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Characterization of a Na^+/Glucose Cotransporter Cloned from Rabbit Small Intestine

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Summary. The Na^+/glucose cotransporter from rabbit intestinal brush border membranes has been cloned, sequenced, and expressed in *Xenopus* oocytes. Injection of cloned RNA into oocytes increased Na^+/sugar cotransport by three orders of magnitude. In this study, we have compared and contrasted the transport properties of this cloned protein expressed in *Xenopus* oocytes with the native transporter present in rabbit intestinal brush borders. Initial rates of ^{14}C-α-methyl-D-glucopyranoside uptake into brush border membrane vesicles and *Xenopus* oocytes were measured as a function of the external sodium, sugar, and phlorizin concentrations. Sugar uptake into oocytes and brush borders was Na^+-dependent (Hill coefficient 1.5 and 1.7), phlorizin inhibitable (K_i 6 and 9 μM), and saturable (α-methyl-D-glucopyranoside K_m 110 and 570 μM). The sugar specificity was examined by competition experiments, and in both cases the selectivity was D-glucose > α-methyl-D-glucopyranoside > D-galactose > 3-O-methyl-D-glucoside. In view of the close similarity between the properties of the cloned protein expressed in oocytes and the native brush border transporter, we conclude that we have cloned the classical Na^+/glucose cotransporter.

Key Words Na^+/glucose cotransporter · intestinal brush border · *Xenopus* oocyte expression system · phlorizin · brush border membrane vesicles

Introduction

We have identified, sequenced, and expressed a cDNA clone for a Na^+/glucose cotransporter from rabbit small intestine (Hediger et al., 1987a). Since there may be more than one intestinal brush border glucose cotransporter (e.g., Honegger & Semenza, 1973; Kaunitz & Wright, 1984; Brot-Laroche et al., 1986, 1987), we have examined the properties of the cloned transporter expressed in oocytes. To distinguish between the endogenous facilitated glucose transporter and the Na^+/glucose cotransporter, we measured the transport of α-methyl-D-glucopyranoside (αMeGlc), which is a substrate for the cotransporter and not the facilitated glucose carrier. The kinetics of αMeGlc uptake into oocytes was then compared and contrasted to the kinetics of αMeGlc uptake into rabbit brush border membrane vesicles. On the basis of ion specificity, substrate specificity, and phlorizin inhibition, we conclude that we have cloned the "classical" brush border Na^+/glucose cotransporter. Preliminary accounts of these results have been presented (Ikeda et al., 1987; Hwang & Wright, 1987).

Materials and Methods

Oocyte Experiments

RNA was synthesized in vitro from the clone pMJC424* using Not I-truncated plasmid DNA and T3 polymerase, and Poly(A)$^+$RNA was isolated from rabbit small intestine as described elsewhere (Hediger et al. 1987*a*,*b*). Oocytes were harvested from *Xenopus laevis* ovaries, injected with RNA, and assayed for Na^+-dependent glucose uptake as described previously (Hediger et al., 1987*a*,*b*). 10–20 ng of RNA were injected into defolliculated oocytes, and then the oocytes were incubated 1–3 days at 18–22°C in Barth's solution. Sugar uptake into oocytes was measured using a radioactive tracer technique (Hediger et al., 1987*b*). We chose αMeGlc as a substrate for the cotransporter because (i) it is handled by the Na^+/glucose cotransporter (*see* Wilson, 1962; Kimmich & Randles, 1984), but not the intestinal facilitated glucose carrier (Wright, van Os & Mircheff, 1980; Kimmich & Randles, 1981); (ii) the native oocyte plasma membranes appear to possess a Na^+-independent D-glucose carrier which does not handle αMeGlc (Hediger et al., 1987*b*; Fig. 5); and (iii) this sugar is poorly metabolized (Barry et al., 1964). Oocytes were incubated in either Na^+ or Na^+-free Ringer solutions containing ^{14}C-D-glucose or ^{14}C-αMeGlc for 1 hr, and the ^{14}C content of each oocyte was determined by liquid scintillation counting. The Ringer solution contained (in mM): 100 NaCl, 2 KCL, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES/Tris, at pH 7.5. In some experiments, NaCl was replaced with choline Cl, KCl, or LiCl. Uptakes were expressed as pmol/oocyte · hr and are given as the mean ± SEM for 4–6 oocytes. In this series of experiments, sugar uptakes were measured at 22°C as a function

* The name of the plasmid was altered from pMC424 to be consistent with the Plasmid Prefix Registry.

