitro transcription. The final concentration of the synthetic cRNA was adjusted based on quantitation by UV spectrophotometry and the integrity of cRNA was verified by electrophoresis. Oocytes, isolated from *X. laevis* (Nasco, Fort Atkinson, WI, USA), were subjected to partial digestion with 1.6 mg/ml collagenase A (Boehringer Mannheim, Indianapolis, IN, USA), in a calcium-free buffer (OR2) for 30 min at room temperature, and manually defolliculated. Defolliculated, mature (stage V–VI) oocytes were selected and maintained at 18°C until the experiment was carried out as previously described [14,17].

A conventional two-microelectrode, voltage-clamp

method was used to study the kinetics of the peptide transporter expressed in oocytes. The membrane potential was clamped at −50 mV. Electrophysiological measurements were made 5–10 days after injection. A standard transport assay buffer (100 mM NaCl-2 mM KCl-1 mM $MgCl_2$-1 mM $CaCl_2$-3 mM HEPES-3 mM MES-3 mM Tris, pH 5.5) was used as the bath solution. The recording chamber was superfused with the bath solution and the testing substrates, applied at desired concentrations. Acquired data were fitted to the following equation, $I = I_{max}[S]^n/[K_{0.5}]^n+[S]^n]$, where I is the substrate-evoked current, I_{max} is the derived current maximum, [S] is the substrate concentration applied, n is the Hill coefficient, and $K_{0.5}$ is the Michaelis-Menten constant, or the substrate concentration at which current is half-maximal [14,17]. Data analyses were performed using software SigmaPlot (Jandel Scientific, San Rafael, CA, USA). To investigate the membrane potential dependence of the transporter activity, step changes to the testing potentials (V_t) from the holding potential (V_h) were achieved by a voltage clamp amplifier (GeneClamp 500 and Digidata-1200A interface, Axon Instruments, CA, USA) controlled by the Clampex computer program of pCLAMP software version 6.0 (Axon Instruments, CA, USA), each for a duration of 100 ms (from +50 mV to −150 mV in 20 mV increments). First, the voltage-jumping protocol was applied in a substrate-free solution, then after superfusing 2–5 min with the test solutions. The substrate-induced current at each test potential was taken as the difference between the steady-state currents, recorded at the end of each voltage pulse, in the presence and absence of the substrate. The current was low-pass filtered at 500 Hz by a built-in Bessel filter in the amplifier, digitized at 50 μs/point, and averaged from three sweeps.

The ratio of H^+:peptide transport was determined by simultaneously measuring inward fluxes of ^{3}H-FX (X = Q, E, K) and net dipeptide-induced inward currents. Substrate-induced currents were recorded in an acquisition system using the Fetchex program within the pCLAMP 6.0 software package. Oocytes were clamped at −50 mV and superfused with transport assay buffer (pH 5.5, ∼3.2 μM of H^+) at a rate of ∼160 μl/min. Current traces were monitored in a chart recorder until they reached a steady baseline. Then, ^{3}H-FX was applied for ∼10 min while the peptide-induced current was recorded. Afterwards, the oocyte was washed with the assay buffer in the absence of the peptide substrate until the current trace returned to the baseline levels. The oocyte was quickly removed from the recording chamber, washed three times in ∼20 ml of ice-cold ND96 buffer, lysed in 10% sodium dodecyl sulfate, and the amount of radioactivity that was present in the oocyte was determined by liquid scintillation counting. The amount of radioactivity in water-injected oocytes incubated with radiolabeled peptides under identical conditions was subtracted from those in the rPEPT2-expressing oocytes. The total inward charge transfer was calculated by integrating the area under the current versus time curve using a transformation protocol in Sigma plot. The ratio of charge transfer:peptide transfer is presented as pmol net inward charge transfer:pmol peptide transfer using the Faraday conversion constant.

The transport assay medium consisted of both cold dipeptides and radiolabeled ^{3}H-dipeptides. The final concentration of the dipeptides in the medium was 100 μM for FQ and FE and 600 μM for FK. The specific radioactivity was ∼20.0–22.0 μCi/μmol peptide in the medium.

