Incorporation into phospholipid vesicles of pore-like properties from Golgi membranes of lactating-rat mammary gland

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1. The ability of rat mammary-gland Golgi membranes to produce monosaccharide-specific pores in phospholipid vesicles was investigated. 2. The apparent ability of Triton X-100 extracts of Golgi membranes to form such pores was re-evaluated, since we have now found that an apparent pore is produced by the detergent alone. 3. We therefore incorporated intact Golgi membranes (1 mg of protein) into egg-yolk phospholipid vesicles by direct sonication in the absence of any detergent. These vesicles retained about 0.6% of the total sucrose, but demonstrated selective permeability towards glucose compared with sucrose, with 19.8% of the glucose being lost during gel filtration on Sepharose 4B. 4. This phenomenon seemed to be enhanced by the presence of acidic phospholipids and lysophosphatidylcholine, but was inhibited by inclusion of cholesterol in the vesicles. The best mixture of phospholipids comprised 6.5 mg of egg-yolk phospholipid, 1 mg of phosphatidylserine and 0.05 mg of lysophosphatidylcholine, where 32.9% of the glucose was lost. 5. By using this optimum phospholipid mixture the pores were shown to be permeable to both glucose and mannitol, whereas sucrose and lactose were retained by the vesicles. 6. Chaps {3-[(3-cholamidopropyl)dimethylammonio]propane-1-sulphonate}-solubilized membranes produced similar permeability in vesicles produced by dialysis of a solution of the phospholipids mixed with the membrane extract. This technique resulted in a greater loss of glucose, 33% loss requiring about 0.25 mg of protein. 7. The pore-forming ability of both intact Golgi membranes and Chaps extracts was sensitive to boiling and proteolysis, indicating that a membrane protein was likely to be involved in pore formation.

INTRODUCTION

Previous investigations have suggested that the Golgi membrane of lactating-rat mammary gland contains pores that allow the passage of glucose, a compound needed to support lactose synthesis within the lumen of this organelle (White *et al.*, 1980, 1981*a,b*, 1984). The presence of pores, rather than specific carriers, was indicated by the apparent reversible, poorly specific and non-saturable permeability of Golgi-membrane vesicles to electrolytes and non-electrolytes of M_r less than 200–300. In particular, the membranes could effectively distinguish between monosaccharides and disaccharides.

Evidence was given for the reconstitution into artificial phospholipid vesicles of heat-stable material solubilized from Golgi membranes with Triton X-100 (White *et al.*, 1984). However, Triton X-100 is not readily removed by dialysis, and in further experiments we have found it difficult to ascribe the pore properties of the extracts to a membrane pore factor rather than to the detergent itself, which, as is shown below, can give very similar effects. In the present paper, therefore, we show that pore-like properties can be incorporated into egg-yolk phospholipid vesicles formed in the presence of Golgi membranes in the absence of any detergent. Reconstituted pore-like properties are also shown to arise when vesicles are formed in the presence of a Chaps extract of the membranes, followed by dialysis to remove the detergent Chaps.

MATERIALS AND METHODS

Rats of a Wistar strain were used between day 14 and day 20 of lactation. Sepharose 4B was from Pharmacia Fine Chemicals. D-[U- 14 C]Glucose, D-[1- 14 C]mannitol, [D-glucose-1- 14 C]lactose and [6,6'- 3 H]sucrose were from Amersham International. Chaps and all phospholipids were from Sigma. The egg-yolk phospholipid (primarily phosphatidylcholine) was purified by precipitation from chloroform solution twice with acetone. All phospholipid solutions were stored under N_2 at -18°C . The non-specific proteinase was Sigma type XIV Pronase E.

Preparation of Golgi membranes

Golgi membranes were prepared as described previously (Kuhn *et al.*, 1980); however, after the final centrifugation the membranes were resuspended in a buffer (A) containing sucrose (50 mM), glucose (50 mM), NaCl (0.1 M), $MgCl_2$ (1 mM) and Tris/HCl buffer, pH 7.4 (10 mM). In certain instances the glucose was substituted with either mannitol or lactose. The membrane suspension was stored at -18°C for not more than 2 days.

Membrane protein was assayed by the method of Goa (1953). The protein content of detergent extracts was determined by the method of Peterson (1977).

