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CARRIER-MEDIATED TRANSFER OF D-GLUCOSE IN BRUSH BORDER VESICLES DERIVED FROM RABBIT RENAL TUBULES

Na^+-DEPENDENT VERSUS Na^+-INDEPENDENT TRANSFER

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SUMMARY

A brush border preparation from rabbit renal tubules containing a high yield of vesicles has been used to study the transfer of D-glucose through the brush border membrane. In the presence of an Na^+ gradient across the vesicular membrane, the vesicles could concentrate D-glucose to a factor of 1.5, whereas in the absence of an Na^+ gradient, only equilibrium with the medium was achieved. Two types of transfer could be distinguished by their requirement of Na^+, their sensitivity to phlorizin and their pH optimum. The Na^+-independent transfer was about 100 times less sensitive to phlorizin than the Na^+-dependent path and exhibited a pH optimum between 7 and 8, whereas the Na^+-dependent transfer was highest at a pH between 8 and 9.

The brush border preparation could be freed of most of the contaminating material derived from the basal and lateral tubular cell membrane by a discontinuous density gradient centrifugation. It still showed both forms of transfer to a similar extent, indicating that both are located in the brush border membrane.

A study of the sensitivity of D-glucose transfer to phlorizin, in the presence and absence of Na^+ at different temperature, suggests a single carrier species functioning in two interchangeable conformational states with different affinities for phlorizin rather than two transfer systems working independently.

INTRODUCTION

The research on transport phenomena in isolated kidney brush border membranes started with the study of membrane-bound receptors known to be involved in transport processes [1–4]. During these studies it became apparent that membrane vesicles present in these preparations were still capable of carrier-mediated substrate translocation. Investigation of this vesicular transport has some advantages over in

Abbreviation: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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vivo studies, because cell metabolism can be excluded and defined conditions not compatible with cellular life, such as non-physiological pH values and temperatures, can be imposed.

Before exploring these vesicular transport processes further with one of the known kidney brush border preparations, it was thought advisable to look for a preparation with a higher yield of vesicles. Therefore, a method for preparing and isolating brush border vesicles has been developed in this laboratory [5].

A number of publications concerned with the uptake of D-glucose by brush border preparations of different origins described an Na^+-dependent transfer of D-glucose [6–8]. An earlier publication by one of us [4] on D-glucose transfer in a crude brush border preparation suggests the presence of a second transport system, which was not dependent upon Na^+. Hopfer et al. [6] and Aronson and Sacktor [8], who worked on highly purified brush border preparations of different origin, have also presented some evidence for a phlorizin-sensitive, Na^+-independent D-glucose transfer, but did not investigate it further. The brush border preparation used gives a several times higher yield of vesicles than any other preparation and has the advantage of being able to estimate the intravesicular concentration of the substance being transported. We thought it worthwhile to reinvestigate the possibility of a second way of transfer of D-glucose across the brush border membrane.

METHODS

Preparation of brush border vesicles

The preparation of brush border vesicles had been described in a previous publication [5]. For this study the preparation was modified in the following way. The hypotonic lysis was performed at pH 6.4. Therefore, before hypotonic lysis the tubule segments were washed once with 1 vol. of a buffer containing 130 mM NaCl/ 5.5 mM KCl/1 mM $CaCl_2$/1.4 mM $MgSO_4$/1.4 mM KH_2PO_4/14mM sodium acetate, final pH 6.4. Then a hypotonic solution containing 1 mM K_2Mg EDTA/1 mM $MgCl_2$/ 1 mM *N*-acetyl-D-glucosamine/1 mM dithioerythritol, final pH 6.4, was added. The procedure of hypotonic lysis and step 2 of the preparation were kept the same as previously described. The solution of Ficoll, which was added to the suspension during step 3, was prepared in 50 mM mannitol instead of glycerol. In step 4, the vesicles were washed in a solution containing 200 mM mannitol/1.4 mM *N*-2-hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES)-Tris/1.4 mM $MgCl_2$, final pH 7.0, instead of an isotonic saline buffer. With the preparation thus modified a 13.5-fold enrichment of trehalase, a marker enzyme for renal brush border, and a 5.3-fold enrichment of $(Na^+ + K^+)$-stimulated ATPase, a marker for the basal and lateral tubular cell membrane, as compared to a total kidney homogenate, was achieved.

