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Rapid report

The human intestinal H^+/oligopeptide cotransporter hPEPT1 transports differently-charged dipeptides with identical electrogenic properties

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Abstract

The human intestinal H^+/oligopeptide cotransporter hPEPT1, expressed in *Xenopus* oocytes, transported neutral, anionic and cationic dipeptides with identical electrogenic properties and maximal evoked currents. Currents were activated by 1 H^+ regardless of the net charge on the driven substrate, and were independent of Na_o^+, K_i^+ and Cl_o^-, calling into question the familiar concept of the origin of the transporter-mediated current.

Keywords: Proton-coupled transport ; Oligopeptide transport ; Intestinal absorption ; Membrane transport protein ; Transporter ; *Xenopus* oocyte

The oligopeptide transporter hPEPT1 (cloned from human intestine [1]) mediates proton-coupled active transport of a broad range of dipeptides and tripeptides, including neutral, anionic and cationic substrates. Previously, using two-microelectrode voltage clamp in oocytes expressing hPEPT1, we determined the kinetic properties of hPEPT1 using the neutral dipeptide glycylsarcosine (Gly-Sar) [2]. The aim of the present study was to investigate how changing the charge on the dipeptide affects hPEPT1 function, with regard to voltage dependence, H^+-coupling and ion dependence of oligopeptide transport.

Oocytes, isolated from *Xenopus laevis* (Nasco, Fort Atkinson, WI or Xenopus One, Ann Arbor, MI), were injected as described with cRNA synthesized from plasmid pHPEPT1 [2] using the Ambion MEGAscript™ kit. A two-microelectrode voltage clamp technique (Geneclamp-500 amplifier and Digidata-1200A interface, Axon Instruments, Foster City, CA) was used to measure dipeptide-evoked currents in oocytes 4–10 days after cRNA injection. Oocytes were superfused at 20–23°C with experimental medium: 100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ (buffered to a range of pH values between 5.0 and 7.5 using Hepes, Mes, Tris each variable at 0–3 mM). For Na^+-free or Cl^--free solutions, Na^+ was replaced by choline, Cl^- by gluconate. The oocytes were held at −50 mV and 100-ms step-changes in membrane potential (V_m) applied as described [2]; dipeptide-dependent currents were fitted to the Hill equation (Eq. (1) of Ref. [2]). First order rate constants describing K^+ efflux in the presence of dipeptide were quantified in ^{86}Rb-loaded oocytes as described [3].

As for Gly-Sar [2], 10 mM L-alanyl-L-valine (Ala-Val) evoked large, H^+-dependent inward currents at pH 6.0 in oocytes expressing hPEPT1 (Fig. 1A). The evoked currents showed a marked dependence on V_m, roughly linear between −10 mV and −130 mV; the currents appeared to saturate with hyperpolarization and approached a zero current asymptote at positive V_m ($\approx$ +50 mV). hPEPT1 also accepted substrates which are predominantly charged at physiological pH. Among these were the anionic dipeptide L-alanyl-L-aspartate (Ala-Asp) and the cationic dipeptide L-alanyl-L-lysine (Ala-Lys): the current–voltage relationships for Ala-Asp and Ala-Lys were identical to that for Ala-Val (Fig. 1A). At pH 6.0, the currents evoked by each of these substrates followed Michaelis–Menten-type kinetics: the apparent affinity constant ($K_{0.5}$) was the same ($\approx$ 0.1 mM) for all three dipeptides (Fig. 1B).

Can differences in H^+-coupling or the involvement of additional ions explain why the dipeptide-evoked currents were identical regardless of the net charge on the driven substrate? We investigated the nature of the H^+-coupling for each of the dipeptides using Hill analysis, measuring

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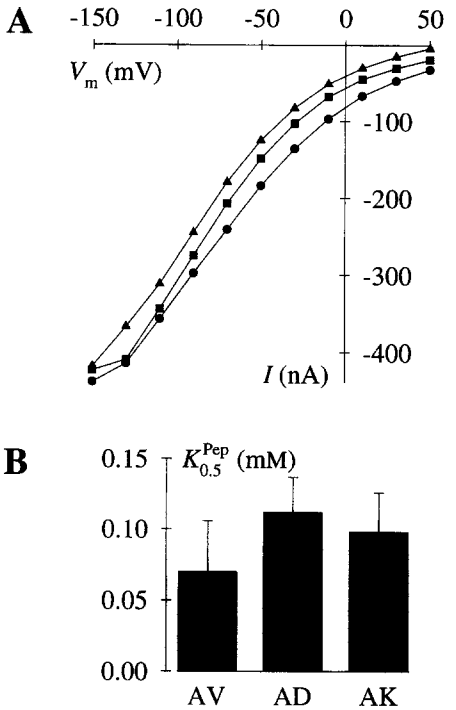