Incorporation of pore-like property from Triton X-100 extracts and intact Golgi vesicles into liposomes

Phospholipid mixtures (see Table 1) were dried as a thin film into the bottom of Bijou bottles under N_2 and then

Table 1. Incorporation of Golgi membrane pores into phospholipid vesicles of various lipid composition: inhibition of pore-forming ability by boiling or treatment with proteinase, and the specificity of this pore towards monosaccharides

Golgi membranes were incorporated into liposomes as described in the Materials and methods section. With the exception of egg-yolk phospholipid, the quantity of each lipid which was used was consistent throughout the experiments and is indicated after its first appearance in the Table. The total lipid was adjusted to 7.5 mg with egg-yolk phospholipid. Samples of these membranes were heated in a boiling-water bath for 5 min or incubated with a non-specific proteinase (see the Materials and methods section). Values are means $\pm$ S.E.M. for six separate Golgi preparations or for four non-Golgi controls. Abbreviations used: EYPL, egg-yolk phospholipid; PS, phosphatidylserine; DPG, diphosphatidylglycerol; lysoPC, lysophosphatidylcholine. The test sugar was 50 mM-glucose, except for *mannitol and †lactose where shown.

Conditions of assay	Golgi present (1 mg of protein) or other conditions	Retention of sucrose (%)	Glucose loss [(^3H - ^{14}C)/ ^3H] (%)
EYPL (7.5 mg)	No	0.60 $\pm$ 0.10	-1.9 $\pm$ 2.4
	Yes	0.58 $\pm$ 0.06	19.8 $\pm$ 3.6
No added lipid	Yes	0.028 $\pm$ 0.005	56.9 $\pm$ 8.4
EYPL + DPG (1 mg)	No	0.83 $\pm$ 0.15	6.6 $\pm$ 2.2
	Yes	0.74 $\pm$ 0.06	21.0 $\pm$ 2.4
EYPL + PS (1 mg)	No	0.62 $\pm$ 0.11	0.4 $\pm$ 1.4
	Yes	0.70 $\pm$ 0.05	25.3 $\pm$ 1.8
EYPL + DPG + PS	No	0.96 $\pm$ 0.18	6.2 $\pm$ 0.7
	Yes	0.79 $\pm$ 0.09	25.8 $\pm$ 3.9
EYPL + PS + lysoPC (0.05 mg)	No	0.87 $\pm$ 0.16	-0.8 $\pm$ 2.1
	Yes	0.64 $\pm$ 0.07	32.9 $\pm$ 3.2
EYPL + cholesterol (1 mg)	No	0.69 $\pm$ 0.05	1.1 $\pm$ 2.1
	Yes	1.05 $\pm$ 0.07	6.7 $\pm$ 1.6
EYPL + PS + lysoPC	100 °C, 5 min	0.81 $\pm$ 0.10	7.5 $\pm$ 0.6
	Proteinase	0.68 $\pm$ 0.10	15.3 $\pm$ 2.7
	*No	0.44 $\pm$ 0.09	4.7 $\pm$ 1.7
	*Yes	0.67 $\pm$ 0.07	27.5 $\pm$ 3.0
	†No	0.35 $\pm$ 0.04	0.5 $\pm$ 1.7
	†Yes	0.70 $\pm$ 0.07	2.2 $\pm$ 2.1

placed in a desiccator under vacuum for at least 2 h in order to remove traces of solvent. To these were then added 1.5 μCi each of [^3H]sucrose and a ^{14}C -labelled test sugar in buffer A described above, and either a Triton X-100 extract prepared as described by White *et al.* (1984) or intact Golgi membranes. The final volume was adjusted to 1 ml with buffer A. Care was taken to ensure that the solution would cover the film of dried lipid. The mixture was then sonicated for 90 s at 0 °C with an MSE-Mullard probe sonicator tuned to give maximum agitation of the liquid.

The vesicles formed in the presence of phospholipid and Triton X-100 extract were heated at 45 °C for 4 h under a N_2 atmosphere. The proteoliposome suspension produced from intact Golgi was sealed under N_2 , frozen to -18 °C and allowed to thaw slowly before being briefly resonicated (10 s). This procedure is reported (Kasahara & Hinkle, 1977) to lead to the formation of vesicles having a larger mean diameter than those prepared solely by sonication and also to facilitate the incorporation of membrane proteins into phospholipid vesicles. In this instance, negative staining of the liposomes with molybdate and subsequent electron microscopy revealed that the vesicles varied in diameter between 20 and 500 nm and were predominantly bi- or tri-lamellar.

