Biochimica et Biophysica Acta, 775 (1984) 121–128
Elsevier

BBA 72166

PARTIAL CHARACTERIZATION OF THE GLUCOSE TRANSPORT ACTIVITY IN THE GOLGI-RICH FRACTION OF FAT CELLS

MELINDA M. SMITH, FRANCES W. ROBINSON, TOMOYUKI WATANABE and TETSURO KONO *

Department of Physiology, School of Medicine, Vanderbilt University, Nashville, TN 37232 (U.S.A.)

(Received December 21st, 1983)
(Revised manuscript received April 16th, 1984)

Key words: Glucose transport; Insulin action; Plasma membrane; Golgi fraction; Liposome; (Rat fat cell)

The glucose transport activity solubilized from the basal and plus insulin forms of the Golgi-rich fraction of adipocytes was partially characterized, and the results were compared with those of the activity obtained from the plus insulin form of the plasma membrane-rich fraction. The transport activity was determined in a cell-free, reconstituted, system. Prior to reconstitution, the activities in the three preparations were all (a) stable at 0°C for at least 4 h, but not at 37°C or above; (b) most stable at pH 7–9, and (c) less stable in Tes than in Tris buffer. After reconstitution, the three activities were all (d) stable at 0°C, (e) most active at pH 5.5, (f) mildly stimulated by divalent cations, (g) unaffected by insulin or 1 mM of several SH-blocking agents, (h) inhibited by heavy metal ions, 10–100 mM of monovalent salts, organic solvents, several sugar isomers, and specific sugar-transport inhibitors. The rates of D-glucose uptake by the three liposome preparations were all inhibited more strongly by 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose than by D-glucose. These data indicate that the general properties of the glucose transport activity in the Golgi-rich fraction are similar to those of the activity in the plasma membrane-rich fraction.

Introduction

It was recently discovered that glucose transport activity in adipocytes is associated not only with the plasma membrane but also with a certain other subcellular structure, which is fractionated by centrifugation into a low-density (Golgi-rich) microsomal fraction. Suzuki and Kono [1] and Cushman and Wardzala [2] theorized (a) that only the activity located in the plasma membrane is physiologically functional while that in the Golgi-rich fraction is in reserve, and (b) that the latter activity is translocated to the plasma membrane when cells are stimulated by insulin. Subsequently, Kono et al. [3,4] and Karnieli et al. [5] suggested that the proposed translocation is reversible; i.e., the glucose transport mechanism in adipocytes may be recycled between the plasma membrane and an intracellular storage site. On the other hand, Carter-Su and Czech [6] proposed that insulin may stimulate the glucose transport activity in the plasma membrane without changing the activity in the low-density microsomal fraction. In addition, Simpson et al. [7] suggested that the glucose transport activity in the low-density fraction is inactive, and that insulin induces both translocation and activation of the transport mechanism.

If the glucose transport activity in the plasma membrane-rich fraction and that in the Golgi-rich

* To whom all correspondence should be addressed.

Abbreviations: Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Mes, 2-(*N*-morpholino)ethanesulfonic acid; DIDS, 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene.

fraction are distinct, or if the proposed translocation is accompanied by chemical modification of the transport mechanism, certain differences might be detectable in the characteristics of the activity in the two subcellular fractions.

Previously, glucose transport activity in the isolated plasma membrane of adipocytes was partially characterized by Carter et al. [8] and Ludvigsen and Jarett [9,10]; however, little is known on the nature of the transport activity in the low-density (Golgi-rich) fraction. Carter et al. [8] and Ludvigsen and Jarett [9,10] carried out their characterization studies using resealed vesicles of the isolated plasma membrane. However, our preliminary attempts to prepare resealed vesicles of the low-density microsomes with functional glucose transport activity have been unsuccessful (Kono, T., unpublished data). Therefore, in our present study, we solubilized glucose transport activity from both plasma membrane-rich and Golgi-rich fractions, reconstituted the activity into crude egg phospholipid liposomes, and compared the characteristics of the activity from the two subcellular fractions.

