The unexpected similarity of intestinal sugar absorption by SGLT1 and apical GLUT2 in an insect (Aphidius ervi, Hymenoptera) and mammals.

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ABSTRACT

Sugars are critical substrates for insect metabolism but little is known on the transporters and epithelial routes that ensure their constant supply from dietary resources. We have characterized glucose and fructose uptakes across the apical and basolateral membranes of the isolated larval midgut of the aphid parasitoid *Aphidius ervi*. The uptake of radiolabelled glucose at the basal side of the epithelium was almost suppressed by 200 µM cytochalasin B, uninhibited by phlorizin and showed the following decreasing rank of specificity for the tested substrates: glucose>glucosamine>fructose, with no recognition of galactose. These functional properties well agree with the expression of GLUT2-like transporters in this membrane. When the apical surface of the epithelium was also exposed to the labelled medium, a cation-dependent glucose uptake, inhibited by 10 µM phlorizin and by an excess of galactose was detected, suggesting the presence in the apical membrane of a cation-dependent cotransporter. Radiolabelled fructose uptakes were only partially inhibited by cytochalasin B. SGLT1-like and GLUT5-like transporters were detected in the apical membranes of the epithelial cell by immunocytochemical experiments. These results, along with the presence of GLUT2-like transporters both in the apical and basolateral cell membranes of the midgut, as we recently demonstrated, allow to conclude that the model for sugar transepithelial transport in *A. ervi* midgut appears to be unexpectedly similar to that recently proposed for sugar intestinal absorption in mammals.

**Key words:** parasitoid wasp; larval midgut; glucose and fructose uptakes; sugar transporters; model for sugar absorption.
INTRODUCTION

The adult females of the entomophagous parasitoid wasp *Aphidius ervi* (Hymenoptera, Braconidae) lay the egg in the haemocoel of the host aphids, which may belong to different aphid species. The larva hatches from the egg and develops in the haemolymph of the living host, which survives for most of the larval development. Just before metamorphosis, *A. ervi* mature larva kills the aphid by eating all the internal tissues and spins a cocoon inside the host body, where it pupates. During larval development, this regulator parasitoid shows intense physiological interactions with the living host, which are similar in terms of complexity to those observed in true parasites and are essential to generate those changes in the host haemolymph required to increase its suitability for the progeny (39).

The physiological and biochemical alterations induced by *A. ervi* in the host, and their nutritional significance to the developing parasitoid larvae, have been investigated in detail in the last few years. Briefly, the host castration induced by venom (18), injected along with the egg at oviposition, is complemented by the action of teratocytes (20, 21), cells present in a few hymenopteran species, deriving from the dissociation of the embryonic membrane (39). The combined action of these maternal and embryonic factors determines a redirection of the host metabolic effort in support of its reproduction, towards the developing parasitoid larva, with the induction of a significant increase of host nutritional suitability (38, 40). These investigations have been recently accompanied by physiological studies addressing the mechanisms and routes of nutrient absorption (9, 17, 24), which has been found to take place both through the body epithelial surface and the midgut, with the latter playing an increasing role as larval development proceeds.

Glucose is the main fuel for insect metabolism, but it is normally present at low concentration in the haemolymph because of its reducing and osmotic properties (5),
and it is converted by the fat body into trehalose (α-D-glucopyranosyl-α-D-glucopyranoside). This non-reducing disaccharide is abundantly available in the haemolymph and represents a permanent source of glucose, that can be continuously obtained by enzymatic hydrolysis mediated by tissue-associated trehalases (59). In aphids, the haemolymph trehalose concentration reaches 255 mM, but, unlike most insects (5), monosaccharides titre is also very high, reaching a concentration of 129 and 60 mM for fructose and glucose respectively (42). The peculiar sugars-rich environment in which *A. ervi* larva develops suggests that these nutrients may play an important role for its development and survival. This premise and the nearly total lack of information on sugar epithelial transport in insects stimulated the idea of investigating the cellular mechanisms involved in glucose and fructose intestinal absorption in *A. ervi*.