The liposomes with their entrapped radioactive sugars were separated from free radioactive sugars by chromatography on a column (0.5 cm $\times$ 22 cm approx.) of Sepharose 4B. This column was equilibrated, and eluted, with buffer A. The liposomes eluted in the void volume, whereas untrapped radioactive sugars were retarded.

Usually the vesicles were collected in a volume of 1.5 ml, a 1 ml portion of which was used for scintillation counting.

Reconstitution of pore property from Chaps extracts

Golgi membranes were pelleted in a polycarbonate centrifuge tube at 110000 g_{av} for 15 min. This pellet was resuspended in buffer A containing 10 mg of Chaps/ml, the membranes were solubilized on ice for 30 min, after which the insoluble residue was sedimented at 180000 g_{av} for 1 h. The supernatant was used for reconstitution of the pore.

A phospholipid mixture comprising egg-yolk phospholipid, phosphatidylserine and lysophosphatidylcholine (130:20:1, by wt.) was dried down as a thin film in a round-bottomed flask. This mixture of lipids was known to produce the greatest selective loss of glucose upon incorporation of intact Golgi membranes into liposomes (see below). The phospholipids were taken up into solution in buffer A containing 10 mg of Chaps/ml at a final concentration of 7.5 mg of phospholipid/ml. Aliquots of the solubilized membranes were mixed with 5 mg of phospholipid mixture in a total volume of 1 ml and then dialysed for 72 h against detergent-free buffer A. This buffer was previously equilibrated with N_2 and a slow stream of N_2 was maintained through the solution throughout the dialysis. Finally 1.5 μCi of each radioactive sugar was added to the dialysis residue. The mixtures were sonicated for 60 s (this was sufficient to incorporate the maximum amount of radiolabel into the vesicles), and the vesicles were then separated from untrapped sugars by gel filtration on the Sepharose 4B columns.

The Chaps remaining after dialysis was assayed with hydroxysteroid dehydrogenase (Talalay, 1960). This assay has been used elsewhere for the determination of bile salts (Coleman *et al.*, 1979) and was applicable with as little as 0.01 mg of Chaps/ml.

Treatment with proteinase

Both intact Golgi membranes (1 mg of protein) and their Chaps extracts (250 μ l) were treated with a non-specific proteinase by incubating them in the presence of up to 1 mg of proteinase/ml for 30 min at 37 °C. After this treatment, 1 ml of ice-cold buffer A was added to the Golgi membranes and they were collected by centrifugation at 14000 g_{av} for 15 min. The pellet was washed once by suspension and re-centrifugation in the buffer and the proteinase-free membranes were used in the pore-detecting assay. However, since it was not possible to remove the proteinase from the solubilized Chaps extracts, there would have been up to approx. 0.25 mg of proteinase/ml present throughout the subsequent dialysis.

Determination of the degree of pore formation

Pore formation in liposomes has previously been expressed as the ratio of trapped solute to permeant solute (Zalman *et al.*, 1980; Linden *et al.*, 1982; White *et al.*, 1984). However, this leads to an upwardly curving plot, since the degree of loss of the permeant solute increases linearly and does not give a mathematically sound way of calculating means, standard deviations etc. We have therefore determined the percentage loss of the permeant or test solute relative to the impermeant solute.

Simple phospholipid vesicles are impermeable to trapped sucrose and glucose (Bangham *et al.*, 1967) and retain these equally after separation by Sepharose 4B. The presence of a selective pore could thus be monitored as a percentage loss of glucose relative to sucrose:

$$\frac{\text{Sucrose retained} - \text{glucose retained}}{\text{Sucrose retained}} \times 100$$

RESULTS

Formation of vesicles by sonication of phospholipids

Fig. 1 shows that egg-yolk phospholipid vesicles, formed in the presence of equimolar solutions of [14 C]glucose and [3 H]sucrose, were eluted in the void volume of Sepharose 4B columns accompanied by about 0.5–0.8% of each sugar. The remaining sugar was eluted later. Radioactive sugar added to preformed vesicles was not eluted in the void volume. These results show that the vesicles contained an internal volume of about 1 μ l/mg of lipid and that they were not selectively permeable to either sugar.