Fig. 1 describes the H^+ activation kinetics of the transport of the dipeptides FQ, FE and FK. At pH 7.5, these dipeptides exist predominantly as a zwitterion, anion and cation, respectively. Nonetheless, the H^+ activation kinetics were similar for all three peptides. The peptide-induced currents were hyperbolically related to H^+ concentration over the range of 10 nM to 10 μM (i.e., pH 8.0–5.0). The concentration of the peptides was 100 μM for FQ and FE and 600 μM for FK. The magnitude of the peptide-induced currents was comparable between FQ and FE, but it was relatively much lower for FK. This prompted the use of higher concentration of FK (600 μM instead of 100 μM). Even under these conditions, FK-induced currents were only ∼40% of the currents induced by FQ or FE. Despite these quantitative differences, the H^+ activation kinetics followed Michaelis-Menten kinetics and the data fit best for a transport model describing a single binding site for H^+. The Hill coefficients (n_H) for the neutral (FQ), anionic (FE), and cationic (FK) dipeptides were 0.7 ± 0.2, 1.1 ± 0.1 and 1.2 ± 0.3, respectively. Therefore, in spite of the differently charged nature of

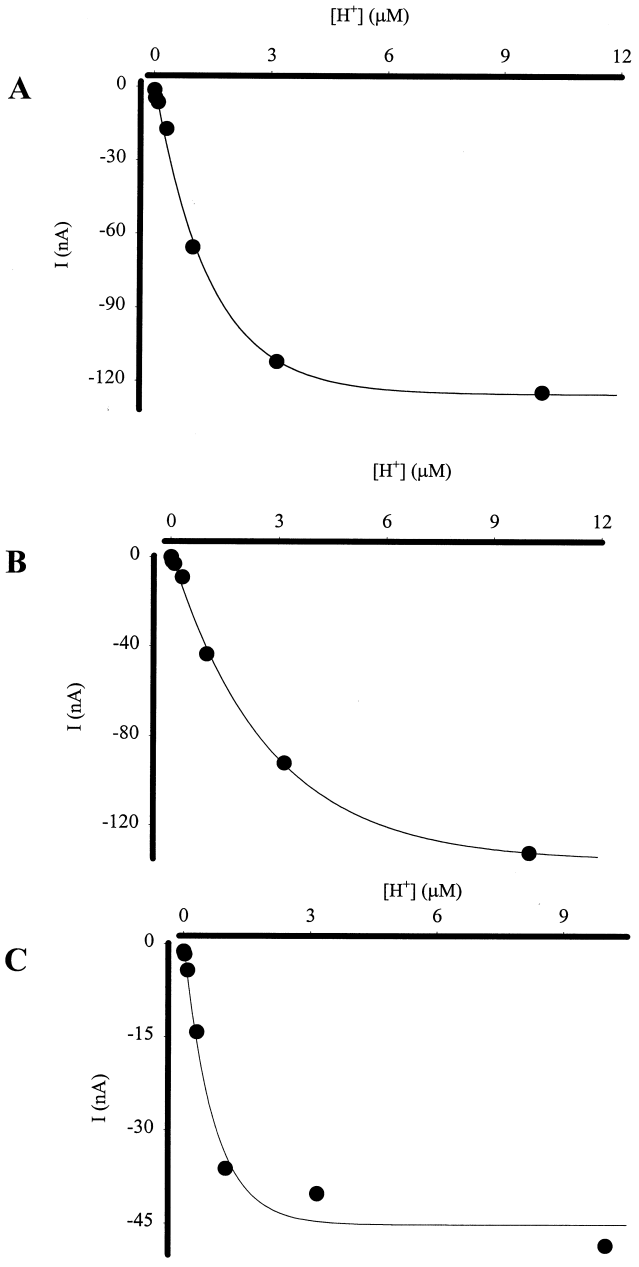

Fig. 1. H^+ activation kinetics of the transport of neutral dipeptide FQ (A), anionic dipeptide FE (B), and cationic dipeptide FK (C) by PEPT2. The peptide-induced currents were monitored at varying concentrations of H^+ in the external medium (10 nM to 10 μM; pH 8.0–5.0) in oocytes expressing rat PEPT2 heterologously. Concentration of the dipeptides was 100 μM for FQ and FE and 600 μM for FK.

