Enhancement of the Reconstituted Glucose Transport Activity from LM Cells by Phosphatidylethanolamine*

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The glucose transport activity from LM cells was solubilized with sodium cholate and reconstituted into liposomes containing phospholipids of varied polar head group composition. The reconstituted vesicles exhibited time-dependent preferential uptake of D- versus L-glucose. Phloretin and mercuric chloride, known inhibitors of glucose transport in the intact cells, inhibited the reconstituted transport activity. The transport activity was found to be sensitive to the phospholipid composition of the reconstituted vesicles. Proteoliposomes containing phosphatidylethanolamine showed increased transport activity. In addition, incubation of reconstituted vesicles containing phosphatidylcholine with phospholipase D plus ethanolamine resulted in vesicles containing phosphatidylcholine plus phosphatidylethanolamine and increased transport activity. These results indicate that the glucose transport system of LM cells is sensitive to polar head group structure of the phospholipids.

The transport of glucose has served for many years as a model system for studying the mechanism of carrier-mediated transport. Since questions concerning transport depend on our comprehension of lipid-protein interactions, studies directed toward discerning the lipid requirements for glucose transport are of fundamental importance. Although several laboratories (1–4) have reported methods for the purification and reconstitution of the glucose transporter from red blood cells, no data concerning the effect of lipid structure on this transporter have yet been obtained. However, Melchior and Czech (5) have examined in reconstituted lipid vesicles the temperature dependence of the partially purified adipocyte glucose transporter and concluded that the transport activity was inactivated by gel state lipids. In addition, Silbert and co-workers (6, 7) found in LM cells that sterol depletion resulted in formation of gel phase lipid structure and inactivation of facilitated glucose transport.

Although there is evidence that facilitated glucose uptake is sensitive to the presence of solid phase lipids, virtually nothing is known concerning the preference of glucose transport for phospholipids of specific polar head group structure. Our approach to this question involves reconstitution of the partially purified glucose transporter from LM cells into liposomes of specific phospholipid polar head group composition. The results of the initial studies demonstrate a dramatic stimulation of transport activity in reconstituted vesicles containing phosphatidylethanolamine.

EXPERIMENTAL PROCEDURES

Materials—D-[1-14C]Glucose and L-[1-14C]glucose were purchased from New England Nuclear, Boston, MA. The scintillation mixture ECP-58 was from Research Products International, Elk Grove, IL. Phloretin was purchased from either Aldrich Chemical Co., Wembly, U. K., or Sigma Chemical Co., St. Louis, MO. The membrane filters used for the transport assays were Acropor An-200, purchased from Gelman, Ann Arbor, MI. Phosphatidylcholine and phosphatidylethanolamine were isolated from crude egg phosphatidylcholine by silicic acid column chromatography (8). The mixed chain phospholipids were kindly provided by Ruth Wilti, Department of Biochemistry, University of Kansas Medical Center. All other chemicals were from Sigma.

Cell Growth and Membrane Extractions—LM cells (mouse fibroblasts) were grown and the plasma membranes isolated as described previously (9). Preparation of the dimethylamino anhydride-treated membranes was similar to that of Steck and Yu (10). One volume of plasma membranes (2 mg/ml) in 10 mM Tris-HCl, pH 7.4, was diluted with 15 volumes of water. Solid 2,2-dimethylamino anhydride (final concentration of 2 mg/ml) was added to the diluted plasma membrane and the pH was maintained between pH 8 and 9 with 0.2 M NaOH. After acid ceased to evolve, the material was centrifuged at 40,000 x g for 1 h at 4°C and the pellet was resuspended at a protein concentration of 2 mg/ml in 10 mM Tris-HCl, pH 7.2. This material could be rapidly frozen and stored at −70°C for several weeks without loss of glucose transport activity. To solubilize the transporter, the dimethylamino anhydride-treated membranes were incubated at 4°C with 2% sodium cholate for about 30 min (at protein concentration of approximately 2 mg/ml). The material was then centrifuged at 40,000 x g for 30 min and the supernatant carefully was removed.