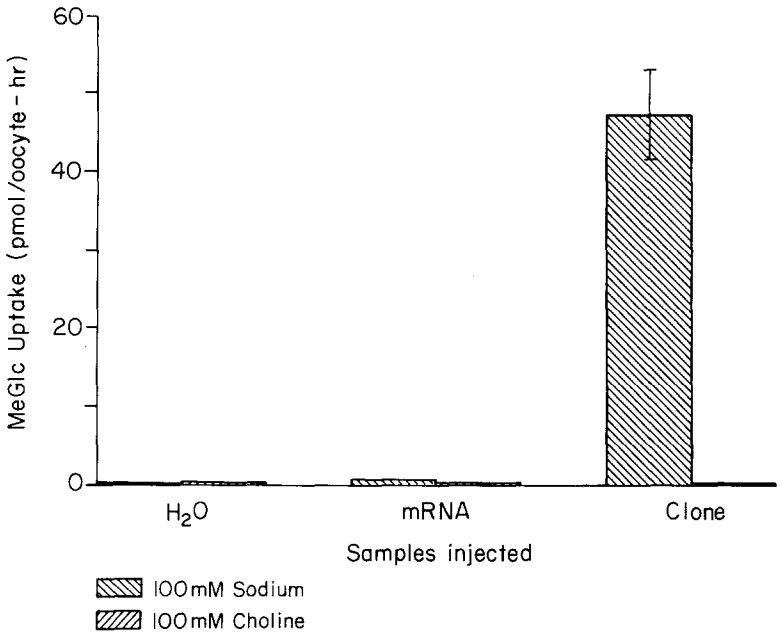


Fig. 1. Expression of the cloned transporter in *Xenopus* oocytes. Uptakes of 50 μM ^{14}C-αMeGlc were measured in the presence and absence of 100 mM NaCl one day after injection of oocytes with (i) RNA synthesized from plasmid pMJC424, (ii) rabbit intestinal poly(A)$^+$RNA, or (iii) water. Sugar uptake was measured into 5–7 oocytes and is expressed as the mean in pmol/oocyte · hr. The bar shows the standard error of the mean where it was greater than 10% of the mean

of the extracellular Na^+ (0–100 meq/liter), sugar (10 μM to 25 mM), and phlorizin (1–100 μM) concentrations.

Vesicle Experiments

Rabbit jejunal brush border membrane vesicles were prepared by a calcium-precipitation procedure (Stevens, Ross & Wright, 1982). Membranes were suspended in 300 mM mannitol and 1 mM HEPES/Tris buffer at pH 7.4 and stored in liquid nitrogen. The vesicles were enriched 25- to 30-fold in alkaline phosphatase over the initial homogenate. Sugar uptake into vesicles was measured at 22°C using a rapid mix/filtration procedure (Stevens et al., 1982; Kaunitz & Wright, 1984). Initial rates of uptake (3 sec) were measured as a function of the external Na^+ (0–100 meq/liter), sugar (10 μM to 25 mM), and phlorizin (1–100 μM) concentrations. Uptakes were expressed as pmol/mg of protein · sec and presented as the mean ± the SEM of 3–8 estimates.

Results

As shown previously (Hediger et al., 1987*a*), oocytes injected with clone RNA exhibited Na^+-dependent αMeGlc uptake while water injected controls did not (Fig. 1). With the cloned RNA, the α-MeGlc uptake was 45 pmol/oocyte · hr, which was 150 times higher than that observed with the intestinal poly(A)$^+$ RNA (0.3 pmol/oocyte · hr). Control oocytes showed no detectable Na^+-dependent sugar uptake. The functional expression of the cloned transporter increased with further incubation of the oocytes. Two days after RNA injection, the rate of Na^+-dependent sugar uptake increased to 350 pmol/oocyte · hr (*see* Fig. 6*A*). At this high rate, sugar uptakes were linear to 60 min (*data not shown*). At this rate of transport, we estimate that the intracellular α-MeGlc concentration at 1 hr is about 20 times higher than the extracellular concentration (the intracellular fluid space of an oocyte is 0.5 μl). αMeGlc cannot readily diffuse out of the oocyte since this sugar is not handled by the facilitated sugar carrier (Wright et al., 1980; Kimmich & Randles, 1981). It should be noted that the Na^+-dependent transport of α-MeGlc across native oocyte membranes is small: the highest J_{max} observed in an earlier series (September–November) of experiments (Hediger et al., 1987*b*) was 4.5 pmol/oocyte · hr, and in the present series (April–September) was frequently undetectable (i.e., <0.2 pmol/oocyte · hr).

Both the cloned and native transporter expressed in oocytes and the native transporter in membrane vesicles show a very specific requirement for Na^+ (Fig. 2). The rates of sugar uptake in choline, K^+, and Li^+ were 5%, or less, of the rates in Na^+. The rates in Li^+ were not significantly higher than those in K^+ or choline. Both systems show a very specific requirement for Na^+.

To obtain information about the number of Na^+ binding sites on each transporter, we measured the Na^+ concentration dependence of αMeGlc uptake (Fig. 3). In both cases, there was a sigmoid-dependence of sugar uptake rate on Na^+ concentration, and the data were fitted by the Hill equation with Hill coefficients of 1.5–1.7. This result suggests that both the native and the cloned transport proteins contain at least two Na^+ binding sites.