Hexoses movement across the small intestine in mammals is almost exclusively transcellular and is known to be mediated by the sodium/glucose cotransporter SGLT1 (SLC5A1) and by two members of the facilitative glucose transporter family, GLUT2 (SLC2A2) and GLUT5 (SLC2A5). The genes encoding these transporters have been identified and the functional properties of the corresponding proteins have been fully characterized (55, 60). The classical model for intestinal sugar absorption in mammals, deemed exhaustive till few years ago, predicted the presence of the two transporters SGLT1 and GLUT5 at the intestinal cell apical membrane favoring the entry of dietary glucose and fructose, and a common exit pathway to the bloodstream for both sugars mediated by GLUT2 transporters, constitutively expressed in the basolateral membrane. The observed total absorption of monosaccharides under a dietary sugar load, that largely exceeded SGLT1 transport capacity (28), was committed to the intestinal paracellular permeability (34). However, recent studies (27, 28) have clearly shown that in the presence of a luminal load of glucose or fructose, GLUT2 transporters are rapidly and transiently recruited to the food-facing luminal membrane. The insertion, stimulated
by hexoses as well as hormones (2), is dependent on the protein kinase C (PKC) βII
isoenzyme (25), that is activated when SGLT1 transports glucose. In this new model for
sugar intestinal absorption (12, 29), the paracellular component plays almost no
nutritional role.

A model for the transcellular movement of sugars across absorbing epithelia in
vertebrates other than mammals and in invertebrates is lacking. SGLT1-like proteins
responsible for intestinal sugar uptake have been detected, and in some instances cloned
and/or functionally characterized, in all vertebrate groups (4, 31, 33, 44), while the
identification of GLUT1-like transporters is extremely limited (26, 48, 49). GLUT5-like
transporters have been studied and detected only in birds (22). In invertebrates, Na⁺-
dependent sugar transport (3, 16, 30, 53, 57) or SGLT1-like proteins (14) have been
described in parasitic platetyhelmints, snail and decapod crustaceans. The molecular and
transport properties of two GLUT2-like transporters present in the apical and basal
membranes of tegumental cells have been well characterized in schistosomes and
cestodes (43, 46, 47), while little information are available for crustaceans (56).

Very little is known on sugars absorption in insects. The few initial studies (15,
45, 51, 52) suggested the absence of sugar transporters in the insect gut and described
the absorption as a passive, possibly diffusional, process sustained by the extremely
rapid transformation of glucose into the disaccharide trehalose. However, functional and
molecular studies have recently demonstrated that sugar transporters belonging to the
GLUT-family are present in insects of different orders (9, 13, 19, 36, 58), although their
membrane localization in the absorbing cells has been reported only in one case (9).

The study here presented, performed in vitro on the incubated A. ervi larval
intestine, allowed us to detect, with functional, immunohistochemical and
immunoblotting approaches, the transport proteins responsible for glucose and fructose
uptake. The results clearly depict, for the first time in an invertebrate, the complete
cellular model for the intestinal absorption of sugars. This model is surprisingly similar to that conventionally described for mammals (i.e. SGLT1-like and GLUT5-like transporters in the apical membrane of the intestinal epithelial cell, and GLUT2-like transporters in the basolateral one), with the expression of GLUT2 transporters also in the apical membrane, which is in full agreement with the model for the transcellular absorption of hexoses recently proposed (28).

MATERIALS AND METHODS

*Insect rearing.* The parasitoid *A. ervi* was reared on pea aphid, *Acyrthosiphon pisum* (Homoptera, Aphididae), colonies maintained on broad bean plants (*Vicia faba* L.). The aphid and parasitoid rearings were carried on in two separate chambers, both kept at 19 ± 1 °C, 75 ± 5 % RH and L16:D8 photoperiod.

*Host parasitization and dissection.* Pea aphid 4th instar nymphs were singly exposed in a glass vial (3 cm × 11 cm) to a few *A. ervi* females. After the oviposition attack, the aphid was removed and transferred to a broad bean plant, where it was kept until dissection, under the environmental conditions indicated above. Superparasitized aphids were discarded.

Parasitoid larvae were carefully synchronized by dissecting the parasitized hosts at a fixed time intervals after oviposition. Under the rearing conditions used, the developmental key-points, expressed in h after parasitization, were the following: hatching 88-91 h, first moult 129-132 h, second moult 149-152 h, beginning of mummy formation 180-185 h. To explant the experimental larvae, after 160 h, the parasitized aphids were surface sterilized for 1-2 sec in a 70% (v/v) ethanol solution in water, rinsed in distilled water and dissected in the buffered medium used for the incubation reported in the following section.
Isolation, incubation of the midgut(s) and sugar uptake measurements. *A. ervi* 3rd

instar larvae were extracted by cutting the cuticle of parasitized host aphids with two
dissecting needles. The whole gut was extracted from the larval body and the midgut
was separated from the foregut and the hindgut. Figure 1A reports a semithin sagittal
section of the entire larva and clearly shows that the midgut accounts for nearly the total
mass of the gut. Details of the larval midgut epithelium are visible in Figure 1B. Figure
1C shows the isolated midgut used for the experiments.

