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K^+-DEPENDENT PHENYLALANINE UPTAKE IN MEMBRANE VESICELS ISOLATED FROM THE MIDGUT OF *PHILOSAMIA CYNTHIA* LARVAE

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Summary

Membrane vesicles prepared from the midguts of *Philosamia cynthia* larvae (Lepidoptera) show a concentrative uptake of phenylalanine in the presence of salt gradients. Unlike mammalian intestines, the highest accumulation of the amino acid occurs with a potassium salt gradient. Glucose is very poorly permeable across the vesicles.

The midgut environment of the phytophagous larvae of Lepidoptera has very peculiar ionic features due to the highly-specialized feeding of these animals. In *Philosamia cynthia* (Saturnidae) larvae, potassium concentrations in the lumen content, gut tissue and haemolymph are 197, 174 and 24 mM respectively, sodium being always below 5 mM in the three compartments [1]. In Vertebrate intestine, the sodium electrochemical gradient across the apical border of the enterocyte seems to provide the driving force for the 'uphill' transport of sugars and amino acids [2—4]. The amino acid transport in Lepidopteran larvae has been investigated by Nedergaard [5—7] who has found an active absorption of some amino acids across the isolated midgut of *Hyalophora cecropia* related to the transepithelial electrical potential difference. In the present work, we investigated the uptake of metabolites across luminal membrane vesicles isolated from the midgut of *P. cynthia*, since the vesicles have been proved to be a simple system for transport studies which avoids the interference of cellular metabolism and of the basolateral membrane [8, 9].

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

The vesicles were prepared from midguts of *P.cynthia* in their last larval instar, according to the procedure of Schmitz et al. [10] as modified by Kessler et al. [9]. 1—2 g of fresh midguts, deprived of the peritrophic membrane and malpighian tubules, were homogenized with a Potter-Elvehjem homogenizer in 50 mM mannitol/2 mM Hepes-Tris buffer, pH 7.1. The pellets obtained from the second and third centrifugations were resuspended in 100 mM ice-cold mannitol/10 mM Hepes-Tris buffer, pH 7.5. The uptake of metabolites was determined by incubating membrane vesicles at room temperature in a mixture of the following composition: 100 mM mannitol; 10 mM Hepes-Tris buffer, pH 7.5, 1 mM D-[U-^{14}C]glucose or L-[U-^{14}C]-phenylalanine and salt gradients as indicated in the legend of Fig. 2.

For the experiments with K^+ or Na^+ inside and outside, the vesicles were pre-equilibrated for 30 min in 100 mM KCl or NaCl before the incubation.

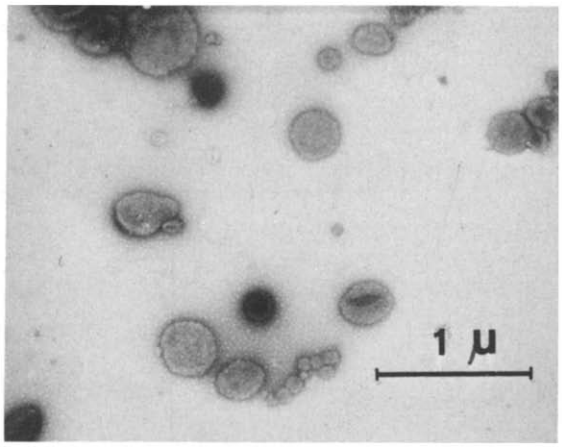
At selected times, 20-μl samples were withdrawn from the incubation mixture, diluted with 1.5 ml ice-cold 150 mM NaCl/1 mM Hepes-Tris (stop solution), filtered through a Sartorius filter (S.M. 11305, 0.6 μm pore-size) and rapidly rinsed with 10 ml ice-cold stop solution. Radioactivity readings were then performed by means of a liquid scintillation spectrometer (Tri-Carb Packard, Model 3385).

Disaccharidase activity was determined according to Semenza and von Balthazar [11] with glucose dehydrogenase (Merck-*o*-test No. 3389); lactate dehydrogenase [12], alkaline phosphatase [13] and cytochrome oxidase [14] were assayed by optical methods. Protein determination was carried out according to Lowry et al. [15].

L-Phenylalanine fluxes were measured on isolated midguts, tied as a tube on an apparatus described elsewhere [16] and perfused with a solution of the following composition: 4 mM NaCl, 25 mM $KHCO_3$, 37 mM $MgSO_4$, 9 mM $CaCl_2$, 156 mM sucrose and 10 mM L-phenylalanine and aerated with 95% O_2 /5% CO_2, pH 7.4. L-[U-^{14}C]Phenylalanine was added either to the luminal or to the haemolymphatic solutions in order to measure the influx (lumen to haemolymph) or the outflux (haemolymph to lumen). Samples were withdrawn after a 30 min equilibration period and after 60 min. Midguts were then removed and dried overnight to obtain the dry weight. Water movement was determined by dilution of inulin [^{14}C] carboxylic acid.