Reconstitution of Glucose Transport—In a standard reconstitution assay, 0.5 ml of cholate-solubilized protein (1.5 mg/ml) was added to 1.0 ml of cholate-dispersed phospholipids, according to the procedure of Brunner et al. (11). The protein-lipid mixture was then passed through a Sephadex G-50 column (1 x 18 cm) which had been equilibrated with 10 mM Tris-HCl, 100 mM NaCl, 2 mM MgSO4, pH 7.4, at a flow rate of approximately 10 ml/h. Under these conditions, the proteoliposomes were found in the void volume of the column. MgSO4 (2 mM) was added to the liposomes and they were then rapidly frozen in dry ice-acetone, thawed at room temperature, and sonicated in an ultrasonic bath Sonifier (Branson B-5) for 5 to 10 s. In the experiments containing defined mixtures of PE and PC, samples were removed and their phospholipid composition was determined as described below.

Transport Assays—Transport was measured at 37°C or as indicated and was initiated by addition of 10 μl of either D- or L-glucose (1 mM, 90 μCi/μmol) to 90 μl of reconstituted vesicles. Transport was stopped by addition of 1.5 ml of cold stopping solution (10 mM Tris-HCl, 100 mM NaCl, 2 mM HgCl2). The solution was then passed through an Acropor membrane filter (0.22 μm) and washed with 4 ml of the stopping solution. The uptake of D- or L-glucose was determined as described previously (9). The rate of vesicle leakage was determined by measuring the differences in radioactivity before and after the addition of the stopping solution.

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† The abbreviations used are: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol.
by measuring both the influx and efflux of L-glucose. Since vesicle leakage did not vary with vesicle composition unless specified, facilitated uptake was determined as the difference between D-glucose influx and L-glucose. For measurements under conditions of equilibrium exchange, the vesicles were first equilibrated with unlabelled (10 mM) D- or L-glucose. Uptake was initiated with 10 mM labeled D- or L-glucose (8 μCi/nmol). For the inhibition studies, the inhibitors were added at the required concentration prior to temperature equilibrium of the liposomes.

**Phospholipase D Treatment**—Reconstituted vesicles were prepared as described above except for the following modifications. The vesicles were prepared at a 2-fold higher concentration in the 10 mM sodium phosphate buffer containing 100 mM NaCl, 50 mM CaCl₂, 30% (v/v) ethanolamine at pH 6.8. After the freeze-thaw and sonication step (see above), 400 μl of proteoliposomes (2 mg/ml) were incubated at 37 °C with 200 μl of phospholipase D (20 mg/ml). Aliquots were withdrawn at various times during this incubation, then passed through a Sepharose 4B column equilibrated with 10 mM sodium phosphate, 2 mM MgSO₄, 2 mM CaSO₄, at pH 6.8, and assayed for transport activity and phospholipid content.

**Lipid Extraction and Analysis**—Phospholipids were extracted by the method of Bligh and Dyer (12). The phospholipids were then chromatographed on Silica Gel G (Analtech) using chloroform/methanol/acetic acid (65:25:4). Chromatographic separation was monitored with phosphatidylcholine and phosphatidylethanolamine standards. The fractions were scraped off the plate and extracted off the plate with methanol, and lipid phosphorus was measured by the ashen procedure of Ames (13).

**Protein Content**—Protein was determined by the method of Peterson (14).

### RESULTS

**Membrane Extraction and Solubilization of Glucose Transport Activity**—Prior to detergent solubilization of the glucose transporter from LM cells, plasma membranes from these cells were treated with dimethylmaleimide anhydride as described under “Experimental Procedures.” This treatment removed about 80% of the membrane protein without significant loss of glucose transport activity (Table I). The remaining protein was extracted with 2% sodium cholate and reconstituted with exogenous lipids as described under “Experimental Procedures.” Vesicles reconstituted in this fashion showed facilitated glucose transport activities (Table I) lower than those found in the plasma membrane and dimethylmaleimide anhydride-extracted membranes. Since the reconstituted activities were lower than expected, it became important to characterize the transport activity.