Incorporation of pore-like material from Triton X-100 extracts into vesicles

In studying the trapping of sucrose and glucose by phospholipid vesicles, White *et al.* (1984) observed a preferential loss of glucose, relative to sucrose, when the vesicles had been formed in the presence of dialysed Triton X-100 extracts of Golgi membranes. However, this was achieved at the expense of considerable loss of sucrose itself. Fig. 2(a) shows the reciprocal relationship between pore activity (preferential loss of glucose) and overall loss of sucrose as the amount of Golgi extract was

Fig. 1. Separation of free and vesicle-trapped radioactive sucrose and glucose by Sepharose 4B chromatography

Phospholipid vesicles (1 ml) were prepared by sonication of EYPL as described in the Materials and methods section. These were percolated through a column of Sepharose 4B and the initial 1 ml of eluent was collected. Subsequently 0.75 ml fractions were taken, and the sucrose and glucose present were determined by liquid-scintillation counting. (a) Elution profiles of sucrose (---) and glucose (·····); (b) turbidity of these fractions (measured as the A_{550} of a 25 μ l sample in 1 ml of buffer) as an indication of the presence of phospholipid vesicles.

varied. Further work has shown that dialysis probably removed only 20–30% the Triton X-100 from such extracts and that it was not possible to separate their pore-forming activity from the detergent micelles by gel filtration or by extraction of the detergent with chloroform. Fig. 2(b) shows that exposure of phospholipid vesicles to comparable solutions of Triton X-100 alone caused a similar reciprocal change in apparent pore activity and sucrose retention. We must conclude that there is a possibility that Triton X-100 itself was responsible for the pore activity reported previously (White *et al.*, 1984). This might also account for the observed heat-stability of the extracts.

Incorporation of pore-like material from intact Golgi membranes into vesicles

In similar experiments with egg-yolk phospholipid vesicles formed in the presence of Golgi membranes that

were not exposed to any detergent there was a 20% loss of glucose relative to sucrose in the isolated vesicles (Table 1). No such loss was observed when Golgi membranes were omitted. This did not appear to have resulted from the presence of a distinct population of Golgi-membrane vesicles, since the formation of vesicles from Golgi membrane alone led to very little trapping of sugar at all. Such trapping as there was, however, also showed a large relative loss of glucose. These findings are consistent with the notion that Golgi membranes are selectively permeable to glucose and can transfer this property to artificial phospholipid vesicles.

Table 1 shows that the selective permeability conferred upon the phospholipid vesicles appeared to be slightly enhanced by inclusion of small amounts of either diphosphatidylglycerol or phosphatidylserine, or of both, but this did not achieve statistical significance (in an unpaired Student's *t* test). Greatest selective permeability seemed to result when small amounts of both phosphatidylserine and lysophosphatidylcholine were included during the preparation of the vesicles, leading to a loss of about 33% of the trapped glucose ($0.050 > P > 0.020$ compared with egg-yolk lipid plus Golgi). By contrast, the inclusion of cholesterol decreased the permeability towards glucose ($0.01 > P > 0.001$). Table 1 also shows

Fig. 2. Reciprocal loss of sucrose, and gain of selective permeability towards glucose, by phospholipid vesicles formed in the presence of (a) Triton X-100 extracts of Golgi membranes, and (b) Triton X-100 solutions alone

The retention of sucrose by these vesicles (○) and the selective loss of glucose (●) are shown. Selective loss of glucose was calculated as the percentage loss relative to total sucrose trapped (see the text). The Triton X-100 solution and the Golgi extract used contained 2% (w/v) and about 1.6% (w/v) Triton X-100 respectively.