these peptides, the H^+:peptide coupling ratio was 1:1. However, there was a significant difference in the shape of the H^+ activation curves among the three peptides even though the data conformed to Michaelis-Menten kinetics in each case. The concentration of H^+ at which the activation of the peptide-induced current reached the maximum was the lowest for FK, highest for FE and intermediate for FQ. This difference was reflected in $K_{0.5}$ values for H^+ activation. This value was 0.7 ± 0.2 μM for FK, 3.1 ± 0.4 μM for FE, and 1.4 ± 0.3 μM for FQ.

We then determined the charge/peptide ratio for each of these three dipeptides at a fixed pH (5.5) and membrane potential (−50 mV). This was done by measuring simultaneously the peptide transported into the oocytes and the charge transfer associated with the transport process. The peptide-induced current was continuously recorded with the Fetchex protocol for 5–10 min while the oocytes were perifused with the peptide solution containing a small amount of radiolabeled peptide (Fig. 2A–C). Integration of the area enclosed by the current versus time curve was performed using the trapezoidal algorithm in SigmaPlot program and the magnitude of the area was used to calculate the charge transfer based on Faraday's constant (9.65×10^4 C/mol). Immediately after the microelectrodes were withdrawn, the oocyte was washed quickly and the radioactivity associated with the oocyte was determined to calculate the amount of the peptide transported into the oocyte during the current recording. This procedure was used independently for 7–9 oocytes for each peptide. The magnitudes of the charge transfer and peptide transfer were then compared for each peptide (Fig. 2D–F). There was a strict quantitative relationship between the charge transfer and the peptide transfer with a charge/peptide ratio of 1 for all three peptides.

The data presented in Fig. 2 show that the transport of the peptide at pH 5.5 is associated with the transfer of one positive charge per peptide molecule irrespective of whether the peptide is zwitterionic, anionic, or cationic at physiological pH. However, the data in Fig. 1 show that the number of H^+ associated with the transport of the peptide is 1 for all three peptides. A 1:1 stoichiometry of H^+ and peptide means that the net charge transfer per peptide molecule would vary depending upon the ionic form of the transported peptide. The value of net charge transfer per peptide molecule would be 1, 0, and 2 for the zwitterionic, anionic and cationic forms, respectively of the peptides transported by PEPT2. However, this is not supported by charge transfer

measurements. The data in Figs. 1 and 2 lead to the conclusion that only the zwitterionic form of the peptides is transported by PEPT2 with a H^+/peptide stoichiometry of 1:1 in each case.

To determine the ionic nature of these peptides at pH 5.5 at which the charge transfer and peptide transfer were measured, we used the Henderson-Hasselbach equation to calculate theoretically the relative concentrations of different ionic species of these peptides at various pH values (Fig. 3). The ionization constants (pK_a) for the dipeptides FQ, FE, and FK were estimated from the values for similar dipeptides in aqueous solution at 25°C [21]. The values used for these calculations were as follows: pK_1 of 3.2 for the α-carboxyl group of glutamine and pK_2 of 8.0 for the α-amino group of phenylalanine in FQ; pK_1 of 3.0 for the α-carboxyl group of glutamate, pK_2 of 4.4 for the γ-carboxyl group of glutamate, and pK_3 of 8.1 for the α-amino group of phenylalanine in FE; pK_1 of 2.2 for the α-carboxyl group of lysine, pK_2 of 7.5 for the α-amino group of phenylalanine, and pK_3 of 11.0 for the ε-amino group of lysine in FK. The relative concentrations of different ionic species of these peptides as a function of pH are given in Fig. 3. Over the pH range of 5.0–8.0, the most predominant ionic species is the zwitterion for FQ, anion for FE, and cation for FK. The concentration of the zwitterionic form as a percent of the total concentra-