In the experiments designed to measure sugar uptake from the basal side of the
epithelium, the midgut was incubated without further manipulations for 60 min at room
temperature (22-24 °C) in small wells containing 100 µl of the following buffered
physiological solution (in mM): 6 MgSO₄, 2 CaCl₂, 13 KCl, 2 Na-citrate, 12 KH₂PO₄,
425 sucrose, 10 Hepes, 5.9 Tris at pH 6.4. The ionic composition, osmolarity and pH of
this medium reproduce those of aphid’s haemolymph (37, 42). In contrast, in those
experiments directed at measuring also the uptake through the luminal surface, prior to
incubation the midgut was carefully cut lengthwise and thoroughly rinsed to eliminate
the peritrophic membrane with the luminal contents. In both cases, one midgut was used
for each incubation.

To measure the amount of sugars or sugar-analogues transported by the larval
midgut, [³H]D-glucose and [³H]3-O-methyl-D-glucose (30 µCi/ml) or [¹⁴C]D-fructose
and [¹⁴C]methyl-α-D-glucopyranoside (15 µCi/ml) (all purchased from Amersham
Biosciences, Italy) were added to the incubation buffer at a final concentration of 0.1
mM. To test the effect of cytochalasin B or phlorizin (Sigma-Aldrich, Italy), midguts
were preincubated for 30 min before the addition of the labelled substrate; control
midguts were also preincubated for 30 minutes with the inhibitor solvent, 2% absolute
ethanol or water respectively. In the experiments performed in the absence of
monovalent cations, osmolarity was kept constant by adding sucrose. The absence of citrate did not affect sugar uptake (not shown).

Incubations were terminated by removing the radio-labeled medium and rinsing the midgut(s) 3 times with 150 µl of fresh physiological solution. After removal of the solution, 50 µl of 1 M NaOH were added to the well to dissolve the tissue, and the suspension was left at room temperature for 2 h. The solution was then carefully recovered, dissolved in Ultima Gold (Packard, UK) liquid scintillation cocktail, and counted for radioactivity in a liquid scintillation spectrometer (Packard Tri-Carb 1600 CA).

Mean values ± s.e. of experimental uptakes were compared by Student’s t test.

**SDS-PAGE and Western blot analysis.** 20-30 midguts were homogenized, with a teflon pestle fitting eppendorf vials, in 50 µl of the following solution: 250 mM sucrose, 10 mM Hepes-Tris at pH 7.4, 1 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride (PMSF), 0.1 mM leupeptin, 0.1 mM pepstatin A, 2 mg/ml aprotinin (all purchased from Sigma-Aldrich, Italy). Protein concentrations were determined by the method of Bradford (8), using bovine serum albumin (BSA) as the standard.

Aliquots of midgut homogenates (50 µg of proteins) were solubilized in sample buffer and resolved by 10% SDS-PAGE. Proteins were transferred to nitrocellulose membranes at the constant voltage of 350 mA for 90 min. Membranes were left overnight at 4 °C in 150 mM NaCl, 50 mM Tris-HCl at pH 7.4, 5 % w/v non fat dry milk, 0.1 % v/v Tween 20, then were subjected to three washings lasting 15 min each, in 150 mM NaCl, 0.1 % v/v Tween 20, 50 mM Tris-HCl at pH 7.4. Immunoblotting was performed by incubations at room temperature for 1 hour with GLUT5-antibody raised in rabbits against a synthetic peptide corresponding to a portion of the C-terminal domain of GLUT5 (residues 490-502), diluted 1:100 in 150 mM NaCl, 2 % w/v BSA, 0.1 % v/v Tween 20, 50 mM Tris-HCl at pH 7.4. The antibody was kindly donated by
Prof. G.L. Kellett (University of York, UK). Membranes were then washed three times (15 min for each washing) and the anti-GLUT5 antibody was detected by the enhanced chemiluminescence method (ECL, Amersham Biosciences, Italy), using peroxidase-conjugated goat anti-rabbit IgG as a secondary antibody (Jackson, Immuno Research Laboratories, PA, USA), at 1:20000 dilution.