Fig. 1. Currents evoked by differently-charged dipeptides in oocytes expressing hPEPT1. (A) Dipeptide-evoked currents as a function of membrane potential (V_m). A single hPEPT1 cRNA-injected oocyte was superfused at pH 6.0 with 10 mM Ala-Val (●), Ala-Asp (■) and Ala-Lys (▲). (B) $K_{0.5}^{Pep}$ (apparent affinity constant for dipeptide) at pH 6.0 for Ala-Val (AV), Ala-Asp (AD) and Ala-Lys (AK), determined from the currents evoked by 0.01, 0.03, 0.1, 0.3, 1, 3 and 10 mM dipeptide (Eq. (1) of Ref. [2]), at −50 mV. The $K_{0.5}$ values for each dipeptide did not significantly vary from one another at each V_m tested. Error bars are the standard error of the estimates.

the currents evoked by 0.2 mM peptide as a function of pH between 7.5 and 5.0. The Ala-Val-evoked currents were dependent upon an inwardly-directed H^+ gradient (Fig. 2A), with Hill coefficient (n_H) for H^+ of 0.8 ± 0.2 at −50 mV, consistent with coupling stoichiometry 1 H^+:1 Ala-Val. n_H did not vary with V_m (Fig. 2D) within the V_m range for which reliable kinetic estimates could be obtained. For the negatively-charged Ala-Asp, H^+ activated the transporter with $n_H = 1.2 \pm 0.1$ at −50 mV (Fig. 2B); for the positively-charged Ala-Lys, $n_H = 1.0 \pm 0.3$ (Fig. 2C). Between −90 mV and −10 mV, n_H (determined in three oocytes) did not differ from 1 for either Ala-Asp or Ala-Lys (Fig. 2E, F). Therefore Hill analysis suggested 1 H^+:1 dipeptide coupling stoichiometry regardless of the net charge on the driven substrate. The Hill coefficient for *dipeptide*-activation of the evoked currents was also 1 in the case of each substrate (not shown; from the experiment in Fig. 1B). Since PEPT1 is of intestinal origin and at least one other H^+/oligopeptide cotransporter is present in the kidney [4], it is probable that PEPT1 is discrete from the system described by Temple et al., who reported $n_H \approx 2$ for transport of the anionic dipeptide phenylalanylglutamate in rat renal cortical brush-border membrane vesicles, and suggested that transport of cationic dipeptides was uncoupled from H^+ [5].

With H^+/dipeptide coupling of 1:1, we might have expected Ala-Asp transport to be electroneutral, and for Ala-Lys to evoke a current twice the magnitude of the Ala-Val current. However, transport of Ala-Asp was electrogenic and the maximal evoked currents as a function of $[H^+]_o$ (I_{max}^H) were identical for each substrate (Fig. 2 A, B,