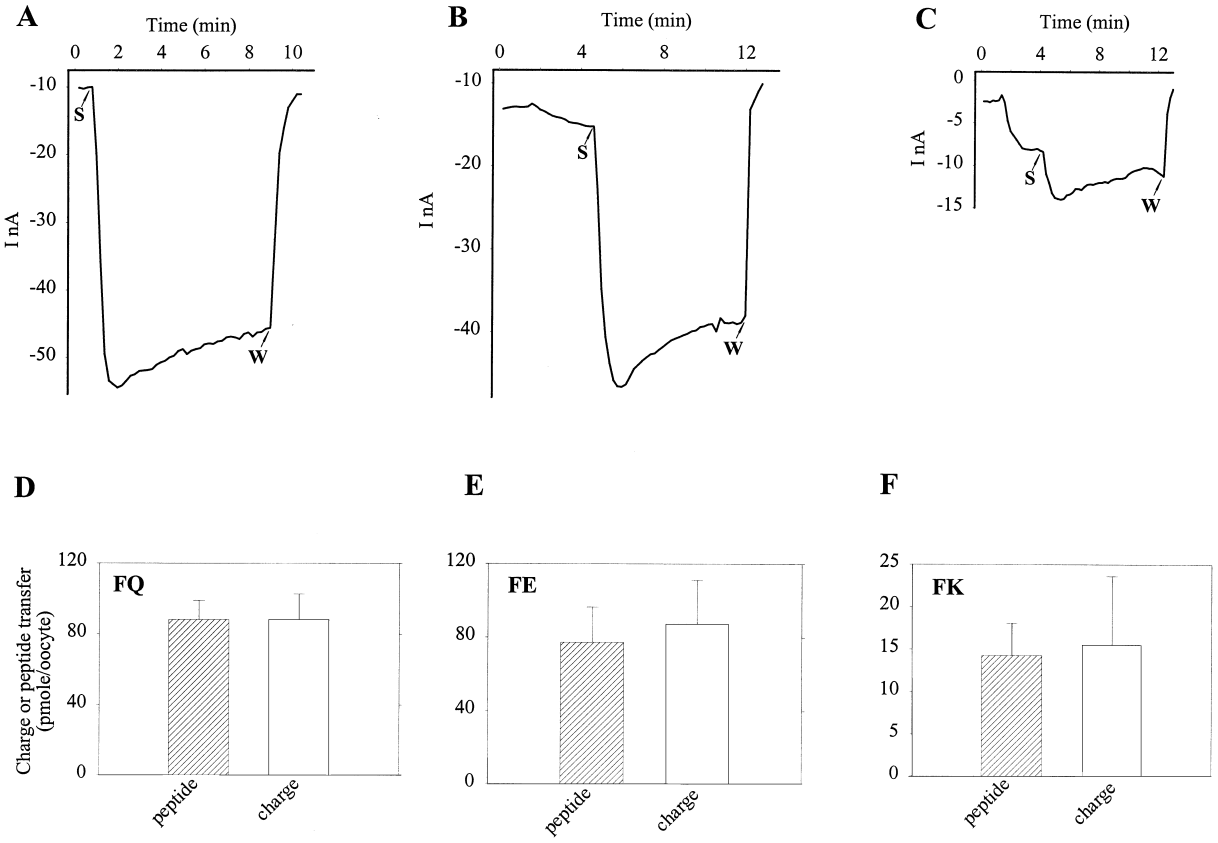

Fig. 2. The charge transfer/peptide transfer stoichiometry for neutral dipeptide FQ (A and D), anionic dipeptide FE (B and E), and cationic dipeptide FK (C and F) in oocytes expressing rat PEPT2 heterologously. (A–C) Representative peptide-induced current traces at pH 5.5 for the dipeptides FQ, FE, and FK, respectively. The points of the current tracing at which the dipeptide substrate was applied (S) and subsequently withdrawn (W) are indicated. Concentration of the dipeptides was 100 μM for FQ and FE and 600 μM for FK. The peptide solutions contained radiolabeled as well as unlabeled peptides which allowed the simultaneous measurements of peptide transfer and charge transfer in the same oocyte. The charge transfer was calculated from the current tracings based on Faraday's constant using the Fetchex protocol and the peptide transfer was calculated by determining the radioactivity associated with the oocyte at the end of the current recording. (D–F) The data on charge transfer and peptide transfer for each dipeptide from multiple oocytes (6–8 oocytes for each peptide).