Membrane proteins, extracted from rat jejunum according to Blakemore et al. (7), were analyzed as a positive control.

Light microscopy. Larvae extracted from parasitized aphids were fixed for 2 h in 2% glutaraldehyde in cacodylate buffer at pH 7.2. Specimens, washed with cacodylate buffer, were post-fixed at 4 °C with 1% osmic acid in cacodylate buffer at pH 7.2 for 2 h. After standard dehydration in ethanol series, specimens were embedded in an Epon-Araldite 812 mixture and sectioned with a Reichert Ultracut S ultratome (Leica, Nussloch, Germany). Semithin sections were stained by conventional methods (crystal violet and basic fuchsin) and were observed with a light microscope (Olympus, Tokyo, Japan).

Immunocytochemistry. A. ervi larvae extracted from parasitized aphids were fixed in 4% paraformaldehyde solution in 0.2 M phosphate buffer at pH 7.2 containing 1% glutaraldehyde, for 2 h at 4 °C. Specimens were washed in phosphate buffer and dehydrated in a graded ethanol series, then sectioned with a Reichert Ultracut S ultratome. After etching with NaOH 3% in absolute ethanol (10), sections (0.7 µm) were treated for 30 min with phosphate-buffered saline solution (PBS) containing 2% BSA and incubated for 30 min at room temperature with the GLUT5 polyclonal antibody described above or with an anti-SGLT1 polyclonal antibody (Chemicon International, Inc., CA, USA) at dilution 1:100 and 1:20 respectively. Samples, washed with PBS, were incubated for 1 h in a dark moist chamber with the appropriate fluorescein isothiocyanate (FITC) conjugated IgG antiserum (dilution 1:100) (Jackson,
Immuno Research Laboratories, PA, USA) as secondary antibody. Coverslips were mounted in Vectashield Mounting Medium for fluorescence (Vector Laboratories, CA, USA) and examined with a confocal laser microscope (laser λ 492 nm) (MRC 1024, Bio-Rad Laboratories, CA, USA). Controls were carried out in the same manner, but omitting the incubation with the primary antibody.

RESULTS

Sugars and sugar analogues uptake. The uptake of D-glucose and D-fructose and of two not metabolized glucose analogues were measured in incubated midguts opened lengthwise to expose both the luminal and the basal surface of the epithelium. 3-O-methyl-D-glucose (3MG) is recognized and transported in vertebrates and some invertebrates by both the Na⁺/glucose cotransporter and GLUT facilitative transporters, while methyl-α-D-glucopyranoside (αMG) is a specific substrate for SGLT1. The use of these analogues would have allowed to minimize the impact of sugar metabolism on the experimental measurements of uptakes, provided that *A. ervi* sugar transporters could have been able to handle them. Unfortunately, the uptakes of both analogues (0.1 mM) were extremely low compared to the uptakes of glucose (Figure 2) and an excess of their unlabelled form (10 mM) was unable to inhibit 0.1 mM glucose uptake (Tables 1 and 2). Therefore we were compelled to use glucose and fructose as substrates.

Glucose uptake across the midgut basolateral membrane. We have previously shown by immunostaining that GLUT2-like transporters are expressed both in the apical and in the basolateral membranes of *A. ervi* midgut epithelial cells (9). On the assumption that, like in mammalian intestine, the only sugar transporter present in the basolateral membrane is GLUT2, whilst more types of transporters could be present in the apical membrane, we functionally characterized glucose uptake across the basolateral membrane in the unopened midgut incubated as a sac (Figure 1 C), a
condition in which the mucosal side of the tissue had negligible access to the incubation solution.

Table 1 reports the effect on labelled 0.1 mM glucose uptake of a 100-fold excess of those substrates that are typically transported by GLUT2 or SGLT1 proteins in other animals, of their specific inhibitors and of the lack of monovalent cations in the medium. The uptake was inhibited by the tested substrates according to the following rank of effect: glucose > glucosamine > fructose, while an excess of galactose did not modify glucose uptake, which was also unaffected by 3MG and α MG. The uptake was inhibited by cytochalasin B at a relatively low dosage but was unaffected by phlorizin, by the absence of Na⁺ and K⁺ in the incubation fluid and by 2% absolute ethanol, the solvent in which cytochalasin B is dissolved.

Since only the carrier-mediated transport can be inhibited by an excess of substrate, the residual glucose uptake measured with a 100-fold excess of cold glucose must be due to simple diffusion and/or to an aspecific binding to the tissue. This value was, then, subtracted from all the uptake values recorded in the different experimental conditions. The % inhibitions of carrier-mediated uptakes are reported in the last column of the table.

