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Glucose Transport in Vesicles Reconstituted from *Saccharomyces cerevisiae* Membranes and Liposomes

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Glucose transport activity was reconstituted into liposomes by the freeze-thaw-sonication procedure from unextracted *Saccharomyces cerevisiae* membranes and preformed phospholipid liposomes. Fluorescence-dequenching measurements with octadecylrhodamine B chloride (R18)-labeled membranes showed that the yeast membrane lipids are diluted by the liposome lipids after the freeze-thaw-sonication procedure. At lipid-to-protein ratios greater than 75:1, vesicles with single transporters were formed. Reconstituted specific activity was increased at least twofold if the liposomes contained 50 mol% cholesterol. A further increase in specific activity, from 3- to 10-fold, was achieved by fractionation of the membranes on a Renografin gradient before reconstitution. Examination of the fractions from the Renografin gradient by sodium dodecyl sulfate-gel electrophoresis showed a parallel enrichment of glucose transport activity and a number of proteins including one with an apparent M_r of ca. 60,000, which might be the glucose transporter. Finally, preliminary kinetic analysis of glucose transport activity in vesicles reconstituted at a high lipid-to-protein ratio gave a V_{max} of ca. 2.8 μmol/mg of protein per min at 23°C and a K_m of ca. 8 mM. The latter value corresponds to the kinase-independent, low-affinity component of glucose transport observed in wild-type cells.

In normal, wild-type *Saccharomyces cerevisiae* cells, glucose is transported by a low apparent K_m (0.5 to 1.0 mM) and a high apparent K_m (10 to 50 mM) process (2, 19, 22). Only the high apparent K_m process is observed in wild-type cells depleted of ATP or in mutants lacking the cognate kinases for glucose (2, 3, 22). Much evidence suggests that the two processes are mediated by the same transporter operating in two different states (11, 18). The same conclusion has been reached for the inducible galactose transport system (11). The mechanism of glucose and galactose transport and the role of the sugar kinases and sugar phosphorylation in the regulation of these transport systems are still not understood. As part of our study of the mechanism and regulation of sugar transport in *S. cerevisiae* (5), we are using three approaches to isolate and characterize the glucose or galactose transporters: (i) reconstitution of the glucose transporter in liposome (6, 7), (ii) affinity labeling of the transporters, and (iii) preparation of antibodies against a hybrid galactose transporter–*E. coli* β-galactosidase protein (21). In this paper we report on improvements of the reconstitution procedure that result in an increase in sugar transport activity by 1 order of magnitude over that previously described (6). The improvements involve a modification of the membrane purification procedure and the use of lipid vesicles containing cholesterol in addition to crude soybean phospholipids (asolectin). Using these improved, reconstituted membrane vesicles, we measured the V_{max} and K_m for glucose transport. The K_m for glucose transport in reconstituted vesicles is ca. 8 mM, which is close to that expected for the high apparent K_m process. Finally we note that certain proteins, seen in sodium dodecyl sulfate (SDS)-gels, are enriched in parallel with glucose transport activity when the membranes are fractionated on Renografin gradients.

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MATERIALS AND METHODS

Membrane isolation procedure. Membranes were purified by a procedure modified from that described previously by elimination of the sonication steps (6). In some experiments these membranes were further purified by centrifugation through a Renografin gradient (20). The modified procedure is as follows. *S. cerevisiae* cells washed in buffer A (250 mM sucrose, 10 mM imidazole, 1 mM EDTA, 2 mM β-mercaptoethanol, 0.02% sodium azide [pH 7.5]) were suspended in 1 volume of buffer A containing 1 mM phenylmethylsulfonyl fluoride and homogenized with 0.5 volume of glass beads by five 1-min bursts (alternating with 1-min rests) in a homogenizer (Bead Beater, Bartlesville, Okla.). After removal of the glass beads by filtration, the homogenate was centrifuged for 3 min at 3,000 rpm in an SS-34 rotor (Ivan Sorvall, Inc., Norwalk, Conn.), producing three layers: a supernatant, a soft pellet, and a hard pellet. The supernatant was saved (S1); the soft pellet was diluted with 0.5 volume of buffer A, vortexed for 3 min in 50-ml centrifuge tubes with 2 g of glass beads, and centrifuged for 3 min at 3,000 rpm in an SS-34 rotor. The supernatant (S2) was combined with the previous supernatant (S1) and recentrifuged for 5 min at 3,000 rpm in an SS-34 rotor. The supernatant (S3) was cleared of mitochondria by the procedure introduced by Fuhrmann et al. (8) by acidification to pH 5.0 followed by two centrifugation steps for 3 min each at 8,000 rpm in an SS-34 rotor. The last supernatant (S4) was adjusted to pH 7.5 and centrifuged for 40 min at 43,000 rpm in a 70 Ti rotor. The pellet of purified membranes was suspended in buffer B (10 mM Tris hydrochloride, 1 mM EDTA, 2 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 0.02% sodium azide [pH 7.5]) by using a Dounce homogenizer and stored at −70°C.

