Stoichiometry and Kinetics of the High-affinity H⁺-coupled Peptide Transporter PepT2*

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Proton-coupled peptide transporters mediate the absorption of a large variety of di- and tripeptides as well as peptide-like pharmacologically active compounds. We report a kinetic analysis of the rat kidney high-affinity peptide transporter PepT2 expressed in Xenopus laevis oocytes. By use of simultaneous radioactive uptake and current measurements under voltage-clamp condition, the charge to substrate uptake ratio was found to be close to 2 for both D-Phe-L-Lys and D-Phe-L-Glu, indicating that the H⁺:substrate stoichiometry is 2:1 and 3:1 for neutral and anionic dipeptides, respectively. The higher stoichiometry for anionic peptides suggests that they are transported in the protonated form. For D-Phe-L-Lys, the charge:uptake ratio averaged 2.4 from pooled experiments, suggesting that Phe-Lys crosses the membrane via PepT2 either in its deprotonated (neutral) or its positively charged form, averaging a H⁺:Phe-Lys stoichiometry of 1.4:1. These findings led to the overall conclusion that PepT2 couples transport of one peptide molecule to two H⁺. This is in contrast to the low-affinity transporter PepT1 that couples transport of one peptide to one H⁺. Quinapril inhibited PepT2-mediated currents in presence or in absence of external substrates. Oocytes expressing PepT2 exhibited quinapril-sensitive outward currents. In the absence of external substrate, a quinapril-sensitive proton inward current (proton leak) was also observed which, together with the observed pH-dependent PepT2-specific presteady-state currents (I_{pss}), indicates that at least one H⁺ binds to the transporter prior to substrate. PepT2 exhibited I_{pss} in response to hyperpolarization at pH 6.5–8.0. However, contrary to previous observations on various transporters, 1) no significant currents were observed corresponding to voltage jumps returning from hyperpolarization, and 2) at reduced extracellular pH, no significant I_{pss} were observed in either direction. Together with observed lower substrate affinities and decreased PepT2-mediated currents at hyperpolarized V_m, our data are consistent with the concept that hyperpolarization exerts inactivation effects on the transporter which are enhanced by low pH. Our studies revealed distinct properties of PepT2, compared with PepT1 and other ion-coupled transporters.

In kidney and intestine, enzymatic degradation of proteins and peptides produces oligopeptides that are absorbed across the brush-border membrane of epithelial cells, followed by breakdown into free amino acids (1–3). The absorption is carried out by proton-driven cotransport systems, as demonstrated by studies using brush-border membrane vesicles, epithelial cells in culture, intact epithelial tubules, and recombined peptide transporters (4–10). A large variety of di- and tripeptides, as well as pharmacologically active peptide-like compounds such as β-lactam antibiotics and angiotensin-converting enzyme inhibitors, are transported (11–13). Peptide transporters have been cloned since 1994 (3, 14–19) and were demonstrated by studies using brush-border membrane vesicles, epithelial cells in culture, intact epithelial tubules, and recombined peptide transporters (4–10). A large variety of di- and tripeptides, as well as pharmacologically active peptide-like compounds such as β-lactam antibiotics and angiotensin-converting enzyme inhibitors, are transported (11–13). Peptide transporters have been cloned since 1994 (3, 14–19) and were shown to accept many oligopeptides and peptide-derived compounds as substrates, highlighting the physiological significance of these transporters in nutrient and drug absorption. Functional studies of these membrane proteins allow a better understanding of the mechanism of substrate-transporter interaction and may help establishing therapeutic strategies involving peptide-based drugs.

An electrophysiological study of PepT2 has been carried out recently on a rabbit homologue (10). However, the H⁺:peptide stoichiometry is still controversial (6, 10, 13, 20). In the present study, we report novel characteristics of the rat PepT2 demonstrated by neutral, anionic, and cationic substrates under various conditions, using the two-microelectrode voltage-clamp technique. We simultaneously measured the radiolabeled peptide uptakes and the PepT2-mediated currents under voltage-clamp conditions in order to determine the H⁺:substrate stoichiometry. We also demonstrated quinapril-sensitive PepT2-mediated outward currents, a proton leak, and pH-dependent presteady-state currents (I_{pss}).

