Vol. 112, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 29, 1983 Pages 444-449

INFLUENCE OF SODIUM IONS ON DETERGENT SOLUBILIZATION OF PIG BRUSH BORDER D-GLUCOSE TRANSPORT SYSTEM FOR RECONSTITUTION EXPERIMENTS

J. C. POIREE, M. STARITA-GERIBALDI and P. SUDAKA

Laboratoire de Biochimie. Faculte de Medecine
Avenue de Vallombrose. 06034 NICE CEDEX - FRANCE

Received March 7, 1983

SUMMARY : The D-glucose uptake by liposomes resulting from the association of egg lecithins with solubilized membrane proteins was measured in order to assess their sodium dependent D-glucose transport activity. Membrane proteins were extracted by Triton X-100 solubilization of pig kidney brush border membrane vesicles,which were suspended either in KCl medium or in NaCl medium. When measured by equilibrium isotope exchange procedure in sodium conditions, the D-glucose uptake by sodium detergent extract associated to liposomes occurred with a higher velocity than that obtained with liposomes reconstituted from potassium detergent extract. No differences were observed in the permeability or in the protein content of two types of liposomes. These results are discussed in terms of activation of D-glucose transport system induced by sodium ions before membrane protein solubilization.

The D-glucose transport by intestinal or renal epithelial cells via their brush border membranes was demonstrated as a coupled passive transport dependent on sodium ions (1, 2). Recent kinetic studies on purified brush border membrane vesicles provided proof of an ordered binding to the cotransporter : Na^+ first, D-glucose or phlorizin second in a gatted channel model (3, 4).

At the same time, experiments undertaken to incorporate the D-glucose transport system in liposomes showed the requirement of D-glucose substrate during detergent solubilization for further optimal reconstitution with respect to transport properties (5).

We report here the influence of sodium ions during detergent extraction procedure for the obtention of an active D-glucose transport system following reintegration in liposomes.

MATERIAL AND METHODS

Solubilization of brush border membrane vesicles : Brush border membrane closed vesicles were isolated from pig kidney cortex according to Vannier

0006-291X/83 $1.50

et al. (6) and suspended either in 100 mM KCl, 1 mM Tris-HEPES pH 7.4 (K buffer) or in 100 mM NaCl, 1 mM Tris-HEPES pH 7,4 (Na buffer); the final concentration was 10 mg/ml of membrane protein. These membrane vesicles in K and Na buffer were incubated with an equal volume of 0.5% Triton X-100 (w/v) respectively in K and Na buffer for 1h at 4°C, then centrifuged for 1h at 200.000g. The resulting clear supernatants were termed potassium and sodium detergent extracts and kept overnight at 4°C.

Preparation of reconstituted proteoliposomes : The procedure used has previously been described by Gerritsen et al. (7). Briefly 10 mg egg yolk L-α-phosphatidylcholine in chloroform/methanol (90:10) (Sigma Chemical Co. St. Louis, Mo) were dried, then 2 mg of potassium or sodium detergent extract were added in 100 mM KCl, 1 mM Tris-HEPES pH 7,4 adjusted to 0.4% Triton X-100 final concentration, found optimal for glucose transport after reconstitution. Total volume was 2.5 ml. This suspension was shaken vigourously and subsequently treated with Biobeads SM-2 (0.3g wet beads/ml) 2h at 4°C. Biobeads were discarded and solutions centrifuged for 10 min at 3000g, and the resulting supernatants, complemented with a large volume of appropriate K or Na buffer for transport measurements, were centrifuged for 90 min at 180.000g. The pellet of proteoliposomes was finally suspended in K or Na buffer at a final concentration of 1 mg/ml of reintegrated proteins.