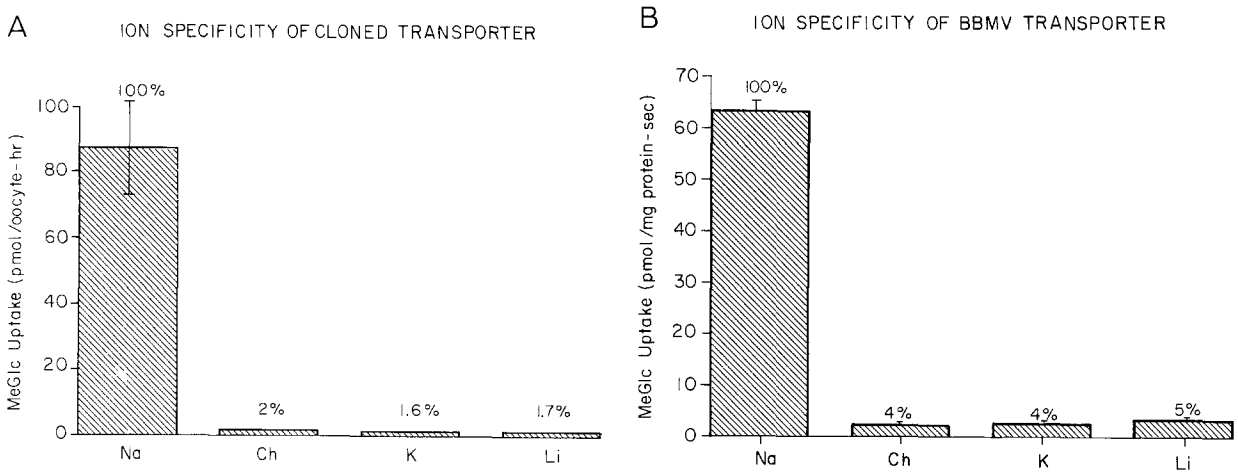


Fig. 2. Ion specificity of sugar transport. (*A*) Cloned transporter. RNA synthesized from clone pMJC424 was injected into oocytes, and the uptakes of 50 μM ^{14}C-α-MeGlc were measured three days later in the presence of 100 mM NaCl, choline Cl, KCl, or LiCl. The uptake rates in pmol/oocyte · hr, are presented as the means of 5–8 estimates, and the error bar represents the SEM (in all other cases, the SEM was less than 10% of the mean). (*B*) Brush border vesicles. The uptake of αMeGlc was measured into rabbit brush border vesicles as a function of the ionic composition of the uptake medium. The vesicles contained 300 mM mannitol and 1 mM HEPES/Tris at pH 7.5, and the uptake medium contained 50 μM ^{14}C-αMeGlc, 100 mM NaCl, Choline Cl, KCl or LiCl, 100 mM mannitol, and 1 mM HEPES/Tris at pH 7.5. Uptake was measured at 3 sec and is expressed as the mean of three estimates in pmol/mg · sec, and the error bars are the SEMs

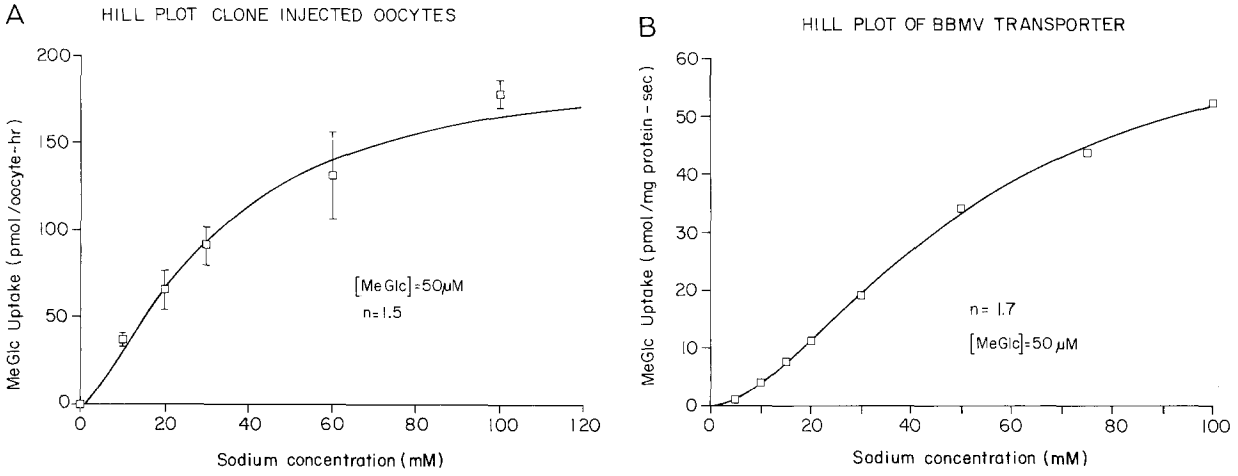


Fig. 3. The Na^+ concentration dependence of α-MeGlc uptake. (*A*) Cloned transporter. RNA synthesized from clone pMJC424 was injected into oocytes and 50 μM ^{14}C-αMeGlc uptakes were measured as a function of the external Na^+ concentration three days later. The Na^+ concentration was varied for 0–100 meq/liter, replacing choline with NaCl. At each Na^+ concentration, uptakes were measured in 5–7 oocytes, and the error bars represent the standard errors. The data were fitted to the Hill equation, $J = J_{max}[Na]^n/(K_{Na}^n + [Na]^n)$ where J_{max} is the maximal uptake at 50 μM αMeGlc, [Na] the sodium concentration, K_{Na} is the [Na] giving 0.5 J_{max}, and n is the Hill coefficient. The curve is the best fit to the equation as determined by a multiple parameter, iterative, nonlinear regression computer program (*see* Kaunitz & Wright, 1984). The Hill coefficient was 1.5, and K_{Na} 32 meq/liter. (*B*) Brush border vesicles. Three-second uptakes were measured in triplicate at each sodium concentration, and the data were fitted to the Hill equation as in *A*. The Hill coefficient was 1.7 and K_{Na} 52 meq/liter. In three such experiments, the coefficients were $n = 1.5 \pm 0.2$ and $K_{Na} = 85 \pm 25$ meq/liter

