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A NOVEL PROLINE, GLYCINE: K^+ SYMPORTER IN MIDGUT BRUSH-BORDER MEMBRANE VESICLES FROM LARVAL *MANDUCA SEXTA*

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Summary

Alkali-cation-dependent uptake of proline and glycine into brush-border membrane vesicles from the midgut of the larval tobacco hornworm *Manduca sexta* was investigated using rapid filtration assays. Uptake of both amino acids was by electrophoretic symport, with K^+ being the favored cation at pH 10. Counterflow accumulation of proline was elicited by glycine and *vice versa*, suggesting that the two amino acids are transported by a common symporter, which we designate the pro, gly: K^+ symporter. L-α-Aminoisobutyric acid was the only other amino acid that elicited the accumulation of both proline and glycine. D-Proline was not symported; L-proline, glycine and L-α-aminoisobutyric acid appear to be the only substrates of the pro, gly: K^+ symporter. Neutral amino acids with relatively short sidechains elicit glycine accumulation, suggesting that glycine may also be symported by the well-established neutral amino acid system. Since proline does not utilize the broad-spectrum, neutral system, its symport appears to be exclusively through the pro, gly: K^+ symporter. Proline symport was found mainly in posterior midgut vesicles, suggesting that the pro, gly: K^+ symporter may be localized in this region of the midgut.

Key words: cotransport, tobacco hornworm, *Manduca sexta*, alkali cation, aminoisobutyric acid, neutral amino acid, symport.

Introduction

Despite considerable effort, the cloning of a metabolic amino acid: cation symporter from an insect has yet to be published. The brush-border membrane (BBM) of a transport model, *Manduca sexta*, may be unusually rich in putative amino acid: alkali cation symport proteins because lepidopteran larvae use amino acids as the primary energy source to sustain a 4 week long, 1000-fold growth spurt from egg to fifth instar (Parenti *et al.* 1985). Indeed, Sacchi *et al.* (1995) have successfully expressed mRNA encoding an amino acid: alkali cation symporter from another larval lepidopteran, *Philosamia cynthia*, in *Xenopus laevis* oocytes. This symporter was identified as a neutral amino acid: alkali cation symporter using a functional assay previously established with vesicle studies (Sacchi *et al.* 1994). Characterization of putative symporters, including their substrate specificities, is needed to interpret further cloning studies. In *P. cynthia*, Giordana *et al.* (1989) described six amino acid: alkali cation symporters that take up neutral amino acids, anionic amino acids, lysine, proline, glycine and D-alanine, respectively. In order to use the midgut tissue of larval *M. sexta* as a source of symporter mRNA or proteins, a similar catalog of the amino acid symporters present in this tissue is required. To this end, we previously described a broad-spectrum, neutral amino acid: cation system (Hennigan *et al.* 1993*a*,*b*) similar to one described by Giordana *et al.* (1989). An arginine: cation symporter (R^+), that uses arginine (but not lysine) as a primary substrate, has also been identified (Z. Liu and W. R. Harvey, unpublished results). No evidence was found for an anionic amino acid: cation symporter (Xie *et al.* 1994). We describe here the uptake of labeled proline and glycine in larval *M. sexta* midgut brush-border membrane vesicles (BBMVs). The results further demarcate differences between the findings on the amino acid uptake systems in *M. sexta* and *P. cynthia*.

The midgut of larval *M. sexta* has several features that influence amino acid uptake. The lumen is alkaline throughout, with a pH maximum of about 11 in the central region, decreasing to about 9.5 and 8.5 in the anterior and posterior regions, respectively (Dow, 1984). Amino acid uptake is driven by a K^+ electrochemical gradient which consists mainly of a voltage ($\Delta\Psi$) of approximately 240 mV (lumen positive) across the BBM (Dow and Peacock, 1989), generated by an H^+ V-ATPase coupled to a $K^+/2H^+$ antiporter in the electrically connected goblet cells (Wieczorek *et al.* 1991; Azuma *et al.* 1995). The activity of K^+, the predominant alkali cation, is approximately the same on both sides of the BBM (Dow, 1984). The cell-negative polarity of $\Delta\Psi$ across the BBM favors the symport of the cationic or zwitterionic amino acid species over

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that of the anionic form. Except for arginine, most amino acids are significantly zwitterionic only below pH 9.5; hence, maximal uptake of amino acids may occur in the posterior midgut (Giordana *et al.* 1994), where the pH stabilizes the zwitterionic form. Intriguingly, the posterior midgut is highly folded and possesses typical microvilli, thus magnifying the available absorptive interface (Cioffi, 1979). However, variable alkali cation: substrate stoichiometry or symporter charge could offset the barrier posed by the cell-negative potential and enable anion uptake in the more alkaline regions as well.

Although distinct proline and glycine systems were found in *P. cynthia* BBMVs (Giordana *et al.* 1989; Hanozet *et al.* 1992), as in many other tissues (Stevens and Wright, 1985; Schwab and Hammerman, 1985; Moseley *et al.* 1988; Satoh *et al.* 1989), imino acids have been shown to share transport systems with glycine (Scriver and Wilson, 1964; Rajendran *et al.* 1987). In fact, defects in a shared renal transport system in humans have been implicated in the benign hereditary disorder iminoglycinuria (Scriver, 1983). We find here that glycine and proline are indeed transported through a common symporter in larval *M. sexta* BBMVs, although glycine is also symported through the broad-spectrum, neutral amino acid system (Hennigan *et al.* 1993*a*).