Transport assay : Glucose uptake by membrane vesicles or proteoliposomes was measured under equilibrium exchange procedure. Membrane vesicles (90 µg proteins/assay) or proteoliposomes (20 to 30 µg proteins/assay) were preincubated for 1h at 25°C in K or Na buffer containing 1 mM D-glucose. The isotope exchange was initiated by addition of 1 mM radioactive D-glucose in the same buffer as preincubation. Transport was stopped with 2 ml of cold stopping solution (K or Na buffer containing 1 mM unlabelled D-glucose). Then the mixture was immediately applied to presoaked Millipore filter (HAMK 0,45 µM) and the filter washed with 2 ml of the stopping solution. Radioactivity retained on the filter was measured in a Packard Tricarb Spectrometer after A.C.S. (Amersham Co) scintillator addition.

Polyacrylamide gel electrophoresis : Protein samples (80 µg) were solubilized in 2% S.D.S. and boiled for 90 sec. S.D.S. polyacrylamide gel electrophoresis was performed according to the procedure described by O'Farrell (8). The running gel was a 5 to 12% linear gradient (140 x 120 x 1.5 mm) and the stacking gel was a 5% gel (140 x 30 x 1.5 mm). Electrophoresis was carried out at 9 mA per plate for 16 h at 14°C in the LKB 2001 vertical electrophoresis unit. The slab gels were stained with 0.05% Coomassie blue in methanol-acetic acid-water (4.5/1/4.5) and destained with several changes in methanol-acetic acid-water (2.5/1/6.5).

D-[^{14}C(U)] glucose was obtained from the Radiochemical Center (Amersham, U.K.).

RESULTS AND DISCUSSION

The D-glucose uptake into pig brush border membrane vesicles and proteoliposomes was measured by equilibrium isotope exchange procedure, useful method for elucidating the mechanism of multireactant systems (9, 10). When performed on brush border membrane vesicles, the uptake of labelled D-glucose was increased in vesicles prepared in NaCl medium in comparison with that observed in vesicles suspended in KCl medium (fig.1A). After 45 s incubation in NaCl medium the equilibrium level of D-glucose

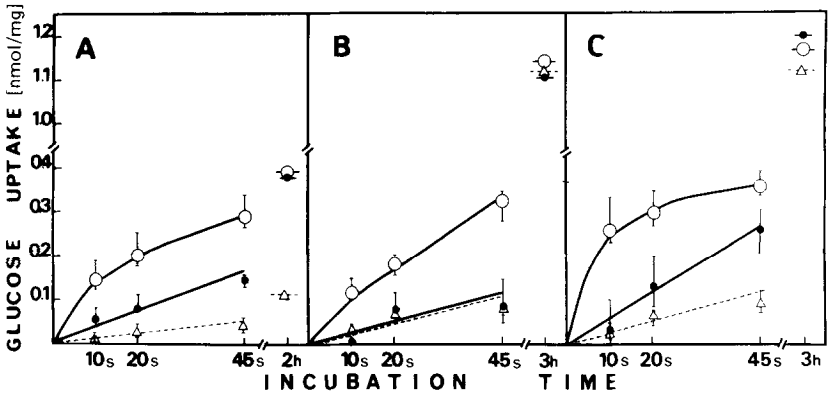

Figure 1 - Equilibrium exchange of D-glucose and maltose in pig kidney brush border vesicles and proteoliposomes reconstituted from Triton solubilized proteins.
Brush border vesicles (A) and liposomes associated with proteins solubilized by 0.25% Triton X-100 in the presence of potassium (B) or sodium ions (C) from brush border membranes (see Material and Methods). They were incubated for 1h at 25°C in 100 mM KCl, 1 mM Tris-HEPES pH 7.4 (●—●) or in 100 mM NaCl, 1 mM Tris-HEPES pH 7.4 (○—○), both containing 1 mM D-glucose, or in 100 mM NaCl, 1 mM Tris-HEPES pH 7.4 containing 1 mM maltose (△--△). The isotope exchange was initiated by addition of 1/7 volume of 1 mM radioactive D-glucose or maltose in the same buffer used during the incubation. Uptake measurements conditions are described in Material and Methods. All measurements were performed in duplicate. Control values for non specific radioactivity retention on the filter were determined using incubation mixture without membrane vesicles. Each value represents the mean of 4 separate experiments (S.E. are indicated by the vertical lines).

isotope was nearly reached, while the equilibrium value of D-glucose in KCl medium was obtained after one hour incubation.

