Permeability of lactating-rat mammary gland Golgi membranes to monosaccharides

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The Golgi-membrane vesicles present in particulate preparations of lactating rat mammary gland were biosynthetically loaded with [^{14}C]lactose. This lactose was effectively retained by particles sedimented after exposure to 0.25 M-disaccharide, but was partly lost after exposure to 0.25 M-glucose or other solutes of similar size. Loss of lactose was time-, concentration- and temperature-dependent and varied with the solute structure. This behaviour is ascribed to the presence of protein in the Golgi membrane, forming a specific carrier or channel that serves to supply glucose for lactose synthesis.

The galactosyltransferase (EC 2.4.1.22) of lactating mammary gland makes lactose from UDP-galactose and β -glucose in the presence of α -lactalbumin and bivalent metal ions. The enzyme is located on the inner face of the Golgi membrane and the lactose apparently accumulates within the Golgi lumen. In crude or purified particulate preparations of rat mammary tissue, which contain pinched-off vesicles of Golgi membrane, 80–90% of [^{14}C]lactose newly synthesized from added radioactive substrates remains trapped within the vesicles and can be sedimented by centrifugation (Hill & Brew, 1975; Kuhn & White, 1975, 1977; Jones, 1978). Since the Golgi membrane is so impermeable to lactose, the question arises as to how it can admit glucose.

The existence of a specific glucose carrier was previously inferred from the inhibition of lactose synthesis in intact vesicles by phlorizin and phloretin (Kuhn & White, 1975). These agents are well-known inhibitors of glucose transport in vertebrate cell membranes. More recently, however, the effect of phlorizin has been shown to be insufficiently specific to constitute reliable evidence for a glucose carrier in the Golgi membrane. On the other hand, experiments with thiol poisons, to distinguish between exposed and unexposed galactosyltransferase in Golgi vesicles, have demonstrated a pool of the enzyme that is more accessible to glucose than to *N*-acylglucosamines (Kuhn & White, 1980).

Being unable to observe the transport of glucose into Golgi vesicles by direct uptake studies, we have applied the osmotic lysis method of Appelmans & de Duve (1955) to crude particulate preparations of rat mammary gland in which the Golgi-membrane vesicles contained newly synthesized [^{14}C]lactose.

The theory behind this method is that permeant solutes to which the vesicles are exposed, moving down a concentration gradient, cross the membrane and enter the vesicle. As a result water is drawn in and the consequent hydrostatic pressure disrupts the membrane and releases the [^{14}C]lactose. The loss of lactose from the particles is a measure of the permeability of the membrane to a solute. In a report that prompted the present study, Docherty *et al.* (1979) have described similar experiments to show that stereospecific sugar uptake by rat liver lysosomes occurs by facilitated diffusion.

Materials and methods

Preparation of [^{14}C]lactose-containing particles

Lactating rat mammary tissue (3–5 g fresh wt.) was chopped and homogenized with 9 vol. of 0.25 M-lactose in a glass homogenizer with a loosely fitting Teflon pestle. After six to ten strokes of the pestle the homogenate was centrifuged at 10000 *g* for 10 min. The pellet was discarded and the supernatant was further centrifuged at 100000 *g* for 30 min. This pellet was re-suspended by gentle homogenization in the original tissue volume of 0.25 M-lactose containing 50 mM-glycylglycine buffer, pH 7.0. These operations were carried out at 4°C.

The Golgi-membrane vesicles of this preparation were loaded with [^{14}C]lactose by mixing 1.7 ml with 0.26 ml of solution containing glucose (81 mM) and MnCl_2 (16 mM). After this had been warmed for 5 min at 37°C, lactose synthesis was initiated by the addition of 0.15 ml of UDP-[^{14}C]galactose solution (5 mM; sp. radioactivity 0.45 Ci/mol) and was allowed to proceed for 3 min. This generated about 250 nmol of [^{14}C]lactose. The incubation mixture

was then rapidly chilled in an ice bath and used immediately.

Osmotic lysis

Particulate preparations loaded with [^{14}C]lactose (0.1 ml) were diluted with ice-cold solution (0.4 ml) containing test solute (0.31 M), EDTA (1 mM) and glycylglycine/NaOH buffer, pH 7.0 (10 mM), to give a final concentration of 0.25 M test solute. After incubation for 15 min the solutions were adjusted to 0.25 M-lactose by the addition of 2.5 ml of ice-cold 0.3 M-lactose. The particle suspension was centrifuged immediately for 15 min at 150 000 *g* to obtain a tightly packed pellet. In some experiments the sugar concentration, the time or the temperature used for lysis was varied.

The pellet obtained above was extracted with 2 ml of 3.5% (w/v) HClO_4 and centrifuged to remove denatured protein. The extracted [^{14}C]lactose was determined by scintillation counting either directly or, in early experiments, after purification by selective precipitation (Kuhn & White, 1975). The latter method was abandoned after it was found that lactose was essentially the only radioactive material that sedimented with the particles.