EXPERIMENTAL PROCEDURES

Isolation of PepT2 Clone and Oocyte Preparation—A kidney cortex cDNA of Xenopus laevis oocytes were prepared as described previously (22) except that oocytes were defolliculated by incubating them in the calcium-free Barth’s solution containing collagenase (3 mg/ml) at 18 °C for 3–3.5 h. Capped cRNA of rat PepT2 was synthesized by in vitro transcription from cDNAs in PTLN2 and injected (with 15–25 ng) into oocytes. The same volumes of H₂O were injected as the control.

Electrophysiology—The two-microelectrode voltage-clamp experiments were performed using a commercial amplifier (Clampator One, model CA-1B, Dagan Co., Minneapolis, MN) and the pCLAMP software (Version 6, Axon Instruments, Inc., Foster City, CA). In experiments involving voltage jumps, currents or membrane potentials were recorded by digitizing at 150 μs/sample and by the Bessel filtering at 10 kHz. When recording currents at a holding potential, digitization at 0.5 s/sample and filtering at 20 Hz were used. Control solutions used for extracellular perfusion contained (in mM): NaCl, 100; HEPES, 10;
MES, Tris base 2.5; KCl, 2; CaCl$_2$, 1; MgCl$_2$, 1; pH 5.0–8.0 by N-methyl-$d$-glucamine or HEPES. After 3–5 min of membrane potential stabilization following microelectrode impalements, the oocyte was clamped to the holding potential ($V_m$) of −50 mV. 100-ms voltage pulses of between −160 and +60 mV, in increments of +20 mV, were then applied, and steady-state currents were obtained as the average values in the interval from 80 to 95 ms after the initiation of the voltage pulses.

Voltage-clamped Tracer Measurements—Substrate-evoked currents and uptake of [4-$^3$H-$d$-Phe]-Ala, [4-$^3$H-$d$-Phe]-Glu, or [4-$^3$H-$d$-Phe]-Lys were simultaneously measured under voltage-clamp conditions, according to a method similar to the one described previously (23). The radiolabeled phenylalanyl-dipeptides were synthesized by Zeneca Cambridge Research Biochemicals (Northwich, Cheshire, United Kingdom) and dissolved in 10% aqueous ethanol. The specific radioactivity is 12 Ci/mmol, and the concentration is 1 μCi/μl (or 83.3 μμ) for all three peptides. The unlabeled Phe-Glu and Phe-Lys were synthesized as described previously (24), and other peptides were purchased from Sigma. The control solution or HEPES-rich solution (in mM: NaCl, 70; HEPES, 60; MES, 2.5; KCl, 2; CaCl$_2$, 1; MgCl$_2$, 1; pH 5.5–6.5 by N-methyl-$d$-glucamine) was used for external perfusion. The uptake solution consisted of the control or HEPES-rich solution plus cold (0.5, 0.5, or 1 μl of Phe-Ala, Phe-Glu, or Phe-Lys, respectively) and hot substrate (1.33, 1.33, or 2.67 μl of radiolabeled Phe-Ala, Phe-Glu, or Phe-Lys, respectively, in 200 μl of solution). The hot substrates contained in the uptake solutions represented only 0.11% of the total substrates. Before starting tracer uptakes, oocyte was clamped at −80 mV and perfused with substrate-free solution. Then the perfusion was stopped, and the uptake solution (200 μl) was added manually using a micropipettor, which washed out the substrate-free solution. The uptake lasted 5 min in the chamber whose volume is about 200 μl and terminated by perfusing (washing) the oocyte with the substrate-free solution.

Statistics and Data Analysis—Experimental results were expressed in the form of mean ± S.E. (n), where n indicates the number of oocytes obtained from at least two different donors. The curve-fitting procedures were performed using the SigmaPlot program (Version 4, Jandel Scientific Software, San Rafael, CA), and each fitted parameter is expressed in the form parameter ± S.E., where S.E. represents the standard error in the fitting estimates.

