Kidney International, Vol. 14 (1978), pp. 279–282

TECHNICAL NOTE

Preservation of renal brush border membrane transport function by storage in glycerol

SHIRLEY A. HILDEN and BERTRAM SACKTOR

Laboratory of Molecular Aging, Gerontology Research Center, National Institute on Aging, National Institutes of Health, Baltimore City Hospitals, Baltimore, Maryland

Various methods for the isolation of renal and intestinal brush border membranes have been reported [1]. The isolated membranes vesiculate and are used as model systems to examine the mechanisms by which solutes, for example, sugar and amino acids, are transported into the epithelial cell [2, 3]. Although these studies have provided valuable insight into the transport systems, their general applicability has been limited because of the necessity to measure uptakes on the same day that the membranes are prepared [4], the time required to prepare the membranes being often as long as 5 to 6 hr [5, 6], and the large numbers of small animals (e.g. 20 rats) needed for each preparation [6]. Moreover, the relatively small yield of brush border membranes with each preparation severely hampers efforts to purify sugar and amino acid carriers since the only assay for the carrier in membrane reconstitution studies is transport function itself, rather than catalytic activity, as with cation transporting adenosine triphosphatases [7]. Thus, a procedure for preserving transport function of these membranes is critically needed. Because glycerol and low temperature have been used in other systems to maintain cell integrity and biologic function [8], this technique was examined with isolated renal brush border membranes. In this communication, the preservation of D-glucose and L-proline transport function in stored membranes is reported.

Rabbit renal brush border membranes were prepared by the calcium chloride precipitation method [9], modified as described elsewhere [10]. The preparation was enriched about tenfold in specific activities of trehalase and γ-glutamyltranspeptidase relative to the cortex homogenate. Brush border membranes (30 to 50 mg of protein) were stored by suspending the membrane pellet in 2 to 4 ml of a solution composed of 20% glycerol, 300 mM mannitol, 1 mM magnesium chloride, 1 mM dithiothreitol, 50 mM Hepes-Tris (pH, 7.5) and were kept for varying periods of time at −20° C. No attempt was made to regulate the rate of freezing or the subsequent thawing. After storage, the membranes were thawed and diluted with 100 to 200 ml of 300 mM mannitol and 50 mM Hepes-Tris (pH, 7.5). After 15 to 30 min of equilibration, the suspension was centrifuged at ×40,000 *g* for 30 min. The resulting membrane pellet was washed at least once in the 300 mM buffered mannitol. The brush borders were finally resuspended in a small volume of the same mannitol medium. Uptake of D-glucose and L-proline was measured by the Millipore filtration technique detailed previously [4, 11] except that the pore size of the filter was 0.3 μ. This filter gave maximum retention of radioactivity after as well as before freezing of the brush border membrane suspension.

Figure 1 describes the uptake of D-glucose by brush border membranes that were stored under different conditions. With freshly prepared membranes (Fig. 1A), the presence of a sodium ion electrochemical gradient induced a marked stimulation of sugar uptake, the initial rate (15 sec) being 20 to 50 times that in the absence of the sodium ion gradient. The final level of uptake (80 min) in the pres-

Received for publication January 16, 1978;
and in revised form March 6, 1978.
0085-2538/78/0014-0279 $01.00