Discontinuous sucrose gradient centrifugation

For the discontinuous gradient centrifugation, the brush border vesicles were kept in 200 mM mannitol/1.4 mM HEPES-Tris/1.4 mM $MgCl_2$, final pH 7.0, at 4 °C overnight and were placed on the top of a 35 ml centrifuge tube containing 7 ml 34 %, 9 ml 29.5 %, 14 ml 24 % and 5 ml 20 % solutions of sucrose (w/w) in 10 ml Tris · HCl, pH 7.5. Centrifugation at 100 000 $\times g$ for 2h followed. 1-ml fractions were taken from

the top using an automatic pipette and analysed for their content of enzymes and of protein and for their transport properties.

Enzymes

The assay for the enzymes trehalase, alkaline phosphatase and $(Na^+ + K^+)$-stimulated, Mg^{2+}-dependent ATPase have been described before [5].

Chromatography

For chromatography, the radioactive material was extracted from the vesicular sediment three times with 60 % ethanol. Aliquots of the extracts and of the incubation medium were spotted on Whatman No 3 paper and chromatographed in two chromatographic systems, ascending in the solvent butanol/ethanol/water (2:1:1, v/v) and descending in the solvent isopropanol/water (4:1, v/v). After chromatography the chromatograms were cut into stripes and analysed for radioactivity in a liquid scintillation counter.

Transport measurements

All transport studies were performed in a cold-room with a thermostatically controlled water bath. The vesicles were resuspended in a buffer containing 140 mM mannitol/1.4 mM $MgCl_2$/1.4 mM HEPES-Tris, final pH 7.4. 50 μl of this suspension was added to 250 μl of a solution containing 1 mM D-[^{14}C]glucose, traces of ^3H_2O, 1.4 mM $MgCl_2$, 1.4 mM HEPES-Tris, final pH 7.4, 140 mM mannitol or 70 mM NaCl, which had been brought to the appropriate temperature before. After incubation, the samples were cooled to 0 °C in an acetone-dry ice bath within 2–4 s. Then 50 μl of 140 mM acetic acid was added rapidly, and after mixing the samples were transferred to a Beckmann microfuge tube, which contained 50 μl of a mixture of dibutylphthalate and dinonylphthalate (3:2, v/v) and centrifuged for 10 min in a Beckman microfuge at about 15 000 $\times g$. After centrifugation, an aliquot of the supernatant incubation buffer was taken and mixed with 1 vol. of a solution containing 10 % trichloroacetic acid and 2 mM D-glucose, saturated with polyethyleneglycol. The rest of the supernatant and the oil were discarded. Then the bottom of the tube containing the vesicular sediment was cut off and placed into 300 μl of a solution containing 5 % trichloroacetic acid and 1 mM D-glucose, half saturated with polyethyleneglycol and resuspended by brief sonication. Both the diluted incubation buffer and the resuspended sediment were centrifuged in an Eppendorf microcentrifuge for 5 min. Aliquots of the supernatants were analysed in a Nuclear Chicago liquid scintillation counter for their content of ^{3}H and ^{14}C. For the estimation of the uptake at zero time of incubation, D-[^{14}C]glucose was added after the sample had been cooled to 0 °C and acidified. Also, in each preparation one sample was reserved for the estimation of the trapped water space in the vesicular sediment by the use of poly-[^{14}C]ethyleneglycol (M_r 4000).

RESULTS

Termination and measurement of uptake

Termination of uptake in transport studies on cells and cell organelles by centrifugation through a layer of oil not only separates them rapidly from the incuba-

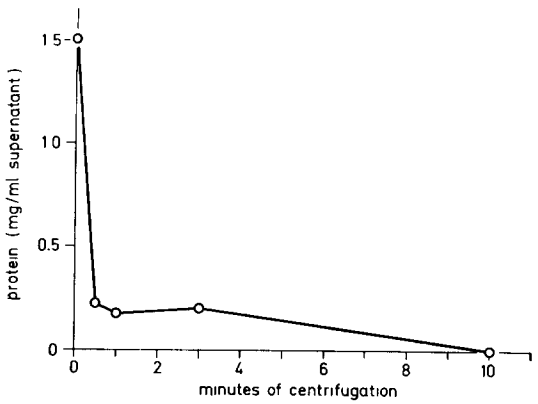

Fig. 1. Centrifugation of brush border vesicles through a layer of oil. 300 μl of a suspension of brush border vesicles in the mannitol medium were cooled to 0 °C, acidified, placed into tubes containing 50 μl of oil and centrifuged for various times. The supernatant was analysed for its content of protein. For details, see Methods.

tion medium but also allows one to work with very small amounts of material, because the method eliminates most of the fluid adhering to the tube wall and, therefore, adds to the accuracy of the measurements.