Materials and methods

Fifth-instar *Manduca sexta* (Johannson) larvae were reared from eggs under constant light at 27 °C on an artificial diet (Carolina Biological Supply Company, Burlington, NC, USA). BBMVs were isolated from fresh midguts by Mg^{2+} precipitation and differential centrifugation (Biber *et al.* 1981; Wolfersberger *et al.* 1987). Anterior-middle or posterior regions of the midgut were identified on the basis of the epithelial sheet folding pattern (Cioffi, 1979). BBM purity was evaluated *via* marker enzyme assays (Eisen *et al.* 1989). Uptake of ^{3}H-labeled amino acids into BBMVs, prepared from whole midguts unless otherwise specified, was measured by the rapid filtration method (Hanozet *et al.* 1980; Wolfersberger *et al.* 1987; Hennigan *et al.* 1993*a*,*b*). Final concentrations, expressed in mmol l^{-1}, and other details are listed in the figure legends. Time points were sampled in duplicate or, especially for the initial uptakes, in triplicate. Initial uptake rates were measured as the slopes of a straight-line fit to uptakes between 3 and 9 s. Data are shown as mean ± S.D. unless otherwise indicated.

^{3}H-labeled amino acids were purchased either from Amersham Corporation (Arlington Heights, IL, USA) or from ICN Biopharmaceuticals (Culver City, CA, USA). Unlabeled amino acids and valinomycin were obtained from Sigma (St Louis, MO, USA) or from Aldrich (Milwaukee, WI, USA). Other chemicals were obtained from Fisher (Pittsburgh, PA, USA). Unless otherwise identified, L-amino acids were used as substrates.

Results

Are proline and glycine symported?

The initial proline and glycine uptake rates at pH 10 were higher with a K^+ gradient than either without the gradient or without K^+ present (Fig. 1A,B), under iso-osmotic conditions in each case. The acceleration imparted by the K^+ gradient is similar for the two amino acids. Representative time courses of uptake for each of the amino acids at pH 10 with a 50 mmol l^{-1} KSCN gradient reveal transient uptake maxima that are about threefold higher than equilibrium values (at 60 min; Fig. 1 insets). Uptake

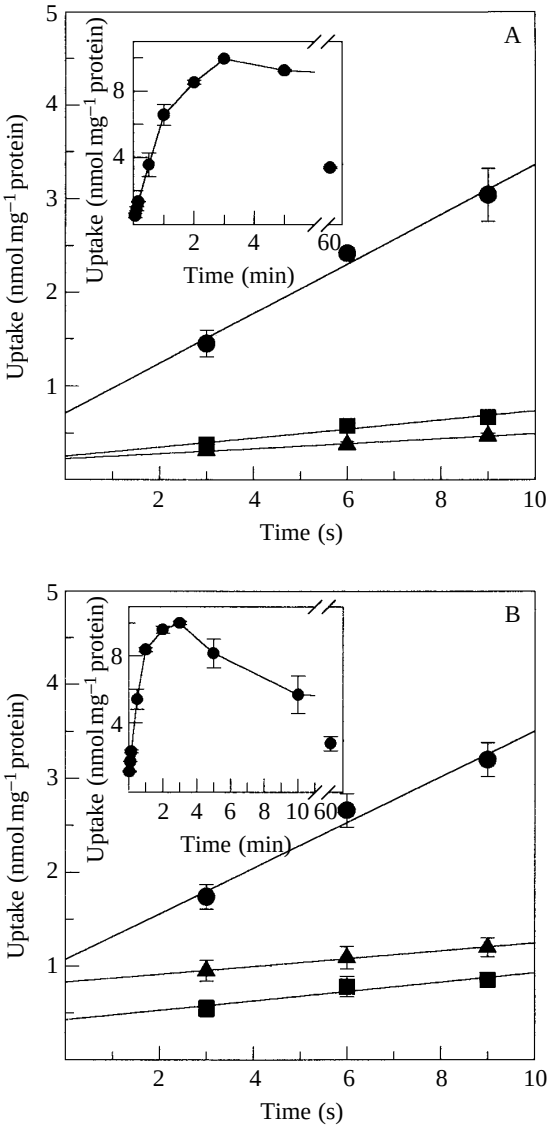

Fig. 1. Initial uptake of (A) proline or (B) glycine at pH 10 *versus* time, with (circles) or without (squares) a potassium gradient or with no potassium present (triangles). The insets show the complete uptake time course for the respective amino acids. Vesicles were suspended in (in mmol l^{-1}, final concentrations) 50 aminomethylpropanediol (AMPD), 200 mannitol (circles, triangles) or in 50 AMPD, 100 mannitol, 100 KSCN (squares) and treated with valinomycin at 8 μg mg^{-1} protein for 30 min. Transport buffer contained (in mmol l^{-1}) 50 AMPD, 100 mannitol, 50 KSCN (circles), or 50 AMPD, 100 mannitol, 100 KSCN (squares) or 50 AMPD, 200 mannitol (triangles) in addition to 0.5 ^{3}H-labeled amino acid. In all the figures, values are given as means ± S.D., *N*=2–3. Data shown are from a representative experiment performed in triplicate.

remained unchanged after 60 min (data not shown), indicating that equilibrium had been reached in this interval.

Is there a preferred cation for symport?

Among the five alkali cation (Li^+, Na^+, K^+, Rb^+ and Cs^+) gradients tested at pH 10, only gradients of K^+ energized proline uptake (Fig. 2A). Na^+ and K^+ both energized glycine uptake at pH 10 (Fig. 2B). K^+, which did not drive glycine uptake in *P. cynthia* at pH 7.4 (Giordana *et al.* 1989), drove glycine uptake in *M. sexta* at this pH, although Na^+ was more effective than K^+ (Fig. 2C). An H^+ gradient in the absence of K^+ failed to drive proline uptake in *M. sexta* BBMVs (Fig. 2D).

Is there an optimal pH for symport?