The substrate specificity of the transporter, its sensitivity to cytochalasin B at a relatively low concentration and its insensitivity to phlorizin and monovalent cations are all functional properties typically described in mammals for GLUT2 (11, 54, 55). Therefore, from our data, a GLUT2-like transporter appears to be the unique carrier for the exit of sugars from midgut epithelial cells to the haemolymph.

**Glucose uptake from the apical and basolateral membranes.** The characterization of glucose uptake into midgut epithelial cells when both the apical and the basal plasma membranes are exposed to the labelled incubation medium is reported in Table 2. It is well known that carrier-mediated transport is affected by low temperature, so we
measured the uptake at 4 °C, observing a large inhibition, that reduced the uptake by 70%. Then, we tested the inhibitory effect of an excess of glucose, to quantify the carrier-mediated component of total uptake. The non-inhibited residual uptake, not significantly different from that measured at 4 °C, was routinely subtracted from all uptakes in the different experimental conditions, to calculate the specific inhibition of the carrier-mediated component.

We examined the effect of an excess of substrates known to be competitors of GLUT2 or SGLT1: glucose uptake was inhibited by glucosamine and fructose, as expected, since we have seen that GLUT2-like transporters are expressed in the basolateral (see Glucose uptake across the midgut basolateral membrane in RESULTS, 9) and apical membranes (9), but it was also inhibited by galactose, a specific substrate of SGLT1. Moreover, when glucosamine and galactose were both present in the incubation medium, glucose uptake was almost entirely suppressed, strongly suggesting the presence in the apical membrane of a SGLT1-like transporter. Neither 3MG nor αMG had any effect.

The incubation of the midgut in the absence of Na⁺ and K⁺ in the medium reduced the uptake and a similar reduction was observed with the addition of a low concentration of phlorizin, the specific inhibitor of SGLT1 (the maximal effect was obtained with 10 µM). An additive inhibition induced by the simultaneous addition of cythocalasin B and phlorizin could not be tested, since ethanol (cytochalasin B solvent) and phlorizin, both active on SGLT1 in vitro (35, 61), are not mutually exclusive inhibitors (35), so ethanol can mask phlorizin effect (61).

Ethanol inhibited Na⁺/glucose cotransport in mammalian intestinal and renal BBMV (32, 35) either by inducing a faster dissipation of Na⁺ electrochemical gradient or as a result of a direct interaction with SGLT1. We observed in A. ervi isolated midgut a similar large inhibitory effect by 2% ethanol (Table 2), that, conversely, had no effect
on glucose uptake at the basal side of the epithelium (Table 1), where only GLUT2 transporters are present. The nearly complete suppression of uptake was obtained with the addition of cytochalasin B (Table 2). A comparable total inhibition of carrier-mediated glucose uptake was also observed by adding cytochalasin B to a medium devoid of monovalent cations (not shown).

Altogether these data are in favor of the presence in the apical membrane of the midgut epithelium of a transport protein with functional properties similar to those of SGLT1. The midgut epithelium, probed with an antibody raised against rat intestinal SGLT1 showed a clear cross-reaction signal in the apical membranes (Figure 3).

Fructose uptakes and immunocytochemical localization of GLUT5. Fructose is taken up by the midgut (Table 3) but its uptake value is markedly lower than that of glucose (Table 2). In line with the presence of GLUT2-like transporters in the epithelial cell plasma membranes, the carrier-mediated component of fructose transport is inhibited by an excess of glucose, unaffected by the absence of monovalent cations and by ethanol, and it is halved by cytochalasin B. The portion of fructose uptake not inhibited by cytochalasin B, about 50% of total uptake, must thus be mediated by a different transport protein and the obvious candidate is GLUT5, the specific facilitative fructose transporter, insensitive to cytochalasin B (55).

Since no specific inhibitor is known for this transporter, we probed the presence of GLUT5 with an anti-GLUT5 antibody. As shown in Figure 3, a strong immunofluorescence signal is detected in the apical membranes of midgut cells. Western blot analysis of A. ervi midgut homogenates revealed a band of 55 kDa, the expected molecular weight for GLUT5, identical to the band detected in rat jejunal membranes (Figure 4). Preabsorption with the antigenic peptide blocked the recognition of the immunoreactive protein (not shown).
DISCUSSION

We started our investigation on nutrient transport in *A. ervi* by studying the absorption of radiolabelled amino acids and sugars in the living larva incubated in a simple saline that mimicked the ionic composition of the host haemolymph (9). The experimental results showed that glucose and fructose were transported by the midgut epithelium from the lumen to the haemocoel, that the transport was inhibited by cytochalasin B and that the two hexoses exerted a mutual inhibition on their uptakes. These functional data well agreed with the presence of GLUT2-like transporters, that were immunolocalized in the basal and in the apical membranes of midgut epithelial cells.