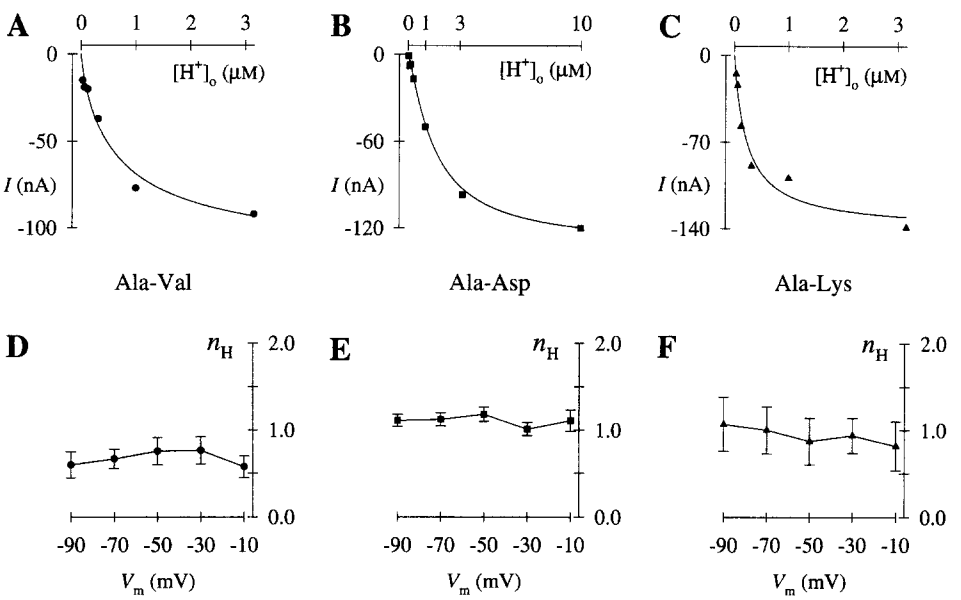

Fig. 2. Hill analysis of H^+ activation of the dipeptide-evoked currents. The currents evoked by the three dipeptides, each at 0.2 mM, were measured at pH 7.5, 7.2, 6.9, 6.5, 6.0, 5.5 and 5.0 in oocytes expressing hPEPT1. (A, B, C) Representative data at −50 mV from a single trial each from the same oocyte. (A) Ala-Val (●, excluding pH 5.0) for which the Hill coefficient for H^+ (n_H) was 0.8 ± 0.2, and the maximal evoked current (I_{max}^H) was 121 ± 32 nA. (B) Ala-Asp (■); $n_H = 1.2 \pm 0.1$; $I_{max}^H = 132 \pm 6$ nA. (C) Ala-Lys (▲, excluding pH 5.0); $n_H = 1.0 \pm 0.3$; $I_{max}^H = 141 \pm 21$ nA. (D, E, F) Hill coefficient (n_H) as a function of membrane potential (V_m); data are means ± S.E.M. from three trials in several oocytes.

C), Despite appreciable differences in the apparent affinity constant for H^+ ($K^H_{0.5}$) between the three dipeptides in this particular oocyte (Fig. 2 A, B, C), we found no statistical differences when we compared the $K^H_{0.5}$ values obtained from multiple trials: $K^H_{0.5}$ for Ala-Val was 1.3 ± 0.5 μM; for Ala-Asp, 1.2 ± 0.1 μM; and for Ala-Lys, 0.5 ± 0.4 μM.

We then considered the involvement of additional ions in order to explain why the current–voltage relationships and maximal evoked currents were identical for each substrate. We found, however, that external Na^+ replacement had no effect on the Ala-Asp-evoked current, and neither did external Cl^- replacement affect the Ala-Lys-evoked current (not shown). A study in membrane vesicles prepared from the LLC-PK_1 kidney cell line expressing the cloned norepinephrine transporter (NET) suggested that transport of dopamine in its cationic form is associated with countertransport of K^+ (together with cotransport of 1 Na^+ and 1 Cl^-) but that K^+ does not play a direct role in activating the transporter [6]. However, the K^+ efflux rate constant in the presence of 10 mM Ala-Lys was no different from that in the presence of Ala-Val (Table 1), excluding countertransport of K^+ as a possible explanation of how Ala-Lys transport was associated with uptake of 1 H^+ and an influx of 1 + net charge. Our data are largely consistent with a preliminary study relating dipeptide-evoked currents to internal acidification: transport of both neutral and cationic dipeptides was coupled to the influx of 1 H^+ [7]. Transport of the anionic dipeptide glycylglutamate however induced twice the internal acidification induced by neutral or cationic dipeptides [7]. These observations may be reconciled with our present findings if transport of anionic dipeptides were associated with countertransport of a pH-changing anion (OH^- or HCO_3^-) such as may be involved in glial cell glutamate uptake [8].

Table 1
Comparison of ^{86}Rb efflux rates in the presence of differently-charged peptides

Peptide	n constant ± S.E.M. (min^{-1}) [a]	K^+ efflux rate
Ala-Val	8	0.118 ± 0.004
Ala-Asp	8	0.126 ± 0.001 [b]
Ala-Lys	9	0.113 ± 0.002 [b]

First-order K^+ efflux rate constants were determined for individual hPEPT1 cRNA-injected oocytes using ^{86}Rb as a K^+ congener. Oocytes were equilibrated with ^{86}Rb (≈ 25 μCi ml^{-1}) in K^+-free buffer at pH 7.4 for 4 h, and the appearance of ^{86}Rb in experimental medium (pH 5.5) containing 10 mM Ala-Val, Ala-Asp or Ala-Lys was measured over 30 min (see Eq. (2) of Ref. [3]).

[a] The K^+ efflux rate constant in hPEPT1 cRNA-injected oocytes without substrate was 0.157 ± 0.004 min^{-1} and was no different in control-injected oocytes; the lower efflux rate constants obtained in the presence of hPEPT1 substrates in cRNA-injected oocytes are attributable to membrane depolarization as a result of hPEPT1-mediated H^+/dipeptide transport.

[b] Not significantly different from Ala-Val.