The initial rates of KSCN-gradient-driven proline and glycine uptake increased dramatically between pH 7.5 and pH 10 (Fig. 3A,B). Proline uptake rates decreased at pH values

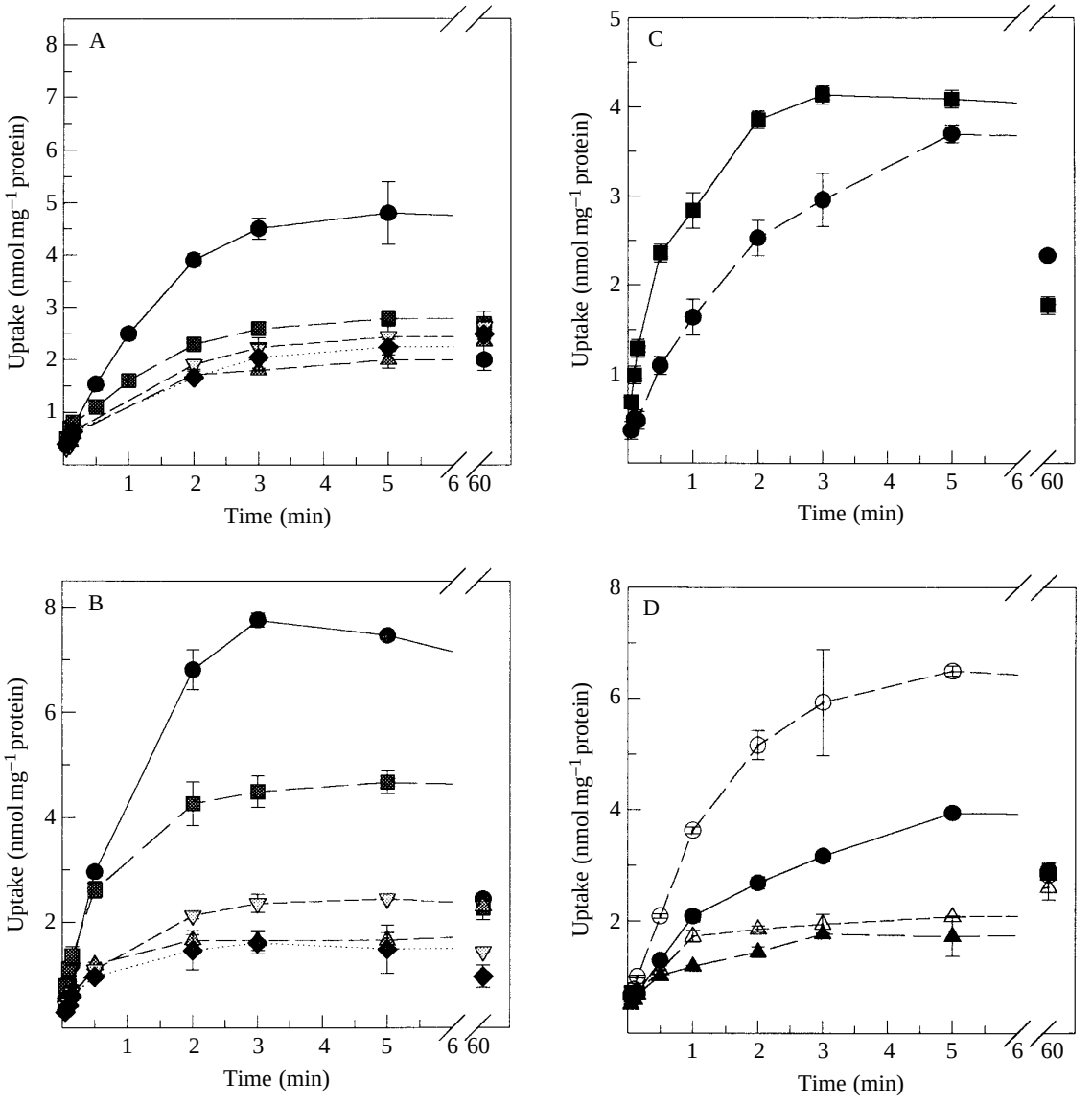

Fig. 2. The uptake timecourse of (A) proline or (B) glycine with different alkali cations at pH 10. Vesicles suspended in (in mmol l^{-1}, final concentrations) 50 AMPD and 100 mannitol were incubated with transport buffer containing 50 AMPD, 100 mannitol, 0.5 ^{3}H-labeled amino acid and 50 KCl (circles), or 50 NaCl (squares), or 50 LiCl (triangles), or 50 RbCl (inverted triangles) or 50 CsCl (diamonds). (C) The uptake time course of glycine at pH 7.4. Conditions were similar to those used at pH 10, except that (in mmol l^{-1}, final concentrations) 20 Hepes–Tris was used instead of 50 AMPD. Circles and squares as in B. (D) The uptake time course of proline with an H^+ gradient (filled symbols) or without an H^+ gradient (open symbols). The conditions were as follows (in mmol l^{-1}, final concentrations): pHi=9.5, pHe=8, 67 KCl, 100 mannitol and 0.67 labeled proline (filled circles); pHi=9.5, pHe=8, 67 LiCl, 100 mannitol and 0.67 labeled proline (filled triangles); pHi=8, pHe=8, 67 KCl, 100 mannitol and 0.67 labeled proline (open circles); pHi=8, pHe=8, 67 LiCl, 100 mannitol and 0.67 labeled proline (open triangles). Vesicles were loaded with 50 Bis–Tris propane and 100 mannitol at the pH values indicated. Data shown are from a representative experiment carried out in triplicate.