RESULTS

Substrate and Proton Affinities—PepT2 mediates the high-affinity transport of most di- and tripeptides at physiological membrane potentials ($V_m$) but not at hyperpolarized $V_m$ (Fig. 1, A and B). Apparent affinities for both glycyl-dipeptides (Gly-Glu, Gly-Leu, Gly-Lys) and $d$-phenylalanyl-dipeptides (Phe-Glu, Phe-Ala, Phe-Lys) were strongly voltage-dependent and decreased about 10-fold when hyperpolarizing from −50 to −160 mV (Table I and Fig. 1B). Although affinities differ largely according to the charge status of glycyl-dipeptides, the maximal currents for glycyl-dipeptides remain approximately the same in the whole voltage range (Fig. 1C). High concentrations of glycyl-dipeptides evoked currents that did not saturate by hyperpolarization, while modest or low concentrations of these substrates did saturate (Fig. 2A). In contrast, saturation at hyperpolarization was observed for phenylalanyl-dipeptides, even at high concentrations (Fig. 2B), presumably due to relatively low substrate affinity (Table I). Depending on pH, substrate type, and substrate concentration, PepT2 may exhibit decreases in current with hyperpolarization (Fig. 2) due to low affinities for substrates. The affinities for cationic peptides are relatively low compared with those for anionic and neutral peptides, in analogy to PepT1 (25, 26).

The proton affinities were determined at various membrane potentials using glycyl-dipeptide concentrations approximately equal to five times that of their $K_m$ values. Substrate-evoked currents generally increase with an increase in $[H^+]$, which is consistent with a proton-driven transport. However, as alluded to above, currents evoked by low substrate concentrations (especially those evoked by cationic substrates) decreased with increasing $[H^+]$ and hyperpolarization (Fig. 2, D and E).

The abbreviations used is: MES, morpholineethanesulfonic acid.

TABLE I

| Substrate          | $K_m^{50} \mu$M | $K_m^{160} \mu$M | $K_m^{160}/K_m^{50}$ |
|--------------------|----------------|----------------|----------------------|
| d-Phe-$d$-Glu      | 48 ± 4         | 520 ± 60       | 11                   |
| d-Phe-$d$-Ala      | 75 ± 6         | 570 ± 70       | 7.6                  |
| d-Phe-$d$-Lys      | 135 ± 6        | 890 ± 230      | 6.6                  |
| L-Gly-$d$-Glu      | 16 ± 1         | 490 ± 80       | 30                   |
| L-Gly-$d$-Leu      | 44 ± 0.5       | 30 ± 2         | 6.9                  |
| L-Gly-$d$-Lys      | 51 ± 3         | 660 ± 10       | 11                   |

$K_m^{50} = K_m$ at −50 mV; $K_m^{160} = K_m$ at −160 mV.
were significantly different, indicating that this method is not accurate for stoichiometry evaluation. In addition, since currents at low pH experienced decreases at hyperpolarized $V_m$ (see Fig. 2F, at $-100$ mV), they no longer obey the Michaelis-Menten or the Hill relationships. Thus, it is inaccurate to evaluate stoichiometric ratios based on Hill coefficients. More direct approaches are necessary to determine the $H^+$:substrate stoichiometry.

**Determination of Stoichiometry by Tracer Method**—The proton:substrate coupling ratio (stoichiometry) can be accurately determined when the proton and substrate fluxes mediated by PepT2 are measured under the same conditions. Simultaneous monitoring of transporter-specific currents and radioactive substrate uptakes from the same oocyte under voltage-clamp conditions is one of the few approaches proven to be accurate. Voltage-clamp condition is critical to monitor the PepT2-specific currents, because background currents (before substrate addition) change due to depolarization that is elicited by substrate addition. We observed that, during the uptake period, substrate-elicited currents significantly decreased after reaching initial peak values (Fig. 3, A–C), which poses the question of whether these decreases interfere with stoichiometry determination. Decreases were less pronounced when the buffer concentration was lower. In the same oocytes, decreases in Gly-Leu-elicited current were also present during continuous perfusion but were accelerated when perfusion was stopped (not shown). These data indicate that decreases in current were partly due to decreased proton concentrations at the immediate proximity of the extracellular membrane. Decreases in current during perfusion may be due to intracellular substrate or $H^+$ accumulation (trans-inhibition), in analogy to observations for other transporters (34, 35). None of these factors are expected to compromise the accuracy of stoichiometry determination.