The kinetics of αMeGlc transport are shown in Fig. 4. In these experiments, the external Na^+ concentration was 100 meq/liter, and uptakes into oocytes and vesicles were measured as a function of the external sugar concentration. The uptakes are presented in the form of Hofstee plots. The Hofstee plot showing uptake of αMeGlc in RNA-injected oocytes was curvilinear and could be described by the sum of a single saturable system (J_{max} 1550 pmol/oocyte · hr, K_m 110 μM) and single nonsatura-

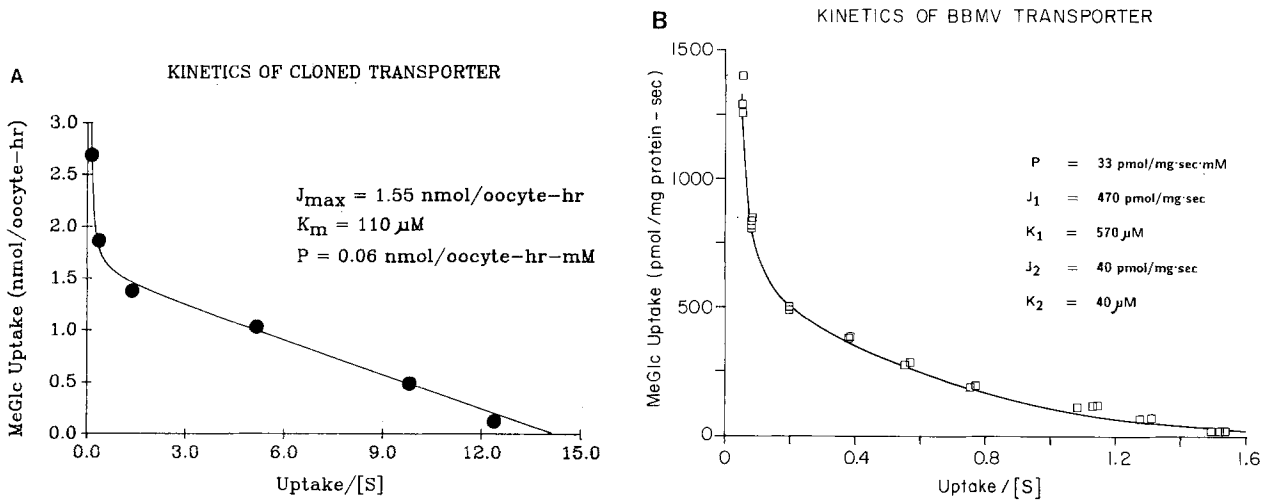


Fig. 4. The concentration dependence of αMeGlc uptake. (*A*) Cloned transporter. RNA synthesized from clone pMJC424 was injected into oocytes, and three days later the uptake of αMeGlc was measured in the presence and absence of 100 mM NaCl. The sugar concentration was varied between 10 μM and 20 mM, and each point represents the mean of 4–5 uptakes into oocytes. The standard errors of the mean were less than 20% of the mean value. The uptakes were fitted to the sum of a diffusional and a saturable uptake process, i.e., $J = J_S + J_D$, where $J_S = (J_{max}S)/(K_m + S)$ with J_{max} = the maximal velocity of the carrier-mediated uptake at 100 mM NaCl, S = sugar concentration, and K_m, the sugar concentration at which $J = 0.5\ J_{max}$, and $J_D = PS$ = where P is the sugar permeability coefficient. The curve represents the best fit to the equation as determined by nonlinear regression analysis (Kaunitz & Wright, 1984). The permeability coefficient was 58 pmol/ooycte · hr · mM, which is equivalent to 6×10^{-8} cm/sec. An identical P was obtained in 100 mM choline Cl (*not shown*). The kinetic constants for the saturable component were J_{max} 1550 pmol/oocyte · hr and K_m = 110 μM. (*B*) Brush border vesicles. Initial rates of uptake into vesicles were measured as a function of the external sugar concentration in the presence and absence of 100 mM NaCl. Uptakes were measured in triplicate at 3 sec, and the initial rates were corrected for the deviation from linearity at 3 sec: The errors were estimated from the time course of uptake and did not exceed 15%. The osmolarity of the uptake medium was maintained constant by substituting αMeGlc for mannitol. Uptakes were fitted to the sum of a diffusional and two saturable (J_1 and J_2) processes, i.e., $J = J_D + J_1 + J_2$ (*see* Kaunitz & Wright, 1984). The permeability coefficient is similar to that obtained previously for D-glucose, 2 *vs.* 3.8 μl/mg/min (Kaunitz & Wright, 1984). The kinetic parameters for the high capacity system were 470 pmol/mg · sec and 570 μM, and for the low capacity system, 40 pmol/mg · sec and 40 μM. At 50 μM αMeGlc, 61% of the uptake occurs via the high capacity system, 36% via the low capacity system, and 3% via diffusion. At a sugar concentration of 500 μM, 80% of uptake occurs through the high capacity system, 14% through the low capacity system, and 6% through diffusion