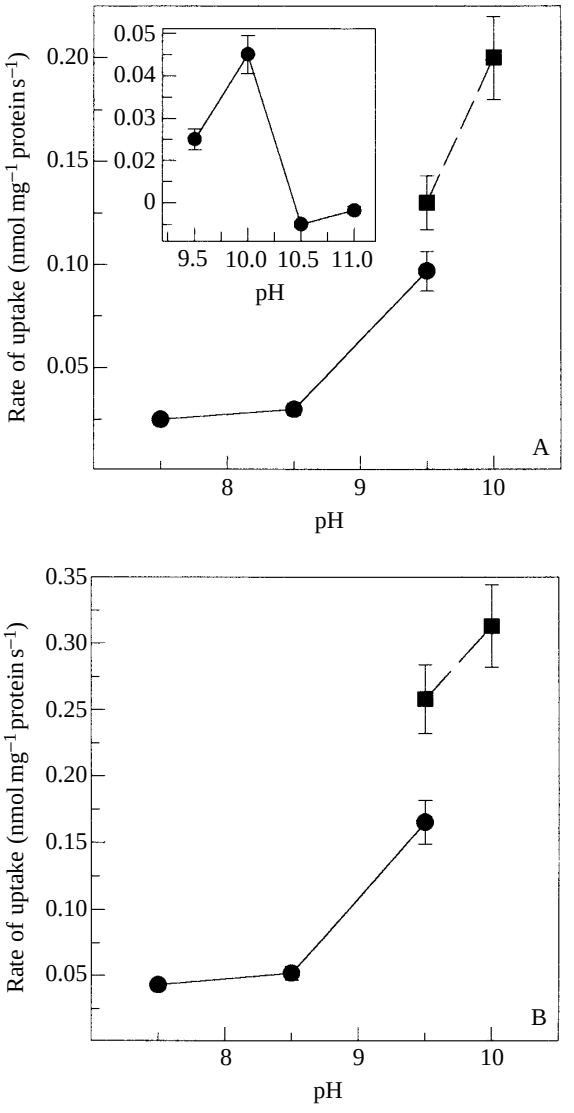

Fig. 3. The initial rate of uptake of (A) proline or (B) glycine as a function of pH. Vesicles were suspended in (in mmol l^{-1}, final concentrations) 50 Bis–Tris propane, 100 mannitol (circles) or 50 AMPD, 100 mannitol (squares). Transport buffer contained (in mmol l^{-1}, final concentrations) 50 Bis–Tris propane, 100 mannitol (circles) or 50 AMPD, 100 mannitol (squares) plus 50 KSCN and 0.5 ^{3}H-labeled amino acid. The inset in A shows the uptake rate of proline *versus* pH in the absence of a potassium gradient. Vesicles were suspended in 20 Caps–KOH, 100 mannitol, 50 KSCN and were treated with valinomycin (see Fig. 1). Transport buffer contained (in mmol l^{-1}, final concentrations) 20 Caps–KOH, 100 mannitol, 50 KSCN and 0.5 ^{3}H-labeled glycine. Stop solutions of the desired pH contained (in mmol l^{-1}) 20 Caps–KOH and 150 NaCl. Data shown are from a representative experiment performed in triplicate.

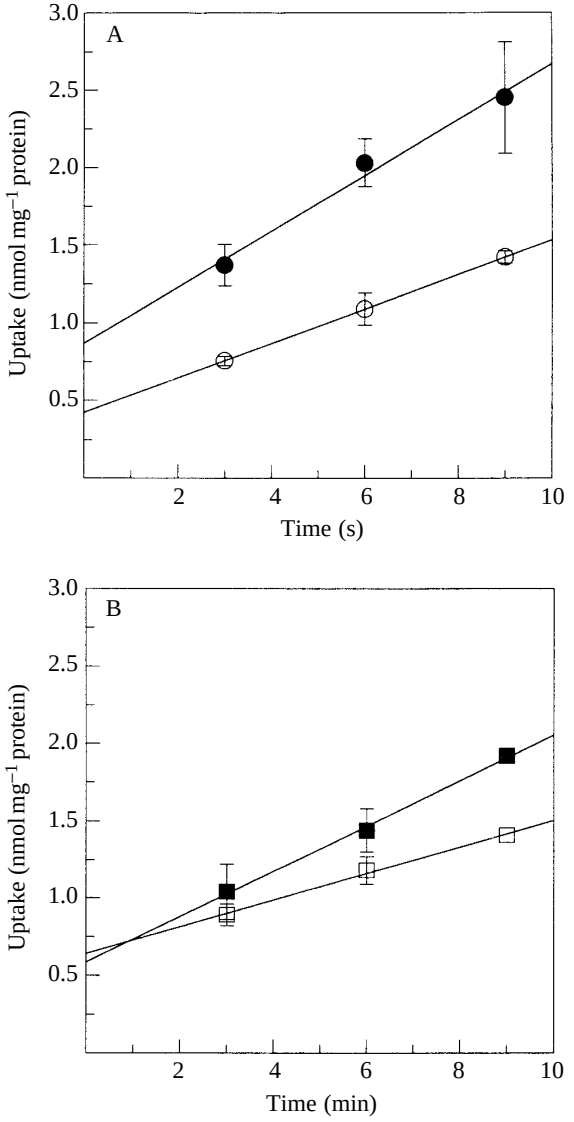

Fig. 4. Initial uptake of (A) proline (circles) or (B) glycine (squares) at pH 10 with a KSCN (filled symbols) or a KCl (open symbols) gradient as a function of time. Vesicles were suspended in (in mmol l^{-1}, final concentrations) 50 AMPD, 100 mannitol. Transport buffer contained 50 AMPD, 100 mannitol, 50 KSCN (or KCl) and 0.5 ^{3}H-labeled amino acid. Data shown are from a single preparation.

greater than 10 (Fig. 3A, inset), suggesting that symport is optimal at pH 10. To obtain pH values greater than 10 required the introduction of an alkali cation into the buffer system and necessitated the measurement of initial uptake rates in the absence of a K^+ gradient.