No significant difference in the charge:uptake ratios were observed when using either control (10 mM HEPES) or HEPES-rich solution (see “Experimental Procedures”). When charge was converted into picomole, the charge to uptake ratios for Phe-Glu, Phe-Ala, and Phe-Lys were determined and averaged $1.91 \pm 0.04$ ($n = 11$), $2.16 \pm 0.02$ ($n = 8$), and $2.42 \pm 0.03$ ($n = 16$), respectively (Fig. 3D). Data obtained using either solution were both plotted in Fig. 3D. These results indicate that the number of protons cotransported is $3$ and $2$, respectively, for each anionic and neutral peptide transported. The cotransported proton number per Phe-Lys molecule is $1.4$, which is significantly larger than $1$, suggesting the possibility that Phe-Lys is transported either under the deprotonated (neutral) form (60%) or under the positively charged form (40%).

**Quinapril-inhibited PepT2-mediated Currents**—Quinapril, an angiotensin-converting enzyme inhibitor, was found to inhibit the PepT2-mediated currents in oocytes expressing PepT2 (Fig. 4, A, B, and D) but had no effects on control oocytes. The inhibition was demonstrated to be noncompetitive with an $IC_{50}$ close to $1$ mM by an independent study (data from our labora-
against radiolabeled uptake. The slopes of the linear fits corresponding to the maximal charge displacement \((Q_{\text{max}})\) at pH 6.0 represents less than 15% of the value at pH 7.0. Surprisingly, at the tested pH range (5.0–8.0), no significant \(I_{\text{pss}}\) were observed for the off-responses (returning from test potentials \(V_{\text{h}}\) to \(V_{\text{o}}\)) (see examples in Fig. 5A–C) and \(I_{\text{pss}}\) at pH 6.0 recovered by addition of substrate (Fig. 5D; steady-state currents were subtracted). These abnormal behaviors of PepT2 are not readily accounted for by previously described kinetic models (see “Discussion”).

Between −160 and −80 mV, relaxation time constants (\(\tau\)) of \(I_{\text{pss}}\) for the on-responses ranged from 10 to 40 ms. With external alkalization, the \(V_{\text{m}}\) corresponding to the maximal \(\tau\) shifted to more negative values (Fig. 5F), similar to other transporters such as PepT1 (9) and SGLT1 (27). At more depolarized potentials, \(\tau\) could not be evaluated due to small \(I_{\text{pss}}\). \(\tau\) was only slightly affected by intracellular acidification using a previously reported approach (9) (not shown), in contrast to \(\tau\) of PepT1 that showed large responses (9).

**DISCUSSION**

Using biophysical approaches, we have revealed unique kinetic properties of rat PepT2 expressed in *Xenopus* oocytes. These include the stoichiometries for different types of substrates determined under voltage-clamp conditions, the proton leak, and the reversed transport mode as well as presteady-state currents.

**Stoichiometry**—The number of proton ions (“\(n\)”) necessary for cotransporting one substrate molecule (stoichiometry “\(n\)”) determines the concentration of intracellular substrate that cells can keep at equilibrium. A high H⁺:substrate ratio allows maintenance of high intracellular substrate concentrations but also requires a relatively large amount of H⁺ electrochemical energy. Earlier studies on isolated intestinal tissue preparations and the Caco-2 cell line derived a H⁺:peptide ratio of greater than 2:1 based on measurements of short circuit currents and peptide fluxes (5, 28). In contrast, according to equilibrium intracellular substrate estimation (14) and Hill plot analysis (13, 26, 29) using *Xenopus* oocytes expressing low affinity PepT1, a 1:1 coupling ratio was deduced for neutral substrates. More recently, our laboratory used current and tracer measurements to study the stoichiometry of PepT1 for neutral and charged peptides (25). These experiments revealed 1:1, 2:1, and 1:1 ratios for neutral, anionic, and cationic peptides, respectively.

Studies of the high-affinity peptide transporter using rat kidney brush-border membrane vesicles (6) and oocytes expressing rabbit renal PepT2 (10, 13) revealed a Hill coefficient \((n_{\text{Hill}})\) close to 1 for charged and neutral peptides, suggesting a 1:1 stoichiometry. However, using brush-border membrane vesicles of rat kidney cortex, Temple et al. (20) found a \(n_{\text{Hill}}\) close to 1 and 2 for neutral and anionic dipeptides, respectively, and suggested a stoichiometry of 1:1 and 2:1, correspondingly. Since \(n_{\text{Hill}}\) value may depend on the substrate concentration and binding cooperativity of different protons in case of coupling to multiple protons, \(n_{\text{Hill}}\) is usually not equal to the actual stoichiometric ratio. Protons are also known to have multiple effects on membrane proteins as well as on substrates (30). The evaluation of \(n_{\text{Hill}}\) necessitates experiments to be performed over a wide pH range. However, proteins and/or substrates might not have the same activity in the whole range, which would significantly influence the profile of current versus proton concentration ([H⁺]). Although in epithelial cells PepT2 is in contact with a luminal unstirred layer, which has a pH ranging between 5.5