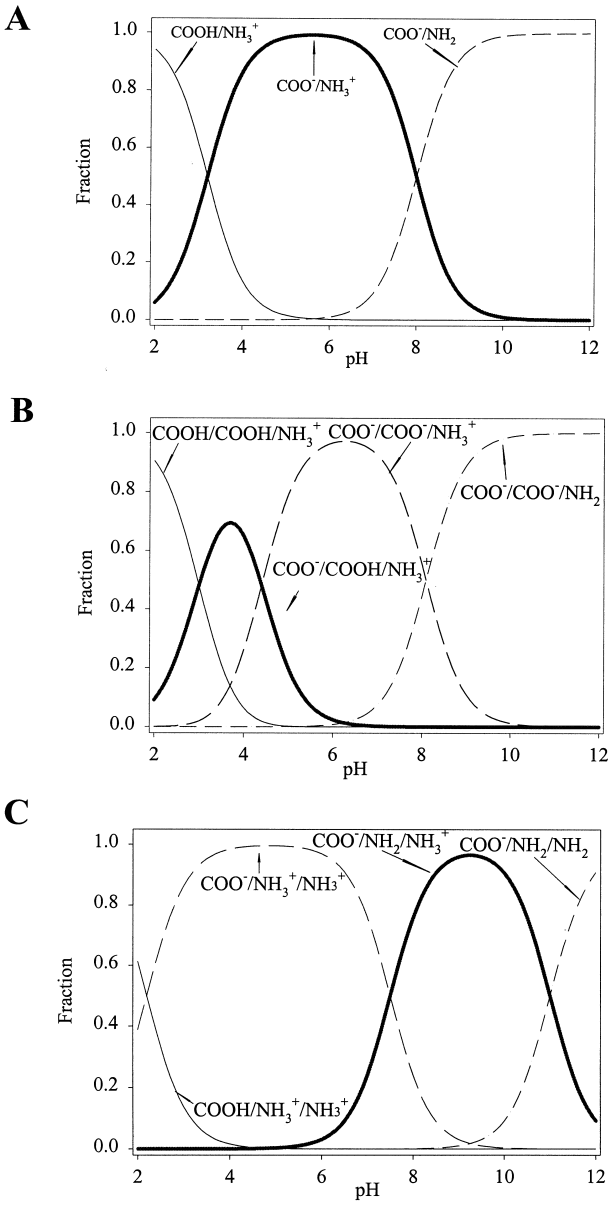

Fig. 3. Concentrations of different ionic species of the dipeptides FQ (A), FE (B), and FK (C) at varying pH. The results are given as fractions of total peptide concentration.

tion of the peptide at pH 5.5 is 99% for FQ, 7% for FE, and 1% for FK. Thus, the H^+ in the external medium has dual effects on the peptide transporter function, influencing the magnitude of the transmembrane H^+ gradient (the driving force for the peptide transporter) as well as the concentration of the transportable substrate. While the influence on the transmembrane H^+ gradient will have the same effect on the transporter function irrespective of the peptide substrate handled by the transporter, the influence on the concentration of the zwitterionic species varies depending on the peptide. A change of the external pH from 7.5 to 5.5 increases the percent of the zwitterionic species from 76% to 99% in the case of FQ, from $<0.1\%$ to 7% in the case of FE, and from 50% to 1% in the case of FK. Thus, while the concentration of the transportable species remains almost the same for FQ when pH is changed from 7.5 to 5.5, it increases more than 70-fold for FE and decreases 50-fold for FK. The H^+ activation kinetics for FQ, FE, and FK therefore reflect the combined effects of the external H^+ on the magnitude of the driving force for the transporter and on the concentration of the transportable ionic species. This may partly explain the significant differences in the $K_{0.5}$ values for H^+ activation among these three peptides. This value is the highest for FE and lowest for FK. An increase in the external H^+ concentration enhances the driving force as well as increases the concentration of the zwitterionic species for FE. In contrast, an increase in the external H^+ concentration enhances the driving force but decreases the concentration of the zwitterionic species for FK. Therefore, the $K_{0.5}$ value for H^+ activation of the transport process is much higher for FE than for FK. In other words, the external pH at which the transport process is activated at the half-maximal level is relatively more acidic for FE (pH 5.2) than for FK (pH 6.2). The corresponding value for FQ (pH 5.9) is intermediate between the values for FE and FK.