Does $\Delta\Psi$ drive the symport of proline or glycine?

Because $\Delta\Psi$ drives amino acid uptake *in vivo*, its ability to drive uptake *in vitro* was studied. The initial uptake rate of both proline and glycine was higher with a SCN^- gradient than with a Cl^- gradient (100 mmol l^{-1} outside, 0 mmol l^{-1} inside, Fig. 4). Because this result implied that a transmembrane voltage, generated by the differential permeability of SCN^- (Carroll and Ellar, 1993), could accelerate amino acid symport, we measured initial uptake rates of proline and glycine under a calculable, time-invariant $\Delta\Psi$. A proton diffusion potential (calculated from the Nernst–Planck–Einstein equation to be approximately −120 mV, interior negative) was generated using a 2 unit pH difference (pHi=7.2, pHe=9.2) in the

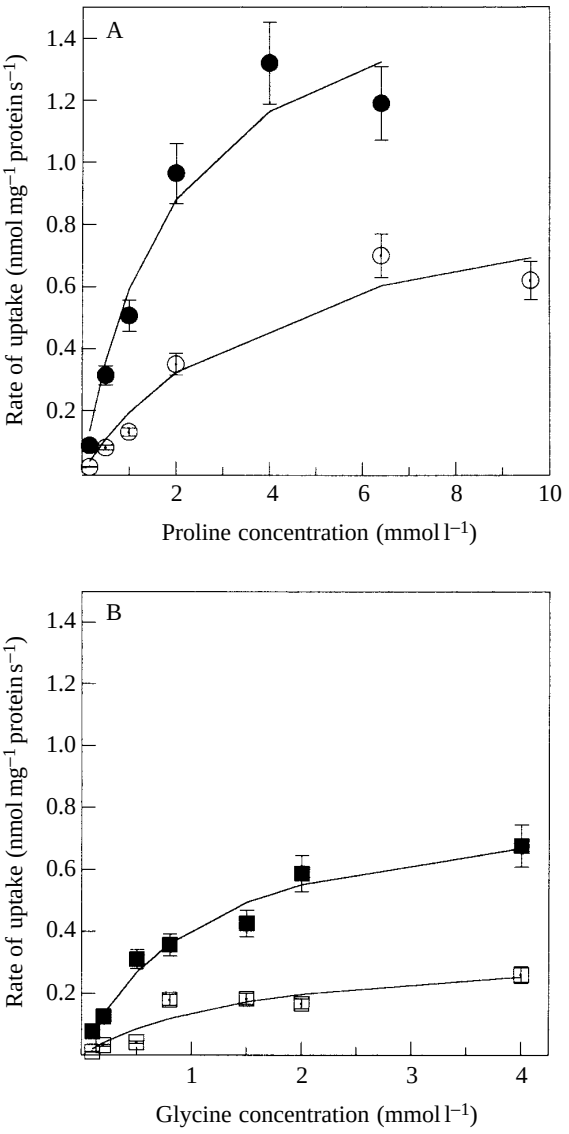

Fig. 5. Initial uptake rates of (A) proline and (B) glycine as a function of proline or glycine concentration with (filled symbols) and without (open symbols) an applied potential difference. Vesicles were suspended in (in mmol l^{-1}, final concentrations) 50 Bis–Tris propane, 100 mannitol at pH 7.2 and treated with 0.1 of the protonophore FCCP (filled symbols), were incubated with transport buffer containing 50 Bis–Tris propane, 100 mannitol, 83 KCl and ^{3}H-labeled amino acid at the concentrations shown at pH 9.2. Treatment with the protonophore was omitted to obtain data in the absence of a potential difference. Stop solutions contained 50 Bis–Tris propane, 150 NaCl at pH 9.2. Data shown are from a representative experiment performed in triplicate.

presence of the protonophore carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). A K^+ gradient/valinomycin couple could not be used because K^+, the physiologically relevant cation, was used to drive amino acid uptake. Initial uptake rates of both amino acids were found to be two- to threefold higher with an imposed voltage than without (Fig. 5).

Table 1. *Accumulations of proline and glycine elicited by typical amino acids*

	Proline	Glycine
AIB	2.20±0.20 (1)	2.91±0.08 (1)
Proline	1.95±0.08 (5)	2.65±0.32 (1)
Glycine	2.53±0.13 (2)	2.44±0.10 (6)
Alanine	1 (2)	2.37±0.04 (1)
Threonine	ND	2.25±0.10 (1)
Lysine	1 (1)	1.76±0.55 (1)
Serine	ND	1.76±0.16 (2)
Phenylalanine	ND	1.48±0.02 (1)
Valine	ND	1.25±0.19 (2)
Leucine	1 (2)	1.32±0.18 (2)
NMeAIB	1 (1)	1 (2)
GABA	ND	1 (1)
Hydroxy-L-proline	1 (2)	ND
D-Proline	1 (2)	ND
Glutamate	1 (1)	1 (1)

Vesicles incubated with valinomycin (8 μg mg^{-1} protein) were loaded with (in mmol l^{-1}, final concentrations) 50 AMPD, 100 mannitol, 100 KSCN and 20 elicitor at pH 10 and diluted 20-fold into transport buffer at pH 10 containing (in mmol l^{-1}, final concentrations) 50 AMPD, 100 mannitol, 100 KSCN, 1 labeled substrate and 1 elicitor.

Accumulation was calculated as the ratio of maximum uptake to the equilibrium value (the value at 60 min).

ND, not determined; AIB, aminoisobutyric acid; NMeAIB, *N*-methyl aminoisobutyric acid; GABA, γ-aminobutyric acid.