Materials

Sugars were obtained from BDH (Poole, Dorset, U.K.) or from Sigma Chemical Co. (Poole, Dorset, U.K.). UDP- ^{14}C galactose was from The Radiochemical Centre (Amersham, Bucks., U.K.). Rats of a Wistar-derived strain were used between days 13 and 18 of their first lactation.

Results

Retention of [^{14}C]lactose by mammary particles

When particulate preparations containing [^{14}C]lactose were diluted into ice-cold 0.25 M-lactose and centrifuged at any time up to 30 min, about 80% of the [^{14}C]lactose was sedimented, in confirmation of a previous report from this laboratory. The remaining lactose was assumed to have been synthesized by leaky vesicles (Kuhn *et al.*, 1980). For the present purposes the [^{14}C]lactose retained under these conditions was assigned an arbitrary value of 100%. When similar preparations were diluted into 0.25 M solutions of maltose, sucrose, cellobiose or raffinose, and compared with dilutions into 0.25 M-lactose in the same experiment, 92–96% of the sedimentable [^{14}C]lactose was retained. Some of these results are shown in Table 1. It appears, therefore, that disaccharides and trisaccharides do not cross the Golgi membrane.

Osmotic lysis by glucose

In contrast with the above findings, dilution of particles into 0.25 M-glucose resulted in markedly

Table 1. Retention of [^{14}C]lactose by Golgi particles exposed to different solutes at 0.25 M concentration for 15 min at 0°C

The results are given as means $\pm$ S.E.M., with the numbers of determinations in parentheses. In each case the retention on exposure to 0.25 M-lactose was arbitrarily set at 100%. *P* gives the significance of differences from the values for D-glucose measured in the same experiment.

Solute	Retention of [^{14}C]lactose (%)	<i>P</i>
Buffer only	25.2 $\pm$ 1.9 (14)	0.001
Glycerol	28.7 $\pm$ 2.3 (7)	0.01
2-Deoxy-D-galactose	37.7 $\pm$ 1.3 (6)	0.01
L-Glucose	40.7 $\pm$ 5.1 (6)	0.7
D-Glucose	41.9 $\pm$ 1.8 (18)	—
D-Xylose	44.6 $\pm$ 2.9 (5)	0.8
2-Deoxy-D-glucose	45.9 $\pm$ 2.9 (7)	0.7
D-Fructose	46.8 $\pm$ 3.4 (8)	0.05
6-Deoxy-D-galactose	48.7 $\pm$ 1.0 (6)	0.01
N-Acetyl-D-glucosamine	49.8 $\pm$ 2.8 (9)	0.05
D-Mannose	50.8 $\pm$ 4.9 (6)	0.01
D-Galactose	51.0 $\pm$ 6.0 (5)	0.05
myo-Inositol	52.6 $\pm$ 2.2 (8)	0.01
3-O-Methyl-D-glucose	62.8 $\pm$ 6.0 (5)	0.01
D-Sorbitol	63.1 $\pm$ 2.3 (9)	0.001
6-Deoxy-D-glucose	63.4 $\pm$ 3.7 (7)	0.01
β -Methyl D-glucoside	64.0 $\pm$ 4.4 (5)	0.001
D-Mannitol	70.9 $\pm$ 3.0 (8)	0.001
α -Methyl D-glucoside	71.5 $\pm$ 3.1 (6)	0.001
N-Butyryl-D-glucosamine	87.2 $\pm$ 1.6 (6)	0.01
Raffinose	92.0 $\pm$ 2.4 (5)	0.001
Cellobiose	93.4 $\pm$ 2.4 (5)	0.001
Sucrose	95.4 $\pm$ 2.9 (5)	0.001
Maltose	96.0 $\pm$ 2.1 (5)	0.001
Lactose	100	—

poorer retention of [^{14}C]lactose. Fig. 1 shows that the loss of [^{14}C]lactose increased with time, and was both more rapid and more extensive at 37°C than at 0°C. Dilution of the particles into 0.25 M-lactose, on the other hand, resulted in the efficient retention of [^{14}C]lactose both at 0°C and at 37°C for up to 30 min. Fig. 2(a) shows that the retention of [^{14}C]lactose over 15 min decreased linearly with increase of glucose concentration.

Specificity of osmotic lysis

A wide range of sugars and their derivatives, of closely similar molecular weight, was tested for osmotic lysis. Table 1 shows the mean percentage ($\pm$ S.E.M.) of [^{14}C]lactose retention by particles exposed to 0.25 M solutions for 15 min at 0°C. Several solutes, always including lactose and glucose, were tested in each experiment and Student's paired *t* test was used to determine whether the degree of lysis caused by a given solute differed significantly from that due to glucose.

