RECONSTITUTION OF SPECIFIC Na^+-DEPENDENT D-GLUCOSE TRANSPORT IN LIPOSOMES BY TRITON X-100-EXTRACTED PROTEINS FROM PURIFIED BRUSH BORDER MEMBRANES OF RABBIT KIDNEY CORTEX

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1. Introduction

In 1960, Crane et al. [1] proposed that active sugar transport at the brush border membrane of the animal small intestine is mediated by a carrier which binds both Na^+ and substrate to form a ternary complex which can respond to the transmembrane electrochemical potential gradient. Over the years numerous studies with intact tissue [2] and vesiculated membrane preparations [3,4] have provided substantial support for the proposal which has come to be called, generally, the Gradient Hypothesis. Studies with kidney cortex slices [5,6] and by microinjection and electrophysiological techniques with intact kidney [7] have indicated that the brush border membrane of the proximal convoluted tubule epithelial cells is very similar in its glucose transport mechanism to that of the epithelial cells of the small intestine. This indication has been confirmed by studies of vesiculated membrane preparations [8,9].

We now wish to report that we have reconstituted Na^+-dependent D-glucose transport in liposome vesicles by the addition of Triton X-100-extracted proteins from brush border membranes of rabbit kidney cortex.

2. Materials and methods

D-[U-^{14}C]glucose (200 mCi/mmole), L-[1-^{3}H(N)] glucose (17.56 Ci/mmole), D-[1-^{14}C]mannitol (47.6 mCi/mmole) and D-[1-^{3}H(N)]mannitol (2.65 Ci/mmole) were from New England Nuclear Corp. Crude soybean phospholipids (Asolectin) were from Associated Concentrates and acetone extracted [10]. Triton X-100 was from J. T. Baker and Co. and Bio-Beads SM2 from Bio-Rad Laboratories. Other chemicals were of known purity. Male rabbits (New Zealand White) were from Perfection Breeders, Inc. Kidney brush border membranes were isolated by the procedures used for intestinal brush border membranes [11]. Kidneys were perfused with 0.154 M NaCl in situ, prior to excision. The cortices were removed, minced and suspended in 20 vols. of 50 mM mannitol–2 mM Tris-Cl, pH 7.0, and homogenized in an Omnimixer for 4 min. Subsequent treatment of the homogenate was as described [11] yielding a brush border membrane fraction at least ten-fold enriched in trehalase, alkaline phosphatase and γ-glutamyl transferase as compared to the homogenate. This fraction was suspended in 0.15 M KCl containing 5 mM HEPES-Tris, pH 7.5, 1 mM dithiothreitol and 0.1 mM $MgSO_4$. Triton X-100 (1% w/v, final) was added and mixed. The suspension was held at 4°C for 60 min then centrifuged at 30 000 *g* for 30 min 65–70% of the membrane proteins were solubilized. Triton X-100 was removed on a column (1 g/ml) of Bio-Beads SM-2 [12] equilibrated with the KCl buffer.

Reconstitution followed Racker [13] with some modifications. The soybean phospholipids, dried in vacuo and kept under dry N_2, were suspended in buffer, kept flushed with N_2, and sonicated for 5 min at 15–30°C (Biosonik, Generator model, 120 W, 20 KHz) to give a clarified suspension of 50 mg/ml phospholipid. The solubilized membrane fraction was

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added to the liposomes (25 mg/ml) phospholipid and 1–2 mg/ml protein) and sonicated for 5 min under a stream of N_2, at 15–30°C. The mixture was centrifuged at 100 000 *g* for 90 min. The pellet was resuspended in a small volume of buffer and tested for transport properties.