Renografin gradients. In some experiments the purified plasma membranes were fractionated by centrifugation on a linear Renografin gradient (20). Renografin (obtained as the 60% commercial solution, Reno-M-60; Squibb Diagnostics, New Brunswick, N.J.) was made up to contain 10 mM

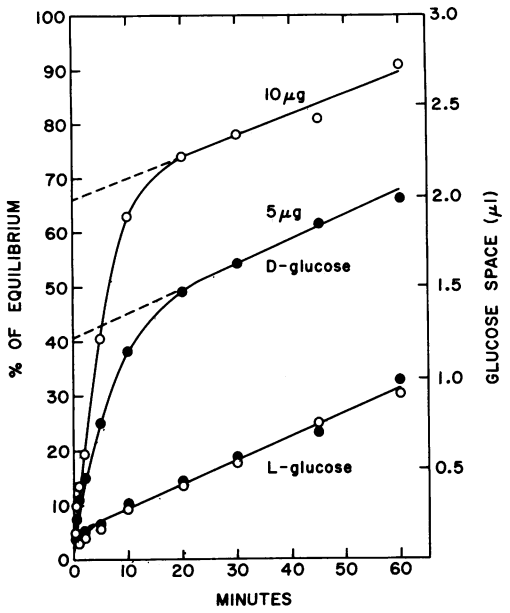

FIG. 1. Glucose uptake as a function of lipid-to-protein ratios. Uptake in vesicles reconstituted from asolectin liposomes: 5 μg (●) and 10 μg (○) of protein per 750 μg of lipid (lipid-to-protein ratios, 150:1 and 75:1, respectively). The uptakes are the average of triplicate samples. The fraction of equilibrium was calculated from the glucose space for each sample (counts per minute per sample divided by counts per minute per microliter of medium) and the total space available for each sample (microliters per milligram of lipid multiplied by milligrams of lipid per sample); an internal volume of 4 μl/mg was used.

potassium phosphate (pH 7.5); 10-ml linear gradients were made from 5 ml each of the highest and lowest concentrations desired. Membrane preparations in 1 ml of buffer B (pH 7.5) were added to the top of the gradient and spun for 60 min at 26,000 rpm in an SW 41 rotor. Fractions were collected from the top, diluted 15-fold with buffer B (pH 7.5), and centrifuged for 40 min at 43,000 rpm in a 70 Ti rotor. The diluted Renografin supernatants were removed by aspiration, and the pellets were suspended in buffer B by using a Dounce homogenizer and stored at −70°C. The Renografin purified plasma membranes are designated R-plasma membranes.

Reconstitution. Reconstitution was carried out by the freeze-thaw-sonication (FTS) procedure (9, 16) using unsolubilized membranes and liposomes as previously described (6). The liposomes were prepared in a bath sonicator. Asolectin alone (50 mg/ml) or mixtures of asolectin (50 mg/ml) and cholesterol (12.5 mg/ml) in 10 mM KP (pH 7.5) were sonicated to clarity at room temperature as follows. The lipids were dissolved in ether in screw-cap Kimax tubes, the ether was removed with a stream of nitrogen gas, and the buffer was added to give the desired concentration; normally, samples of 500 μl are sonicated in nitrogen-flushed and sealed Kimax tubes. Reconstitution was carried out by mixing membranes and liposomes in 10 mM KP (pH 7.5) in the amounts to produce the desired lipid-to-protein ratio, frozen in an alcohol-dry ice mixture with gentle mixing, thawed at room temperature (10 to 15 min), and subjected to bath sonication for 7 s. Reconstitution was usually carried out in a final volume of 300 μl.