ble system. In two control experiments with water-injected oocytes, we also obtained a saturable sugar uptake, but the J_{max} was less than 5 pmol/oocyte · hr, and the K_m was between 300 and 400 μM (*see* Hediger et al., 1987*b*). The J_{max} of the cloned transporter varied between 200 and 1500 pmol/oocyte · hr depending upon the amount of mRNA injected and the number of days after mRNA injection. As noted previously (Kaunitz & Wright, 1984), sugar uptake into rabbit intestinal brush border membrane vesicles exhibits complex kinetics. Figure 4*B* shows that αMeGlc uptake into rabbit vesicles in the presence of Na^+ appears to consist of three components: two saturable and one diffusional. The diffusional component was identical to that observed in the absence of Na^+ and is comparable in magnitude to that reported previously for D-glucose (Kaunitz & Wright, 1984). The saturable components consist of a high-capacity, low-affinity system (J_{max} 470 pmol/mg · sec, K_m 570 μM), and a low-capacity, high-affinity system (J_{max} 40 pmol/mg · sec, K_m 40 μM). Both saturable systems are Na^+-dependent as evidenced by the nonsaturable kinetics of uptake in choline chloride (*not shown*).

The substrate specificity of the transporters was examined by measuring D-glucose uptake. Figure 5 shows 50 μM D-glucose uptake into oocytes three days after injection of RNA. The rate of Na^+-dependent glucose uptake (300 pmol/oocyte · hr) was comparable to that of αMeGlc uptake (Fig. 6), indicating that the clone transports both sugars equally well. As noted previously (Hediger et al., 1987*b*), oocytes contain a Na^+-independent glucose uptake, which is probably due to the presence of an endogenous facilitated glucose carrier. Injection of intestinal poly(A)$^+$ RNA increased the Na-independent D-glucose uptake [from 7.1 ± 0.9(6) to 9.1 ± 0.7(6) pmol/oocyte · hr], and this may be due to the expression of the intestinal basolateral glucose transporter. There was no significant increase in the facilitated glucose uptake with injection of cloned RNA (11.3 ± 2.0(6) pmol/oocyte · hr) compared to the control.

Specificity of sugar uptake was further exam-

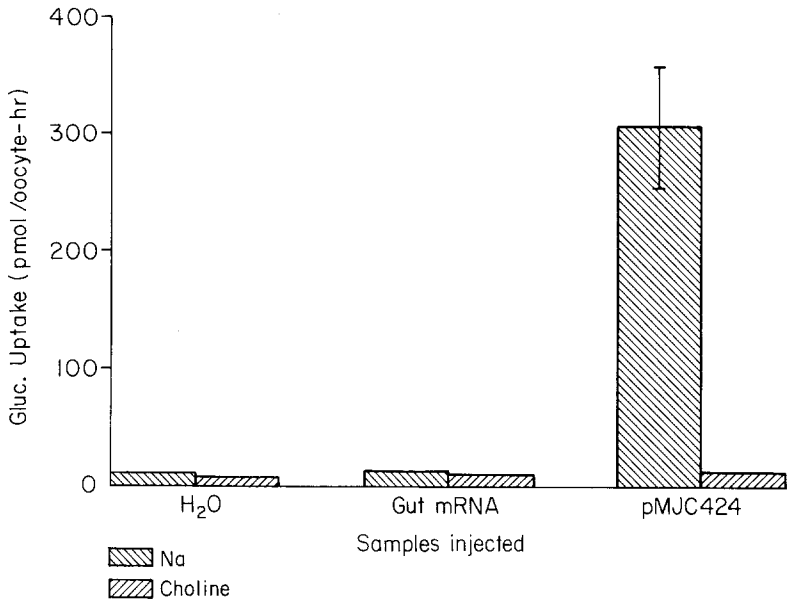


Fig. 5. D-glucose uptake by the rabbit cotransporter expressed in oocytes. RNA synthesized from clone pMJC424, *m*RNA from rabbit intestinal mucosa or water were injected into oocytes and 50 μM D-glucose uptake was measured in the presence and absence of 100 mM NaCl two days later as described in Fig. 1. The error bars, where indicated, represent the SEM of 6–8 measurements

