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TEMPERATURE DEPENDENCE OF SOLUTE TRANSPORT AND ENZYME ACTIVITIES IN HOG RENAL BRUSH BORDER MEMBRANE VESICLES *

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The temperature dependence of sodium-dependent and sodium-independent D-glucose and phosphate uptake by renal brush border membrane vesicles has been studied under tracer exchange conditions. For sodium-dependent D-glucose and phosphate uptake, discontinuities in the Arrhenius plot were observed. The apparent activation energy for both processes increased at least 4-fold with decreasing temperature. The most striking change in the slope of the Arrhenius plot occurred between 12 and 15°C. The sodium-independent uptake of D-glucose and phosphate showed a linear Arrhenius plot over the temperature range tested (35–5°C). The behavior of the transport processes was compared to the temperature dependence of typical brush border membrane enzymes. Alkaline phosphatase as intrinsic membrane protein showed a nonlinear Arrhenius plot with a transition temperature at 12.4°C. Aminopeptidase M, an extrinsic membrane protein exhibited a linear Arrhenius plot. These data indicate that the sodium-glucose and sodium-phosphate cotransport systems are intrinsic brush border membrane proteins, and that a change in membrane organization alters the activity of a variety of intrinsic membrane proteins simultaneously.

Introduction

The renal brush border membrane represents a highly specialized plasma membrane of the proximal tubule cell. In recent years it has been demonstrated that this membrane, in contrast to the basal-lateral plasma membrane of the same cells, contains a large number of sodium cotransport systems including the well-studied sodium-glucose and sodium-phosphate cotransport system [1]. This polarity of the two membranes has also been observed in the presence of enzymes such as amino peptidases, hydrolases and cyclases. Also, the lipid composition of the membranes has been investigated [2], however, little attention has been paid to the lipid-protein interaction in the renal brush border membrane.

Two reports analyzing the temperature dependence of sodium cotransport systems in renal brush border membranes have been published. In rat kidney brush border membranes [3] as well as in rabbit kidney brush border membranes [4] a nonlinear Arrhenius plot for sodium-dependent D-glucose uptake has been observed. These studies were performed under sodium gradient conditions and therefore the effect of the temperature on the dissipation of the sodium gradient driving the transport, and on the transport systems proper cannot be distinguished.

In the present paper, the question of the tempera-

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* A preliminary report of this work has appeared [27].

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

ture dependence of the sodium-D-glucose transport system was therefore reinvestigated under tracer exchange conditions, where no gradient of sodium exists across the membrane. In addition, the behavior of another sodium cotransport system, the phosphate-sodium cotransport system, was investigated in order to answer the question of whether the effect of temperature is different for various transport systems. Lastly, the response of the transport systems was compared to the response of intrinsic and extrinsic brush border membrane enzymes. This approach should elucidate the possibility of the existence of specific lipid domains around the various membrane proteins [5–7].

Our results clearly indicate that the sodium cotransport systems show a temperature dependence, irrespective of whether investigated under sodium gradient or equilibrium exchange conditions. The temperature range in which major changes of the transport occur is similar for the two sodium cotransport systems and the intrinsic membrane protein alkaline phosphatase. This result might suggest that, as also recently postulated by Chapman et al. [7], the temperature-dependent alterations in the membrane protein-lipid interaction are relatively unspecific and affect a variety of membrane proteins simultaneously.