Sugar transport. Sugar transport was carried out in open tubes containing 1 to 10 μg of membrane protein in reconstituted vesicles diluted with 10 mM KP (pH 7.5). Uptake was initiated by the addition of an equimolar mixture of D-[^{14}C]glucose and L-[^{3}H]glucose to produce the desired final concentration; usually 20-μl samples of sugar were mixed with 100-μl samples of reconstituted vesicles at room temperature (ca. 23°C). Samples (100 μl) were removed after the desired incubation times and transferred to centrifuged Sephadex G-50 microcolumns (15) equilibrated with a stop solution consisting of 10 mM KP and 1 mM $HgCl_2$ (pH 7.5). The samples were processed as described by Baldwin et al. (1). The radioactivity in the samples from the Sephadex columns and from a 10-μl sample of the incubation mixture was measured by scintillation spectroscopy. Sugar uptake was variously calculated as nanomoles per milligram of protein, nanomoles per sample, the glucose space, or fraction of equilibrium. The last of these was determined from the sugar concentration in the incubation mixture (in nanomoles per microliter), the amount (milligrams) of lipid per sample, and the trapped volume per milligram of lipid; the last of these was found to be ca. 4 μl/mg for asolectin and ca. 6 μl/mg for asolectin-cholesterol vesicles sonicated for 7 s after the freeze-thaw step as measured by Pick (16). The glucose space was calculated as microliters per microgram of protein from the amount of glucose taken up by the vesicles (i.e., nanomoles per microgram protein) and the glucose concentration in the incubation medium (i.e., nanomoles of glucose per microliter of medium).

The use of Sephadex microcolumns was required because the amount of lipid present in samples containing a high lipid-to-protein ratio clogged the membrane filters used in our previous study (6).

Fluorescence dequenching. Fusion of yeast plasma membranes with liposomes induced by the freeze-thaw procedure was detected by fluorescence dequenching of membranes labeled with octadecylrhodamine B chloride (R18) as described by Citovsky et al. (4). Briefly, 5 ml of a 6-mg/ml ethanolic solution of R18 was rapidly injected into 400 μl of purified membranes (2 mg/ml in 10 mM KP buffer [pH 7.5]). The mixture was incubated for 15 min at room temperature in the dark and centrifuged in an Eppendorf microfuge for 2 min. The pellet was washed twice and suspended in buffer at a concentration of 2 mg/ml and stored at −70°C until used.

The labeled membranes and liposomes were either frozen and thawed together as described above or frozen and thawed separately and then mixed in the same proportions. The degree of fluorescence after thawing was measured with an MPF-4A spectrofluorometer (The Perkin-Elmer Corp., Norwalk, Conn.) (excitation at 560 nm and emission at 590 nm). Fluorescence in the presence of 0.1% Triton X-100 was taken to represent 100% dequenching.

Polyacrylamide gel electrophoresis and silver staining. The proteins of membrane preparations were analyzed by polyacrylamide gel electrophoresis by the method of Laemmli (12); the gels were silver stained by the method of Merril et al. (13).

Other procedures and materials. ATPase and protein were assayed as previously described (6). Radioactively labeled sugars D-[^{14}C]glucose and L-[^{3}H]glucose were purchased from New England Nuclear Corp., Boston, Mass. Other procedures and materials were as previously described (6).

RESULTS AND DISCUSSION

The data in Fig. 1 show the time course of D- and L-glucose uptake by vesicles containing two ratios of purified plasma membrane protein to asolectin, namely 5 and 10

μg/750 μg of lipid, representing lipid-to-protein ratios of 150:1 and 75:1, respectively. At these ratios of membrane protein to lipid, the uptakes for D-glucose show two phases: an initial, rapid phase, which is proportional to the amount of membrane protein, and a slow phase, which is independent of the amount of protein present and which occurs at the same rate as the uptake of L-glucose. We interpret the first and second phases to represent uptake into vesicles containing transporters and uptake into vesicles without transporters, respectively. Although uptake into vesicles with transporters occurs by both facilitated diffusion and simple diffusion, the rate of equilibration by facilitated diffusion is so rapid that the contribution of simple diffusion is small. In several experiments in which glucose uptake was measured in vesicles reconstituted at different protein-to-asolectin ratios, it was found that if the protein-to-lipid ratio was kept below 20 μg per mg of lipid, the glucose space when the fast phase of uptake was completed was less than the total internal volume of the vesicles. Thus at levels of membrane protein up to 20 μg per mg of lipid, not all vesicles contain transporters.