However, the experiments with the living larvae did not allow to shed light on the different types of sugar transporters involved in glucose and fructose transepithelial transport and on their functional properties. These issues could be investigated in depth when we developed a protocol to operate on isolated midguts extracted from early 3rd instar larvae, when the absorbing capacity of the epithelium is still relevant (9). The midgut is a small sac (Figure 1 C) and when it is incubated in this form exposes to the radiolabelled medium only the basal side of the epithelium, allowing the functional characterization of glucose transport at the cell basolateral membrane, which is responsible *in vivo* for the exit from the midgut cell to the haemolymph of the sugar molecules absorbed at the apical side. The experiments presented in this paper revealed that one transporter only is involved in this task (Table 1), and that this transporter shares many of the functional features of mammalian GLUT2 (55), from which it differs for a lower affinity for glucosamine and for its inability to recognize galactose and 3MG (Table 1).

With the exposure to the medium of the luminal surface of the epithelium in the midgut incubated as a flat sheet, a cation-dependent glucose uptake emerged, which was
inhibited by galactose, 10 µM phlorizin and 2% ethanol (Table 2), functional features in common with SGLT1 (60). In the experiments carried out in the absence of cations, we removed both Na⁺ and K⁺, because in some insect orders organic molecules cotransport may take place with K⁺ as a driver cation, in addition to Na⁺, as it happens, for instance, for the secondary active uptake of amino acids in lepidopteran larval midgut (23). Although ethanol does not affect SGLT1 transport efficiency in rat or mouse whole intestine \textit{in vivo} unless administered at very high concentrations (5% v/v) (1), it does inhibit Na⁺-dependent glucose transport in simplified \textit{in vitro} systems like BBMV (32, 35), either because of the increased membrane fluidity (6) or because regulatory mechanisms possibly active \textit{in vivo} are lacking. In \textit{A. ervi} midgut we detected a large inhibition of glucose uptake by ethanol only when the mucosal surface was exposed, i.e. only when the cation-glucose cotransporter was accessible. No effect of ethanol was detected on glucose transport mediated by GLUT2-like transporters (Table 1) or on fructose uptake (Table 3).

According to our data, a major functional difference of the cation–dependent glucose transporter of \textit{A. ervi} compared to SGLT proteins of vertebrate tissues is its extremely poor capacity to recognize methyl-\textgreek{i}-D-glucopyranoside (Fig. 2 and Table 1).

A cross-reaction signal to a commercial antibody raised against mammalian SGLT1 was clearly detected in the apical membranes of midgut cells (Figure 3). Actually, a sodium gradient across the apical membrane, fuelling the secondary active glucose accumulation in the cell and its active transcellular flux, could be effectively generated and maintained by the Na⁺/K⁺-ATPase that we have previously immunolocalized in the basolateral membrane of \textit{A. ervi} midgut cells (17).

The amount of glucose that appears to be mediated by the SGLT1-like transporter in the isolated midgut is about 40-50% of the total carrier-mediated uptake, while the remaining amount is apparently mediated by the GLUT2-like transporter of the basal
membrane. We are aware that the GLUT2-like transporters are expressed also in the apical membrane of *A. ervi* larvae *in vivo* (9), but their contribution could not be discriminated in our experimental condition. However, an almost complete inhibition of labelled glucose uptake was recorded with the co-presence in the medium of an excess of the preferred substrate of each transporter, i.e. galactose and glucosamine. Similarly, a total inhibition of the carrier mediated uptake was observed in the absence of cations and the concomitant addition of cytochalasin B (Table 2).

The presence of GLUT2 transporters in the apical and basal membranes is in line with the ability of the midgut epithelium to transport fructose (Table 3), but fructose carrier-mediated uptake was only halved by cytochalasin B, the inhibitor of GLUT transporters known to be ineffective on the fructose transporter GLUT5 (41). So, fructose must cross the apical cell membrane also through the GLUT5-like transporters that we have immunodetected in that membrane domain (Figure 3).