Fig. 3. Increase of selective permeability of phospholipid vesicles formed with various amounts of intact Golgi membranes or of Chaps-solubilized membranes

Freshly prepared Golgi membranes were assayed for protein content and stored overnight at -18°C without apparent loss of pore-forming ability. Golgi membranes, containing known amounts of membrane protein, were then incorporated into artificial phospholipid vesicles by sonication and freeze-thaw as described in the text. Retention of sucrose (□) and selective loss of glucose (●) from such mixed proteoliposomes are shown. Values are means $\pm$ S.E.M. for four separate Golgi preparations except for those marked *, which are means of six (results from Table 1). Aliquots of freshly prepared Chaps extracts of Golgi membranes were mixed with solubilized phospholipid and dialysed against a detergent-free buffer (see the text). Protein was assayed at a later date after storage of the extract at -18°C . The mean protein content in a particular size of aliquot was determined (S.E.M. indicated by horizontal error bars). The retention of sucrose (○) and selective loss of glucose (●) from the vesicles are shown. Values are means $\pm$ S.E.M. for four extracts of separate Golgi preparations.

that the ability of Golgi membranes to promote the selective loss of glucose was almost abolished by heating them for 5 min at 100°C , and was markedly impaired after their treatment with proteinase ($P < 0.001$ and $0.01 > P > 0.001$ respectively). Both these findings point to the possible involvement of a protein. Table 1 shows, furthermore, that mannitol was selectively lost from these vesicles, but that lactose was not. Thus the mixed Golgi/phospholipid vesicles clearly distinguished between disaccharides and monosaccharides, but failed to show a specificity towards glucose that might have suggested the incorporation of a plasma-membrane-type monosaccharide carrier into the vesicle membrane.

Under each of the above conditions, selectivity of glucose loss was dependent upon the presence of Golgi membrane ($P < 0.01$, except in the presence of cholesterol, where $0.1 > P > 0.05$). Moreover, in contrast with the use

Table 2. The effect of boiling or proteinase treatment upon the apparent pore-forming ability of Chaps extracts of Golgi membranes

Chaps-solubilized Golgi extracts (250 μ l) were heated in a boiling-water bath for 5 min or incubated with a proteinase at 37 °C for 30 min. These treated extracts were then reconstituted into artificial phospholipid vesicles and assessed for pores as described in the Materials and methods section. Five separate extracts from different Golgi membrane preparations were used; the results shown are the means $\pm$ S.E.M., with the number of observations shown in parentheses.

Incubation conditions	Retention of sucrose (%)	Loss of glucose relative to sucrose (%)
Untreated extract (5)	0.12 $\pm$ 0.02	46.9 $\pm$ 10.0
Boiled (5)	0.14 $\pm$ 0.02	19.3 $\pm$ 8.1
Proteinase		
1 mg/ml (5)	0.12 $\pm$ 0.01	2.9 $\pm$ 2.5
0.1 mg/ml (3)	0.12 $\pm$ 0.01	7.1 $\pm$ 3.6
0.01 mg/ml (2)	0.12 $\pm$ 0.02	30.0 $\pm$ 7.4

Table 3. Retention of sucrose and glucose by phospholipid vesicles dialysed against buffer containing low concentrations of Chaps

Vesicles were formed by dialysis of a solution of phospholipids in 10 mg of Chaps/ml (see the Materials and methods section) with various concentrations of Chaps added to the dialysis buffer. Data shown are from a single experiment.

[Chaps] in dialysis medium (mg/ml)	Retention of sucrose (%)	Loss of glucose relative to sucrose (%)
0.0	0.13	0.0
0.01	0.12	0.0
0.025	0.15	-1.3
0.05	0.13	-6.0

of Triton X-100 extracts, addition of Golgi membrane did not significantly impair the trapping of sucrose ($P > 0.2$ by analysis of variance).

Fig. 3 shows that the relative loss of glucose from the vesicles increased with the amount of added Golgi membrane, but approached a maximum at about 1.5 mg of membrane protein/assay. This limit might have been due in part to the addition of endogenous cholesterol, since as little as 0.5 mg of added cholesterol was found to have a clear inhibitory effect upon the incorporation of membrane pores into the vesicles (results not shown). Golgi membranes contain approx. 0.2–0.25 mg of cholesterol/mg of membrane protein (calculated from the data of White *et al.*, 1981a). However, as this assay does not measure an initial rate of loss of glucose, one might not expect to observe a linear relationship between the quantity of protein added and the amount of glucose

evacuated from the vesicles. Rather, we envisage that the presence of one pore per vesicle permits complete exchange of radioactive glucose with the unlabelled elution buffer (this view was supported by the observation that when the chromatographed vesicles were passed down a second column, no further selective loss of glucose was observed; results not shown). We therefore believe that the assay gauges the proportion of the vesicle population which contains at least one pore. However, some of these vesicles may contain more than one pore, and where greater amounts of protein are added, the likelihood of further pores being incorporated into vesicles which already contain one or more pores will increase. Thus one would predict that a curve of the form shown in Fig. 3, rather than a linear relationship between selective glucose loss and protein added, is to be expected.