Another point of interest is the relative transport rates of these three peptides. The FE-induced current at an external pH of 5.5 is comparable to the FQ-induced current but is much higher than the FK-induced current. The peptide-induced currents are comparable for FQ and FE despite the fact that the concentration of the transportable zwitterionic species of FQ is at least one order of magnitude greater than that of FE at this external pH. It is possible that the influence of internal pH on the ionization of these peptides affects their dissociation from the transporter-substrate complex on the internal surface. Since the transporter preferentially recognizes the zwitterionic species as the substrate, the transported zwitterionic FE in the transporter-FE complex ionizes to become an anion at the internal pH of 7.4 on the cytoplasmic surface of the oocyte

and consequently loses its affinity for the transporter. This facilitates the release of the substrate from the transporter. In contrast, this process is not expected to have a significant influence on the dissociation of FQ from the transporter because the concentration of the zwitterionic species remains approximately the same at pH 7.4 (internal pH) and at pH 5.5 (external pH). The facilitation of the release of FE from the transporter on the internal surface as a result of the transmembrane pH gradient might be expected to enhance the transport process and compensate for the relatively decreased concentration of the transportable zwitterionic species compared with FQ on the external side. This would explain the comparable transport rates of FQ and FE despite the differences in the concentration of the transportable zwitterionic species between these two peptides in the external medium. In case of FK, the existence of the zwitterionic species is favored at the internal pH of 7.4 and therefore the release of the transported zwitterionic species of FK is not expected to change due to the transmembrane pH gradient. This situation is similar to that of FQ. Therefore, the difference in the concentrations of the transportable zwitterionic species between FQ and FK in the external medium is the major contributing factor for the difference in the transport rates of these two peptides.

Steel et al. [18] have reported that, in the case of PEPT1, the H^+/substrate coupling ratios are 1:1, 2:1, and 1:1 for neutral, anionic, and cationic dipeptides, respectively. These data are very similar to the data presented here for PEPT2. The H^+/peptide stoichiometry for the neutral dipeptide FQ and the cationic dipeptide FK is 1:1 for PEPT2 as is the case for PEPT1. The H^+/peptide stoichiometry of 2:1 reported for PEPT1 in the case of anionic peptides is the same as the H^+/peptide stoichiometry of 1:1 observed in the present study in the case of PEPT2 for the anionic dipeptide FE if only the zwitterionic form of FE is the transportable substrate. It has to be pointed out that the ratio of charge transfer/peptide transfer was 1 for the anionic peptide FE in the case of PEPT1 [18] as is the case in the present study for PEPT2, but the number of H^+ cotransported with FE via PEPT1 was deduced to be 2 based on the assumption that the predominantly occurring anionic form of FE was the transportable species. An alternative interpretation of the data with PEPT1 for the anionic peptides is that one H^+ is required to convert the anionic peptide into a zwitterion and the other H^+ is required for coupling as the cotransported ion.

The present findings with rat PEPT2 expressed heterologously in *X. laevis* oocytes are different from the findings by Temple et al. [22] with rat PEPT2 studied using rat renal brush border membrane vesicles. Peptide transport in these vesicles is predominantly due to PEPT2 activity. The H^+/peptide stoichiometry for the neutral, anionic, and cationic peptides FQ, FE, and FK in these membrane vesicles was found to be 1:1, 2:1, and 0:1, respectively. These stoichiometry values were obtained by analyzing the H^+ activation kinetics of the transport of the three peptides in radiolabeled form. In the case of the cationic peptide FK, the transport rates were very small in membrane vesicles and this might have made it difficult to detect the activation of transport by extravesicular H^+. With the same peptide, we were able to detect the activation of transport by external H^+ in *X. laevis* oocytes expressing PEPT2 heterologously. The reasons for the difference in the stoichiometry values for the anionic peptide FE obtained by Temple et al. [22] and by us (present study) are not known because in both experimental approaches the transport rates of FE were easily measurable and H^+ activation of transport was easily detectable. But, the H^+ activation of FE transport in membrane vesicles was sigmoidal with a Hill coefficient of 2 whereas the H^+ activation of FE transport in *X. laevis* oocytes expressing PEPT2 was hyperbolic with a Hill coefficient of 1.