In conclusion, the transepithelial net transport of sugars in *A. ervi* larval midgut, schematically outlined in Figure 5, appears to be sustained by a SGLT1-like transporter at the apical membrane, where the chemical Na\(^+\)-gradient generated by the Na\(^+\)/K\(^+\)-ATPase in the basolateral membrane energizes glucose transepithelial net absorption. Fructose enters the cell from the lumen through GLUT5-like transporters, that may also recognize glucose. The exit of the two sugars at the basal side of the cell to the haemolymph is mediated by GLUT2-like transporters. This model, unexpectedly similar to that elucidated over the years for mammalian intestine, is the first one proposed for the intestinal absorption of sugars in an insect. However, it has not to be considered as a model universally valid for insects belonging to the different orders, in which the midgut is characterized by a wide variety of morphological, biochemical and physiological adaptations.
The immunodetection of GLUT2-like transporters in the apical membrane of midgut cells in vivo (9) is intriguing, especially since a careful scrutiny of various specimens from second and third instar larvae revealed that the apical signal is frequent but of variable intensity and sometimes is absent (B. Giordana, unpublished observations). It is tempting to speculate that in this species, as in mammals, the apical transporters are not constitutive, but stored in intracellular vesicles and recruited to the cell membrane in response to specific external and internal signals, as it is now well described for mammalian intestine (2, 29). This is certainly a very interesting hypothesis that is worth of further research efforts.

Finally, the study of nutrient transport and its regulation in A. ervi larvae will produce solid information of crucial importance to define and/or refine artificial diets for this species, largely used in insect pests biocontrol and for which the continuous in vitro rearing is not yet achieved, as for all other endophagous parasitoid species considered so far. It will be essential to figure out how the host biochemical changes are matched by a parallel regulation of absorption mechanisms during development. The lack of a detailed knowledge of this dynamic process is probably one of the main limits preventing their successful artificial rearing (50).

**GRANTS**

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REFERENCES


Figure legends

Figure 1. Semithin sagittal section of the entire larva stained with fuchsin-crystal violet (A). The midgut (arrowhead) occupies a large portion of the body. In B, details of midgut epithelial cells, presenting a well developed brush border (arrowheads), are visible. C shows the isolated pouchlike midgut. l: midgut lumen.

Figure 2. Uptakes of 0.1 mM D-glucose, its analogues 3-O-methyl-D-glucose (3MG) and methyl-α-D-glucopyranoside (αMG), and D-fructose. Above each bar, mean uptake values ± SE are reported along with the number of replicates in parenthesis.

Figure 3. Immunocytochemical localization of SGLT1- and GLUT5-like transporters. The immunofluorescence signal is located in the apical membranes of midgut cells. l: midgut lumen.

Figure 4. Western blot analyses with GLUT5 antibody of A. ervi larval midgut homogenates (A) and of rat crude jejunal membranes (B).

Figure 5. Model for sugar absorption in A. ervi larval midgut cells. Explanation in the text.
Table 1. Uptake of 0.1 mM glucose from the basolateral side of the epithelium in different experimental conditions.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>pmoles/midgut</th>
<th>Inhibition %</th>
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<tbody>
<tr>
<td>control</td>
<td>22.8 ± 0.8 (17)</td>
<td></td>
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<tr>
<td>+ D-glucose</td>
<td>3.6 ± 0.3 (4)**</td>
<td>100</td>
</tr>
<tr>
<td>+ glucosamine</td>
<td>8.0 ± 0.7 (4)**</td>
<td>77</td>
</tr>
<tr>
<td>+ D-fructose</td>
<td>11.2 ± 0.7 (4)**</td>
<td>60</td>
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<tr>
<td>+ D-galactose</td>
<td>19.5 ± 2.7 (4)</td>
<td>0</td>
</tr>
<tr>
<td>+ 3-O-methyl-D-glucose</td>
<td>19.9 ± 1.4 (6)</td>
<td>0</td>
</tr>
<tr>
<td>+ methyl-α-D-glucopyranoside</td>
<td>20.7 ± 1.9 (7)</td>
<td>0</td>
</tr>
<tr>
<td>+ 2 % ethanol</td>
<td>20.0 ± 1.3 (11)</td>
<td>0</td>
</tr>
<tr>
<td>+ cytochalasin B (100 μM)</td>
<td>12.3 ± 3.8 (3)*</td>
<td>55</td>
</tr>
<tr>
<td>+ cytochalasin B (200 μM)</td>
<td>5.9 ± 0.9 (7)**</td>
<td>88</td>
</tr>
<tr>
<td>+ phlorizin 50 μM</td>
<td>24.2 ± 4.7 (3)</td>
<td>0</td>
</tr>
<tr>
<td>+ phlorizin 200 μM</td>
<td>23.1 ± 0.2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>absence of Na⁺ and K⁺</td>
<td>19.2 ± 3.5 (3)</td>
<td>0</td>
</tr>
</tbody>
</table>

The concentration of competitor substrates was 10 mM. Values are means ± SE, number of experiments in parenthesis.