Sugar transport was measured by adding 50 μl of suspended liposomes into buffers containing the radioactive (^{14}C or ^{3}H) sugars in a final volume of 350 μl. Aliquots (50 μl) were removed at intervals and diluted into 1 ml of cold stopping solution (0.154 M NaCl) + 0.5 mM phlorizin at 4°C) containing tracer amounts of radioactive (^{3}H or ^{14}C) mannitol which served as the extravesicular marker. The mixture was rapidly filtered through 0.3 μm Millipore filters and washed with 5 ml of stopping solution. The radioactivity remaining on the filters was measured in a Beckman LS counter.

Protein was assayed according to Lowry et al. [14].

3. Results

As also observed in liposomes reconstituted with solubilized intestinal brush border membrane proteins [15], the kidney brush border membrane protein-reconstituted liposomes showed a time-dependent non-specific uptake of D- and L-glucose from 0.1 M KCl (fig.1). When K^+ was replaced with Na^+ and the lipophilic anion, SCN^-, was used to provide a high mobility counterion for Na^+, marked enhancement of D-glucose uptake was observed whereas there were no discernible effects on L-glucose uptake. Phlorizin (5 mM) completely inhibited D-glucose uptake in the presence of Na^+ while uptake of L-glucose was unaffected.

Sugars that share the same transport mechanism as D-glucose viz. D-galactose, 6-deoxyglucose and α-methyl glucoside [7] also substantially inhibited the uptake of D-glucose in the presence of Na^+ and SCN^- (fig.2). L-glucose which does not compete for the D-glucose binding site in the kidney [7] had no effect on D-glucose uptake. Cl^- is a usable, though not ideal, counterion for Na^+ influx in this liposome system. Replacing KCl with NaCl enhanced D-glucose uptake although to a markedly lesser extent as compared to NaSCN.

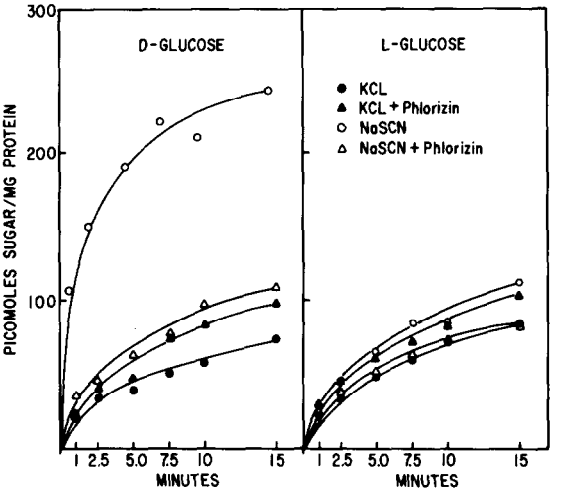

Fig.1. Reconstitution with kidney brush border membrane proteins: effects of NaSCN and phlorizin. Triton X-100 extractable membrane proteins were reconstituted with soybean phospholipid vesicles in 0.15 M KCl–5 mM HEPES-Tris–1 mM DTT–0.1 mM $MgSO_4$. Incubation was at room temperature (~ 25°C) in a total volume of 350 μl. The incubation medium contained 0.1 mM D-[U-^{14}C]glucose or 0.1 mM L-[1-^{3}H(N)]glucose, 0.15 m KCl or NaSCN 5 mM HEPES-Tris (pH 7.5) and 0.1 mM $MgSO_4$. The reaction was initiated by addition of 50 μl reconstituted liposomes. Phlorizin was added at 5 mM concentration.

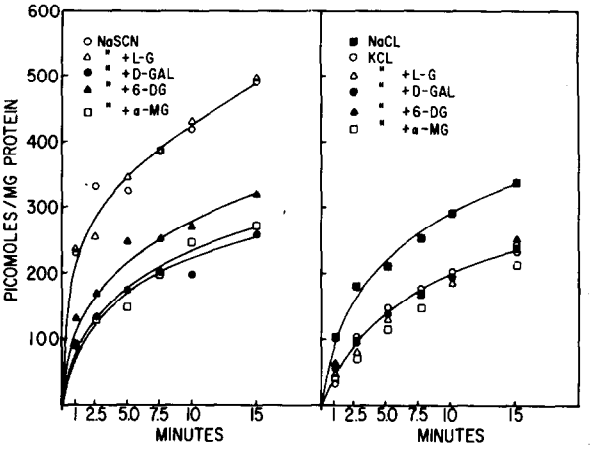

Fig.2. Reconstitution with kidney brush border membrane proteins: effects of other substrates. Experimental details were the same as in fig.1. NaCl (0.15 M replaced KCl where indicated). L-glucose, D-galactose 6-deoxy-glucose and α-methyl glucoside were used at a final concentration of 5 mM.