Incorporation of pore-like material from Chaps extracts into vesicles

The above findings encouraged us to employ Golgi extracts prepared with Chaps, a zwitterionic detergent that diffuses on dialysis faster than Triton X-100 on account of its higher critical micellar concentration.

Vesicles formed by detergent dialysis in the absence of Golgi extract trapped approximately equal amounts of sucrose and glucose. About 0.1–0.2% of the total sucrose was found associated with the vesicles. This value is noticeably lower than that seen in vesicles produced by simple sonication of phospholipids. However, the detergent-dialysis technique yielded smaller vesicles (mean diameter 25–50 nm) and thus these vesicles will, for a given quantity of phospholipid, entrap a smaller internal volume. The vesicles produced by this technique were predominantly uni- or bi-lamellar.

When Golgi extract was added to the dialysis mixtures, glucose was subsequently seen to be selectively lost (Fig. 3). This loss of glucose increased with the amount of Golgi protein added. The evacuation of glucose from the vesicles required the addition of less protein than did that when proteoliposomes were formed with intact Golgi membranes.

Incubation of the Chaps extract at 100 °C for 5 min or with proteinase markedly decreased the apparent pore-forming ability without affecting the retention of sucrose (Table 2). In a series of five experiments using different membrane preparations, heating reduced the measured selective glucose loss from 46.9 to 19.3% (0.02 $> P > 0.01$ for a paired *t* test), whereas the presence of 1 mg of proteinase/ml decreased the pore-forming ability to only 2.9% (0.01 $> P > 0.001$ paired *t* test). The paired *t* tests were necessary, since the protein content of the different extracts varied, leading to a considerable variation in the degree of glucose loss observed in each individual experiment. Lower concentrations of the proteinase, although less effective, also seemed to impair the pore-forming ability of these extracts (Table 2), but 0.1 mg of proteinase/ml had little or no effect upon the pore-forming ability of intact Golgi membranes (results not shown). This increased sensitivity could imply that an intrinsic pore protein has been extracted from the membrane and thus rendered more accessible to proteolytic attack. However, one should recall that, whereas the intact membranes were washed free of the proteinase, it was not removed from the Chaps-solubilized extracts, hence this difference may simply reflect the prolonged exposure of the pore-forming material to the proteinase.

In the light of our knowledge that Triton X-100 could generate an apparent pore in lipid membranes, it was important for us to ensure that a similar effect was not responsible for these observations with Chaps extracts. Vesicles formed in the absence of Golgi membrane extracts, by the dialysis of Chaps-solubilized lipid against up to 0.05 mg of Chaps/ml, retained both sucrose and glucose on subsequent gel filtration (Table 3). Indeed, we have often found a small selective retention of glucose in vesicles formed by detergent dialysis in the absence of membrane extract (up to about 10% more glucose than sucrose was trapped). Our data have therefore been normalized to allow for this, such that a control preparation is ascribed a value of 0% glucose loss. Conversely, we found that, although the concentration of Chaps inside the dialysis sac fell slightly more slowly if membrane extract was present, by 72 h it had reached a value (about 0.01 mg/ml) that was easily tolerated by the vesicles. Finally, the decrease in pore-forming activity caused by heating or by proteinase treatment suggested that a protein was involved in pore formation.