**Characterization of Glucose Transport in Reconstituted Vesicles**—Several lines of evidence indicate that the rapid time-dependent uptake of D-glucose into the reconstituted vesicles (Fig. 1) is due to carrier-mediated transport. As was observed in the intact cells and isolated plasma membranes, L-glucose is taken up much slower than D-glucose (Fig. 1). The uptake of D- but not L-glucose was dependent on the solubilized protein reconstituted into the vesicles (data not shown). Mercuric ions inhibited facilitated D-glucose uptake at concentrations comparable to those observed in the whole cells and isolated membranes (Table II). Phloretin, an inhibitor in whole cells, inhibited net efflux of glucose from the reconstituted liposomes with a Kᵢ of about 25 μM.

**Enhancement of Facilitated Glucose Transport by Phosphatidylethanolamine**—Previous studies with the whole cells (5) had shown that glucose transport was sensitive to modification of lipid composition and structure. As an initial approach to determining the effects of lipid composition and structure on the solubilized transporter, reconstitution was carried out in vesicles of selected lipid compositions. In addition, since the orientation of the transporters in the reconstituted vesicles was not known, this was further approached by identifying the orientation of the transporter in the reconstituted vesicles by examining its reaction with a number of specific probes.
cles is likely mixed, equilibrium exchange measurements which should not depend on sidedness (15) were carried out (Fig. 2). Proteoliposomes which contained approximately equimolar amounts of phosphatidylcholine (isolated soya, egg, or synthetic 1-16:0, 2-18:1 PC) and phosphatidylethanolamine (isolated soya, or 1-16:0, 2-18:1 PE) showed facilitated glucose uptake comparable to that measured in vesicles containing total soya lipids (Fig. 2). In addition, proteoliposomes containing an equimolar mixture of phosphatidylethanolamine (1-16:0, 2-18:1 PE) plus phosphatidylglycerol (isolated from Escherichia coli) showed similar facilitated glucose transport (Fig. 2). Reconstituted vesicles made either with 1-16:0, 2-18:1 PC or with 1-16:0, 2-18:1 PC plus phosphatidylglycerol (E. coli) showed facilitated glucose uptake activities about 2-fold lower than the vesicles containing PE (Fig. 2). Since small vesicles were found to transport glucose at a lower rate than large vesicles, the proteoliposomes were fractionated on a Bio-Gel A-150m column to obtain a fairly homogeneous population of large (1000 to 700 Å in diameter) vesicles (16) which were then assayed for glucose uptake. Moreover, when comparing vesicles of comparable size, proteoliposomes containing PE plus PC always gave facilitated uptake values higher than those containing PC as the only lipid (data not shown).

The Effect of the Ratio of PE/(PE + PC) on the Glucose Transport Activity in Reconstituted Vesicles—Since Cullis and DeKrujff (17) have shown that phospholipid vesicles containing PE/(PE + PC) ratios greater than 50% can form monolayer structures, it was important to determine whether the transport activity was enhanced in vesicles containing low levels of phosphatidylethanolamine. Proteoliposomes with PE/(PE + PC) ratios below 0.1 showed increased facilitated glucose uptake (Fig. 3). In addition, at PE/(PE + PC) ratios greater than 0.5, facilitated uptake decreased dramatically (Fig. 3). The decreased activity was due to a significant increase in passive diffusion as measured by the uptake of L-glucose and indicates an increase in the leakiness of the vesicles. Therefore, the decrease in glucose transport observed at PE/(PE + PC) ratios might not reflect any effect on the carrier per se.