A method which had been used for mitochondrial transport studies [9] was modified for our purposes. A mixture of phthalic acid esters, which were preferable because of their higher surface tension [10], replaced the silicon oil. Since the brush border vesicles do not sediment as fast as mitochondria do, the transport reaction had to be stopped before centrifugation. For this, the samples were cooled to 0 °C in

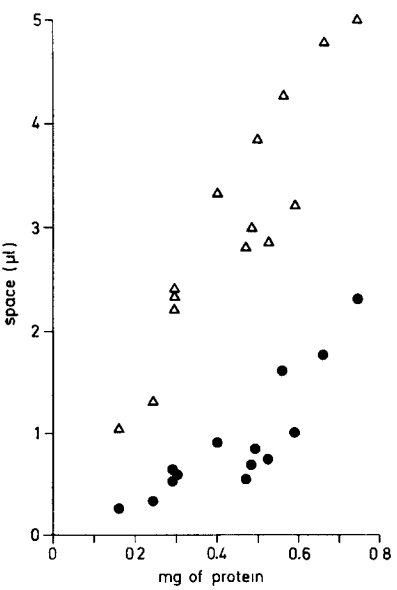

Fig. 2. Total aqueous spaces (△) and trapped water spaces (●) of the sediments of 14 different brush border preparations after centrifugation through a layer of oil. For details, see Methods.

a dry ice-acetone bath within 2-4 s and brought to pH 4 by the addition of acetic acid. Immediate centrifugation brought more than 80 % of the vesicles through the layer of oil within 0.5 min as shown in Fig. 1 and reduced non-carrier-mediated diffusion to negligible levels. As shown later (Fig. 8) this method gave reproducible results for incubation times as short as 15 s. The use of ^3H_2O as a second label, marking the aqueous space of each sediment, contributed to the experimental accuracy and made it possible to calculate a distribution ratio. Fig. 2 shows the aqueous spaces determined by ^3H_2O and the trapped water spaces determined by poly-[^{14}C]ethyleneglycol (M_r 4000) of the sediments of 14 different brush border preparations and demonstrates a quite reproducible yield of vesicles in these preparations.

Chromatography

After incubating the vesicles for 3 min at 25 °C with 1 mM D-[^{14}C]glucose in the NaCl medium, extracts were prepared from the medium and from the vesicular sediment and checked for a possible degradation of D-[^{14}C]glucose chromatographically. In both extracts, 98 % of the radioactivity recovered from the chromatogram was found in a region having an identical R_F to that of authentic D-glucose.

Transport of D-[^{14}C]*glucose*

In order to get a value of uptake for a contact time as short as possible (zero time of incubation), D-[^{14}C]glucose was added after the membrane suspension had been cooled and acidified. This treatment resulted in a distribution ratio of 0.6–0.7 for D-[^{14}C]glucose as can be seen in Figs 3 and 4. The same experiment performed with [^{14}C]mannitol gave a similar distribution ratio of 0.55 (data not shown). These unexpected findings will be discussed in detail later.

Longer incubation times, from 30 s to 12 min at 15 °C, showed a time-dependent uptake of D-[^{14}C]glucose and [^{14}C]mannitol. The uptake of both compounds reached an equilibrium at a distribution ratio of 0.9, when the mannitol medium was used for the incubation. But with D-[^{14}C]glucose the equilibrium was reached 3–4 times as fast (data not shown) and the time to equilibrium could be delayed from 3 to more than 6 min by the addition of phlorizin (Fig. 3).