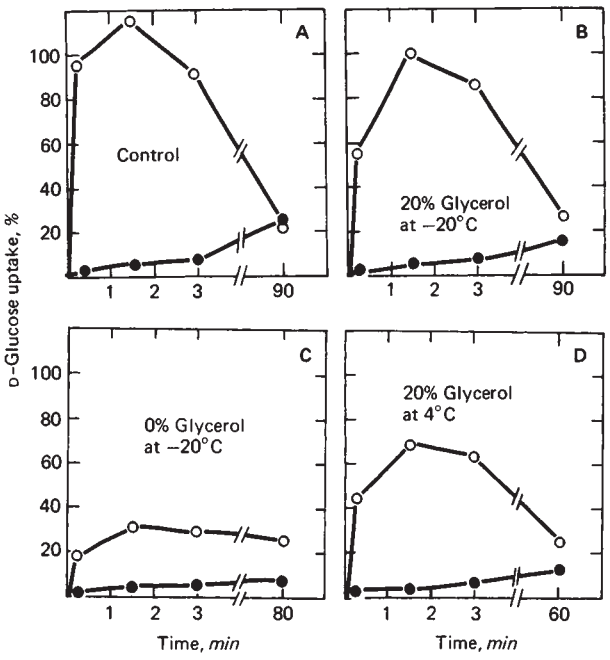

Fig. 1. *Uptake of D-glucose by brush border membranes stored under different conditions.* The sugar concentration was 50 μM. The extravesicular medium for the experiments with the sodium ion gradient (○) was 100 mM sodium chloride and 100 mM mannitol, and without the gradient (●) it was 300 mM mannitol. **Panel A.** *Freshly prepared membranes.* **Panel B.** *Membranes stored in 20% glycerol at −20° C for 19 days.* **Panel C.** *Membranes stored at −20° C for 5 days in buffered mannitol in the absence of glycerol.* **Panel D.** *Membranes stored in 20% glycerol at 4° C for 5 days.* Typical experiments are illustrated. The data have been normalized so that the maximum uptake induced by the sodium ion gradient in brush border membranes stored in 20% glycerol at −20° C for 19 days (1B) is set at 100%.

ence and absence of the sodium ion gradient was essentially the same, indicating that equilibrium had been established. At the peak of the "overshoot" (1.5 min) the uptake of D-glucose was five- to six-fold the final equilibrium value, suggesting that the imposition of a large extravesicular to intravesicular sodium ion electrochemical gradient effected the transient movement of the sugar into the membrane vesicles against its concentration gradient (uphill transport). Similar observations with freshly prepared brush border membranes have been reported [10, 12, 13]. When the membranes were stored in 20% glycerol at −20° C for 19 days (Fig. 1B), the sodium ion gradient stimulation and "overshoot" were essentially the same as when the membranes were assayed on the day of preparation. This demonstrates that membranes frozen under these conditions retained their original conductance towards sodium ions, thus enabling the sodium ion electrochemical gradient to drive the uptake of D-glucose against its concentration gradient. In contrast, when the membranes were stored at −20° C for 5 days in buffered mannitol in the absence of glycerol (Fig. 1C), the uptake of sugar did not exceed the equilibrium value. To be noted, however, was the substantial stimulation in the initial rate of D-glucose uptake, due to the presence of sodium ions. This finding suggests that freezing of the membranes in the absence of glycerol provokes the rapid equilibration of sodium ions between the extravesicular and intravesicular media. Fig. 1D shows that the sodium ion gradient-dependent uptake of D-glucose was partially maintained when the brush border membranes were stored in 20% glycerol for 5 days at 4° C. Preservation under these conditions would be useful in experiments in which non-freezing conditions were essential. Membranes stored overnight at 4° C in the absence of glycerol did not take up D-glucose above equilibrium (data not shown).

Other properties of the D-glucose uptake system in freshly prepared brush border membranes were also maintained in the membranes frozen in glycerol. Figure 2 shows that the sodium ion gradient-dependent stimulation of the sugar was stereospecific, as the uptake of L-glucose was not enhanced. The uptake of D-glucose was not stimulated

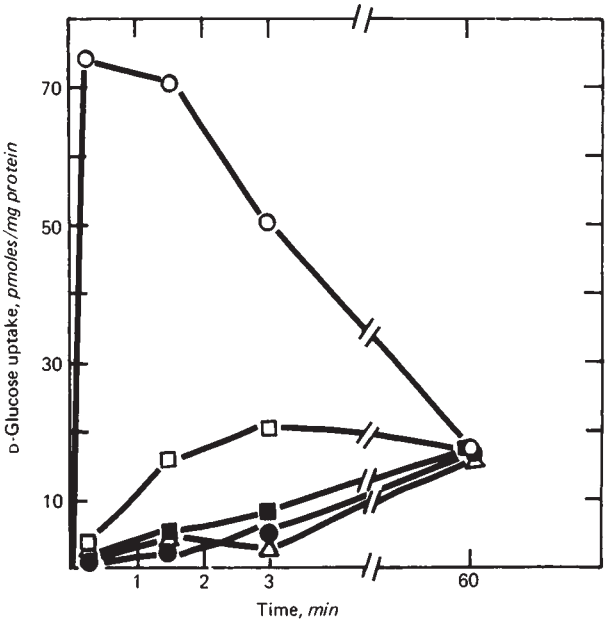

Fig. 2. *Uptake of D-glucose (50 μM) by brush border membranes stored in 20% glycerol at −20° C for 7 days in the presence of 100 mM sodium ion gradient (○), mannitol (●), 100 mM sodium ion gradient and 0.5 mM phlorizin (□), 100 mM potassium ion gradient (■), and 100 mM sodium ion gradient (△), with L-glucose substituting for D-glucose.*

by a potassium ion gradient. Also, the sodium ion gradient-dependent transport of D-glucose was inhibited by phlorizin.