Enhancement of the Reconstituted Transport Activity by Modification of the Polar Head Group Composition of the Vesicles by Phospholipase D treatment—To determine whether the increased activity found in the reconstituted liposomes containing phosphatidylethanolamine (Fig. 2) is caused by differential incorporation of the transporters into these vesicles or is due to activation of pre-existing transporters, proteoliposomes which were composed of phosphatidylcholine were treated with phospholipase D in the presence of ethanolamine (Table III). After 60 min, the vesicles were found to have a PE/PC ratio of 0.25 (Table III). In addition, there was an approximately 2-fold increase in glucose transport activity. Further incubation with phospholipase D plus ethanolamine had little effect on either the PE/

![Fig. 2. Enhancement of facilitated transport by proteoliposomes containing phosphatidylethanolamine. Proteoliposomes were prepared as described in Fig. 1 except for the following modifications. In the vesicles containing PC and PE, synthetic 1-16:0, 2-18:1 PC and 1-16:0, 2-18:1 PE were employed. In the vesicles containing PG, PG isolated from E. coli was employed. Transport was measured at 35°C as described (see Fig. 1). Facilitated uptake was measured at 35°C as described (see Fig. 1). Facilitated uptake is shown).](http://www.jbc.org/)

![Fig. 3. Facilitated glucose uptake in reconstituted vesicles containing different PE/(PE + PC) ratios. Proteoliposomes were as described in Fig. 1 except for the following modification. Synthetic 1-16:0, 2-18:1 phosphatidylcholine and synthetic 1-16:0, 2-18:1 phosphatidylethanolamine were employed. Transport was measured at 35°C as described (see Fig. 1). Facilitated uptake is defined as the difference of D-glucose uptake minus L-glucose uptake. The open and closed symbols represent measurements from different membrane preparations.](http://www.jbc.org/)

| Incubation time | D | Phospholipase composition | Activity |
|-----------------|---|--------------------------|----------|
|                 |   | PC | PE | mmol/min/mg |
| 0 h             | + | 100 |    | 0.51 ± 0.12 |
| 1 h             | + | 80 | 20 | 1.21 ± 0.09 |
| 2 h             | 83 | 17 | 1.14 ± 0.08 |
| 2 h             | None | 100 |    | 0.59 ± 0.11 |

**Table III**

Enhancement of glucose transport activity by modification of polar head group composition of the vesicles by phospholipase D treatment—To determine whether the increased activity found in the reconstituted liposomes containing phosphatidylethanolamine (Fig. 2) is caused by differential incorporation of the transporters into these vesicles or is due to activation of pre-existing transporters, proteoliposomes which were composed of phosphatidylcholine were treated with phospholipase D in the presence of ethanolamine (Table III). After 60 min, the vesicles were found to have a PE/PC ratio of 0.25 (Table III). In addition, there was an approximately 2-fold increase in glucose transport activity. Further incubation with phospholipase D plus ethanolamine had little effect on either the PE/
PC content of the liposomes or transport. This result shows that the stimulation of transport activity found in liposomes which contain phosphatidylethanolamine is not caused by the inability of the transporters to reconstitute into vesicles which do not have phosphatidylethanolamine.

**DISCUSSION**

Previously, it had been shown (6, 7) in whole cells and reconstituted vesicles (5) that glucose transport activity was sensitive to the formation of gel phase lipids. The current studies focus on the effect of polar head group composition on glucose transport activity. As is shown in Fig. 2, proteoliposomes which contain phosphatidylethanolamine demonstrate that vesicles containing phosphatidylcholine plus phosphatidylethanolamine have dramatically increased activities. This result was observed when both reconstitution and the transport assays were performed at temperatures above the gel to liquid crystalline phase transitions of the phospholipids of the proteoliposomes. Thus, in addition to being sensitive to the gel-liquid crystalline phase properties of the phospholipids, the glucose transport activity of LM cells appears to have a specific requirement for phosphatidylethanolamine.