Numbers of independent preparations are given in parentheses.

Data shown are averaged over all of the preparations and are expressed as mean ± S.E.M. (*N*=number of independent preparations).

Do proline and glycine use the same system?

Glycine and proline elicited 2.5-fold accumulations of each other (Table 1), suggesting that the two amino acids are transported by a common system (which we term the pro, gly: K^+ symporter). Leucine, a preferred substrate of the broad-spectrum, neutral amino acid system (Hennigan *et al.* 1993*a*), did not elicit accumulation of proline (Fig. 6A) and elicited only a very small accumulation of glycine (Fig. 6B). Substrates for other epithelial transport systems were also tested as elicitors of proline or glycine uptake (Table 1). The model substrates *N*-methyl aminoisobutyric acid (NMeAIB, System A, IMINO) and L-α-aminoisobutyric acid (AIB, neutral brush border) were included (Stevens *et al.* 1984). Substrates of the broad-spectrum system, especially those with a short sidechain, were found to elicit accumulations of glycine (Table 1). However, only proline, glycine and AIB elicited proline accumulation. Apparently, proline is taken up only by the pro, gly: K^+ symporter, whereas glycine may be taken up by both the pro, gly: K^+ and the broad-spectrum systems. The putative pro, gly: K^+ symporter is stereospecific in that D-proline did not function as a substrate. NMeAIB elicited neither glycine nor proline uptake, whereas AIB elicited uptake of both substrates.

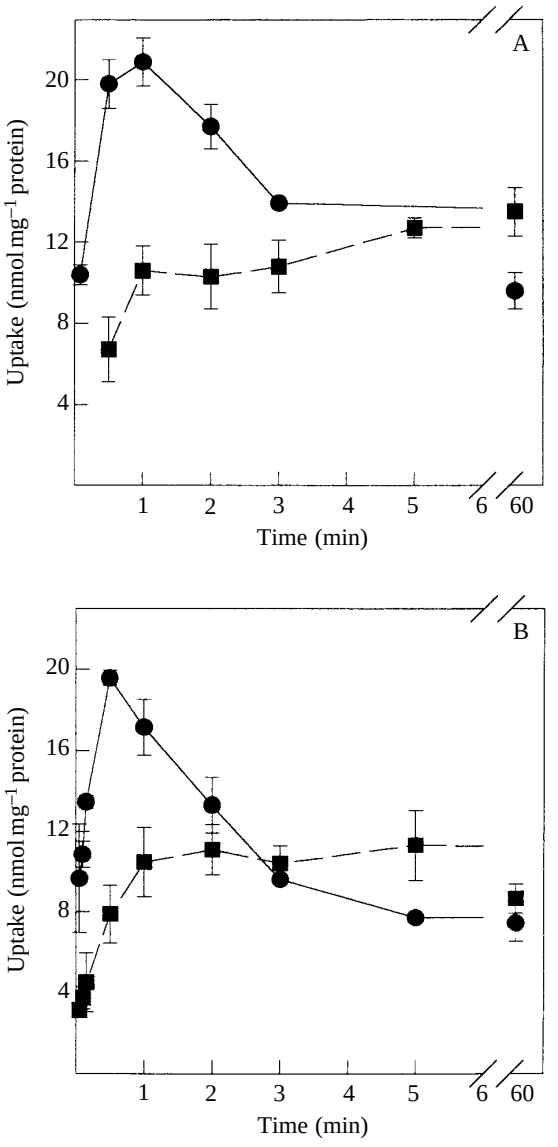

Fig. 6. Countertransport at pH 10 with (A) glycine (circles) or leucine (squares) eliciting proline transport and with (B) proline (circles) or leucine (squares) eliciting glycine transport. Vesicles suspended in (in mmol l^{-1}, final concentrations) 50 AMPD, 100 mannitol, 50 KSCN, 20 elicitor and treated with valinomycin (see Fig. 1), were incubated with transport buffer containing 50 AMPD, 100 mannitol, 100 KSCN, 1 ^{3}H-labeled substrate and 1 elicitor. Data shown are from a representative experiment performed in triplicate.

