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Transport of D-galactose by the gastrointestinal tract of the locust, *Locusta migratoria*

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Abstract

Due to exoskeleton, the absorption of nutrients in adult insects takes place across the gastrointestinal tract epithelium. In most physiological studies, sugar intestinal absorption has been described as a diffusional process and to date no sugar transporter has been cloned from the digestive tract of insects. In the present work, the existence of a saturable transport system for galactose in the gastric caeca of *Locusta migratoria* is clearly demonstrated. This transport shows a relatively high affinity for galactose (apparent $K_{0.5}=2-3$ mM) and is inhibited by glucose, 2-deoxyglucose and with less potency by fructose and α-methyl-D-glucoside. The absence of sodium or the presence of phloridzin hardly affects galactose absorption, indicating that it is not mediated by a SGLT1-like transporter. The absence of K^+, Cl^-, Mg^{2+} and Ca^{2+} or changes in the pH do not modify galactose absorption either. Nevertheless, phloretin, cytochalasin B and theophylline (inhibitors of facilitative transporters) decrease sugar uptake around 50%. *Xenopus laevis* oocytes microinjected with poly A^+ RNA isolated from gastric caeca show sodium-independent galactose uptake that is three times higher than in non-injected oocytes, further supporting the existence of a mRNA coding for at least one equilibrative sugar transporter in *L. migratoria* gastric caeca.

Keywords: Cytochalasin B; Equilibrative sugar transport; Galactose intestinal absorption; Gastric caeca; GLUT; *Locusta migratoria*; Phloretin; Phloridzin

1. Introduction

Arthropods are the only invertebrate phylogenetic group where aquatic species are not able to absorb nutrients directly across the integument. Therefore, acquisition of exogenous nutrients in adult insects relies on the activity and properties of the gastrointestinal system that is divided into three major areas with single-layer epithelium: foregut, midgut and hindgut (Wright and Ahearn, 1997). Most insects need carbohydrates, with glucose the main energy source, but little is known about the way of incorporation of sugars in these animals.

The absorption of carbohydrates in the small intestine of mammals occurs by two kinds of membrane transporters: concentrative and equilibrative. In the apical membrane of the enterocyte, the sodium-dependent glucose cotransporter SGLT1 is able to accumulate glucose or galactose into the cytosol using the electrochemical gradient of sodium (Wright and Turk, 2004). Also apically located, GLUT5 transports fructose from the lumen to the cell down its chemical gradient. In the basolateral membrane, GLUT2 is the facilitative translocator for exit of sugars to the interstitial space (Cheeseman, 1993), although GLUT5 has been also found in this membrane (Blakemore et al., 1995). Recent studies have demonstrated the presence of GLUT2 in the apical membrane of the enterocytes during the absorptive periods, suggesting that this transporter would mediate the entry of sugars from the lumen to the cell down their concentration gradient (Kellett and Helliwell, 2000).

Intestinal SGLT1-like transporters have been described, and some of them cloned, in all vertebrate groups: fishes (Kipp et al., 1997), amphibians (Nagata et al., 1999), reptiles (Pajor et al., 1992), birds (Garriga et al., 1999) and mammals (Hediger et al., 1987). In invertebrates, Na^+-dependent sugar intestinal transport has been detected in mollusks (Barber et al., 1979; Gerencser and White, 1980; Stewart and Bamford, 1976), echinoderms (Lawrence et al., 1967) and crustacean (Ahearn and Maginniss, 1977), although no transporter has been cloned.

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GLUT-type intestinal facilitative transporters have been found also in fishes (Krasnov et al., 2001) and birds (Garriga et al., 1999).

In plants and other organisms like bacteria and yeast, sugar absorption often depends on proton gradient (Miller et al., 1994). However, in *Vibrio parahaemolyticus*, a sodium–glucose cotransporter has been cloned and functionally characterized in *Xenopus laevis* oocytes (Leung et al., 2002) and in plasmodium parasite, facilitative hexose transporters that present high homology with GLUT-type proteins have been described (Woodrow et al., 1999).