Materials and Methods

For this study, we purchased cytochalasin B from Aldrich, phloretin and phlorizin from ICN Pharmaceutical, Tes and Mes from Calbiochem-Behring, and 4,4′-diisothiocyano-2,2′-disulfonic acid stilbene (DIDS) from Sigma. The sources of other materials were listed in our previous publications [3,4].

Isolated fat cells were prepared from epididymal adipose tissue of Sprague-Dawley rats (180–230 g) by the collagenase method [11]. Unless otherwise stated, freshly prepared cells were kept at 37 °C for 30 min, then treated with or without insulin for 10 min, washed, and homogenized, as previously described [3,4]. The cell homogenate was fractionated by modification of our previous method (Method B [4]). The modified method (referred to as Method C) employed discontinuous, rather than continuous, sucrose density gradient centrifugation; as a result, the fractionation was rendered considerably simpler and less time-consuming than the previous one. In brief, cells from 2 to 6 rats were homogenized with 10 ml of Buffer A (0.25 M sucrose containing 1 mM sodium EDTA (pH 7.5) and 10 mM Tris-HCl (pH 7.5)). The homogenate was centrifuged in a Beckman JA-20 rotor for 2 min at a maximum speed of 7000 rpm ($6000 \times g$max). The time for centrifugation includes the acceleration period but not the deceleration time, all the centrifugations were carried out at 2 °C. The infranatant solution was withdrawn from under the fat layer and centrifuged for 30 min at 14000 rpm ($23500 \times g$max). The supernatant solution (S-2) was saved for preparation of the Golgi-rich fraction, while the pellet was suspended in 5 ml of Buffer A, homogenized in a Dounce tissue grinder (2 strokes), and placed in a 15 ml Corex test tube. To the bottom of this test tube was gently added 2 ml of 15.0% (w/w) sucrose solution followed by 3 ml of 32.5% (w/w) sucrose solution; both of these sucrose solutions contained 1 mM sodium EDTA and 10 mM Tris-HCl (pH 7.5). The test tube was then centrifuged for 30 min at 13000 rpm ($26860 \times g$max) in a Beckman JS-13 (swinging bucket) rotor. The band that appeared at the interface between 15.0 and 32.5 percent sucrose solutions was withdrawn along with approx. 2 ml of the sucrose solution, and the syringe was rinsed with 1 ml of Buffer A. The rinse and the main solution were mixed together, diluted with 2 ml of 1 mM sodium EDTA buffered with 10 mM Tris-HCl (pH 7.5), and centrifuged for 30 min at 20000 rpm ($48000 \times g$max). The resultant pellet was referred to as the plasma membrane-rich fraction. The supernatant solution (S-2) that was saved at an earlier step was centrifuged for 60 min at 40000 rpm ($150000 \times g$max) in a Beckman 75 Ti rotor. The pellet obtained was referred to as the Golgi-rich fraction. The glucose transport activities in the plasma membrane-rich and Golgi-rich fractions were separately dissolved in 0.22 ml of 20 mM sodium cholate in 10 mM Tris-HCl (pH 7.5), and stored at −70 °C for 1 to 7 days prior to use. The solubilized glucose transport activity was reconstituted into crude egg phospholipid liposomes and assayed, basically as described previously [4,12]. However, certain modifications were made as needed and as specified in the figure legends. In all cases, however, the final volume of the incubation mixture was 25 μl, the final crude egg phospholipid concentration was 20 μg/ml; and the

final (substrate) glucose concentration was 1 mM. The final protein concentration was usually 200–400 μg/ml, depending on the initial protein concentration and the subsequent dilution.

Unless otherwise stated, the carrier-mediated D-glucose transport activity (simply referred to as 'glucose transport activity') was estimated from the difference in the amounts of D-[^{3}H]glucose and L-[^{14}C]glucose taken up by the reconstituted liposomes during a 10-s incubation at 37 °C, as previously described [3,4]. In certain experiments, however, it was necessary to measure the rate of transport of sugar isomers that were contaminated with permeable radioactivity (presumably tritiated water). On such occasions, the mediated transport activity was estimated from the difference in the rates of uptake of the sugar isomers in the presence or absence of 20 μM cytochalasin B. As reported previously [3,4], the results obtained by the two methods were identical.