For electron microscopy a negative staining procedure was used: the vesicles (diluted to above 1 mg protein per ml) were directly stained with 1% uranyl acetate for 30 s.

A micrograph of the vesicles obtained from the midgut of *P. cynthia* can be seen in Fig. 1: the vesicular size and shape are not very different to those previously observed in preparations from mammalian intestine [9, 17]. Table I lists the specific activities of some enzymes: sucrase and alkaline phosphatase, typical marker enzymes of the brush border membrane, show an enrichment factor higher than 1, whilst mitochondrial and cytosolic contamination seem to be lacking. (Na^+ + K^+)-ATPase has not been considered, since this enzyme is not present in the midgut of Lepidoptera [18—20]. The unidirectional fluxes of L-phenylalanine have been measured in the isolated midgut in the absence of a chemical gradient and in the presence



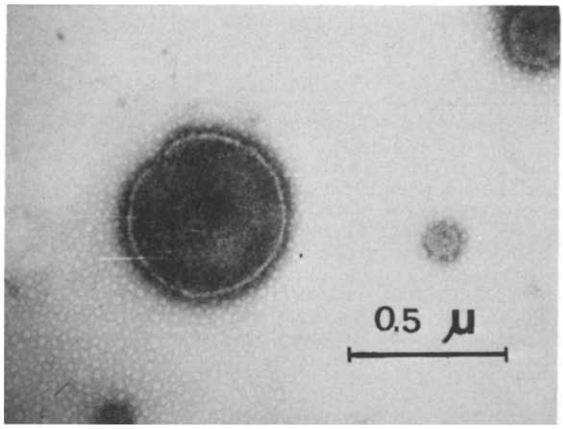


Fig. 1. Electron micrographs of negatively-stained membrane vesicles from *Philosamia cynthia* midgut.

TABLE I

SPECIFIC ACTIVITY OF SOME ENZYMES IN THE CRUDE HOMOGENATE AND IN THE MEMBRANE VESICLES FROM *PHILOSAMIA CYNTHIA* MIDGUT

Midguts of *P. cynthia* were homogenized in 50 mM mannitol/2 mM Hepes-Tris buffer, pH 7.1 and membrane vesicles were isolated according to Kessler et al. [9]. Enzyme activities were measured at 30°C and are expressed as nmol • min^{-1} • mg $protein^{-1}$ for cytochrome oxidase and μmol • min^{-1} • mg $protein^{-1}$ for the other enzymes.

	Sucrase	Lactate dehydrogenase	Cytochrome oxidase	Alkaline phosphatase
Crude homogenate	0.242	0.096	4.66	0.064
Membrane vesicles	0.646	0.005	0.19	0.306
Enrichment factor	2.67	0.05	0.04	4.78

of the spontaneous transepithelial electrical potential difference (approx. 90 mV, lumen positive to haemolymph). The influx is 67.0 ± 2.9 μmol • g dry $wt.^{-1}$ • h^{-1} (three experiments) and the outflux 10.4 ± 2.9 μmol • g dry $wt.^{-1}$ • h^{-1} (three experiments): no net water movement takes place across the midgut. Therefore, an active transport of L-phenylalanine occurs across the tissue. In order to clarify the mechanism involved in this transport, L-