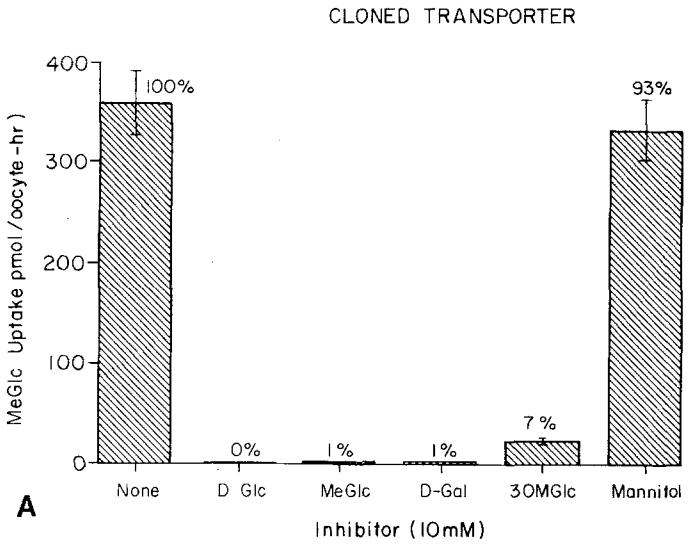
ined by competition experiments (Fig. 6). In the case of the cloned transporter, the addition of a sugar analog (10 mM) to the external medium inhibited 50 μM αMeGlc uptake if the analog was a hexopyranose in the Cl conformation with a hydroxyl group on carbon #2 in the equatorial position. 10 mM D-glucose, αMeGlc, D-galactose, and 3-O-methyl-D-glucoside inhibited the uptake of 50 μM αMeGlc over 90% (Fig. 6*A*), while mannitol, D-mannose (*not shown*), and L-glucose (*not shown*) did not inhibit αMeGlc uptake. At lower concentrations of the competing sugar analogs, for example, 1 mM (Fig. 6*B*), the potency of the inhibitory effect was D-glucose > αMeGlc > D-galactose > 3-O-methyl-D-glucoside. The specificity of the transporter in rabbit brush borders was virtually identical to that of the clone (*see* Fig. 6*C*). Glucose (1 mM) inhibited αMeGlc uptake 90%, whereas D-mannose and L-glucose had virtually no effect. Intermediate inhibitions were observed with 1 mM αMeGlc, D-galactose, and 3-O-methyl-D-glucoside. Increasing the αMeGlc, D-glucose, and D-galactose concentration to 25 mM eliminated the Na^+-dependent uptake of αMeGlc, whereas 25 mM 3-O-methyl-D-glucoside only reduced uptake to 13% of the control. This result suggests that D-glucose, αMeGlc, D-galactose, and 3-O-methyl-glucoside are substrates for both the high and low affinity brush border uptakes.

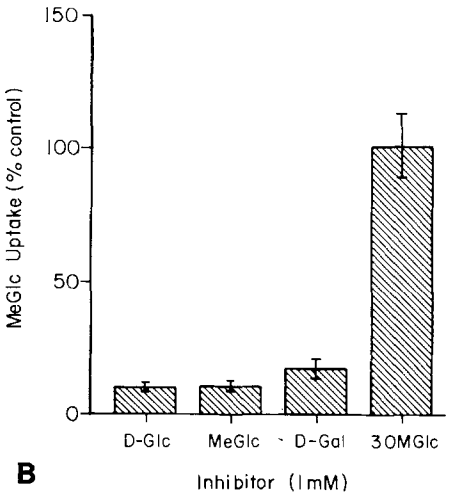
Phlorizin is a competitive inhibitor of Na^+-dependent sugar transport both in the intestine and kidney. We examined the effect of this inhibitor on αMeGlc uptake into brush border membranes and oocytes. In the case of the brush border membranes, we measured the effect of phlorizin on uptakes of 50 and 500 μM αMeGlc. Uptakes are shown in Fig. 7*A* as a function of phlorizin concentration in the form of Dixon plots. Phlorizin acts as a competitive inhibitor with a K_i of 9 μM. The estimated J_{max} for phlorizin-sensitive αMeGlc uptake obtained from the Dixon plot corresponds to 520 pmol/mg · sec, while the measured values were 470 and 40 pmol/mg · sec for the high and low capacity systems (Fig. 4*B*). These results reinforce the conclusion that phlorizin is a competitive inhibitor of both αMeGlc uptake systems. Figure 7*B* compares phlorizin inhibition of sugar transport in brush border membrane vesicles and in oocytes. In each system, 100 μM phlorizin inhibited transport by more than 90%, and about 50% inhibition was produced by 5–15 μM phlorizin. Hence, the cloned and the native transporters are equally sensitive to phlorizin.

Discussion

The goal of the present series of experiments was to characterize the Na^+/glucose cotransporter we have cloned from the rabbit intestinal mucosa. Since our cloning strategy rested on the use of a D-glucose analog, we have compared and contrasted the transport kinetics of the glucose analog by the native transport protein in rabbit brush border membranes with the cloned transporter expressed in oocyte plasma membranes.

The Table summarizes the kinetic characteristics of αMeGlc transport across brush border membranes and oocyte plasma membrane containing the cloned transporter. It is clear that there are qualitative and quantitative similarities between the cloned