Materials and Methods

Brush border membranes were isolated from hog-kidney cortex, according to the method described by Vannier et al. [8,9] with some modifications. Hog kidneys obtained from a local slaughter house were perfused with ice-cold Ringer's solution. The kidney cortex was dissected and was stored at −70°C. For each experiment, 100 g of the tissue were thawed and homogenized in 6-times its weight of a hypotonic buffer (2 mM Tris/HCl, pH 7.1 containing 10 mM mannitol). $CaCl_2$ was then added to a final concentration of 10 mM. After a low speed centrifugation (1500 X g; 12 min) the supernatant was removed carefully from the soft pellet, and centrifuged at 30 000 X g for 3 h (Beckman JA14 rotor). The pellets were homogenized in a Potter-Elvehjem Teflon-glass homogenizer (seven strokes at 1320 rev./min) in 170 mM NaCl solution. Per 3 vol. of this suspension, 7 vol. of Tris-HCl, pH 7.8, were added. After centrifugation at 10 000 X g for 10 min, the supernatant was removed from the soft pellet and the membranes were pelletted by centrifugation in a Beckman ultracentrifuge (Ti 45 rotor) at 100 000 X g for 1 h. The pellets were resuspended in the appropriate transport buffer. The centrifugation at 100 000 X g for 1 h was repeated and the final pellets were resuspended in the same buffer. The final protein concentration was about 10 mg/ml. The membranes were stored in 300 μl aliquots at −70°C. The D-glucose uptake of the membranes in a NaSCN gradient of 100 mM showed an overshoot of 300% versus equilibrium. This activity remained constant over several months of storage at −70°C.

Purity. The membranes obtained were assayed as previously described for enzymes shown to be characteristic of brush-border microvilli, trehalase (EC 3.2.1.28) [10], alkaline phosphatase (EC 3.1.3.1) [9], aminopeptidase M (EC 3.4.11.2) [9] and basal-lateral membranes ($Na^+ + K^+$)-ATPase (EC 3.6.1.3) [10].

Protein was determined after precipitation of the membranes with 10% trichloroacetic acid by the method of Lowry et al. [11].

The enrichment of the brush-border markers versus the original homogenate was 10-fold. The enrichment of ($Na^+ + K^+$)-ATPase was 0.9-fold.

Transport studies. Uptake experiments were carried out in 1.0 ml disposable Eppendorf tubes according to the method of Hopfer et al. [12]. A portion of 20 μl membrane suspension was added at zero time to 120 μl of incubation medium at the appropriate temperature. Uptake was stopped by withdrawing 20 μl of the incubation mixture and adding this to 1.0 ml of ice-cold stop solution.

The resultant suspension was rapidly filtered through a Sartorius filter (SM 11 305, pore size 0.65 μm) and washed with 3–5 ml of ice-cold stop solution. The filters were placed in scintillation fluid and counted by standard techniques in a liquid scintillation counter (Mark III, Searle Analytic).

The uptake experiments were carried out under tracer exchange conditions [13]. This means that the incubation solution (medium outside the vesicles) was identical with the solution used for homogenization and resuspension of the membranes (medium inside the vesicles), except for the presence of the isotope.

This medium contained 100 mM NaSCN, 100 mM mannitol, 20 mM Hepes-Tris buffer, pH 7.4 (25°C), 15 mM KSCN, and 0.1 mM D-glucose or 0.1 mM K_2HPO_4. The medium outside contained in addition 20 μCi per sample of D-[^{3}H]glucose (New England Nuclear, Chicago, U.S.A.) or 7 μCi per sample of $KH_2^{32}PO_4$ (New England Nuclear, Chicago, U.S.A.), the concentration of the unlabeled substrate was reduced accordingly. The stop solution contained 150 mM NaCl, 100 mM mannitol, 20 mM Hepes-Tris buffer, 15 mM KSCN and 0.5 mM phlorizin (D-glucose transport) or 10 mM arsenate (phosphate transport). Samples were taken, as described above, at appropriate time intervals ranging from 15 s to 6 min, depending on the temperature.

Two equilibrium values, at two consecutive incubation times, were determined for each sample after 1–4 h for D-glucose transport and 2–7 h for P_i transports.

For D-glucose transport, the phlorizin-insensitive uptake was determined in identical control samples that contained in addition 0.5 mM phlorizin.

For phosphate transport, the Na-independent transport was determined in samples that contained KSCN instead of NaSCN. All uptake experiments were carried out in quadruplicate.

Temperature studies. For the transport experiments, the media were incubated during 10 min at the appropriate temperature before addition of the membranes. The samples remained incubated at this temperature during the whole uptake experiment, including the sampling at equilibrium time.

For the enzyme activities, an Aminco D.W. II spectrophotometer was used, equipped with a thermostatically cell holder.

The pH of the buffers in all experiments was adjusted to 7.4 at 25°C. We did not correct for the change in pH due to temperature. The shift of the Hepes buffer used is –0.14 pH units for a ΔT of +10°C.