Protein concentration was determined by the method of Bradford [13]; the standard used was crystalline bovine serum albumin. The 5′-nucleotidase activity was assayed by the method of Avruch and Wallach [14], and UDPGal:*N*-acetylglucosamine galactosyltransferase was determined by the method of Fleischer [15] as described previously [3]. Succinate-cytochrome *c* reductase was assayed from the change in the absorbance at 550 nm [16].

As is well known, metabolic activities of fat cells are slightly, but significantly, different from batch to batch of cell preparations. Therefore, if possible, the data to be compared were obtained from aliquots of a pooled cell suspension. When the data of more than one set of experiments were to be compared, we included one or more common controls in each set and made certain that all the control values were in good agreement. In addition, all the results reported in this communication were confirmed by carrying out identical experiments on different occasions using at least one other batch of adipocytes.

Results

Levels of cross contamination of the two subcellular fractions associated with glucose transport activity

As shown in Fig. 1, the fractions referred to as

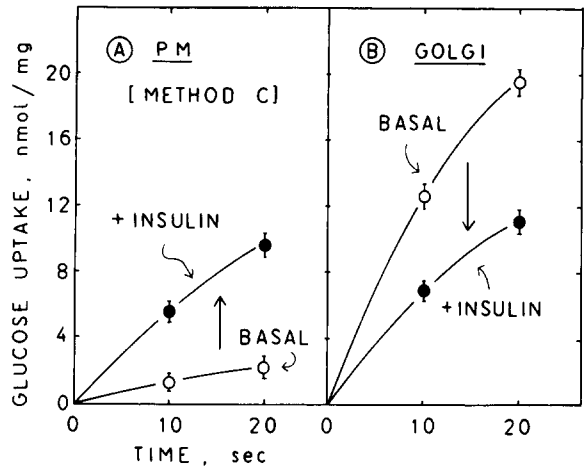

Fig. 1. Time-course of glucose uptake by reconstituted liposomes. The plasma mebrane-rich fraction (PM) and the Golgi-rich fraction (Golgi) were prepared by Method C (see Materials and Methods) from the basal and plus insulin forms of fat cells. The glucose transport activity was solubilized and reconstituted into crude egg phospholipid liposomes. The carrier-mediated uptake of D-glucose by the reconstituted liposomes (glucose uptake) was measured at two time points as previously described [3]. The figure presents a typical set of data obtained with aliquots of a pooled cell preparation. Each point shows the mean value and the standard error of three observations made in three subfractions divided just before the step of reconstitution.

plasma membrane-rich and Golgi-rich fractions contained glucose transport activity. According to the data in Table I, the plasma membrane-rich fraction was enriched in 5′-nucleotidase (7.6-fold), while the Golgi-rich fraction showed a higher activity of UDPGal:*N*-acetylglucosamine galactosyltransferase (6.8-fold). Of these two enzymes, 5′-nucleotidase appears to be localized almost exclusively in the plasma membrane in adipocytes [3,4]. Therefore, the level of contamination of the Golgi-rich fraction by the plasma membrane-rich fraction may be calculated to be approx. 10% from the distribution of 5′-nucleotidase in the two fractions ($4.43 \times 100/(38.6 + 4.43) = 10.3\%$). On the other hand, the galactosyltransferase, which is a specific Golgi marker in the liver [15], appears to be associaed not only with the Golgi apparatus but also with the plasma membrane in fat cells [3,4]. Therefore, it may be expected that the contamination of the plasma membrane-rich fraction by the Golgi-rich fraction was less than the value sug-

TABLE I

RESULTS OF FRACTIONATION

The fat cell homogenate was fractionated as described in Materials and Methods. The data show the mean values and standard errors of three separate experiments.