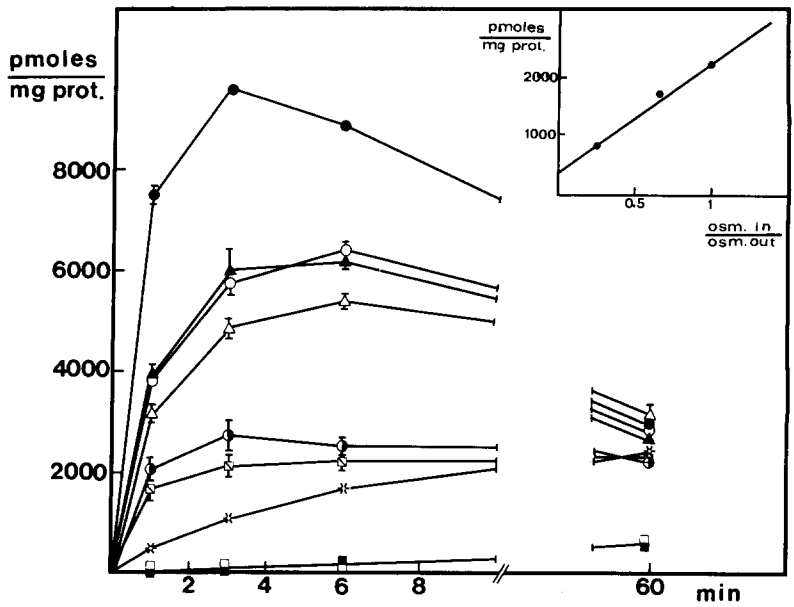


Fig. 2. Uptake of L-phenylalanine and D-glucose by membrane vesicles from *Philosamia cynthia* midgut. L-Phenylalanine (1 mM) uptake in the presence of an initial gradient (100 mM outside and 0 inside) of: KSCN (●), NaSCN (○), KCl (▲) and NaCl (△). L-Phenylalanine (1 mM) uptake in the absence of any salt gradient: without salt (☆) and with KCl (◑) or NaCl (⊡) (100 mM outside, 100 mM inside). D-Glucose (1 mM) uptake in the presence of an initial gradient (100 mM outside and 0 inside) of KSCN (■) or NaSCN (□). The buffer was in all cases 10 mM Hepes-Tris, pH 7.5/100 mM mannitol. The results are the mean ± S.E. of a typical experiment carried out in triplicate. When not given, S.E. values were smaller than the symbols used. In the upper part of the figure, the equilibrium uptake after 60 min incubation in the presence of 20 mM KSCN is plotted against the inside/outside osmolarity ratio obtained on increasing the outside concentration of mannitol.

phenylalanine uptake was determined in a subcellular system of isolated plasma membrane vesicles. The results are shown in Fig. 2. It is evident that, unlike mammalian intestine [21], L-phenylalanine uptake by these vesicles is potassium-sensitive. A concentrative transport of L-phenylalanine in the presence of both sodium and potassium salt gradients is apparent: however, the 'overshoot' is much more remarkable when a KSCN concentration gradient is provided. Without a salt concentration gradient, but in the presence of K^+ or Na^+, no accumulation takes place, the uptake being only equilibrative: however, the uptake of the amino acid is enhanced as compared to the uptake obtained in the absence of any salt, thus suggesting a direct interaction between K^+ or Na^+ and the amino acid transport system. It can be observed that when equilibrium is attained, the same uptake values are reached, whatever the initial condition.

D-Glucose uptake is extremely low and even after 1 h of incubation it does not attain the phenylalanine value at equilibrium.

In the upper part of the same figure, it is shown that the amount of phenylalanine uptake at equilibrium is in inverse proportion to the osmolarity of the medium: extrapolation to infinite medium osmolarity shows a very small binding value. Therefore, the observed uptake of phenylalanine can be accounted for almost completely by transport into an osmotically-active space.

TABLE II

Effect of cations and anions on L-phenyalanine uptake into membrane vesicles of *Philosamia cynthia* midgut (amounts taken up during the first 3 min). The incubation medium was in all cases 10 mM Hepes-Tris, pH 7.5; 100 mM mannitol; 1 mM L-phenylalanine and different salt gradients as indicated in the table. Mean ± S.E. of three experiments.

Salt in the incubation medium	(M)	pmol L-phenylalanine per mg protein
KSCN	0.10	9589 ± 85
KCl	0.10	6017 ± 457
K_2SO_4	0.05	2839 ± 180
NaSCN	0.10	5734 ± 357
NaCl	0.10	4842 ± 302
Na_2SO_4	0.05	3320 ± 25
Choline chloride	0.10	1145 ± 126
—		1086 ± 66

The different effects of SCN^- and Cl^- on the transient concentrative uptake of phenylalanine reported in Fig. 2 support the hypothesis of an involvement of the membrane electrical potential in the transport mechanism. Therefore, experiments were carried out to confirm such a dependence and the results are reported in Table II. The higher rate observed with SCN^- can be accounted for by the fact that SCN^- is more permeable than Cl^- across the plasma membranes and so it causes a higher polarization, inside negative, across the membrane vesicle [9, 22]. SO_4^{2-} is very poorly permeable [23] so it produces a smaller potential than SCN^- and Cl^-, or even a reversed potential. In the absence of K^+ or Na^+, the transmembrane potential due to a choline chloride gradient has practically no effect on the uptake, as compared to the control.

It seems, therefore, that phenylalanine is co-transported with K^+ or Na^+ and that there is an involvement of the membrane potential in the transmembrane movement of the amino acid, provided that K^+ or Na^+ is present.

These data are in agreement with those reported by Nedergaard [5—7] and in addition they provide a basic rationale of the dependence on the potential difference of the amino acid uptake.

These experiments suggest that in *P. cynthia* midgut in vivo, whose luminal environment contains K^+ as the main cation, the driving force for the absorption of amino acids could be provided by a potassium electrochemical gradient. Thus, the co-transport hypothesis proposed by Crane [2] seems to acquire a more general significance.

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