The data were analyzed using a computer program described recently by Livingstone and Schachter [14,15]. No two-line fit or breakpoint temperature was accepted unless the *F*-test indicated statistical significance ($p < 0.05$).

Results

Temperature dependence of D-glucose transport

Fig. 1 shows the uptake of D-glucose into brush border membrane vesicles in the absence and in the presence of phlorizin. The difference in uptake reflects the uptake via the phlorizin-sensitive, sodium-dependent transport system [16]. For the determination of the rate constants, the first time point had to be omitted in order to obtain – within the experimental error – a straight line. During the very early period of the experiment, equilibration of small vesicles and binding to the membranes probably prevail. This gives rise to a series of exponential functions [13]. In all further experiments, therefore, only the period between 30 and 85% equilibration was taken into account. The effect of changes in temperature on the rate constants of phlorizin-sensitive and phlorizin-insensitive glucose uptake are compiled in Fig. 2. The Arrhenius plot for the

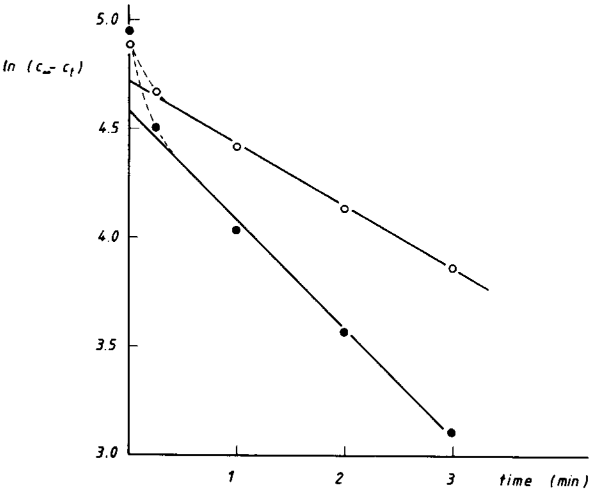

Fig. 1. Uptake of D-glucose by hog kidney brush border membrane vesicles (Tracer exchange). Experimental conditions: temperature 25°C; the composition of the medium inside and outside the vesicles was: 100 mM NaSCN, 20 mM Tris-Hepes (pH 7.4), 15 mM KSCN, 100 mM mannitol, 0.1 mM D-glucose. 20 μCi per sample of (120 μl) D-[^{3}H]glucose was added only to the outside of the vesicles. The experiments were performed in quadruplicate and the symbols given represent mean values in the presence (○) or in the absence (●) of 0.5 mM phlorizin. ln(C_∞ – C_t) is plotted as a function of time. C_∞ represents the equilibrium concentration of D-glucose tracer in the vesicles. From the slopes, the rate constants (in min^{-1}) for the total (●) and for the phorizin-insensitive (○) equilibration are derived. The dashed lines represent the deviation from linearity in the early part of the equilibration process.

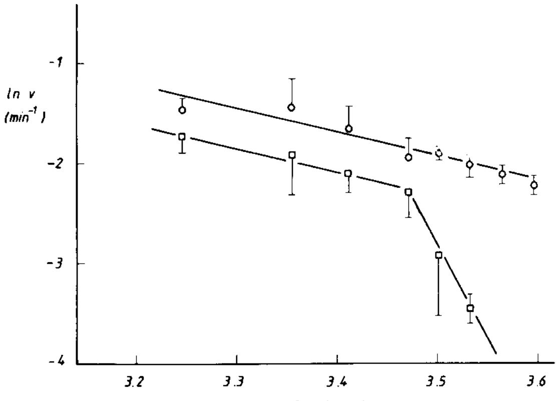

Fig. 2. Arrhenius plot for D-glucose tracer exchange. The conditions are as described in the legend to Fig. 1. The natural logarithm of the rate constant for equilibration is plotted as a function of the inverse of absolute temperature. The symbols represent mean values (±S.D.) for three to five experiments. For the phlorizin-sensitive part of the process (□) the best fit yielded two lines with one breakpoint. For the phlorizin-insensitive part of the process (○), the best fit yielded one line with no breakpoint.