The question as to whether the uptake of D-glucose by the stored brush border membranes represents transport into membrane vesicles or simply binding to the membrane was examined by determining the effect of the osmolarity of the medium on uptake. At equilibrium (60 min), uptake of D-glucose was inversely proportional to medium osmolarity, varying from 0.33 to 2.0 M with sucrose, a relatively impermeable solute which is not hydrolyzed by the kidney [14], and, thus, directly relating to intravesicular space. Little, if any, uptake was estimated by extrapolation to infinite medium osmolarity. Therefore, D-glucose uptake into the stored brush border membranes could mostly be accounted for by transport across the membrane into an intravesicular space, which is in agreement with the uptake of the sugar by freshly prepared renal membranes [12].

The effect of glycerol in preserving membrane transport was concentration-dependent. A 20% glycerol medium gave the fastest rate of D-glucose uptake. Concentrations higher than 30% were inhibitory. The quantity of brush border membranes in the 20% glycerol solution was varied from 7 to 25 mg of protein/ml without affecting the sodium ion gradient-dependent uptake of D-glucose. The membranes were not stable when stored at a concentration less than 1 mg of protein/ml. In experiments to test the effect of time of storage in 20% glycerol on the preservation of membrane transport function, it was found that although there was considerable variability, at least 50% of the original rate of uptake of the sugar was retained after 3 to 4 weeks of storage.

Brush border membranes were also stored for 10 to 14 days in solutions containing 20% dimethyl sulfoxide or 20% ethylene glycol instead of glycerol. Dimethyl sulfoxide gave partial protection (about half), compared to the maximal protection found with 20% glycerol, whereas ethylene glycol afforded only slight protection. Increasing the mannitol concentration of the stabilizing medium to 0.75 M in the absence of glycerol resulted in no preservation of the sodium ion gradient-stimulated D-glucose uptake.

Figure 3 shows that the uptake of L-proline by the stored brush border membranes was sodium ion gradient-dependent, the initial rate (15 sec) being increased about 20 times. Moreover, the maximum uptake which was transiently greatly in excess of the equilibrium uptake indicates that the sodium ion

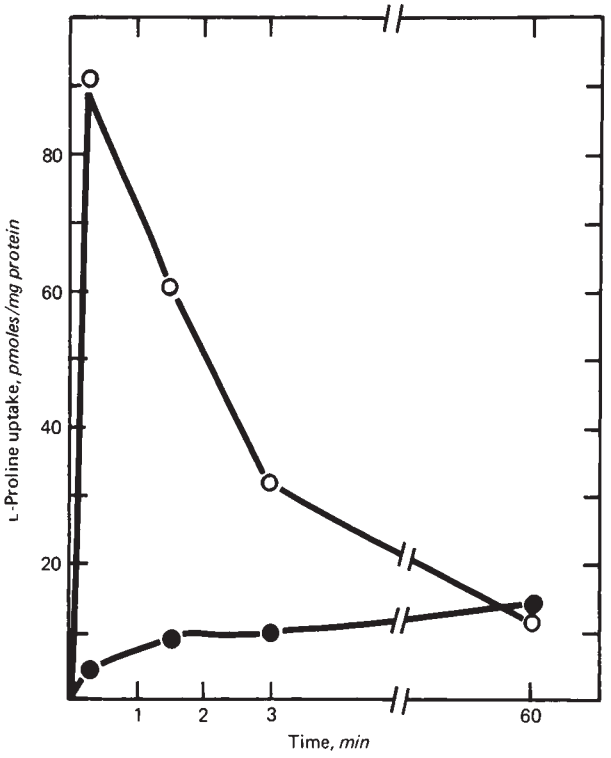

Fig. 3. *The sodium ion gradient-dependent uptake of L-proline by stored brush border membranes.* A typical experiment is shown. This preparation was stored in 20% glycerol at −20° C for 19 days. The concentration of amino acid was 25 μM. Extravesicular medium was 100 mM sodium chloride and 100 mM mannitol (○), or 300 mM mannitol (●).

electrochemical driving force effected the uphill transport of the amino acid in these membranes, similar to that found with nonstored renal membranes [15].