Component	Fat-free cell homogenate	Plasma membrane-rich fraction	Golgi-rich fraction
Protein total (μg)	4344 ±32	221 ±12	258 ±15
5′-Nucleotidase			
Total (nmol/min)	27.51 ± 1.81	10.63 ± 0.22	1.22 ± 0.27
Distribution (%)	100.0	38.6	4.43
Specific activity (nmol/min/mg)	6.33	48.1	4.73
Enrichment	1.00	7.60	0.75
UDPGal: *N*-acetylglucosamine galactosyltransferase			
Total (pmol/min)	168.0 ±25.2	14.5 ± 5.2	67.9 ± 5.9
Distribution (%)	100.0	8.63	40.4
Specific activity (pmol/min/mg)	38.7	65.6	263.2
Enrichment	1.00	1.70	6.81
Succinate-cytochrome *c* reductase			
Total (nmol/min)	25.45 ± 2.74	1.98 ± 0.26	0.20 ± 0.00
Distribution (%)	100.0	7.78	0.78
Specific activity (nmol/min/mg)	5.85	8.96	0.78
Enrichment	1.00	1.53	0.13

gested by the distribution of the transferase activity ($8.63 \times 100/(8.63 + 40.4) = 17.6\%$). This view was also supported by the transport data described below.

Fig. 1 shows the time-courses of D-glucose uptake by crude egg phospholipid liposomes reconstituted with glucose transport activity solubilized from subcellular fractions of adipocytes. According to the data, insulin (added to cells prior to homogenization) increased the glucose transport activity in the plasma membrane-rich fraction while decreasing the activity in the Golgi-rich fraction. This observation is consistent with our previous data [1,3,4]. The apparent basal activity of the plasma membrane-rich fraction measured at 10 s of incubation was approx. 1 nmol/mg protein, and that of the Golgi-rich fraction was about 12.5 nmol/mg. It would appear, therefore, that the former fraction was not contaminated by any more than 7% of the latter ($(1 \times 0.221) \times 100/(12.5 \times 0.258) = 6.9\%$; the protein data were obtained from Table I).

Based on the considerations presented above, we suggest that the two subcellular fractions used in our present study (see below) were cross contaminated by each other approx. 10% or less.

Characterization of glucose transport activities solubilized from the Golgi-rich and plasma membrane-rich fractions

In the following presentation, we first describe the characteristics of glucose transport activity solubilized from the basal form of the Golgi-rich fraction, and then consider whether they were significantly different from those of the transport activity solubilized from the plus insulin form of the Golgi-rich or plasma membrane-rich fraction. The basal form of the plasma membrane-rich fraction was excluded from our present studies simply because its activity was too weak to be measured reliably (see Fig. 1).

In the first set of experiments reported in Fig. 2, we studied the heat stability of the reconstituted glucose transport activity. The activity obtained from the basal form of the Golgi-rich fraction was stable at 0 °C for at least 4 h. Unexpectedly, the activity was increased at 37 °C between 0.5 and 3 h of incubation; however, no such stimulation was

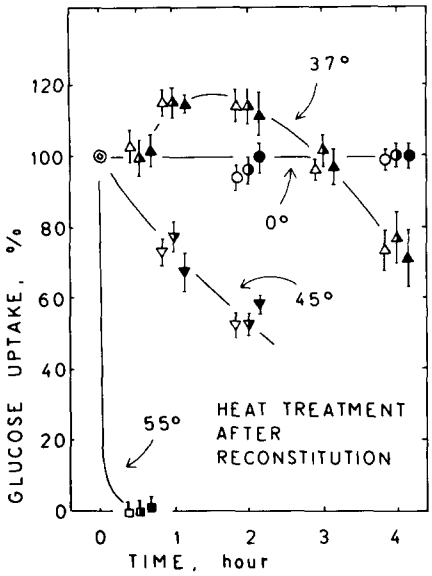

Fig. 2. Heat resistance of reconstituted glucose transport activity. The reconstituted glucose transport activity was exposed to the designated temperatures for the indicated periods prior to the transport assay, which was carried out at 37 °C. Open symbols represent the basal form of the Golgi-rich fraction; half-closed symbols, the plus insulin form of the Golgi-rich fraction; and closed symbols, the plus insulin form of the plasma membrane-rich fraction. Each point in the figure shows the mean value and the standard error ($n = 3$ or 4). For the sake of clarity, crowded points are shown slightly shifted horizontally.