Unfortunately, unlike the glucose transporters of erythrocytes and other animal cells, the glucose transporters of yeast cells cannot be enumerated by cytochalasin B binding. In the absence of an independent means of enumerating the yeast glucose transporters, it cannot be determined whether vesicles containing transporters contain one or more transporters per vesicle. Especially when unsolubilized yeast membranes are used for the reconstitution, one might expect reconstituted vesicles to contain patches of membranes and hence to contain more than one transporter per vesicle. However, Wheeler (24) has demonstrated that the glucose transporters of erythrocyte membranes do not remain clustered when unsolubilized membranes are fused with liposomes by the FTS procedure. The number of glucose transporters in erythrocyte membranes can be determined by cytochalasin B binding; at a lipid-to-protein ratio greater than 50:1, not all vesicles contain transporters, but those that do contain only a single transporter. A similar conclusion was reached by Weber et al. (23), who also studied the reconstitution of intact erythrocyte membranes with crude soybean phosphatidylcholine liposomes by the freeze-thaw-sonication procedure. Thus the membrane proteins of

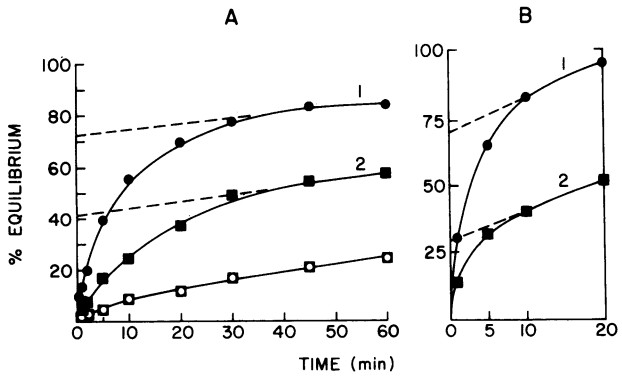

FIG. 2. Glucose uptake in vesicles as a function of lipid composition. The vesicles were prepared from 8 (A) or 10 (B) μg of protein and liposomes containing asolectin alone (■) (lipid-to-protein ratio, 150:1) or asolectin plus 50 mol% cholesterol (●) (lipid-to-protein ratio, 187.5:1). Other conditions were as described in the legend to Fig. 1; an internal volume of 5.6 μl/mg was used for asolectin plus cholesterol. Open symbols, L-glucose.

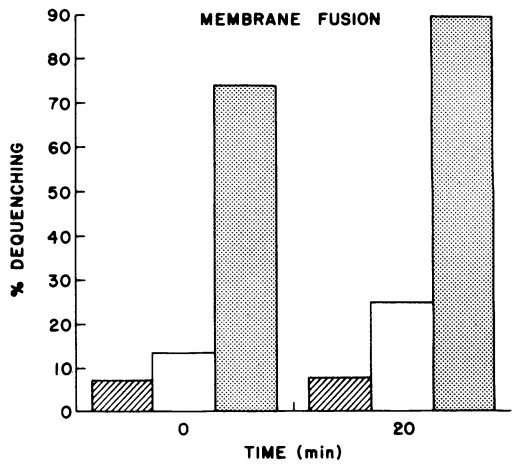

FIG. 3. Fluorescence dequenching of octadecylrhodamine B chloride (R18). Yeast plasma membranes (10 μg), labeled with R18, were subjected to FTS alone or together with asolectin liposomes (300 μg) at 37°C (lipid-to-protein ratio, 30:1). Symbols: ▨, membranes alone; ▭, membranes and asolectin liposomes mixed after separate FTS; ▩, membranes and asolectin liposomes FTS together. Time indicates length of incubation at 37°C after FTS before fluorescence measurement. Fluorescence of samples diluted in 0.01% Triton X-100 was taken as 100% dequenched.

unsolubilized erythrocyte membranes are distributed uniformly after fusion with liposomes.