Preliminary studies with the reconstituted glucose transporter indicate that the kinetic bases for the sensitivity of the transport activity to the gel-liquid crystalline phase properties of the phospholipids and to phosphatidylethanolamine are different. Silbert and co-workers (6, 7) have shown in LM cells that formation of gel phase lipids resulted in the reduction of the V<sub>max</sub> values for 3-O-methylglucose transport. Recent results with the reconstituted transporter<sup>7</sup> show that the enhancement of glucose transport activity observed in proteoliposomes which contain phosphatidylethanolamine is due to a change in the apparent K<sub>m</sub> values.

Although our results suggest that the effect of phosphatidylethanolamine on the reconstituted transporter is due to a specific interaction between phosphatidylethanolamine and the transporter, it is difficult to conclude this at the present time. Cullis and DeKruijff (18) have demonstrated that vesicles which contained mixtures of phosphatidylycholine plus phosphatidylethanolamine adopt the hexagonal phase when the PE/PC ratio is above 50%. Since activation of transport was observed at PE/PC ratios below 50% (see Fig. 3 and Table III), it seems reasonable to conclude that the proteoliposomes in this study do not adopt the hexagonal II phase. In addition, as mentioned above, the effect of phosphatidylylethanolamine on the transport activity appears to be different from that observed when there are changes of the gel-liquid crystalline phase properties of the membrane. However, it is not clear whether the glucose transport activity has a specific requirement for phosphatidylethanolamine, or, as was found (19) with the exchange activity of the reconstituted ADP-ATP carrier, phosphatidylethanolamine can be replaced by certain negatively charged phospholipids, namely phosphatidylserine or diphasphatidylglycerol. Presently, we are addressing these questions with both the reconstituted transporter and isolated plasma membranes from LM cells.

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**REFERENCES**

1. Kasahara, M., and Hinkle, P. C. (1977) *J. Biol. Chem.* 252, 7384-7390
2. Kahlenberg, A., and Zala, C. A. (1977) *J. Supramol. Struct.* 7, 287-300
3. Baldwin, S. A., Baldwin, J. M., and Lienhard, G. E. (1982) *Biochemistry* 21, 3836-3842
4. Shanahan, M. F., and Czech, M. P. (1977) *J. Biol. Chem.* 252, 8341-8345
5. Melchior, D. L., and Czech, M. P. (1979) *J. Biol. Chem.* 254, 8744-8747
6. Rintoul, D. A., Chou, S.-M., and Silbert, D. F. (1979) *J. Biol. Chem.* 254, 10070-10077
7. Baldassare, J. J., and Silbert, D. F. (1979) *J. Biol. Chem.* 254, 10078-10083
8. Litman, E. J. (1973) *Biochemistry* 12, 2545-2549
9. Baldassare, J. J., Saiko, Y., and Silbert, D. F. (1979) *J. Biol. Chem.* 254, 1108-1113
10. Steck, T. L., and Yu, J. (1973) *J. Supramol. Struct.* 1, 220-232
11. Brunner, J., Skrobot, P., and Hauser, H. (1976) *Biochim. Biophys. Acta* 455, 322-351
12. Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* 37, 911-917
13. Ames, B. M. (1966) *Methods Enzymol.* 8, 115-118
14. Peterson, G. L. (1977) *Anal. Biochem.* 83, 346-356
15. Eilam, Y., and Stein, W. D. (1974) *Methods Membr. Biol.* 2, 283-351
16. Rhoden, V., and Goldin, S. M. (1979) *Biochemistry* 18, 4173-4176
17. Cullis, P. R., and DeKruijff, B. (1979) *Biochim. Biophys. Acta* 539, 399-420
18. Cullis, P. R., and DeKruijff, B. (1978) *Biochim. Biophys. Acta* 513, 31-42
19. Kramer, R., and Klingenberg, M. (1980) *FEBS Lett.* 119, 257-260

<sup>7</sup>J. J. Baldassare, unpublished results.
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