In summary, the present investigation demonstrates that PEPT2 transports neutral, anionic, and cationic dipeptides with a H^+/peptide stoichiometry of 1:1. In all cases, the transport process is H^+-dependent and electrogenic. The charge/peptide stoichiometry is 1:1 for all three classes of dipeptides. Taken collectively, these data lead to the conclusion that PEPT2 recognizes only the zwitterionic forms of the dipeptides as transportable substrates. These findings with PEPT2 are similar to those reported previously for PEPT1.

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References

[1] V. Ganapathy, F.H. Leibach, Curr. Opin. Cell Biol. 3 (1991) 695–701.
[2] D.M. Matthews, Protein Absorption. Development and Present State of the Subject, Wiley-Liss, New York, 1991.
[3] V. Ganapathy, M. Brandsch, F.H. Leibach, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, Raven Press, New York, 1994, pp. 1773–1794.
[4] V. Ganapathy, F.H. Leibach, Am. J. Physiol. 251 (1985) F945–F953.
[5] D. Meredith, C.A.R. Boyd, J. Membr. Biol. 145 (1995) 1–12.
[6] H.Y. Steiner, F. Naider, J.M. Becker, Mol. Microbiol. 16 (1995) 825–834.
[7] V. Ganapathy, F.H. Leibach, J. Biol. Chem. 258 (1983) 14189–14192.
[8] V. Ganapathy, G. Burckhardt, F.H. Leibach, J. Biol. Chem. 259 (1984) 8954–8959.
[9] V. Ganapathy, Y. Miyamoto, F.H. Leibach, Contr. Infusion Ther. Clin. Nutr. 17 (1987) 54–68.
[10] F.H. Leibach, V. Ganapathy, Annu. Rev. Nutr. 16 (1996) 99–119.
[11] H. Daniel, J. Membr. Biol. 154 (1996) 197–203.
[12] S. Nussberger, M.A. Hediger, Exp. Nephrol. 3 (1995) 211–218.
[13] Y.J. Fei, V. Ganapathy, F.H. Leibach, Prog. Nucleic Acid Res. Mol. Biol. 58 (1998) 239–261.
[14] H. Wang, Y.J. Fei, V. Ganapathy, F.H. Leibach, Am. J. Physiol. 275 (1998) C967–C975.
[15] R. Dringen, B. Hamprecht, S. Broer, J. Neurochem. 71 (1998) 388–393.
[16] S. Amasheh, U. Wenzel, M. Boll, D. Dorn, W. Weber, W. Clauss, H. Daniel, J. Membr. Biol. 155 (1997) 247–256.
[17] B. Mackenzie, Y.J. Fei, V. Ganapathy, F.H. Leibach, Biochim. Biophys. Acta 1284 (1996) 125–128.
[18] A. Steel, S. Nussberger, M.F. Romero, W.F. Boron, C.A.R. Boyd, M.A. Hediger, J. Physiol. 498 (1997) 563–569.
[19] S. Amasheh, U. Wenzel, W. Weber, W. Clauss, H. Daniel, J. Physiol. 504 (1997) 169–174.
[20] N. Lister, A.P. Sykes, P.D. Bailey, C.A.R. Boyd, J.R. Bronk, J. Physiol. 484 (1995) 173–182.
[21] M.A. Sober, CRC Handbook of Biochemistry, CRC Press, Boca Raton, FL, 1968.
[22] C.S. Temple, J.R. Bronk, P.D. Bailey, C.A.R. Boyd, Pflugers Arch. 430 (1995) 825–829.