TABLE I

RATE CONSTANTS FOR TOTAL AND PHLORIZIN-SENSITIVE D-GLUCOSE AND FOR TOTAL AND SODIUM-DEPENDENT PHOSPHATE UPTAKE AT DIFFERENT TEMPERATURES

The rate constants are derived from experiments shown in Fig. 1. The transport mediated by the sodium co-transport systems is indicated in percentage of the total uptake. For D-glucose, this value is derived from the transport rate in the presence of 0.5 mM phlorizin. For phosphate transport, it is derived from the transport rate in the presence of K$^+$ instead of Na$^+$, as described in Materials and Methods.

Temperature (°C)	Rate constant (min^{-1})			
	D-Glucose uptake		Phosphate uptake	
	Total	% Phlorizin-sensitive	Total	% Na$^+$-dependent
5	0.14	25	0.07	70
10	0.16	19	0.13	89
15	0.24	42	0.27	84
20	0.31	39	0.32	75
25	0.39	38	0.39	82
30			0.43	85
35	0.40	43	0.68	75

phlorizin-insensitive uptake is linear over the temperature range studied. The Arrhenius activation energy derived from this plot is 4.9 ± 0.7 (S.D.) kcal/mol. The points obtained for the phlorizin-sensitive part cannot be fitted by a straight line. If one assumes that the curve is composed out of two straight lines, a transition temperature of 15°C can be calculated. Below 10°C, the phlorizin-sensitive part can no longer be determined accurately, since it only represents about 20% of the total uptake (see Table I). Therefore, at present no reliable values are available for the low temperature range. The activation energy for the sodium-D-glucose transport above the transition temperature is equal to the activation energy of the sodium-independent part; below the transition temperature it amounts to about 33 kcal/mol.

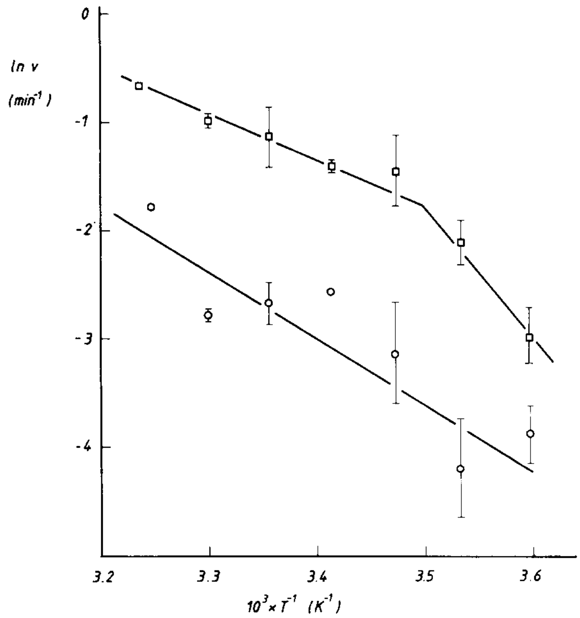

Fig. 3. Arrhenius plot for phosphate tracer exchange. Conditions: the medium inside and outside the vesicles contained 100 mM mannitol; 20 mM Tris-Hepes (pH 7.4), 0.1 mM K_2HPO_4 and 100 mM NaSCN + 15 mM KSCN (□) or 115 mM KSCN (○). In addition, 7 µCi per sample (120 µl) of $KH_2^{32}PO_4$ was added to the outside of the vesicles only. The natural logarithm of the rate constant for equilibration is plotted as a function of the inverse of absolute temperature. The symbols represent mean values (± S.D.) for three to five experiments. Table II lists the apparent energy of activation and the temperature of the breakpoint.