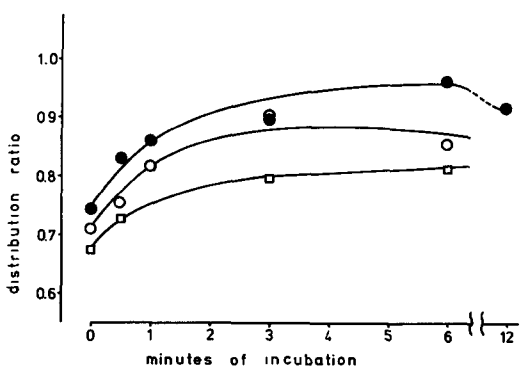

Fig. 3. Distribution ratio of D-[^{14}C]glucose in the brush border vesicles after various times of incubation at 15 °C. Brush border vesicles were resuspended in the mannitol medium and injected into the NaCl medium (●), into the mannitol medium (○) and into the mannitol medium containing 0.5 mM phlorizon (□). For details, see Methods.

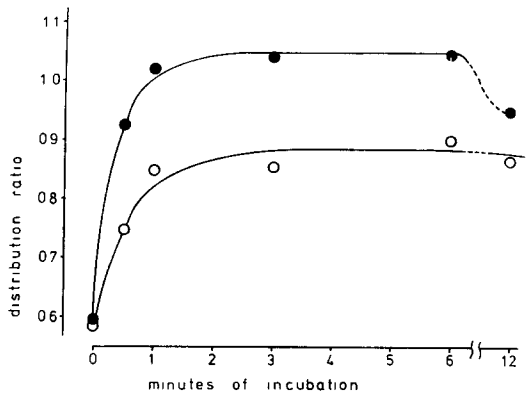

Fig. 4. Distribution ratio of D-[^{14}C]glucose in the brush border vesicles after various times of incubation at 25 °C. Brush border vesicles were resuspended in the mannitol medium and injected into the NaCl medium (●) and into the mannitol medium (○). For details, see Methods.

When the vesicles were injected into the NaCl medium at 15 °C, a slightly higher distribution ratio of D-[^{14}C]glucose was reached between 6 and 12 min of incubation and the initial slope of uptake increased to a small extent. A higher temperature of incubation potentiated this effect quite markedly. At 25 °C the initial slope of uptake was more than doubled by the presence of Na^+ and between 2 and 6 min of incubation a distribution ratio of 1.05 was achieved, which after 12 min approached equilibrium again with a distribution ratio of 0.92 (Fig. 4). As shown in Fig. 3, the uptake of D-[^{14}C]glucose could be inhibited by phlorizin even when Na^+

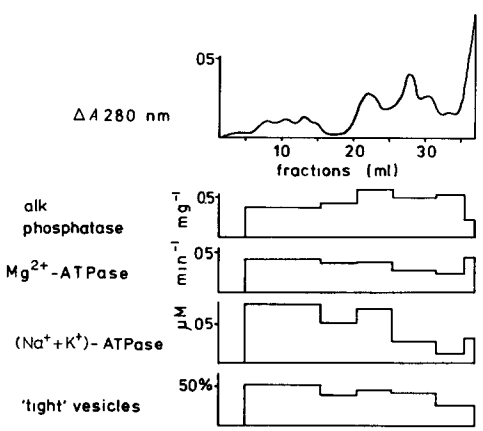

Fig. 5. Further purification of brush border vesicles in discontinuous sucrose gradient centrifugation. 1-ml fractions were taken from the top using an automatic pipette and analysed for their content of protein (absorbance at 280 nm). The fractions were combined according to the protein peaks of the gradient and analysed for the enzymes alkaline phosphatase, Mg^{2+}-dependent and ($Na^+ + K^+$)-stimulated ATPase. The discrimination between tight and leaky vesicles was achieved by [^{14}C]mannitol, which was added to the vesicular suspension immediately before centrifugation through a layer of oil. The distribution ratio obtained was assumed to represent the fraction of leaky vesicles. This gradient centrifugation represent one typical example out of 4 observations.