Studies on intestinal absorption of sugars in insects are scarce and the classical theories of Treherne (1967) are still accepted. Based on the results in *Schistocerca gregaria* (Treherne, 1957) and *Periplaneta americana* (Treherne, 1958), he concluded that sugars are passively absorbed from the lumen to the hemolymph down their concentration gradient which is established by the net flux of ions and water in the same direction and, above all, by the rapid conversion of the ingested monosaccharides, namely glucose, to the disaccharide trehalose. This conversion takes place mostly in the fat body which surrounds the gut that maintains a high sugar gradient across the gut wall and correlates with the high concentration of the disaccharide in the hemolymph. Later results in other species of insects continue supporting that, apparently, only simple diffusion of sugars could occur in the gut (Crailsheim, 1988; Droste and Zebe, 1974; Gelperin, 1966; Turunen and Crailsheim, 1996). However, as it was pointed out by Treherne (1967), the condition of high trehalose and low glucose concentration in the hemolymph is not universal in insects.

The uptake of D-glucose by midgut epithelium of *Ostrinia nubilalis* larvae has been found to be higher than that of L-glucose, suggesting the presence of a transporter that appears on feeding and disappears after starvation and during diapause (Trabelsi and Labanseau, 1994). In isolated Malpighian tubules of *Locusta migratoria*, the glucose reabsorption towards the hemolymph is inhibited by phloridzin, phloretin and ouabain (Mordue and Rafaeli-Bernstein, 1978). In Kc cell line from *Drosophila melanogaster*, a facilitative cytochalasin B-sensitive glucose transporter has been described (Wang and Wang, 1993) and in the endophagous parasitoid *Aphidius ervi* larvae, there is evidence of absorption of 3-*O*-methyl-D-glucose through the epidermis, which is not affected by the absence of sodium or by phloridzin but is inhibited by phloretin and cytochalasin B in a significant way (Giordana et al., 2003).

In 1999, a 2168 base pair cDNA was cloned from *D. melanogaster*, with a coding region of 480 amino acids. The protein presents a homology of 68% with the human sugar transporter GLUT1, although the functional studies are still lacking (Escher and Rasmuson-Lestander, 1999).

In summary, there are no recent data about intestinal sugar absorption in insects and it is difficult to accept that it may be mostly explained by simple diffusion of the monosaccharides across the plasma membrane. Since previous immunohistochemical studies using anti-SGLT1 antibodies demonstrated immunoreactivity in midgut and gastric caeca of *L. migratoria* (Zudaire, 1999, personal communication), the aim of the present work was to study the functional characteristics of the intestinal absorption of sugars in the locust in order to demonstrate the occurrence of some transport mechanisms, which would indicate the presence of sugar transporters.

2. Material and methods

2.1. Materials

[1-^{14}C]-Galactose (47 mCi/mmol) was purchased from Perkin Elmer, Life Sciences, USA. D-galactose and D-glucose were from Merck and α-methyl-D-glucoside, D-fructose, 2-deoxy-D-glucose, phloridzin, phloretin, theophylline, cytochalasin B and ouabain were from Sigma Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial sources.

2.2. Animals

All experiments were performed on mature adult locusts of both sexes obtained from a colony of *L. migratoria* reared at 30 °C under crowded conditions. The locusts were kept on a 12 h light/12 h dark photoperiod and fed on fresh wheat seedlings supplemented with bran.

2.3. Measurement of galactose absorption in vitro

To determine the intestinal absorption of sugars in *L. migratoria*, substrate accumulation was measured using a radiotracer method (Barrenetxe et al., 2001) adapted to the insect physiological conditions. In order to reduce metabolic transformations, galactose was used as substrate instead of glucose. Animals were killed by decapitation, dissected and the whole gut was isolated. Then, the selected gut regions were longitudinally opened in order to expose the apical surface to the medium (including each one of gastric cecum) and suspended in cold (4 ± 1 °C) physiological solution (158.4 mM NaCl; 12.3 mM KCl; 4 mM $MgCl_2$, 4.5 mM $CaCl_2$; 5–5.5 mM Tris–HCl, pH 7.0). Four similar segments obtained from 4 animals were incubated (30 ± 0.2 °C) in 10 mL of physiological solution containing galactose and radiolabeled sugar (0.5 μCi) as marker, with continuous stirring and oxygen bubbling. After the incubation time, each piece was removed, carefully washed in ice-cold physiological solution and blotted on wet filter paper to eliminate extracellular sugar trapped by the tissue. Then, it was weighed and the radioactivity was extracted by treating the tissue for 24 h with HNO_3 (0.5 mL, 0.1 N). Aliquots of 200 μL were taken from the incubation solution and the tissue extracts and radioactivity was counted in a β-scintillation counter. Results, estimated from the relation between cpm of the incubation medium and cpm of the HNO_3 aliquots, are expressed as μmol galactose per g of wet mass. In the kinetic experiments, the apparent values of $K_{0.5}$ and V_{max} for the sugar transport were obtained after fitting the