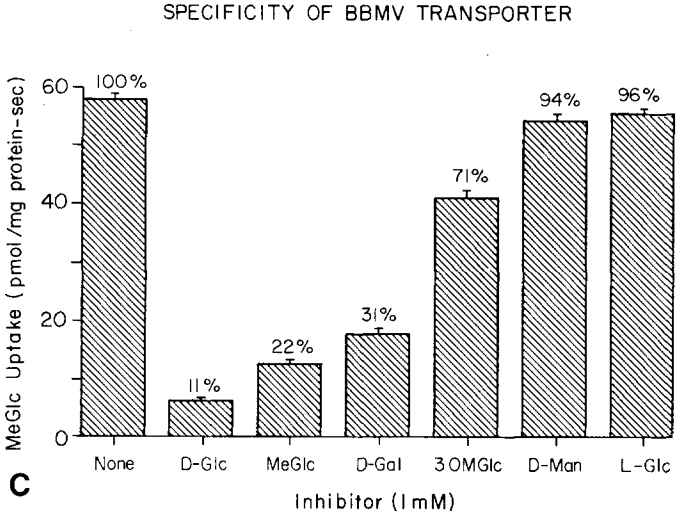


Fig. 6. The specificity of sugar transport. (*A*) Cloned transporter. *m*RNA synthesized from clone pMJC424 was injected into oocytes, and the uptake of 50 μM αMeGlc was measured 2 days later in the presence or absence of 10 mM sugar analogs. Uptakes represent the mean of 6–8 estimates, and the error bars indicate the standard errors. Uptakes in the absence of Na^+ were less than 0.5 pmol/oocyte · hr. These were the same batch of oocytes used in Fig. 1. (*B*) Cloned transporter. These oocyte experiments were similar to those in *A* except that the uptakes were measured 3 days after the injection of RNA in the presence and absence of 1 mM sugar analogs. The 100% control uptake was 220 ± 16(4) pmol/oocyte · hr. (*C*) Brush border vesicles. Initial rates of 50 μM αMeGlc transport were estimated in triplicate for 3 sec uptakes, and the error bars indicate the SEM. Uptakes were measured in the presence and absence of 1 mM sugar analogs. Similar experiments were also conducted on these membranes in the presence of 25 mM sugar analog. In this case, the uptakes in the presence of D-glucose, αMeGlc, and D-galactose were less than 2% of the control uptakes

transporter expressed in oocytes and the high-capacity transporter detected in rabbit brush borders. Transport by both systems is driven specifically by Na^+. It has long been recognized that the unique requirement for Na^+ is a diagnostic feature of brush border sugar transport (*see* Schultz & Curran, 1970). As judged by the Hill coefficients for Na^+ (1.5–1.7), the stoichiometry for Na^+/αMeGlc transport in both cases is 2 : 1. This agrees with the direct estimates obtained by Kimmich (Kimmich & Randles, 1984; Restrepo & Kimmich, 1985) for chick enterocytes. The K_m's for αMeGlc uptake are comparable in oocytes and brush borders (0.1 and 0.4–0.6 mM), but these are lower than the 2 mM previously reported for guinea pig brush border vesicles (Brot-Laroche et al., 1987) and chick enterocytes (Restrepo & Kimmich, 1985). These quantitative differences may be due to different post-translational processing of the protein, different membrane lipids in these cells and tissues, and different driving forces, e.g., membrane potentials. Preliminary experiments (Coady, Umbach & Wright, 1988; Birnir et al., 1989) in fact show that the kinetics of the cloned transporter are indeed voltage dependent. The sugar specificity observed for the clone and brush borders, D-glucose > αMeGlc > D-galactose > 3-O-methyl-glycoside ≫ mannose, L-glucose, is entirely consistent with the initial reports that all sugars transported are hexoses in the Cl conformation with a free equatorial -OH group on carbon number 2 (Crane, 1960; Wilson, 1962). Finally, phlorizin inhibits αMeGlc uptake more than 90% in

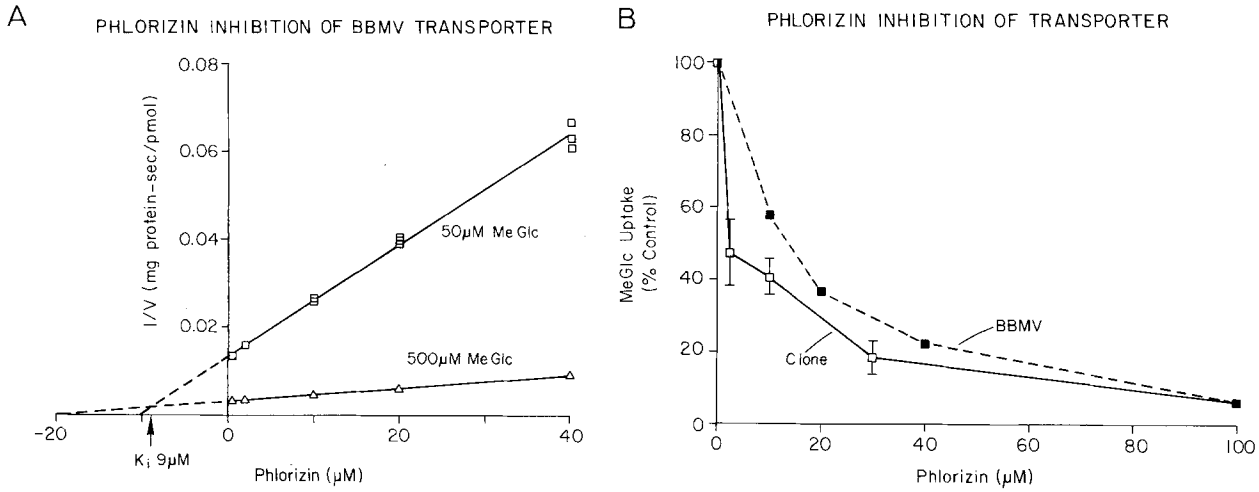


Fig. 7. Phlorizin inhibition of sugar transport. (*A*) Brush border vesicles. A Dixon plot of initial rates of αMeGlc uptake as a function of phlorizin concentration. At each sugar concentration, the data were fitted to