If the same were true for reconstitution of the yeast glucose transporter from unsolubilized yeast cells, under conditions in which the volume into which glucose equilibrates rapidly is less than the total intravesicular volume, the majority of vesicles with transporters would contain single transporters. The number of functional transporters per milligram of membrane protein can be calculated from the fraction of vesicles containing transporters and the total number of vesicles per milligram of lipid. From the intravesicular volume of 4 μl/mg of asolectin and an estimated average vesicle diameter of 100 nm determined from negatively stained preparations (data not shown), one can calculate that there are ca. 8×10^{12} vesicles per mg of lipid. Therefore, 6×10^{12} vesicles were present in the experiment described in Fig. 1, which contained 0.75 mg of asolectin per sample. Since the percentage of vesicles containing single transporters varied between 40 and 65% for 5 and 10 μg of membrane protein, respectively, this represents about 4×10^{11} transporters per μg of membrane protein. If the molecular mass of the transporter protein is ca. 50 kilodaltons, as estimated by Kreuzfelt and Fuhrmann (10), a pure preparation of transporter would contain 1.2×10^{13} transporters per μg of membrane protein. Thus according to this estimate, the transporter represents about 3% of the plasma membrane protein.

It would be expected that the number of transporters per microgram of protein would be independent of the lipid; however, vesicles reconstituted from liposomes containing a mixture of asolectin and 50 mol% cholesterol showed a significant increase in the specific activity of sugar transport. In the experiment shown in Fig. 2, glucose uptake was measured in vesicles reconstituted from liposomes containing asolectin alone or asolectin plus 50 mol% cholesterol; 10 μg of membrane protein was added to 1,500 μg of asolectin plus 375 μg of cholesterol (lipid-to-protein ratio, 150:1). The fraction of equilibrium in vesicles formed from asolectin plus cholesterol is about twice that in vesicles containing

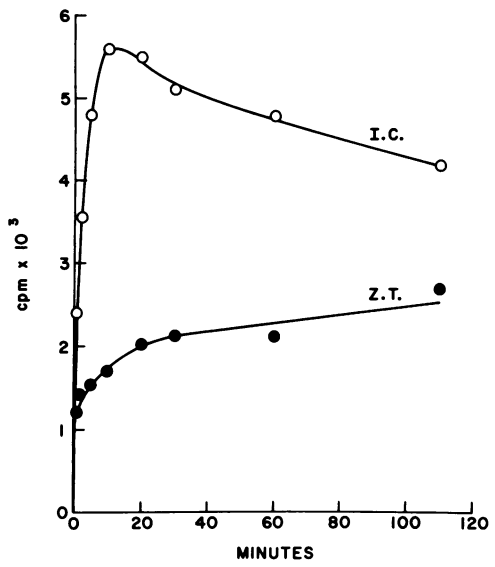

FIG. 4. Influx counterflow in vesicles reconstituted from asolectin plus 50 mol% cholesterol. Vesicles incubated for 1 h at 30°C with an equimolar mixture of D- and L-glucose (100 mM) were diluted to 5 mM sugar by addition to a mixture of carrier-free D-[^{14}C]glucose and L-[^{3}H]glucose in 10 mM KP buffer (pH 7.5). The uptakes are the averages of triplicate samples containing 1.25 μg of protein per 187.5 μg of asolectin and 46.9 μg of cholesterol (lipid-to-protein ratio, 187.5:1) removed at the times indicated. Abbreviations: I.C., uptake by influx counterflow into vesicles after equilibration; Z.T., zero *trans* uptake into vesicles without prior equilibration.

asolectin alone. Thus the estimate of the number of transporters per microgram of membrane protein based on reconstitution with asolectin-cholesterol liposomes is twice as large as that from reconstitution with liposomes containing asolectin alone. The simplest explanations for this difference is either that only a fraction of the transporters are active in the asolectin or that more than one transporter is present per vesicle in asolectin-containing vesicles. The latter seems unlikely, since the relative rate of equilibration in the asolectin-containing vesicles is no greater than in asolectin-cholesterol vesicles (Fig. 2A and B). At present the former explanation seems the most plausible, although no reason can be offered.