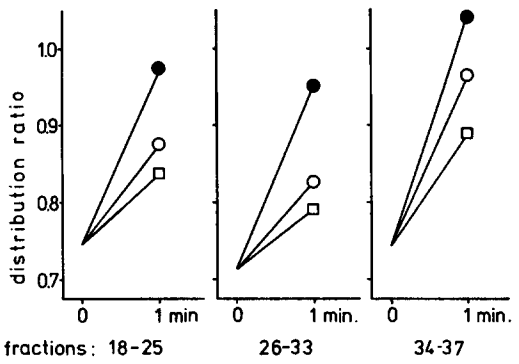

Fig. 6. D-[^{14}C]Glucose transfer in brush border vesicles with different degrees of contamination by basal and lateral tubular cell membranes. After separation by discontinuous sucrose gradient centrifugation, as depicted in Fig. 5, the fractions 18–25, 26–33 and 34–37 were combined, washed free of sucrose, resuspended in the mannitol medium and incubated with D-[^{14}C]glucose for 0 and 1 min under 3 different conditions at 25 °C. The vesicles were injected into the NaCl medium (●) into the mannitol medium (○) or into the mannitol medium containing 1 mM phlorizin (□). Each point represents the mean of 4 observations obtained on 4 different days.

was absent. This suggests a carrier-mediated transfer of D-glucose not strictly dependent upon Na^+ or two transfer systems, one dependent and the other independent of Na^+. The possibility that the Na^+-independent transfer of D-glucose was mediated by a system localized in contaminating basal and lateral tubular cell membranes [11, 12] had also to be considered. Therefore, it was decided to repeat a part of the transport experiments in a more purified brush border preparation. Fig. 5 shows the separation of the membrane vesicles after centrifugation in a discontinuous sucrose gradient. The concentration of brush border in the subfractions, as indicated by the specific activity of alkaline phosphatase, did not change very much throughout the gradient, as could be expected for a component which contributes the largest part of the starting material. The specific activity of Mg^{2+}-dependent ATPase, which is both a plasma membrane and a mitochondrial enzyme, showed a similar distribution pattern to that of alkaline phosphatase but was increased in the last fraction, suggesting that some mitochondria still present in the starting material had been concentrated at the bottom of the tube. More than 70 % of the basal and lateral tubular cell membrane, as indicated by its marker ($Na^+ + K^+$)-stimulated ATPase, were recovered in the first part of gradient until fraction 25, leaving the other fractions with a 3 times lower specific activity of this enzyme. Surprisingly, three quarters of the membranes came to equilibrium with a sucrose solution of a concentration lower than 34 %. This peculiar behaviour, quite different from that of other brush border preparations [13], will be discussed later in detail.

When incubated with D-[^{14}C]glucose, both kinds of transfer, Na^+-dependent and Na^+-independent, could be demonstrated in all three regions of the gradient to a similar extent (Fig. 6). This observation indicates that both types of carrier-mediated transfer observed are located in the brush border membrane.

In order to validate the suggestion of the presence of two different types of transfer, the sensitivity of D-glucose uptake to phlorizin and to changes in the pH of the incubation buffer was investigated. As shown in Fig. 7, 0.5 mM phlorizin was

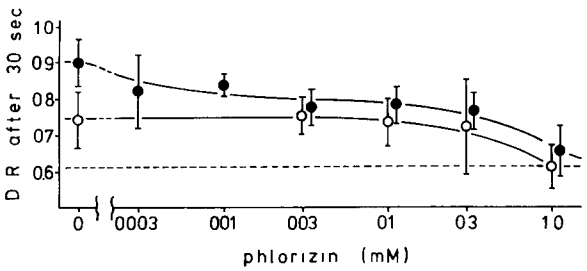

Fig. 7. Inhibition of initial D-[^{14}C]glucose transfer by phlorizin at 25 °C. Brush border vesicles were injected into the mannitol medium (○) and into the NaCl medium (●), to which different concentrations of phlorizin had been added. The data depicted represent the distribution ratio ± standard deviation of D-[^{14}C]glucose obtained after an incubation of 30 s. The broken line represents the distribution ratio obtained when D-[^{14}C]glucose was added after the suspension had been cooled and acidified (zero time of incubation). For details, see Methods.

needed to inhibit D-glucose transfer by half, with the vesicles incubated in the mannitol medium. In the presence of Na^+, however, a part of the transfer was about 100 times more sensitive to phlorizin. Further differences were seen when the vesicles were injected into buffers of different pH values (Fig. 10). In the mannitol medium, D-glucose uptake was optimal at a pH between 7 and 8, whereas in the presence of Na^+ the optimum was shifted to a pH between 8 and 9.