Temperature dependence of phosphate uptake

The effect of temperature on the sodium-dependent and the sodium-independent phosphate uptake is shown in Fig. 3. The rate constants were obtained from the uptake between 25 and 85% of the equilibrium because binding and vesicle inhomogeneity affected phosphate uptake the same way as glucose uptake. It is evident that for the sodium-dependent phosphate uptake, a discontinuity occurs in the temperature range between 11 and 13°C. If again it is assumed that the curve can be fitted with two straight

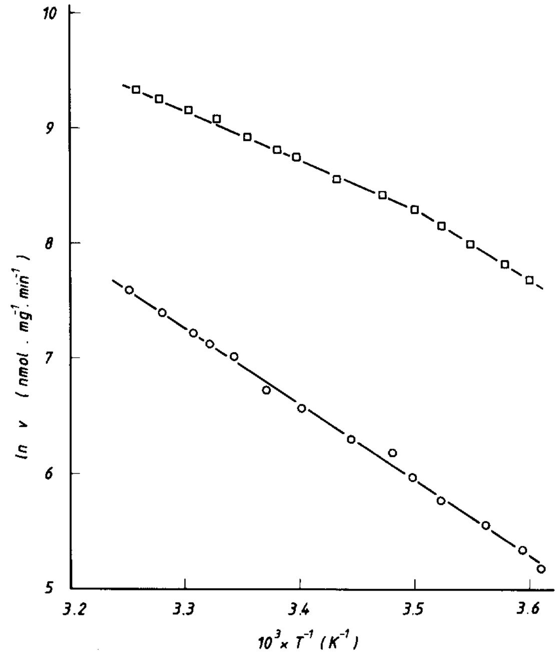

Fig. 4. Arrhenius plot for brush border membrane enzyme activities. The natural logarithm of the enzyme activity in nmol mg^{-1} · min^{-1} is plotted as a function of the inverse of absolute temperature. The symbols represent mean values obtained from three determinations; alkaline phosphatase (□), aminopeptidase M (○). Table II lists the apparent energy of activation and the temperature of the breakpoint.

TABLE II

TEMPERATURE DEPENDENCE OF BRUSH BORDER TRANSPORT SYSTEMS

Membrane	Apparent breakpoint	Apparent activation energy (kcal/mol)		
		Above breakpoint	Below breakpoint	Ref.
1. Sodium glucose cotransport				
Hog kidney	15°C	4.2 ± 0.7	32.6 ± 5.5	a
Rat kidney	15°C	5.5	18	3
Rabbit kidney	curvilinear (12–14°C)	<11.3	>37.6	4
Rat small intestine	27.2 ± 1.3	4.1 ± 2.4	15.2 ± 6.1	17
2. Sodium-independent glucose transport				
Hog kidney	None	4.9 ± 0.7		a
Rabbit kidney	None	9.8		4
Rat small intestine	28.8	4.7 ± 0.3	18.1 ± 1.9	17
3. Sodium-phosphate cotransport				
Hog kidney	12.5	8.6 ± 2.7	24.6 ± 4	a
4. Sodium-independent phosphate uptake				
Hog kidney	None	12.5 ± 2.7		a
5. Alkaline phosphatase				
Hog kidney	12.5	8.8 ± 0.2	11.6 ± 0.36	a
Rat small intestine	29.1 ± 0.7	6.9 ± 1.1	11.6 ± 1.1	17
6. Aminopeptidase M				
Hog kidney	None	13.1 ± 0.2		a
Rat small intestine	None	16.1 ± 1.5		17

[a] This study. Values of the energies of activation in this study are ± S.D.

lines, a transition temperature of 12.5°C and apparent activation energies of 8.6 and 24.6 kcal/mol can be calculated. For the sodium-independent phosphate uptake, no definite breakpoint is evident. Unfortunately, the scatter of the latter data is quite high because the sodium-independent part only represents a small quantity of the total phosphate uptake (see Table I).

Temperature dependence of alkaline phosphatase and aminopeptidase M activity

The effect of temperature on the activities of the two brush border enzymes, alkaline phosphatase and aminopeptidase M, is shown in Fig. 4. For alkaline phosphatase, the computer analysis revealed a significant breakpoint at 12.4°C. For the aminopeptidase M activity, no breakpoint was observed. The respective apparent activation energies are given in Table II.

Discussion

The studies presented above differ in one main aspect from studies on the temperature dependence of sodium cotransport systems published so far [3,4,17]. The investigations were performed under conditions where no sodium gradient was present across the membrane and, thus, the temperature dependence of the transport systems proper could be determined without interference of other transport processes related to the dissipation of the sodium gradient such as the Na/H exchange system or conductive pathways for sodium or anions. The results of the experiments clearly demonstrate a strong temperature dependence both of the sodium-D-glucose and of the sodium-phosphate cotransport system. The change in apparent activation energy occurs at the same temperature as the activity change in the alkaline phosphatase. Thus, these studies reconfirm the notion of the intrinsic nature of the transport systems in the brush border membrane [18,19].