DISCUSSION

An original reason for attempting to reconstitute pore-like properties of the Golgi membrane into artificial phospholipid membranes was to place the evidence for such pores on a firmer basis. Confirmation of their existence would have valuable implications for the access of small cytosolic molecules to the lumen of the Golgi apparatus and, in the case of the mammary gland, subsequently to milk (Naccarato *et al.*, 1975; Faulkner, 1980; Faulkner *et al.*, 1981). Indeed, the evidence for such pores has already prompted a reconsideration of the proposed activation of galactosyltransferase by bivalent metal ions in the Golgi apparatus of lactating tissue (Kuhn *et al.*, 1985). Therefore, in the face of doubts about the validity of our previous results with Triton X-100 extracts of Golgi membranes (White *et al.*, 1984), it was important to incorporate such properties in the total absence of any detergent. Table 1 shows that this was possible and that, as with the reconstitution of cytochrome *c* (Eytan *et al.*, 1976), the process was aided by acidic phospholipids. A small amount of lysophosphatidylcholine was additionally advantageous, but cholesterol inhibited. In particular, the introduction of pores was not accompanied by general leakiness as evidenced by retention of sucrose. The selective loss, from a vesicle, of glucose or mannitol (hydrodynamic radius 0.42 nm and 0.43 nm respectively) relative to sucrose or lactose (hydrodynamic radius 0.52 nm and 0.54 nm respectively; Schultz & Solomon, 1961) must be seen as a rather stringent test of pore size, so that it is remarkable that Triton X-100 alone can partly achieve the effect.

Our subsequent use of Chaps extracts of Golgi membranes to incorporate pore properties into phospholipid vesicles stands on firmer ground than that of Triton X-100 extracts. The greater 'dialysability' of Chaps reflects its higher critical micellar concentration (approx. 4–6 mM) over that of Triton X-100 (approx. 0.15–0.3 mM), and we have observed that 48 h dialysis leaves only about 0.01 mg of Chaps/ml (results not shown), a concentration well below the 0.05 mg/ml that can still easily be tolerated in the assay. The pore-forming ability of both intact vesicles and Chaps extracts of them was sensitive to heating and proteolysis.

In summary, the present data support the previous evidence, on transport properties of Golgi-derived vesicles, for a pore-like permeability to monosaccharides but not to disaccharides in these membranes.

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REFERENCES

- Bangham, A. D., de Gier, J. & Greville, G. D. (1967) *Chem. Phys. Lipids* **1**, 225–246
- Coleman, R., Iqbal, S., Godfrey, P. P. & Billington, D. (1979) *Biochem. J.* **178**, 201–208
- Eytan, G. D., Matheson, M. J. & Racker, E. (1976) *J. Biol. Chem.* **251**, 6831–6837
- Faulkner, A. (1980) *Biochim. Biophys. Acta* **630**, 141–145
- Faulkner, A., Chaiyabutr, N., Peaker, M., Carrick, D. T. & Kuhn, N. J. (1981) *J. Dairy Res.* **48**, 51–56
- Goa, J. (1953) *Scand. J. Clin. Lab. Invest.* **5**, 218–222
- Kasahara, M. & Hinkle, P. C. (1977) *J. Biol. Chem.* **252**, 7384–7390
- Kuhn, N. J., Wooding, F. P. B. & White, A. (1980) *Eur. J. Biochem.* **103**, 377–385
- Kuhn, N. J., White, M. D., Ward, S. & Singh, S. (1985) *Proc. Int. Symp. Glycoconjugates 8th*, Houston, TX, p. 489
- Linden, M., Gellerfors, P. & Nelson, B. D. (1982) *Biochem. J.* **208**, 77–82
- Naccarato, W. F., Ray, R. E. & Wells, W. W. (1975) *J. Biol. Chem.* **250**, 1872–1876
- Peterson, G. L. (1977) *Anal. Biochem.* **83**, 346–356
- Schultz, S. G. & Solomon, A. K. (1961) *J. Gen. Physiol.* **44**, 1189–1199
- Talalay, P. (1960) *Methods Biochem. Anal.* **8**, 119–143
- White, M. D., Kuhn, N. J. & Ward, S. (1980) *Biochem. J.* **190**, 621–624
- White, M. D., Kuhn, N. J. & Ward, S. (1981a) *Biochem. J.* **194**, 173–177
- White, M. D., Ward, S. & Kuhn, N. J. (1981b) *Biochem. J.* **200**, 663–669
- White, M. D., Ward, S. & Kuhn, N. J. (1984) *Biochem. J.* **217**, 297–301
- Zalman, L. S., Nikaido, H. & Kagawa, Y. (1980) *J. Biol. Chem.* **255**, 1771–1774