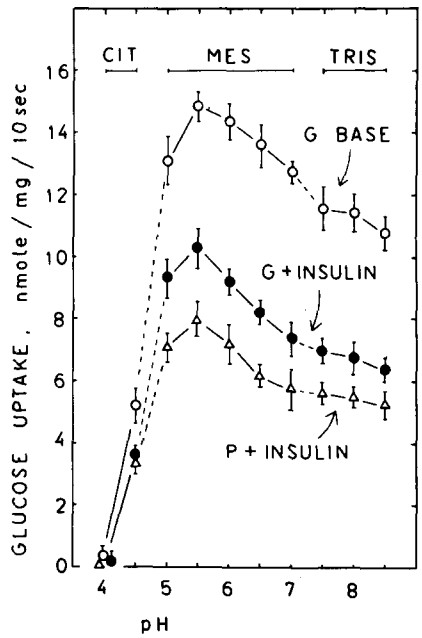

Fig. 3. Effects of pH on the reconstituted glucose transport activity. The glucose transport activity in 10 mM NaCl was reconstituted into crude egg phospholipid liposomes. The reconstituted liposomes in 10 mM NaCl were mixed with the indicated buffers (10 mM in final concentration), warmed up to 37 °C, and (approx. 4 min later) supplemented with labeled glucose in water for the transport assay. Each point shows the mean value and the standard error ($n = 6$–12). CIT stands for citrate; G for the Golgi-rich fraction, and P for the plasma membrane-rich fraction.

observed at 45 or 55 °C. The data in Fig. 2 also show that no significant difference was detectable among the characteristics of the aforementioned three preparations of glucose transport activity. Although the data are not presented, unreconstituted glucose transport activity (i.e., the activity solubilized from the basal Golgi-rich fraction into 20 mM sodium cholate solution buffered with 10 mM Tris-HCl (pH 7.5)) was also stable at 0 °C for at least 4 h. Unlike the reconstituted activity, however, the solubilized activity was gradually denatured at 37 °C and lost approx. 40% of its potency in 4 h. So far as tested (at 0, 37, and 45 °C), no significant difference was discovered among the heat stabilities of the three preparations of solubilized glucose transport activity (data not shown).

As reported in Fig. 3, the glucose transport activity reconstituted from the basal form of the Golgi-rich fraction was most active at pH 5.5 in Mes buffer. In contrast, the solubilized activity (in 20 mM sodium cholate solution containing 10 mM of various buffers) was considerably more stable at pH 7–9 than at pH 5–7 (tested at 37 °C, data not shown). When incubated for 2 h at 37 °C, the activity solubilized from the basal Golgi preparation retained approximately twice as much activity in 10 mM Tris-HCl than in 10 mM Tes buffer, at pH 7–8 (data not shown). In all these experiments, no significant difference was detectable in the properties of the three preparations of glucose transport activity (data not shown).

In the next series of experiments, we studied effects of 21 agents at different concentrations on the reconstituted glucose transport activity. Although the individual data are not presented for brevity, the glucose transport activity solubilized and reconstituted from the basal form of the