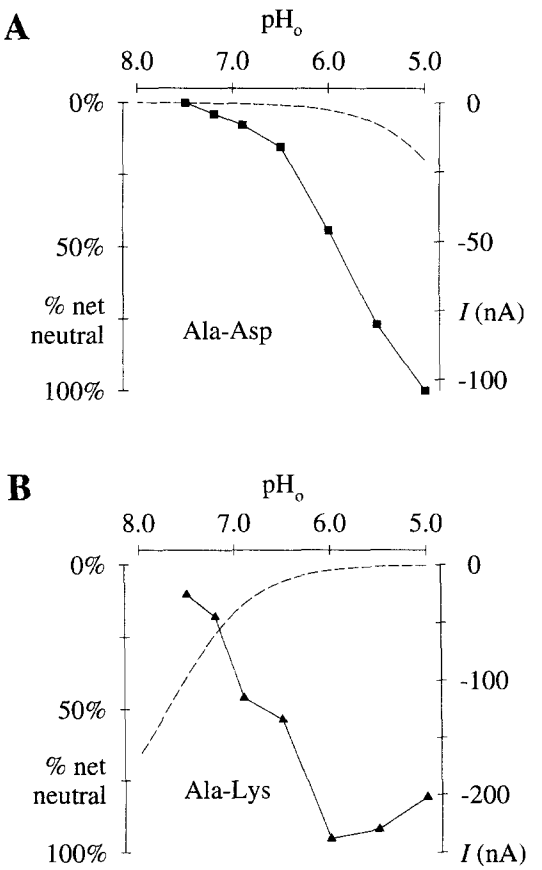

Fig. 3. Evoked currents (−50 mV), together with the concentrations of the net neutral species of the dipeptide, as a function of external pH (pH_o). (A) The Ala-Asp-evoked currents (■, solid line) were plotted as a function of pH_o and related to the fraction of Ala-Asp existing as the net neutral form (broken line), estimated according to the Henderson–Hasselbalch equation and the pK_a values of 3.0, 4.4 and 8.1 [10]. (B) Ala-Lys-evoked currents (▲, solid line), related to the fraction of Ala-Lys in the net neutral form (broken line), using pK_a values 3.2, 7.7 and 10.5 [10]. In the case of the zwitterionic dipeptide Ala-Val (not shown), > 90% was in the net neutral form over the entire pH_o range used.

Wenzel et al. [9] have suggested, for transport of the tripeptide-like β-lactam antibiotics mediated by rabbit PEPT1, that only the net neutral species of the driven substrate was transported. If this was the case, however, it would mean that an acidic pH activates transport solely by increasing the concentration of the transportable neutral species. Since, according to the authors, the transport rate strictly paralleled the concentration of the neutral species of cefixime [9], there seems to be no role for a transmembrane H^+ gradient. If we accept this, PEPT1 should be a facilitative transporter rather than an active transporter. This conclusion is at odds with the fact that the Ala-Asp-evoked currents were activated at pH_o considerably higher than the pH at which we expect an appreciable increase in the fraction present as the neutral species (Fig. 3A). Even more convincing however are the observations (i) that Ala-Lys transport was H^+-coupled, not facilitative (see also Ref. [7]), and (ii) that maximal transport of Ala-Lys

occurred at a pH_o (6.0–5.5) at which less than 2% of the substrate was in the net neutral form (Fig. 3B). Furthermore, $K_{0.5}$ was the same for each dipeptide regardless of net charge (Fig. 1B) whereas (since the I_{max} values were also the same) we should have expected the $K_{0.5}$ values for Ala-Asp and Ala-Lys to be 20- and 50-fold higher than the $K_{0.5}$ for Ala-Val at pH 6.0 if only $\approx 5\%$ and $\approx 2\%$ of the substrate (respectively) were in a transportable form.

In conclusion, our data indicated that hPEPT1 mediates the active transport of neutral, cationic and anionic dipeptides. Dipeptide transport was H^+-coupled and activated by a single H^+ regardless of net charge. The biological significance of this finding is that transport of neutral, cationic and anionic oligopeptides (i) will always be concentrative, and (ii) the energy cost to the cell in terms of the H^+ electrochemical potential is the same, regardless of the charge on the driven substrate. That the electrogenic properties of H^+-coupled transport of charged dipeptides were identical to those of the neutral dipeptides cannot be explained in terms of Na^+ or Cl^- cotransport, nor K^+ countertransport.

With regard to the transport mechanism of hPEPT1, for which H^+/oligopeptide cotransport is viewed as a series of ligand-induced conformational changes (with H^+ binding before the oligopeptide – see the kinetic model in Ref. [2]), the identity of the current/voltage curves for differently-charged dipeptides indicates that membrane voltage has little effect on the translocation of the fully-loaded transporter; instead, the major effect of voltage is on the conformational change of the empty transporter from the internal to external surface of the membrane. We conjecture that this conclusion will have general applicability to transporters serving charged substrates.

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