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**Fig. 3. Stoichiometry determination by simultaneous measurements of substrate-evoked currents and tracer uptakes under voltage-clamp conditions** (<math>\(V_{\text{h}} = -80 \text{ mV}\)). A, representative examples of currents generated by 500 μM Phe-Glu (cold + hot). Recordings were obtained using solutions containing 10 mM (left panel) or 60 mM HEPES (right panel) (see “Experimental Procedures” for detail). The charge moved was calculated by integrating the Phe-Glu-evoked current over the uptake period. B, representative examples using 500 μM Phe-Ala. C, representative examples using 1 mM Phe-Lys (see the scale difference). D, charge moved was converted to pmol and plotted against radiolabeled uptake. The slopes of the linear fits corresponding to Phe-Lys (▼), Phe-Ala (○), and Phe-Glu (▲) are 2.44 ± 0.02, 2.15 ± 0.02 and 1.88 ± 0.03, respectively.

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and 6.0 (31), and proton affinity constants of PepT2 are around pH 6.0 (see "Results"), PepT2-mediated currents decreased when pH, approached 5.0–6.5 (Fig. 2). This means that PepT2 currents no longer obey the Michaelis-Menten or Hill relationships.

Our results from simultaneous measurements of radiolabeled peptide uptakes and PepT2-mediated currents under voltage-clamp condition show that the charge:uptake ratios are close to 2 for both anionic and neutral substrates. These data indicate that PepT2 possesses two H⁻- and one substrate-binding sites. In the case of anionic substrates, one additional proton is needed, most likely for substrate protonation before or during binding. Because of the hydrophobic environment provided by the membrane and the transporter, the charges that the loaded transporter is permitted to carry within the membrane should be well controlled and relatively constant (+2 for neutral and anionic dipeptides). In the case of cationic substrates (S⁺⁻) such as Gly-Lys and Phe-Lys, the charges on the loaded transporter (2H⁻ and 1S⁺) are +3, and the cotransport might be unfavorable. The lysine residue under physiological pH range (pH 5.0–8.5) predominantly carries one positive charge. However, PepT2 protein might help in deprotonating (neutralizing) the lysine residue even at external physiological pH and, thereby, transport the resulting neutral form of dipeptide by coupling to two protons. This process is apparently equivalent to the stoichiometry of 1H⁻:1S⁺ and corresponds to carried charges of +2. Observed charge:Phe-Lys ratio of 2.4 is consistent with the interpretation that both cationic and neutral forms of Phe-Lys are transported. The former (2H⁻:1S⁺) accounts for 40% and the latter (1H⁻:1S⁺) accounts for 60% of observed Phy-Lys-evoked currents. However, neither of these two coupling mechanisms satisfies both the 2:1 H⁻-substrate stoichiometry requirement and the 2:1 charge:substrate ratio requirement, which may explain observed significantly lower affinities for cationic substrates compared with neutral or anionic substrates.

The characteristics of high stoichiometry and overall high affinity of PepT2 as compared with PepT1 are consistent with its S3 localization in the kidney (32), where PepT2 can efficiently reabsorb peptides using higher electrochemical energy of protons. In contrast, the 1:1 stoichiometry and low affinity of PepT1 allow economic and efficient substrate absorption in the intestine and early parts of renal proximal tubules.

The Reversed Transport Mode—As required by the principle of microscopic reversibility, reversed transport must occur provided that substrates are available in the intracellular side of the membrane. Usually, forward and reversed transports are not symmetrical in terms of substrate/ion affinity. Transport by SGLT1 exhibits a strong inward rectification in both cotransport and Na-leak modes, as revealed using the cut-open oocyte technique (33). Reversed transport has been demonstrated by using transporter-specific inhibitors in oocytes expressing SGLT1 (by phloretin, Ref. 33), EAAC1 (by kainate, Ref. 38), and SDC1 (by phloretin, Ref. 23), etc. Reversed transport depends on the availability of sufficient levels of substrates inside the oocyte. However, no significant levels of dipeptides were observed in Xenopus oocytes (39) as they are efficiently digested by intracellular peptidases. The observed quinapril-inhibitable outward currents of PepT2 might originate either from re-
versed cotransport of unknown PepT2 substrates or from proton-leak current.