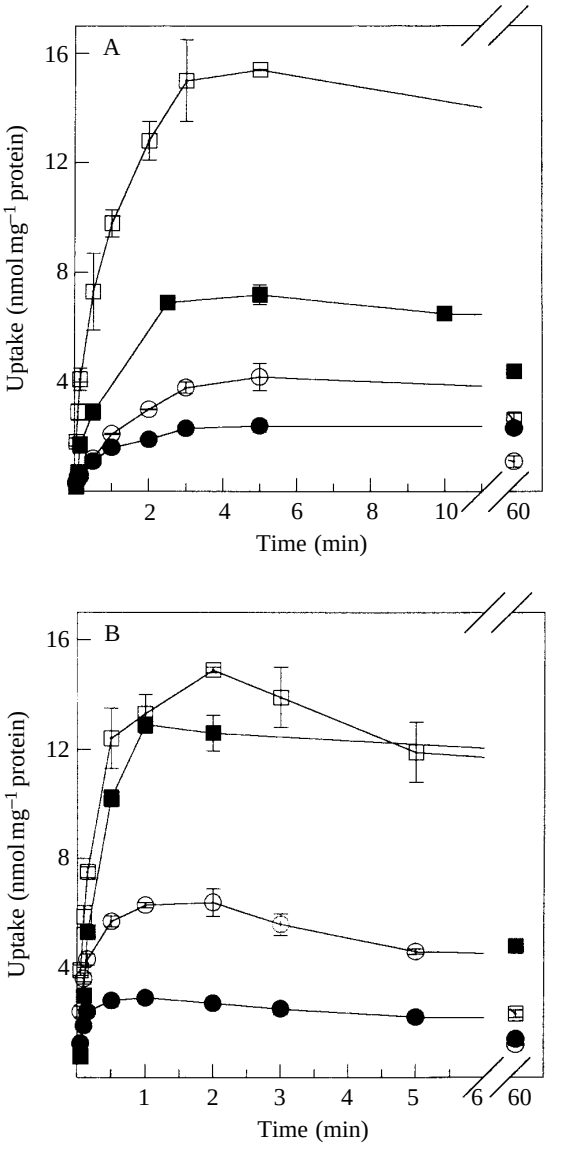

Fig. 7. The uptake time course of (A) proline and (B) leucine in BBMVs prepared from different regions of the midgut. Vesicles prepared from the anterior-middle (filled symbols) or posterior (open symbols) regions of the midgut were suspended in (in mmol l^{-1}, final concentrations) 50 Bis–Tris propane at pH 8.5 (circles), or 50 AMPD at pH 10 (squares), and 100 mannitol. Transport buffer contained either 50 Bis–Tris propane at pH 8.5 (circles) or 50 AMPD at pH 10 (squares), in addition to 100 mannitol, 50 KSCN and 0.5 ^{3}H-labeled amino acid. Stop solutions contained 150 NaCl, 50 of the appropriate buffer at the appropriate pH. Data shown are from a representative experiment performed in triplicate.

Are the pro, gly: K^+ symporter and neutral amino acid system distributed uniformly along the midgut?

To investigate the possibility that the pro, gly: K^+ symporter and the broad-spectrum system are differentially localized, the uptakes of proline (Fig. 7A) and leucine (Fig. 7B) were measured at pH 8.5 and 10 in BBMVs prepared from the anterior-middle (AM) and posterior (P) regions of the midgut. These pH values were chosen to approximate the *in vivo* conditions found in the two regions of the midgut lumen. At pH 8.5, proline uptake was region-dependent, being considerably higher in P BBMVs than in AM BBMVs (where no accumulation was detected); this difference was more pronounced at pH 10. Leucine uptake was not region-dependent at pH 10, whereas uptake seemed to be slightly higher in P BBMVs than in AM BBMVs at pH 8.5. Uptake of both amino acids increased with pH (cf. Fig. 3), irrespective of region. The uptake time courses of glycine and leucine at pH 10 were similar in both regions (Fig. 8).

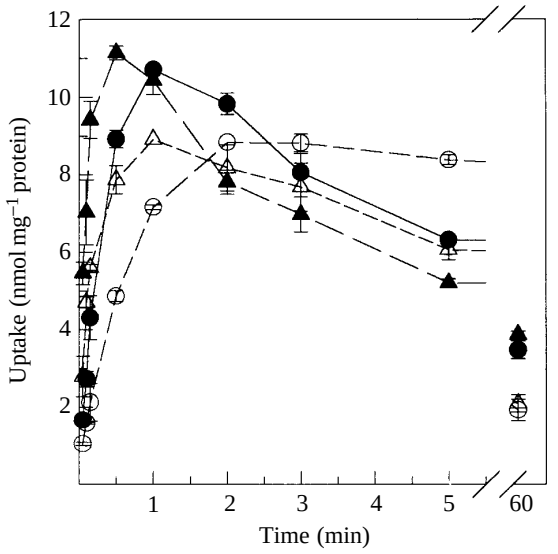

Fig. 8. The uptake of glycine (circles) or leucine (triangles) in BBMVs prepared from the anterior-middle (filled symbols) or posterior (open symbols) regions of the midgut at pH 10. The experimental conditions used are detailed in the legend to Fig. 7. Data shown are from a representative experiment performed in triplicate.

Discussion

In larval *M. sexta* midgut BBMVs, proline and glycine both appear to be symported because (a) the rate of amino acid uptake is higher with a K^+ gradient than without the gradient and (b) transient uptake maxima exceeding equilibrium values are observed only in the presence of the gradient. At pH 10, symport was faster with K^+ than with Na^+, although Na^+ can function as a surrogate cation with glycine, especially at pH 7.4. In contrast to the uptake of neutral amino acids (Hennigan *et al.* 1993*a*), anionic amino acids (Xie *et al.* 1994) or cationic amino acids (Z. Liu and W. R. Harvey, unpublished results), proline uptake at pH 10 is not driven by a Na^+ gradient. However, Na^+ drives proline uptake in another lepidopteran, *P. cynthia* (Giordana *et al.* 1989).

Glycine and proline symport are optimal at pH 10, as is the symport of neutral and cationic amino acids, which is consonant with the known alkalinity of the midgut *in vivo*. However, the striking increase in uptake rate as pH is increased from neutrality is quite different from that observed in the broad-spectrum system and hints at the possibility that proline and glycine together use a distinct symport mechanism. Glycine and proline have pK_2 values of 9.6 and 10.6, respectively; thus, the zwitterionic fraction of proline drops by only 20 % between pH 7 and pH 10. The zwitterionic fraction of glycine, in contrast, drops by 72 % over the same range. A working hypothesis is that the putative pro, gly: K^+ symporter recognizes only the zwitterionic form of its substrates and accommodates the scant amount of zwitterionic glycine at pH 10 through either a low K_m or a high V_{max} for this substrate. The low uptake rates seen at near-neutral pH, despite saturating zwitterion concentrations, suggest pH-induced effects on the symporter.