Evidence that the vesicles reconstituted from asolectin liposomes do not contain patches of yeast membrane comes from the fluorescence-dequenching measurements. Fluorescence-dequenching methods were developed to study fusion between enveloped viruses and the cell membranes of host cells (4). In a separate study, using the fluorescence-dequenching technique, we demonstrated that the freeze-thaw procedure results in the dilution of *Mycoplasma* membrane lipids by asolectin liposome lipids (V. P. Cirillo, A. Katzenell, and S. Rottem, Isr. J. Med. Sci., in press). In a similar manner, the data presented in Fig. 3 show that freezing and thawing yeast membranes containing quenched R18 resulted in an immediate and almost complete dequenching. That the dequenching resulted from fusion and subsequent dilution of the R18 is demonstrated by the relatively low level of dequenching seen when the quenched membranes and liposomes were mixed after being frozen and thawed separately. The increase in dequenching in the control experiment after 15 min of incubation at 37°C is a measure either of the exchange of the label between the membranes and the liposomes or of a slow fusion process which occurs without freezing and thawing. Almost no fluorescence dequenching was observed when labeled membranes were frozen and thawed alone.

The data in Fig. 4 show the results of an influx counterflow (6) experiment in vesicles reconstituted from liposomes containing asolectin plus cholesterol. The data show that this important transporter function is observed in vesicles containing cholesterol.

We have been able to increase the specific activity of reconstituted vesicles by treating the membranes in different ways prior to reconstitution. For example, the specific activity of vesicles reconstituted from purified membranes washed with 1% cholate was reproducibly increased about twofold (A. J. Franzusoff and V. P. Cirillo, data not shown). However, of the various procedures used, fractionation of the plasma membranes on a Renografin gradient produced the most significant effect. The specific activities of the vesicles reconstituted from the fractions from a Renografin gradient are presented in Fig. 5. The enrichment of trans-

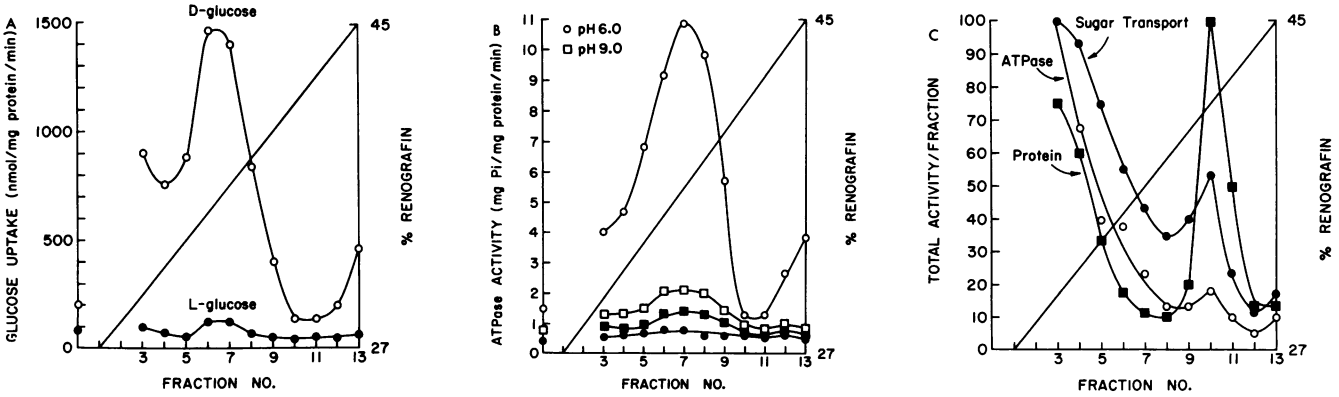

FIG. 5. Glucose transport and ATPase activity in fractions from a linear 27 to 45% Renografin gradient prepared in 10 mM KP buffer (pH 7.5). Each fraction was diluted 15-fold with KP buffer, pelleted by centrifugation for 40 min at 140,00 × *g*, and assayed. (A) Glucose transport specific activity. Membrane protein (10 μg) from each fraction was reconstituted with liposomes containing 1,500 μg of asolectin and 375 μg of cholesterol (lipid-to-protein ratio, 187.5:1). Glucose uptake was measured for 1 min at 30°C at 5mM. (B) ATPase specific activity. Membrane protein (10 μg) was assayed for ATPase activity at pH 6 (plasma membrane) and pH 9 (mitochondria). Inhibition by sodium vanadate (●) was measured at pH 6, and inhibition by oligomycin was measured at pH 9 (■). (C) The total amount of protein and activity per fraction. The fraction with the highest activity is set at 100.