Student’s t test versus control: *P < 0.05, **P < 0.001.
Table 2. Uptake of 0.1 mM glucose from the apical and basolateral side of the epithelium in different experimental conditions.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>pmoles/midgut</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>34.5 ± 2.7 (16)</td>
<td></td>
</tr>
<tr>
<td>low temperature (4 °C)</td>
<td>10.2 ± 2.5 (5)**</td>
<td>70</td>
</tr>
<tr>
<td>+ D-glucose</td>
<td>6.2 ± 0.6 (4)**</td>
<td>100</td>
</tr>
<tr>
<td>+ glucosamine</td>
<td>21.7 ± 3.3 (4)*</td>
<td>45</td>
</tr>
<tr>
<td>+ D-fructose</td>
<td>19.3 ± 1.3 (4)**</td>
<td>54</td>
</tr>
<tr>
<td>+ D-galactose</td>
<td>18.2 ± 1.7 (6)**</td>
<td>58</td>
</tr>
<tr>
<td>+ D-galactose plus glucosamine</td>
<td>8.8 ± 1.1 (7)**</td>
<td>92</td>
</tr>
<tr>
<td>+ 3-O-methyl-D-glucose</td>
<td>36.2 ± 5.7 (8)</td>
<td>0</td>
</tr>
<tr>
<td>+ methyl-α-D-glucopyranoside</td>
<td>35.9 ± 4.6 (8)</td>
<td>0</td>
</tr>
<tr>
<td>absence of Na(^+) and K(^+)</td>
<td>20.8 ± 1.9 (10)**</td>
<td>48</td>
</tr>
<tr>
<td>+ phlorizin 5 µM</td>
<td>21.1 ± 1.1 (5)**</td>
<td>47</td>
</tr>
<tr>
<td>+ phlorizin 10 µM</td>
<td>16.8 ± 1.4 (6)**</td>
<td>62</td>
</tr>
<tr>
<td>+ phlorizin 100 µM</td>
<td>16.2 ± 1.1 (7)**</td>
<td>64</td>
</tr>
<tr>
<td>+ 2 % ethanol</td>
<td>17.4 ± 0.8 (4)**</td>
<td>60</td>
</tr>
<tr>
<td>+ cytochalasin B (200 µM)§</td>
<td>8.4 ± 0.5 (6)**</td>
<td>80</td>
</tr>
</tbody>
</table>

The concentration of competitor substrates was 10 mM. Values are means ± SE, number of experiments in parenthesis.

Student’s t test versus control: *P < 0.01, **P < 0.001.

§ Student’s t test and % inhibition versus uptake with 2% ethanol.
Table 3. Uptake of 0.1 mM fructose from the apical and basolateral side of the epithelium in different experimental conditions. The concentration of competitor substrates was 10 mM.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>pmoles/midgut</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>13.0 ± 1.2 (9)</td>
<td></td>
</tr>
<tr>
<td>+ D-fructose</td>
<td>3.8 ± 0.6 (4)**</td>
<td>100</td>
</tr>
<tr>
<td>+ D-glucose</td>
<td>6.9 ± 1.1 (4)**</td>
<td>66</td>
</tr>
<tr>
<td>absence of Na⁺ and K⁺</td>
<td>14.4 ± 2.7 (4)</td>
<td>0</td>
</tr>
<tr>
<td>+ 2% ethanol</td>
<td>12.2 ± 2.4 (5)</td>
<td>0</td>
</tr>
<tr>
<td>+ cytochalasin B (200 µM)</td>
<td>8.8 ± 1.0 (7)*</td>
<td>46</td>
</tr>
</tbody>
</table>

Values are means ± SE, number of experiments in parenthesis.

Student’s t test versus control: *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 2, Caccia et al
Figure 4, Caccia et al
Figure 5, Caccia et al.