Brush border membrane vesicles prepared in KCl and NaCl medium were solubilized by 0.25% Triton respectively in the presence of potassium and sodium ions and the extracted proteins associated with egg phosphatidylcholine (1/5 ratio) in KCl medium to give proteoliposomes. Then the resulting proteoliposomes were analyzed for D-glucose equilibrium exchange either in KCl medium or NaCl medium. Protein concentration of the potassium or sodium extracts varied from 45 to 50% of the total protein vesicle content and nearly 20% of extracted proteins were reincorporated into liposomes. By this direct incorporation procedure used in our experiments, reconstituted proteoliposomes were sealed in closed vesicles as proved by electron microscopy determination (M. Boudouard, unpublished observations).

In KCl equilibrium conditions with proteoliposomes reconstituted from potassium detergent extract, a low linear exchange of D-glucose was observed for 45 s, whereas this isotope exchange in the presence of sodium ions resulted in a more pronounced accumulation of D-glucose (fig, 1B). Proteoliposomes obtained from sodium detergent extract and equilibrated in KCl medium took up the D-glucose isotope (fig.1C) more quickly than when it

was reconstituted from potassium detergent extract, but in the same linear way. Sodium equilibration of proteoliposomes reconstituted from sodium detergent extract resulted in a very rapid exchange of D-glucose isotope (fig.1C); the exchange rate after 10 s incubation was twice that reached by proteoliposomes derived from potassium detergent extract equilibrated with sodium ions. In addition, the equilibrium value of D-glucose isotope in proteoliposomes under sodium or potassium transport conditions was obtained after less than 3h incubation and reached a higher level than that observed after 45 s incubation. The most likely explanation is that the D-glucose transport system may be linked to a small fraction of the liposomes as noted by others (11).

Finally, it seems that proteoliposomes prepared with sodium detergent extract accumulated D-glucose more quickly than those obtained from potassium detergent extract.

The differences observed in D-glucose uptake with potassium and sodium extracts could be explained by differential permeabilities of potassium and sodium reconstituted proteoliposomes. In order to test this hypothesis, equilibrium exchange of maltose was performed with the two kinds of proteoliposomes. This disaccharide is neither transported by pig brush border membrane vesicles, nor hydrolyzed in D-glucose and its exchange in equilibrium conditions was so low that the equilibrium level was not reached after 2h incubation. Whatever the medium used in equilibrium exchange, radioactive maltose penetrated the two kinds of proteoliposomes to the same extent and the equilibrium level occured after 3h incubation as shown above for D-glucose exchange. Moreover, in comparison with the equilibrium value of maltose in brush border vesicles and proteoliposomes, a similar exchange rate was observed during 45 s incubation, which seems to point to an identical permeability rate vis a vis this disaccharide.

From these data it appears that the presence of sodium rather than potassium ions during detergent solubilization of brush border membranes could allow better extraction of D-glucose transporter. Nevertheless, it cannot be ruled out that sodium ions interact with the D-glucose transport system to give it some conformation better suited to the transport function.

S.D.S. polyacrylamide gel electrophoresis experiments performed on proteoliposomes reconstituted from sodium or potassium detergent extracts showed no differential patterns whatever the medium used, in extraction or transport (Fig.2).

These results support the latter hypothesis of conformational dependence on sodium ions. However, they do not entirely exclude