**Presteady-state Properties of PepT2—Transporters and channels possess charged residues within the membrane which, in response to voltage changes (jumps) applied across the membrane, move (or relax) to new equilibrium positions, thus generating electrical signals (i.e., currents). These currents vanish when the new equilibrium (steady state) is reached and are therefore called presteady-state currents ($I_{\text{pss}}$). Binding/dissociation of coupling ions or substrates are often voltage-dependent and contribute to the observed $I_{\text{pss}}$. Characterization of these currents provides unique information on properties of membrane transporters.

We propose a kinetic model to help understanding how PepT2 $I_{\text{pss}}$ are associated with conformational changes from one steady state to another (Fig. 6). For simplicity, the model was drawn with ordered binding and mirror symmetry, in analogy to models proposed for PepT1 (9, 29). At low external driving-ion concentrations and in the absence of external substrates, the transporter should be predominantly inward facing (states $I$ to $IV$, Fig. 6). $I_{\text{pss}}$ elicited under these (or similar) conditions have been interpreted as being associated with conformational changes of the unloaded transporter from the inward-faced to the outward-faced configurations (27, 36). Conversely, when $[\text{H}^+]_{\text{i}}$ is high, previous kinetic models predict that the transporter is predominantly outward facing (states $II$ and $III$) and that voltage jumps to depolarized $V_m$ generate positive $I_{\text{pss}}$. However, these predictions were not applicable to PepT2 (see below), even though they were extensively verified in a number of transporters such as rat PepT1 (9), human PepT1 (29), and SGLT1 (27, 36). The reported models for these transporters also predict that 1) the maximal charge displacement ($Q_{\text{max}}$) is independent of driving-ion concentration provided that the driving ion is still present and 2) the charge movement associated with the on-response from $V_h$ to a test potential $V_m$ ($Q_{\text{on}}$) is equal to that corresponding to the off-
response from $V_m$ to $V_h$ ($Q_{\text{eff}}$), consistent with strict charge conservation.

As alluded to above, these predictions are not applicable to PepT2. First, $Q_{\text{max}}$ was greatly reduced at low pH ($V_m$) (Fig. 5E). Second, in contrast to $Q_{\text{sw}}$, $Q_{\text{eff}}$ (from hyperpolarized $V_m$ to $V_h$ of $-50$ mV) was negligible at any pH tested. Third, in contrast to previous observations that the presence of external substrate diminishes or eliminates $I_{\text{pss}}$ (see SGLT1, Ref. 36; the Na$^+$-iodide transporter, Ref. 34; PepT1, Ref. 9), PepT2-specific $I_{\text{pss}}$ were increased by substrate addition at low pH, (Fig. 5D). Finally, when the time interval separating two consecutive voltage jumps ($V_h = -50$ mV) was reduced from 650 to 200 or 50 ms, $I_{\text{pss}}$ were largely reduced after the first jump (to $-160$ mV), whereas there was no significant reduction in $I_{\text{pss}}$ when using time intervals of 650 ms or longer (not shown).

These observations indicate that hyperpolarization results in transporter inactivation, which can be reversed by depolarization ($-50$ mV) during a 650-ms period. Hyperpolarization appears to have inactivation effects on steady-state properties as well. At low pH, hyperpolarization resulted in decreases in substrate-evoked current (Fig. 2), substrate affinity (Table I and Fig. 1B), and proton leak (Fig. 4E). External substrate appears to prevent the transporter from being inactivated by hyperpolarization, as we can see from increases in $I_{\text{pss}}$ by substrate addition (Fig. 5D) and from increases in maximal steady-state currents evoked by saturating substrate concentrations (Fig. 1C). Mechanisms underlying such a hyperpolarization-stimulated inactivation are still unclear.

Although PepT2 possesses considerable sequence homology to PepT1 ($-50\%$ identity), our findings revealed profound differences in several aspects such as stoichiometry, substrate affinity, effects of hyperpolarization, and presteady-state properties. While PepT1 was shown to exhibit remarkable symmetry and cold phenylalanyl-dipeptides were kindly provided by Dr. C. A. R. Boyd.

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