The similarity in the temperature range at which the transition in the apparent activation energy occurs for the three intrinsic membrane proteins does not provide evidence that the proteins are surrounded by specific lipids that do not participate in the changes taking place in the bulk lipids. Thus, a functional role of boundary-layer lipids remains to be demonstrated in the brush border membrane. Reconstitution experiments performed with a brush border membrane extract and various lipids (DaCruz, M.E.M., Lin, J.T. and Kinne, R., unpublished results) support this view. The transition temperature for sodium-dependent D-glucose transport closely reflected the transition temperature of the lipid used for reconstitution.

In Table II our studies on hog kidney brush border membranes are compared with studies on rat and rabbit renal brush borders on the one hand and with studies on rat intestinal brush border membranes on the other. Within the studies on renal brush border membranes, a close agreement exists with regard to the sodium-independent D-glucose uptake that occurs predominantly via simple diffusion. It shows a linear Arrhenius plot, as expected for such a process. With respect to the sodium-dependent D-glucose transport, Kippen et al. [4] observed a curvilinear Arrhennius plot; whereas in the studies reported by our group, a biphasic plot seems to prevail. The latter interpretation of the data is certainly an oversimplification but seems to be a reasonable first approximation to the problem under study. The curvilinear nature in the studies with rabbit brush border might also be due to the fact that the studies were performed under sodium gradient conditions. Under these experimental conditions, a variety of factors, and especially their temperature dependence, enter into the data. Nevertheless, the maximum apparent activation energy obtained for the sodium-D-glucose cotransport system is quite similar to the value obtained in this study. For rat kidney and rat small intestine, the activation energy seems to be lower. Whether this represents a species difference with respect to the sodium-D-glucose cotransport system or not, has to be tested in further experiments. Two observations might be of interest in this respect: (1) the apparent activation energies for alkaline phosphatase and aminopeptidase M in hog kidney and in rat small intestine are quite similar. Thus, if there is a species difference, it might be rather specific for the D-glucose transport system; and (2) sodium-D-glucose cotransport systems have been purified successfully so far only from rabbit kidney (and intestine) [20,21] and hog kidney [22]. The apparent higher activation in the latter membranes might indicate a more stable conformation of the transport protein that facilitates the isolation.

The most striking difference between the renal brush border membranes and the intestinal brush border membranes is that the apparent transition temperatures lie in the range of 27–29°C for the intestinal brush borders, whereas the apparent transition temperatures for the renal brush border lie in the range of 12–15°C, i.e., there is a difference of about 15°C. If one assumes that similar processes are responsible for the appearance of transition points in both membranes, these data can be interpreted to indicate that the renal brush border membrane is more fluid than the intestinal brush border membrane. In a comparison between the intestinal brush border and intestinal basal-lateral plasma membranes, where similar differences in the transition temperature were observed [23,24], higher protein/lipid, cholesterol/phospholipid and sphingomyelin/lecithin ratios were found in the brush border membrane and were discussed as determinants for the fluidity of the membranes. The lipid composition of the hog kidney brush border has recently been determined [25] and a cholesterol/phospholipid ratio of 0.72 and a sphingomyelin/lecithin ratio of 0.5 have been found. Both values are lower than the values found in the intestinal brush border (0.87 and 0.67, respectively) [23]. These findings would provide a partial explanation for the observed differences. Two other factors should be considered additionally. One concerns the outer surface of the membrane that is covered by the glycocalyx. Carbohydrate-carbohydrate and electrostatic interactions can stabilize the membrane from the outside. The other factors concern the control of fluidity via cytoskeletal elements from the inner or cytoplasmic face of the membranes. The microvilli contain highly structured actin filaments that are anchored into the membrane at the tip and along the sides of the microvilli [26]. Their role in the control and possible regulation of protein mobility, membrane fluidity, and transport activity is just beginning to be realized.

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