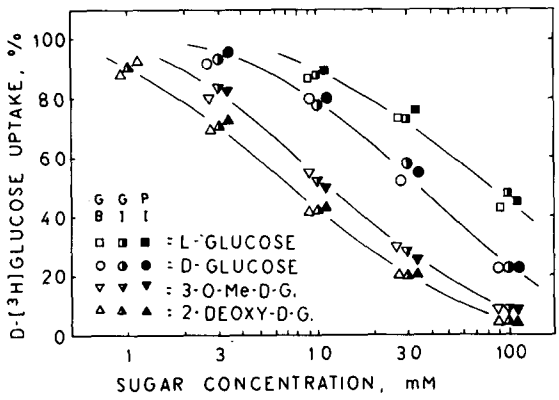

Fig. 4. Effects of hexose isomers on the rate of D-[^{3}H]glucose uptake by reconstituted liposomes. Reconstituted liposomes were mixed with the designated hexose isomers. Each preparation was incubated for approx. 4 min at 37 °C before the addition of labeled glucose for the transport assay; no carrier glucose was added in this test. No adjustment was made for changes in the osmotic pressure caused by the addition of hexose isomers. Each point shows the mean value (n = 3 or 4). For the sake of clarity, no standard errors are presented; crowded points are slightly shifted horizontally. 3-O-Me-D-G, 3-*O*-methyl-D-glucose; and 2-deoxy-D-G, 2-deoxy-D-glucose.

Golgi-rich fraction was: (a) mildly (5–15%) stimulated by 1–5 mM of divalent cations such as Mn^{2+}, Mg^{2+}, or Ca^{2+}, (b) considerably inhibited by salts of monovalent cations at 10–100 mM (e.g., 63–67% inhibition by 100 mM NaCl, KCl, or Tris-HCl), (c) inhibited by salts of heavy metal ions (Pb^{2+}, Cu^{2+}, or Hg^{2+}), especially by mercuric chloride (70–80% at 1 mM), (3) not significantly affected by 1 mM of SH-modifiers (*N*-ethylmaleimide and iodoacetamide) including redox agents (hydrogen peroxide and dithiothreitol), except for *p*-chloromercuriphenyl sulfonate (44–47% inhibition at 1 mM), (e) inhibited almost completely by 20 μM cytochalasin B, more than 90% by 1 mM phloretin, and approx. 55% by 1 mM phlorizin, (f) unaffected by 20 μl DIDS (an inhibitor of anion transport) or 2 nM insulin (added to the reconstituted liposomes) and (g) inhibited approx. 10% by 2% ethanol and about 20–30% by 2% dimethylsulfoxide. Under all these conditions, no significant difference was observed in the characteristics of the three preparations of glucose transport activity.

In the next set of experiments summarized in Fig. 4, we studied effects of hexose isomers on the uptake of a tracer quantity of D-[^{14}C]glucose by reconstituted liposomes. The data show that the uptake of labeled D-glucose was inhibited more strongly by 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose than by unlabeled D-glucose at the same concentration. Unexpectedly, the uptake of labeled D-glucose was considerably inhibited by 30–100 mM L-glucose. The apparent K_i value of L-glucose estimated from the hexose concentration at 50% of inhibition was approx. 80 mM, which was only 2.5-times as large as that of D-glucose (32 mM, also estimated from the figure). Because of this observation, we next examined the rates of uptake

TABLE II

THE RATES OF UPTAKE OF CERTAIN SUGAR ISOMERS BY RECONSTITUTED LIPOSOMES

Glucose transport activity was solubilized from the indicated fractions and reconstituted into crude egg phospholipid liposomes. The reconstituted liposomes were incubated for 10 s at 37 °C with the indicated sugar isomers (all at 1 mM in final concentration) either in the presence or absence of 20 μM cytochalasin B, and the difference in the amounts of uptake was assumed to indicate the carrier-mediated transport activity, as in some of our previous experiments [1,3,4]. The data show the mean value ± S.E. (n = 9–12).