As demonstrated in Fig. 7, in the presence of Na^+ two types of D-glucose transfer could be distinguished by their sensitivity to phlorizin. The use of phlorizin made it possible to estimate the fraction of D-glucose transferred by each of the two. The amount of D-glucose transfer remaining after the Na^+-dependent highly phlorizin sensitive D-glucose transfer had been blocked by intermediate concentrations of phlorizin, should be the fraction of transfer which is not coupled to Na^+. When the temperature was raised from 25 to 37 °C, the amount of D-glucose transfer by the uncoupled pathway stayed the same (Fig. 9, spotted area), whereas the translocation coupled to Na^+ increased three times (Fig. 9, oblique shaded area). Expressed as fraction of total uptake, uncoupled transfer decreases from one half at 25 °C to

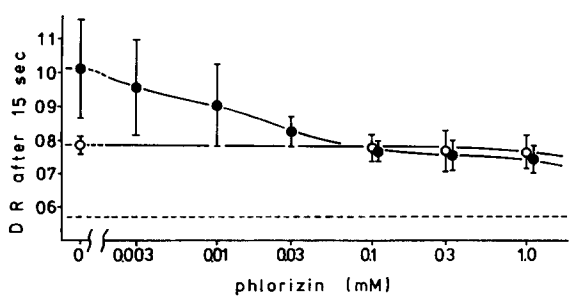

Fig. 8. Inhibition of initial D-[^{14}C]glucose transfer by phlorizin at 37 °C. Brush border vesicles were injected into the mannitol medium (○) and into the NaCl medium (●), to which different concentrations of phlorizin had been added. The data depicted represent the distribution ratio ± standard deviation of D-[^{14}C]glucose obtained after an incubation of 15 s. The broken line represents the distribution ratio obtained when D-[^{14}C]glucose was added after the suspension had been cooled and acidified (zero time of incubation). For details, see Methods.

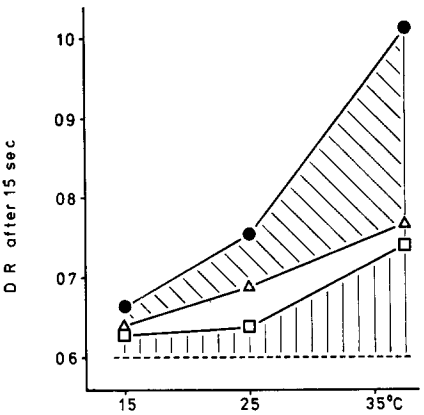

Fig. 9. Effect of temperature upon initial D-[^{14}C]glucose transfer. Brush border vesicles were resuspended in the mannitol medium and injected into the NaCl medium with 1 mM phlorizin (□), 0.1 mM phlorizin (△) and no phlorizin (●). The broken line represents the distribution ratio obtained when D-[^{14}C]glucose was added after the suspension had been cooled and acidified. Part of the data depicted was taken from Figs 7 and 8. Each point presents the mean of at least 4 observations. For details, see Methods.

about one quarter at 37 °C. At 37 °C, the latter could not be estimated very accurately because at this temperature a significant part of D-glucose uptake is not blocked, even with high concentrations of phlorizin (1 mM), indicating that non-carrier-mediated diffusion through the membrane is facilitated by this high temperature of incubation (Fig. 9, vertical shaded area).

At 37 °C in the presence of Na^+, D-glucose transfer not coupled to Na^+ is smaller than D-glucose transfer in the absence of Na^+ (Fig. 8). This observation was statistically highly significant at 37 °C, when the data obtained in the presence and absence of Na^+ for each experimental day were compared, but at 25 °C the two

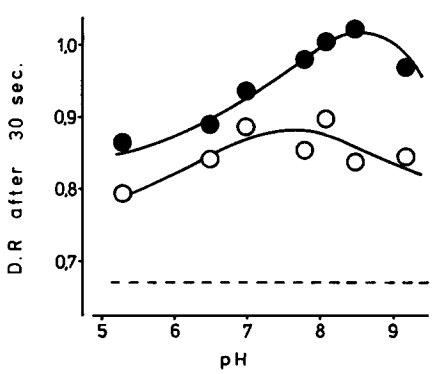

Fig. 10. Effect of pH upon the initial D-[^{14}C]glucose transfer in the presence and the absence of Na^+ at 25 °C. Brush border vesicles were injected into the mannitol medium (○) and into the NaCl medium (●), which had been adjusted to different pH values by 1.4 mM HEPES-Tris. The pH depicted represents the pH measured after the vesicular suspension had been added to the incubation medium. Each point represents the mean of at least 4 observations. The broken line presents the distribution ratio obtained when D-[^{14}C]glucose was added after the vesicular suspension had been cooled and acidified (zero time of incubation). For details, see Methods.