4. Discussion

To the extent studied thus far, our results are consistent with those obtained with vesiculated kidney brush border membrane preparations [8,9]. Na^+-dependent glucose transport by intact kidney cells [7] and by membrane vesicles is electrogenic [8,9]. That is Na^+ bound to the carrier moves without a counterion. With intact cells, entering Na^+ may be removed by the sodium pump or may exchange with intracellular K^+. With membrane vesicles which lack the pump and appear not to exchange cations readily [8,9] Na^+-dependent substrate entry can be best observed when Na^+ movement is induced by movement of an anion in the same direction or a cation in the opposite direction. The same is true for liposome vesicles as indicated by the greater enhancement of D-glucose entry with SCN^- than with Cl^- as the counterion for Na^+.

In the experiments of figs.1 and 2, the excess entry of D-glucose in the presence of Na^+ is maintained over the time period studied. We have not tested whether an equilibrium value is finally achieved and whether it is the same for all substrates. Also, demonstrable movement of sugar against a gradient has not yet been achieved. Further studies of this new system are needed in order to understand how to manipulate it.

The use of a heterogeneous protein extract has probably reconstituted a number of other systems. For example, preliminary experiments appear to demonstrate Na^+-dependent L-alanine uptake. Also, we have not yet identified the molecular weight range or other characteristics of the membrane protein(s) responsible. However, it may be recognized that a hindrance to isolation and characterization of transport modalities has been the need to use chemical rather than functional tests, e.g. [16]. This demonstration of reconstitution is equally a demonstration that the much needed in vitro functional assay system has been achieved.

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References

[1] Crane, R. K., Miller, D. and Bihler, I. (1961) in: Membrane transport and Metabolism, (Kleinzeller, A. and Kotyk, A., eds.) Academic Press, London, p. 439–439.
[2] Schultz, S. G. and Curran, P. F. (1970) Physiol. Rev. 50, 637–717.
[3] Hopfer, U., Nelson, K., Perrotto, J. and Isselbacher, K. J. (1973) J. Biol. Chem. 248, 25–32.
[4] Murer, H. and Hopfer, U. (1974) Proc. Nat. Acad. Sci. 71, 484–488.
[5] Kleinzeller, A. (1970) Biochim. Biophys. Acta. 211, 277–292.
[6] Rosenberg, I. H., Coleman, A. L. and Roseberg, L. E. (1965) Biochim. Biophys. Acta 102, 161–171.
[7] Ullrich, K. J. (1976) Kidney International 9, 134–148.
[8] Beck, J. C. and Sactor, B. (1975) J. Biol. Chem. 250, 8674–8680.
[9] Evers, J. Murer, H. and Kinne, R. (1976) Biochim. Biophys. Acta 426, 598–615.
[10] Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5177–5187.
[11] Schmitz, J., Preiser, H., Maestracci, D., Ghosh, B. K., Cerda, J. J. and Crane, R. K. (1973) Biochim. Biophys. Acta 323, 98–112.
[12] Holloway, P. W. (1973) Anal. Biochem. 53, 304–308.
[13] Racker, E. (1973) Biochem. Biophys. Res. Comm. 55, 224–230.
[14] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[15] Crane, R. K., Malathi, P. and Preiser, H., Biochem. Biophys. Res. Comm., in the press.
[16] Thomas, L. (1973) Biochim. Biophys. Acta 291, 454–464.