Hexose	Transport activity					
	Basal Golgi-rich fraction		Plus insulin Golgi-rich fraction		Plus insulin Plasma membrane-rich fraction	
	nmol/mg/10s	%	nmol/mg/10s	%	nmol/mg/10s	%
D-[^{3}H]Glucose	14.41 ± 0.78	100	8.01 ± 0.13	100	5.77 ± 0.11	100
3-*O*-[^{3}H]Methyl-D-glucose	17.16 ± 0.56	119	10.00 ± 0.26	125	7.04 ± 0.39	122
2-Deoxy-D-[^{3}H]glucose	17.34 ± 0.81	120	9.65 ± 0.23	121	6.78 ± 0.34	118
L-[^{14}C]Glucose	0.19 ± 0.35	1	0.23 ± 0.07	3	0.07 ± 0.21	1

of several sugar isomers by reconstituted liposomes. The results summarized in Table II indicate that the apparent rates of transport of 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose were approx. 120% of that of D-glucose while that of L-glucose was only 1–3%. Again, no significant difference was detectable in the properties of the three preparations of glucose transport activity in any of the experiments reported in Fig. 4 and Table II.

Discussion

So far as tested in our present study, the characteristics of the glucose transport activity solubilized from the basal form of the Golgi-rich fraction appear to be identical to those of the activity obtained from the plus insulin form of either the Golgi-rich or the plasma membrane-rich fraction. This finding is consistent with the translocation hypothesis of insulin action [1,2]. According to the hypothesis, insulin increases glucose transport activity in intact adipocytes by causing relocation of the glucose transport mechanism from an intracellular storage site to the plasma membrane. It should be noted, however, that the data obtained in our present study are all 'negative observations' and do not necessarily prove that the transport activities from the two subcellular fractions are identical. Also, the data do not exclude the possibility that certain properties of the glucose transport mechanism might have been altered by solubilization and reconstitution. Therefore, the final identification of the activities must be carried out without solubilization by certain non-kinetic methods, such as immunochemical analyses, after the activities are sufficiently purified.

The characteristics of glucose transport activity observed in our present work in the reconstituted liposome system are similar to those described by others in their studies with resealed vesicles of the plasma membrane. Thus, in agreement with our present study, it was previously reported that glucose transport activity in resealed vesicles of the plasma membrane of rat adipocytes was: (a) mildly stimulated by Ca^{2+} [10], (b) not significantly affected by 1 mM *N*-ethylmaleimide [10], and (c) either considerably or strongly inhibited by 1 mM phlorizin [8], 50 mM 2-deoxy-D-glucose [8], 50 mM 3-*O*-methyl-D-glucose [8], 10 μM cytochalasin B [10], and 150 mM of NaCl or KCl [8]. As a possible explanation of the last observation, Carter et al. [8] suggested that NaCl or KCl at a high concentration might cause an aggregation of lipid vesicles. In apparent disagreement with our present data, however, Carter et al. [8] reported that 50 mM L-glucose had no significant effect on the uptake of D-glucose by the resealed plasma membrane vesicles. In our liposome experiments, 50 mM L-glucose was inhibitory (Fig. 4) although the rate of transport of this sugar isomer was considerably slower than that of D-glucose (Table II). Ludvigsen and Jarett [10] reported that the optimum pH for the uptake of glucose by the resealed vesicles was 7.4. Since the value recorded in our present study is fairly low (pH 5.5, see Fig. 3), the optimum pH might have been shifted as a result of reconstitution of the transport activity into crude egg phospholipid liposomes. Both Carter et al. [8] and Ludvigsen and Jarett [10] reported that the apparent glucose transport in resealed vesicles of the plasma membrane was considerably impaired by 10–20 mM *N*-ethylmaleimide. However, these observations should be interpreted with caution since we have found previously that adipocytes were rendered 'leaky' by the agent at 10 mM (Kono, T., unpublished data).

In agreement with our present data (Fig. 4), the glucose transport mechanism in intact adipocytes is known to have a greater affinity for 3-*O*-methyl-D-glucose (which is nonphysiological) than for D-glucose [17,18]. However, the dissociation constants estimated in our present study are significantly larger than those previously determined in the whole cell experiments. Thus, the K_i values estimated from the data in Fig. 4 for 3-*O*-methyl-D-glucose and D-glucose are 10 and 32 mM, respectively, while the corresponding values determined in the whole cell system by Loten et al. [17] are 3.4 and 14.8 mM, respectively, and those measured by Pederson and Gliemann [18] are 3.8 and 7.7 mM. This discrepancy might have been caused by the fact that the transport activity estimated in our present study did not show the exact initial rate of reaction. As seen in Fig. 1, the time-course of glucose uptake by the reconstituted liposomes is curvilinear. In this connection, Ludvigsen and Jarett reported that the K_m value of D-glucose uptake by resealed vesicles of the

plasma membrane was 9 mM in one set of experiments [9] and 26 mM in another [10].