porter activity in the fractions from the top of the gradient is quite dramatic, increasing both the specific activity of D-glucose uptake and the discrimination between D- and L-glucose. The plasma membrane ATPase activity (i.e., vanadate-sensitive pH 6 activity) (17) is enriched in parallel with transporter activity (Fig. 5). (The low activity of oligomycin-sensitive pH 9 activity of the original purified membranes and the Renografin fractions attests to the effective elimination of mitochondria by the pH 5 step in the purification of the plasma membranes.) The data on the total transporter activity, ATPase activity, and protein per fraction are shown in Fig. 5. The enrichment of transporters and ATPase in fractions from the top of the gradient is the result of the removal of inactive protein which sinks to the bottom of the gradient; the relative amounts of active and inactive protein varies widely from preparation to preparation. It should be noted that although most of the transporter activity is at the top of the gradient (Fig. 5C), the highest specific activity is in fraction 7 (Fig. 5A). This fraction may represent a distinct membrane subfraction. The existence of distinct subfractions of yeast membranes on Renografin gradients has been reported by Tschopp and Schekman (20). On the basis of the sedimentation characteristics seen in Fig. 5, subsequent fractionations involved the use of a shallow 10-ml linear gradient prepared from 5 ml each of 36 and 37.8% Renografin. On such a gradient, the transporter and ATPase-enriched fractions do not enter the gradient and are separated from the inactive material, which enters the gradient. The transporter activity of the membranes isolated from the top of the gradient may be enriched about 10-fold (data not shown).

When SDS-polyacrylamide gel electrophoresis gels from fractions across either the steep or shallow gradient were examined, a number of bands showed a change in intensity (by silver staining) which paralleled the changes in transporter and ATPase activity. The gel patterns for the three fractions obtained in the shallow Renografin gradient and their transporter activities are presented in Fig. 6. A number of bands are enriched in fraction 1, which is enriched for the glucose transporter and the ATPase (arrows in Fig. 6). The band with an apparent M_r 100,000 has been shown, by immunoblotting with antibodies against the *Neurospora* ATPase, to contain the plasma membrane ATPase (data not shown). It may also contain a glucose transporter, on the basis of the recent observations of Bisson et al. (2a) indicating that a protein of this molecular weight may contain a transporter. It is also tempting to guess that the bands with an apparent M_r ca. 50,000 or 60,000 might be the transporter because their mobility in SDS-gels is similar to that of the protein identified by Kreuzfelt and Fuhrmann (10) in their study on affinity labeling of the yeast glucose transporter and to that of the human (8a) and rat (14) glucose transporters. Efforts are now under way to purify these bands and to test their transporter activity in reconstituted proteoliposomes. Whether the yeast and mammalian glucose transporters are similar or dissimilar in size, the yeast transporter is both pharmacologically and antigenically different from that of

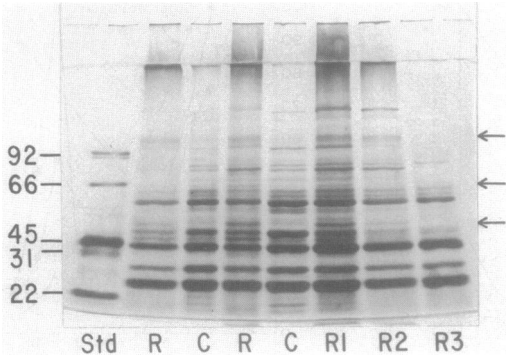

FIG. 6. SDS-polyacrylamide gel electrophoresis gels of fractions from Renografin gradient. Membrane protein (10 μg) was subjected to SDS-polyacrylamide gel electrophoresis and silver stained. Abbreviations: C, crude membrane; R, Renografin fraction 3 from the gradient shown in Fig. 5; R1, R2, and R3, fractions from the top, body, and pellet of a linear 36 to 37.8% Renografin gradient; Std, molecular weight standards. The arrows on the right identify bands which are enriched in R1. See text for discussion.