So far, only 2 brush border preparations out of a total of 30 failed to show significant sodium ion gradient-stimulated sugar or amino acid transport relative to the 60 min equilibrium value, indicating that this procedure had a very good success rate. These two had been stored for 28 days in 20% glycerol before uptake was tested. It should also be noted that all preparations described in this study were stored in the freezer compartment of a self-defrosting refrigerator. It is likely that storage at lower temperatures or under conditions in which the periodic warming of the freezer compartment was avoided might give even better results than those reported here. Nevertheless, the present findings demonstrate that sugar and amino transport function can be preserved in renal brush border membranes and that application of this technique will greatly facilitate studies in which storage of membranes is required.

Reprint requests to Dr. B. Sacktor, Laboratory of Molecular Aging, Gerontology Research Center, NIA, NIH, Baltimore City Hospitals, Baltimore, Maryland 21224, U.S.A.

References

1. SACKTOR B: The brush border of the renal proximal tubule and the intestinal mucosa, in *Mammalian Cell Membranes,* edited by JAMIESON GA, ROBINSON DM, London, Butterworths, 1977, Vol. 4, p. 221
2. KINNE R: Properties of the glucose transport system in the renal brush border membrane, in *Current Topics in Membranes and Transport,* edited by BRONNER F, KLEINZELLER A, New York, Academic Press, 1976, Vol. 8, p. 209
3. SACKTOR B: Transport in membrane vesicles isolated from the mammalian kidney and intestine, in *Current Topics in Bioenergetics,* edited by SANADI R, New York, Academic Press, 1977, Vol. 6, p. 39
4. CHESNEY RW, SACKTOR B, ROWEN R: The binding of D-glucose to the isolated luminal membrane of the renal proximal tubule. *J Biol Chem* 248:2182–2191, 1973
5. BERGER SJ, SACKTOR B: Isolation and biochemical characterization of brush borders from rabbit kidney. *J Cell Biol* 47:637–645, 1970
6. HEIDRICH HG, KINNE R, KINNE-SAFFRAN E, HANNIG K: The polarity of the proximal tubule cell in rat kidney: Different surface charges for the brush-border microvilli and plasma membranes from the basal infoldings. *J Cell Biol* 54:232–245, 1972
7. CRANE RK, MALATHI P, PREISER H: 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. *FEBS Lett* 67:214–216, 1976
8. WOLSTENHOLME GEW, O'CONNOR M: *The Frozen Cell.* London, J and A Churchill, 1970, p. 149
9. SCHMITZ J, PREISER H, MAESTRACCI D, GHOSH BK, CERDA JJ, CRANE RK: Purification of the human intestinal brush border membrane. *Biochim Biophys Acta* 323:98–112, 1973
10. BECK JC, SACKTOR B: The sodium-electrochemical potential-mediated uphill transport of D-glucose in renal brush border membrane vesicles. *J Biol Chem,* in press
11. ARONSON PS, SACKTOR B: The Na^+ gradient-dependent transport of D-glucose in renal brush border membranes. *J Biol Chem* 250:6032–6039, 1975
12. BECK JC, SACKTOR B: Energetics of the Na^+-dependent transport of D-glucose in renal brush border membrane vesicles. *J Biol Chem* 250:8674–8680, 1975
13. SLACK EN, LIANG CT, SACKTOR B: Transport of L-proline and D-glucose in luminal (brush border) and contraluminal (basal-lateral) membrane vesicles from the renal cortex. *Biochem Biophys Res Commun* 77:891–897, 1977
14. SACKTOR B: Trehalase and the transport of glucose in the mammalian kidney and intestine. *Proc Natl Acad Sci USA* 60:1007–1014, 1968
15. HAMMERMAN MR, SACKTOR B: Transport of amino acids in renal brush border membrane vesicles: Uptake of L-proline. *J Biol Chem* 252:591–595, 1977