$$\frac{1}{V} = \frac{K_m}{V_{max}(S)K_i} I + \frac{1}{V_{max}} 1 + \frac{K_m}{(S)}$$

by linear regression analysis. The kinetic constants were extracted from the uptakes at the two sugar concentrations: the V_{max} and K_M for sugar transport were 520 pmol/mg · sec and 420 μM, and the K_i for phlorizin was 9 μM. In these membranes, the kinetic constants obtained from Hofstee plots (Fig. 4*B*) were 470 pmol/mg · sec and 570 μM. Uptakes were measured in triplicate at 3 sec. (*B*) Phlorizin inhibition of sugar uptakes in oocytes and brush border vesicles. In each experiment, 50 μM αMeGlc uptakes were measured in the presence of 100 mM NaCl as a function of the external phlorizin concentration. The uptakes in the absence of phlorizin were 51 pmol/mg · sec and 76 pmol/oocyte · hr, and the results in the presence of phlorizin were expressed as the percentage of those in the absence of inhibitor

Table. Properties of the α-methyl-D-glucopyranoside transporter

	BBMV	Clone
Ion specificity	Na ≫ Li, Ch, K	Na ≫ Li, Ch, K
Hill coefficient	1.7	1.5
Kinetics		
K_m	420–570 μM	110 μM
V_{max}	470 pmol/mg · sec	150–1550 pmol/oocyte · hr
Substrate specificity	D-glucose > α-methyl-D-glucopyranoside > D-galactose > 3-O-methylglucoside ≫ D-mannose = L-glucose	D-glucose > α-methyl-D-glucopyranoside > D-galactose > 3-O-methylglucoside ≫ D-mannose = L-glucose
Phlorizin K_i	9 μM	6 μM

Summary of the transport properties of rabbit brush border membrane vesicles (BBMV) and the cloned transporter expressed in oocytes. The BBMV system refers to the high-capacity, low-affinity transporter (*see* Fig. 4).

brush border membranes, oocytes (Fig. 7), and chick enterocytes (Kimmich & Randles, 1984). Phlorizin acts as a competitive inhibitor of αMeGlc uptake by oocytes (Fig. 7) and rabbit and guinea pig brush border membrane vesicles with a K_i of 6, 9 and 18 μM (Fig. 7; Brot-Laroche et al., 1987). These similarities lead to the conclusion that we have cloned the classical Na+/glucose cotransporter.

The kinetics of αMeGlc uptake by brush border membrane vesicles (Fig. 4*B*) suggests the presence

of two saturable uptake systems for αMeGlc: a high-capacity, low-affinity system and a minor component with a capacity and affinity constant about an order of magnitude lower than the major system. At the sugar concentration used in most of the experiments in this study (50 μM αMeGlc), 61% of the uptake occurred by the high-capacity system, 36% by the low-capacity system, and 3% by diffusion. Since more than 94% of the 50-μM αMeGlc uptakes were eliminated by (i) replacing Na^+ into choline (Fig. 2*B*), (ii) phlorizin (Fig. 7*A*), and (iii) by sugar analogs (Fig. 6*C*), we conclude that both uptake systems share the same properties. One interpretation of these results is that the two components of αMeGlc uptake by rabbit brush border vesicles are simply different manifestations of a single transporter, i.e., binding and transport. The phlorizin inhibition experiments (Fig. 7*A*) are consistent with this conclusion, since the Dixon plots were linear, and there was good agreement between the predicted and measured J_{max} values for αMeGlc uptakes. A likely reason for the discrepancy between the uptake kinetics into oocytes and rabbit brush border vesicles is the low turnover number of the transporter (~5 sec^{-1}, Peerce & Wright, 1984) and the fast time required to measure linear uptakes with brush border vesicles. We have previously noted that the low-capacity, high-affinity (K_m = 30 μM) D-glucose uptake by brush border vesicles may be attributed to binding rather than transport (Kaunitz & Wright, 1984). Uptakes of αMeGlc into guinea pig intestinal brush border vesicles appear to be mediated by a single Na^+-dependent, phlorizin-sensitive transporter with a K_m of 2 mM (Brot-Laroche et al., 1987).

There may indeed be more than one Na^+/sugar cotransport system in the small intestine. For example, Brot-Laroche et al. (1986) have identified a low-affinity (K_m = 24 mM), high-capacity D-glucose transporter in guinea pig brush border membranes that does not handle αMeGlc. In a preliminary attempt to identify other intestinal sugar transporters in the small intestine, we have screened a rabbit intestinal lambda gt10 cDNA library with the cloned Na^+/glucose cotransporter as a probe. Although we isolated eight positive clones with inserts greater than 1.6 kb, we concluded that all were identical to the cloned transporter, i.e., identical restriction fragments were obtained upon digestion of all nine clones with Bgl I and Mlu I. This suggests that if indeed there are other Na/sugar cotransporters in the rabbit intestine, they are not closely homologous to the one transporter we have already cloned.

In conclusion, we have shown by a direct comparison that the Na^+/glucose cotransporter cloned and expressed in *Xenopus* oocytes is kinetically very similar to the classical Na^+/glucose cotransporter observed in rabbit brush border membranes. The oocyte expression system will now permit us to carry out a study of the electrophysiological properties of the cotransporter that has been difficult to perform in the native enterocyte.

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