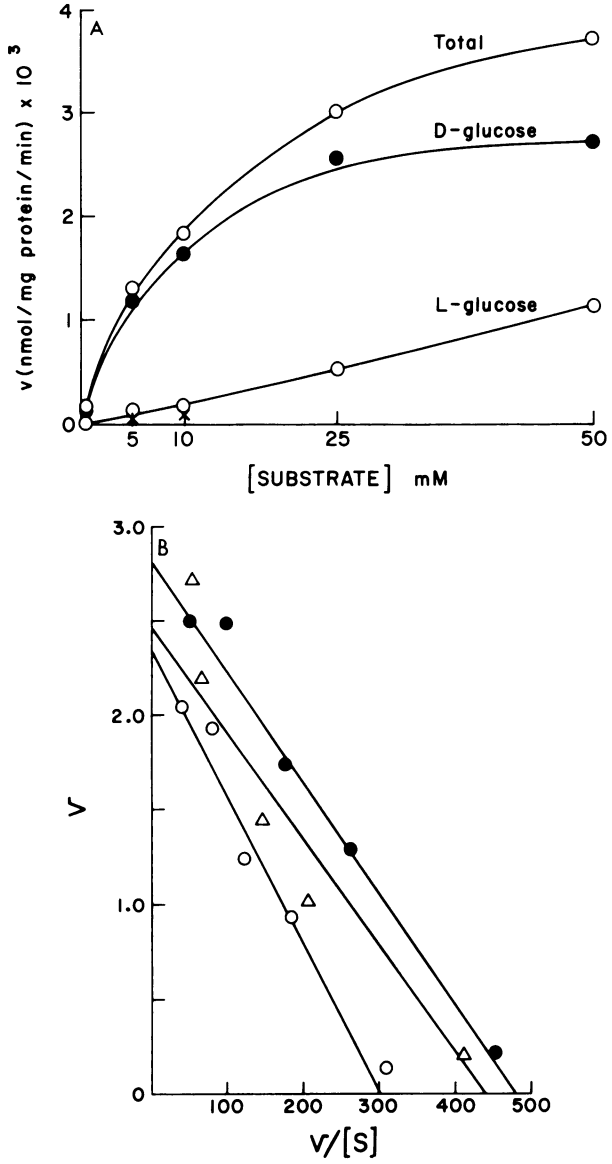

FIG. 7. Kinetics of glucose transport. D- and L-Glucose uptake was measured in vesicles prepared from asolectin plus 50 mol% cholesterol liposomes. Triplicate samples each contained 10 μg of membrane protein, 1,500 μg of asolectin, and 375 μg of cholesterol (lipid-to-protein ratio, 187.5:1). Uptake was measured for 1 min at 30°C. (A) Michaelis plot. (B) Eadie Hofstee plot of data from panel A (●) and two other experiments (○, △).

the mammalian transporter. The yeast glucose transport system is not inhibited by phlorizin, phloretin, or cytochalasin B (unpublished results), and antibodies against the human erythrocyte transporter do not cross-react with components of the yeast plasma membrane (S. Cushman, personal communication).

By using Renografin-purified membranes isolated from the top of the shallow gradients, vesicles were prepared from asolectin plus 50 mol% cholesterol at a lipid-to-protein ratio of 187.5:1 and used in experiments to determine the V_{max} and K_m for transport. The data from these experiments are presented in Fig. 7. Figure 7A presents the results of a representative experiment in which uptake rates were determined from 1-min uptakes of D- and L-glucose at concentrations from 0.5 to 50 mM. Figure 7B presents an Eadie-Hofstee plot of the data of Fig. 7A, as well as those of two other experiments. The V_{max} is 2.8 ± 1 nmol/mg of protein per min, and the K_m is ca. 8 mM. The K_m value is closer to that of the high apparent K_m (10 to 50 mM) than to that of the low apparent K_m (0.5 to 1 mM) process for whole cells. Thus the reconstituted vesicles seem to carry out the unregulated, high apparent K_m process which is observed in wild-type cells depleted of ATP or in mutants lacking cognate kinases for glucose (2, 18, 22). Guided by the assumption that both the high- and low apparent K_m processes of glucose transport are carried out by the same transporter in different states, we tested the effect of trapping either sugar phosphates or yeast hexokinase plus ATP inside the vesicles on the apparent K_m of glucose uptake by reconstituted vesicles. (Trapping was accomplished by including the material in the buffer used in the FTS step; the untrapped material was removed by passing the vesicles over gel filtration columns.) So far, none of the solutes or proteins which we have trapped inside the vesicles have had any demonstrable effect on the kinetics of sugar transport.

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