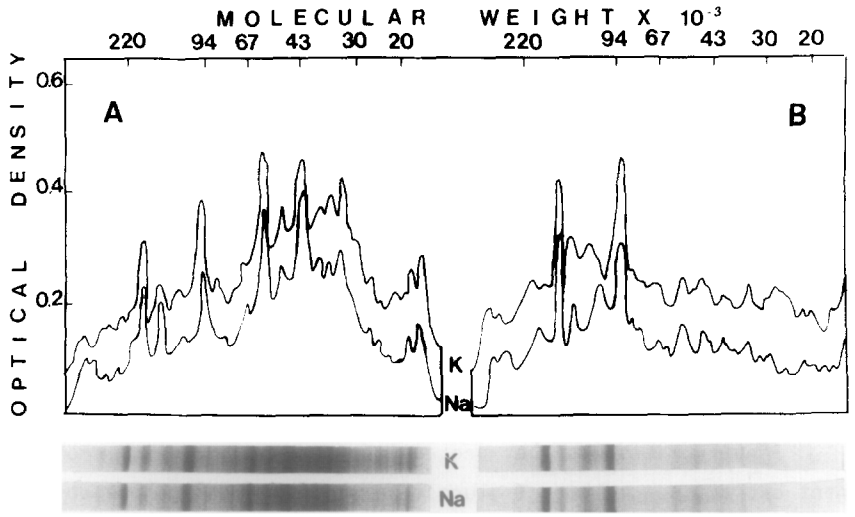

Figure 2 - Electrophoretic analysis of Triton X-100 extracted and liposomes associated brush border membrane proteins.
Protein pattern and related optical density scanning provided by brush border membranes solubilized by 0.25% Triton X-100 in KCl medium or in NaCl medium (A) and respective extracted proteins associated with liposomes (B). Electrophoresis conditions were described in Material and Methods. Ferritin (220.000 daltons), phosphorylase b (94.000 daltons), albumin (67.000 daltons), ovalbumin (43.000 daltons), carbonic anhydrase (30.000 daltons) and trypsin inhibitor (20.000 daltons) were used as protein markers.

preferential extraction of D-glucose transporters, nor, likewise better incorporation of them in liposomes prepared with a sodium detergent extract.

Finally, the data are consistent with the idea that sodium ions could energize the D-glucose transport system before detergent solubilization, probably by induction of a conformational change as previously described for intestinal brush border membranes (4); then the exchange of D-glucose isotope could be accelerated when proteoliposomes are equilibrated with sodium ions.

The absence of rapid D-glucose accumulation in the presence of sodium ions with proteoliposomes obtained from potassium detergent extract could be due to a hindrance in carrier conformational mobility due to used lipids, since it has been proven that the nature of lipids in reconstituted vesicles influences D-glucose transport (12).

ACKNOWLEDGMENTS : We gratefully thank Dr. C. Vannier for helpful discussions and Mrs. Morgan for the revision of this manuscript.

REFERENCES

1. Crane, R. K. (1965) Federation Proc. 24, 1000-1006.
2. Aronson, P. S. and Sacktor, B. (1974) Biochim. Biophys. Acta 356, 231-243
3. Hopfer, U. and Groseclose, R. (1980) J. Biol. Chem. 255, 4453-4462.
4. Toggenburger, G., Kessler M. and Semenza, G. (1982) Biochim. Biophys. Acta 688, 557-571.
5. Koepsell, H. and Menuhr, H. (1982) Transport in Biomembranes : Model Systems and Reconstitution, pp. 169-176, Raven Press, New York.
6. Vannier, C., Louvard, D., Maroux, S. and Desnuelle, P. (1976) Biochim. Biophys. Acta 455, 185-199.
7. Gerritsen, W. J., Verkley, A. J., Zwaal, R. F. A. and Van Deenen, L. L. M. (1978) Eur. J. Biochem. 85, 255-261.
8. O'Farrell, P. H. (1975) J. Biol. Chem. 250, 4007-4021.
9. Segel, I. H. (1975) Enzyme Kinetics, pp. 846-883, John Wiley and Sons, New York, N. Y.
10. Hopfer, U. (1977) J. Supramol. Struct. 7, 1-13.
11. Koepsell, H., Menuhr, H., Ducis, I. and Wissmuller, T. F. (1983) J. Biol. Chem. in press.
12. Koepsell, H., Menuhr, H., Wissmuller, T. F., Ducis, I. and Haase, W. (1980) Am. N. Y. Acad. Sci. 358, 267-281.