The greatest loss of [¹⁴C]lactose occurred when the particles were exposed to only the buffer in which the solutes were dissolved, or to glycerol. L-Glucose, D-xylose, and 2-deoxy-D-glucose did not differ significantly from D-glucose in the lysis they

caused, but the epimer galactose and all other solutes in Table 1 were significantly inferior. The open-chain compounds sorbitol and mannitol, and the two methyl glucosides, were among the poorest. It is also noteworthy that 6-deoxy-D-glucose was significantly inferior to 2-deoxy-D-glucose ($P < 0.001$) and 6-deoxy-D-galactose was significantly inferior to 2-deoxy-D-galactose ($P < 0.001$). In separate experiments (not shown) 0.125 M-glucosamine chloride or acetate, and 0.083 M-sodium glucose 6-phosphate, were markedly less permeant than glucose tested at the same concentration, NaCl being used to make up 0.25 osmolarity.

Although specificity of lysis was observed at 0°C, this appeared to be lost at 37°C. The experiment illustrated in Figs. 2(b) and 2(c) shows that mannitol, β -methyl glucoside and glucose differed in the lysis they produced over 15 min at 0°C, when this was tested at three different concentrations. When the same measurements were repeated at 37°C the lytic activities of the three solutes were not only greater, but identical with one another.

Discussion

An advantage of osmotic lysis as a method of studying solute transport across the Golgi membrane is that, being based here on the release of [¹⁴C]lactose, the observed transport relates unambiguously to the same Golgi membrane that supplies glucose for lactose synthesis. For this reason one can use relatively crude particulate preparations. A disadvantage of the method is that if the putative carrier is saturated at low solute concentrations, relative rates of [¹⁴C]lactose loss may reflect the V_{max} of the carrier system more than the relative K_m values. The method may therefore not discriminate efficiently between solutes of widely

Fig. 1. Variation of osmotic lysis with time and temperature
Retention of [¹⁴C]lactose by Golgi particles was measured after their exposure for different times to 0.25 M-glucose at 0°C (●) and 37°C (○), or to 0.25 M-lactose at 0°C (■) and 37°C (□).

Fig. 2. Variation of osmotic lysis with concentration of solute, and effect of temperature on specificity
(a) Effects of different concentrations of glucose. (b) Comparison of lysis by different concentrations of glucose (●), β -methyl glucoside (○) and mannitol (▲) at 0°C. (c) As for (b), but at 37°C. Lysis was carried out for 15 min in each case. Lactose was added, where appropriate, to adjust test solute concentrations to 0.25 M.

differing affinity for the system. This has been discussed by Docherty *et al.* (1979).

The lysis of any one vesicle, and the consequent loss of [^{14}C]lactose, is likely to be an all-or-none effect. Therefore the variation in loss of [^{14}C]lactose that is seen under different incubation conditions probably reflects a variation, throughout the vesicle population, in the ability to withstand a given osmotic pressure.

The results in Table 1 show that the Golgi membrane is very impermeable to disaccharides and to a trisaccharide, markedly permeable to D-glucose, and even more permeable to glycerol. By contrast artificial phospholipid bilayers, although highly permeable to glycerol, are extremely impermeable to both sucrose and glucose (Bangham *et al.*, 1967). The Golgi membrane is further able to discriminate between solutes of similar size to glucose. Thus *N*-acetylglucosamine is rather less permeant, as was previously concluded from an entirely different approach (Kuhn & White, 1980), and so were nearly all the solutes placed below this sugar in Table 1. The relative permeabilities of 2- and 6-deoxy-D-glucose, and of 2- and 6-deoxy-D-galactose, show that a 6-hydroxy group is important but a 2-hydroxy group is not. The permeabilities of 3-*O*-methyl-D-glucose and of D-galactose, relative to that of glucose, show the value of a free 3-hydroxy group and a correctly oriented 4-hydroxy group. The unexpectedly great permeability of *myo*-inositol, and the poor permeabilities of sorbitol and mannitol, indicate the preference for a ring structure. The occurrence of both free and galactosylated inositol in rat milk (Naccarato & Wells, 1974) suggests that inositol may actually penetrate the Golgi membrane *in vivo*. It can also be seen from Table 1 that the lytic activity of a solute appears to decrease with increasing hydrophobicity, as from the introduction of a methyl group or of an *N*-butyryl group instead of an *N*-acetyl group.

The above observations are most simply explained by postulating the presence of a single carrier or channel, straddling the Golgi membrane, that is hydrophilic in nature and selects on the basis of size and ability of the solute to form particular hydrogen bonds. The degree of specificity seen in Table 1 suggests the involvement of a protein in this process.

At the same time it is not easy to envisage how the rapid movement of a solute through a channel can be compatible with recognition based on firm hydrogen bonding. It is therefore noteworthy that the increased permeability of glucose, mannitol and β -methyl glucoside at higher temperature was accompanied by an apparent loss of carrier discrimination between them.

This permeability of the mammary Golgi membrane to uncharged solutes of molecular weight less than 200–300 differs from that of hepatic 'microsomes' (microsomal fraction), which are reported to admit sucrose (Nilsson *et al.*, 1973), and more nearly resembles that of hepatic lysosomes (Lloyd, 1969). Even so, the Golgi membrane clearly differs from both the lysosomal membrane, and from bilayers of extracted erythrocyte lipids, in the rank order of penetration by different solutes (Lloyd, 1969; Jung, 1971; Docherty *et al.*, 1979).

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