transfers were not significantly different from each other (Fig. 7). In other words, at 37 °C Na^+ decrease the Na^+-independent mode of D-glucose transfer. The latter observation and the observation that, in the presence of Na^+, the transfer not coupled to Na^+ stays the same with increasing temperature, led us to suspect two interchangeable conformational states of a single carrier species, rather than two different transfer systems working independently. The relative fractions of the carrier in one or the other conformational state would depend upon the presence of Na^+ and the temperature of incubation.

DISCUSSION

D-glucose transport in isolated brush border vesicles from the intestine and the kidney of the rat and the rabbit has already been investigated several times [6–8, 14]. We had the advantage to start with a preparation which had a several times higher yield of vesicles than in previous studies (Fig. 2), even when we disregard the "leaky" vesicles in our preparation.

Plasma membrane vesicles do not necessarily have the same volume when resuspended in isoosmolar buffers containing different solutes. The nature of the solutes on both sides of the selectively permeable membrane also have some influence upon the vesicular volume. Therefore, the uptake of an identical amount of a substance by the vesicles in two different isotonic media does not imply an identical intravesicular concentration. We consider the estimation of intravesicular concentrations and the expression of uptake in relation to vesicular volume by the above-described method to be a significant advance. To our knowledge, this has not been achieved in bacterial membrane vesicles or in submitochondrial particles, which have already been under investigation for about a decade.

Using this method, we became aware of a large fraction of vesicles that are not permeable to large molecules like polyethyleneglycol (M_r 4000) and raffinose but do not present a barrier, as far as observed by our limited methods, for smaller molecules like glucose and mannitol. A similar leakage of small molecules has been described, when membrane ghosts were prepared from erythrocytes [15]. The tight and the leaky vesicles show a different pattern of sedimentation during centrifugation in a sucrose gradient and can be partly separated as demonstrated in Fig. 5. Unfortunately the intravesicular volume (μl/mg protein) is decreased in all fractions of the gradient. There seems to be a considerable breakdown of vesicles during storage, centrifugation and resuspension, which makes this time-consuming method unprofitable.

The uptake of small molecules by isolated plasma membranes represents either binding to a membrane-bound receptor or uptake into the vesicular space. The latter can take place either by simple diffusion or by a saturable specific process. In general, all three kinds of uptake are observed simultaneously. Binding to a membrane-bound receptor can contribute a significant portion of the total uptake only at very low substrate concentrations, because the number of specific binding sites is limited. In order to avoid interference with binding, we performed all transport measurements at a relatively high concentration of D-glucose (1 mM) and did not attempt a kinetic analysis of D-glucose transport. This precaution had not been taken in a previous study by one of us [4], so that the results obtained at very low substrate

concentrations are difficult to interpret and to compare with the present study.

The distribution ratio calculated for D-glucose represents a mean value for all vesicles, including the 'tight' and the 'leaky' vesicles. Only the 'tight' vesicles are capable of maintaining the intravesicular concentration of a low molecular weight substance higher than the outside concentration. All D-glucose accumulated above the equilibrium of 0.9 is taken up by these 'tight' vesicles. Since they contribute only 30 % of the total vesicular space, the concentration inside the 'tight' vesicles at a mean distribution ratio of 1.05 can be estimated as about 1.5 fold above the outside concentration. These results confirm previous studies, which reported an overshoot of D-glucose uptake in the presence of an Na^+ gradient, which was about 1.5-fold above the later achieved equilibrium [6, 7].