Contrary to findings in the renal BBMVs of the rat (Chesney *et al.* 1991) and in the antennal gland BBMVs of the lobster (Behnke *et al.* 1990), Cl^- plays no special role in symport of proline and glycine in midgut BBMVs of larval *M. sexta*. This finding is not surprising since Cl^- is abundant in mammalian intestine and sea water but scarce in *M. sexta* midgut (Dow *et al.* 1984). In fact, the higher proline and glycine uptake observed with SCN^- than with Cl^- (Fig. 4) suggests that the symport of the two amino acids is electrophoretic. With a constant $\Delta\Psi$ of -120 mV (interior negative), the V_{max} for glycine is almost tripled by the voltage whereas that for glycine is doubled. In a fluorescence spectroscopic study, Parthasarathy and Harvey (1994) demonstrated that the application of $\Delta\Psi$ increases the V_{max} of proline uptake but has little effect on the $K_{0.5}$. The role of K^+-independent mechanisms may be minor since the K^+-independent uptake rate of proline is only one-tenth of that in the presence of a cation gradient; the ratio for glycine uptake is one-fifth (Fig. 1) when 0.5 mmol l^{-1} of the amino acids is present in the transport buffer.

The counterflow accumulations of proline and glycine elicited by each other are similar to the accumulations of each amino acid elicited by itself (Table 1). Proline symport is elicited only by glycine, AIB and itself. Since AIB (pK_2=10.2) is also significantly zwitterionic at pH 10, we conclude that the substrate repertoire of the pro, gly: K^+ symporter is limited to the zwitterionic forms of the amino acids that elicit proline symport. Furthermore, proline seems to be symported neither through the broad-spectrum system (Hennigan *et al.* 1993*a*) nor through the R^+ symporter (Z. Liu and W. R. Harvey, unpublished results). Glycine symport, however, is elicited by nearly all of the amino acids tested except glutamate and NMeAIB. The small accumulation of glycine elicited by leucine implies that glycine is a weak substrate of the broad-spectrum system (Hennigan *et al.* 1993*a*). Therefore, glycine symport must occur through the broad-spectrum system as well as through the pro, gly: K^+ symporter. The highest accumulations of glycine are elicited by amino acids with relatively small sidechains (cf. the accumulation elicited by alanine *versus* that by leucine; Table 1), suggesting that a constituent form of the broad-spectrum system may be adapted to the uptake of the smaller amino acids. However, although glycine is a substrate for the broad-spectrum system, leucine appears not to be a substrate for the pro, gly: K^+ symporter, because glycine elicits a higher accumulation of leucine than leucine does of glycine (Hennigan *et al.* 1993*a*).

Proline accumulates in P BBMVs but not in AM BBMVs at pH 8.5 (Fig. 7A); however, leucine accumulates in both regions at this pH (Fig. 7B), although the accumulation seems to be higher in P BBMVs than in AM BBMVs, as in *Bombyx mori* (Giordana *et al.* 1994). Even at pH 10, the uptake of zwitterionic proline is favored in P BBMVs over AM BBMVs (Fig. 7A), suggesting that the pro, gly: K^+ symporter is localized in the posterior region of the midgut. In contrast, leucine uptake seems not to be region-specific at pH 10 (Fig. 7B), suggesting that leucine uptake occurs along the

length of the midgut at this pH. An increase in pH evidently affects proline uptake more in P BBMVs than in AM BBMVs, but affects leucine uptake to similar extents in both midgut regions. This finding is also consistent with the hypothesis that there is little proline symport in the anterior midgut region.

Differential localization of proline and leucine symport supports the idea that they use distinct symporters, as suggested above from the differences in the pH–symport rate profile and cation specificity. To the extent that glycine is symported by both the broad-spectrum and the proline–glycine symporters, no spatial resolution of glycine symport is seen along the midgut (Fig. 8).

Differential localization of cohorts of carriers specific to certain substrates is not new and has been demonstrated, for example, in rabbit small intestine (Munck and Munck, 1992*a*,*b*). However, the Na^+-dependent symport of imino acids in the rabbit small intestine is competitively inhibited by aliphatic amino acids such as leucine (Munck, 1985; Munck and Munck, 1992*b*). The pro, gly: K^+ symporter described here differs from the rabbit imino acid carrier in that it transports only glycine among the common aliphatic amino acids.

Although the pro, gly: K^+ symporter superficially resembles the rabbit imino acid carrier, it is quite different from other amino acid symporters. It differs from system A because it does not transport NMeAIB, and from system ASC because it transports glycine readily. Differences between the broad-spectrum system of *M. sexta* and the pro, gly: K^+ symporter have been discussed above. Finally, proline and glycine interact very weakly with lysine and arginine uptake mediated by system R^+ in *M. sexta* (Z. Liu and W. R. Harvey, unpublished results). It appears that the pro, gly: K^+ symporter is different from the other systems described in BBMVs from the midgut of this organism.

Most importantly, the pro, gly: K^+ symporter is relevant *in vivo* because the symport of both amino acids is strongly voltage-dependent. Proline uptake in a variety of tissues is electrophoretic (Roigaard-Petersen and Sheikh, 1984; Behnke *et al.* 1990) and may be Cl^--activated (Behnke *et al.* 1990). Glycine symport is also thought to be electrophoretic and to be driven by an H^+ gradient (Rajendran *et al.* 1988). In *M. sexta* midgut, especially in the AM region, symport is unlikely to be proton-driven given the unfavorable proton electrochemical gradient. This prediction is supported by the failure of a proton gradient to drive proline accumulation in the absence of K^+ (Fig. 2D). By the same token, Cl^- is a minor component of the lumen contents (Dow *et al.* 1984) and therefore is not a likely symport cosubstrate. An early report by Scriver and Wilson (1964) suggested that uniport of imino acids and glycine across rat kidney slices used a common translocation step, rather than the same binding site. Elucidation of this important detail in the case of the pro, gly: K^+ symporter from *M. sexta* awaits purification of the protein and its reconstitution into model vesicular systems.

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