It was found in our present study that the reconstituted glucose transport activity is transiently stimulated at 37 °C (Fig. 2). For lack of any other reasonable explanation, we suggest that the stimulation is caused by a certain change in the (morphological?) nature of liposomes. If unchecked, this transient stimulation would introduce a serious error in the estimation of glucose transport activity. We have been avoiding this problem by keeping the reconstituted liposomes in ice until shortly before the transport assay [3,4]. The cause of the toxic effect of Tes buffer (data not shown) is also unknown. However, use of this buffer should be avoided in the solubilization, purification, and reconstitution of glucose transport activity from adipocytes. The injurious effects of organic solvents on the reconstituted glucose transport activity (data not shown) might be secondary, at least in part, to their effects on liposomes; however, ethanol (> 2%) is a known inhibitor of glucose transport in adipocytes [19]. The observation that insulin does not stimulate glucose transport activity in reconstituted liposomes (data not shown) is consistent with the view that the action of the hormone on glucose transport may be indirect and may involve certain intermediate reactions [20].

Acknowledgments

We are thankful to Dr. Charles R. Park and Dr. Sidney P. Colowick for their continuous interest in our study. This work was supported by United States Public Health Service Grant 5R01 AM 19925 from the National Institutes of Health.

References

1 Suzuki, K. and Kono, T. (1980) Proc. Natl. Acad. Sci. USA 77, 2542–2545
2 Cushman, S.W. and Wardzala, L.J. (1980) J. Biol. Chem. 255, 4758–4762
3 Kono, T., Suzuki, K., Dansey, L.E., Robinson, F.W. and Blevins, T.L. (1981) J. Biol. Chem. 256, 6400–6407
4 Kono, T., Robinson, F.W., Blevins, T.L. and Ezaki, O. (1982) J. Biol. Chem. 257, 10942–10947
5 Karnieli, E., Zarnowski, M.J., Hissin, P.J., Simpson, I.A., Salans, L.B. and Cushman, S.W. (1981) J. Biol. Chem. 256, 4772–4777
6 Carter-Su, C. and Czech, M.P. (1980) J. Biol. Chem. 255, 10382–10386
7 Simpson, I.A., Martin, M.L. and Cushman, S.W. (1982) Diabetes 31, 29A
8 Carter, J.R., Jr., Avruch, J. and Martin, D.B. (1972) J. Biol. Chem. 247, 2682–2688
9 Ludvigsen, C. and Jarett, L. (1978) J. Biol. Chem. 254, 1444–1446
10 Ludvigsen, C. and Jarett, L. (1980) Diabetes 29, 373–378
11 Rodbell, M. (1964) J. Biol. Chem. 239, 375–380
12 Robinson, F.W., Blevins, T.L., Suzuki, K. and Kono, T. (1982) Anal. Biochem. 122, 10–19
13 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
14 Avruch, J. and Wallach, D.F.H. (1971) Biochim. Biophys. Acta 233, 334–347
15 Fleischer, B. (1974) Methods Enzymol. 29, 180–191
16 Tisdale, H.D. (1967) Methods Enzymol. 10, 213–215
17 Loten, E.G., Regen, D.M. and Park, C.R. (1976) J. Cell. Physiol. 89, 651–659
18 Pedersen, O. and Gliemann, J. (1981) Diabetologia 20, 630–635
19 Sauerheber, R.D., Esgate, J.A. and Kuhn, C.E. (1982) Biochim. Biophys. Acta 691, 115–124
20 Kono, T. (1983) Recent Prog. Horm. Res. 39, 519–557