The sensitivity of D-glucose transfer in brush border vesicles to phlorizin has been studied twice before by Hopfer et al. [6] and by Aronson and Sacktor [8]. Both studies report a significant inhibition only by relatively high concentrations of phlorizin (0.5–1.0 mM), an observation which contradicts in vivo micropuncture experiments [16] and binding studies of phlorizin to isolated brush border [17, 18]. In the presence of Na^+ we could distinguish a second kind of D-glucose transfer (Figs 7 and 8), whose high sensitivity to phlorizin came closer to the one observed by Vick et al. in their in vivo study [16]. In an earlier work, the same group of authors differentiated between an active and a passive component of D-glucose transport [19]. When the active transport was blocked completely by 0.1 mM phlorizin, a passive net flux of D-glucose was observed, which was proportional to the concentration difference between intra- and peritubular fluid. Although these authors came to the conclusion that the passive component of D-glucose transport represented simple diffusion through leaks, it might very well correspond to the low phlorizin sensitive transfer observed in this study.

An additional difference of the two types of transfer was found between their pH optima as depicted in Fig. 10.

The membrane vesicles used in this transport study were derived mainly from the brush border of the tubule cell but contained also some basal and lateral tubular cell membranes as shown by the enrichment of their marker enzyme (Na^+ K^+)-stimulated ATPase (see under Methods). It is not possible to determine the fraction of both kinds of membranes in the preparation unless they can be separated completely from each other. A complete separation was not achieved by the sucrose gradient centrifugation employed in this study. But assuming that other contaminations were negligible, we estimated the fraction of basal and lateral cell membrane in the preparation to be between 10 and 20 % by comparing the changes of specific activity of alkaline phosphatase and ($Na^+ + K^+$)-stimulated ATPase in the different subfractions of the gradient centrifugation. Na^+-dependent and Na^+-independent D-glucose transfer were present in membrane vesicles to a similar extent, when the membrane fractions used differed 3-fold regarding their specific activity of ($Na^+ + K^+$)-stimulated ATPase (Fig. 6). This observation makes a significant participation in vesicular transport by making contaminating basal and lateral tubular cell membranes unlikely [11, 12] and suggests that both types of transfer are located in the brush border membrane.

Two observations suggest the presence of two interchangeable conformational states of a single carrier species rather than two transfer systems. Firstly, at 37 °C Na^+

diminishes the transfer with the low sensitivity to phlorizin, i.e. under the influence of Na^+ more carriers are transformed into the highly phlorizin-sensitive state and not available for the low phlorizin-sensitive D-glucose translocation at this temperature (Fig. 8). Secondly, D-glucose transfer less sensitive to phlorizin does not increase with increasing temperature, suggesting a higher rate of transformation into the highly phlorizin-sensitive state with increasing temperature (Fig. 9). However, the possibility of two separate transfer systems present in the brush border membrane cannot be excluded, although the experiments which indicate an increase of one type of transfer at the cost of the other would be difficult to interpret under these circumstances. In a recent micropuncture study, Ullrich et al. [20] showed that the K_m of D-glucose transport decreases with increasing intratubular Na^+ concentrations, while V remained almost constant. Frömter et al. [21] found a D-glucose-induced Na^+ flux across the luminal brush border of the tubule cell by electrical measurements. These observations on the renal proximal tubule support a mechanism first postulated by Crane [22, 23] for hexose transport in the intestine, in which Na^+ greatly affects the affinity between sugar and carrier and the Na^+ gradient across the brush border is the asymmetry providing the driving force for active sugar transport. Our observations, which indicate a shift in the distribution of the conformational states of the carrier towards the highly phlorizin-sensitive state under the influence of Na^+ and temperature, adds support for the mechanism proposed by Crane and could also give an explanation of the nature of a low affinity, so called unspecific binding site for phlorizin found in binding studies on purified brush border membranes [17, 24, 25]. Thomas (Thomas, L., personal communication), who worked on a purified phlorizin binding protein was able to change the affinity of this protein for phlorizin from high affinity to low affinity and vice versa with the help of Na^+. This feature of the protein makes it an almost certain candidate for involvement in active sugar transport and is consistent with our obserations, which suggest two interchangeable conformational states of the glucose carrier. In the model proposed by Crane, the sugar could be translocated by the ternary complex (carrier-sugar-Na^+) and possibly by the binary complex (carrier-sugar) as well, a question which was left open for the intestinal sugar transport [22]. This study supports the idea that, at least in isolated brush border vesicles, the binary complex can translocate D-glucose.

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