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The Effects of Triton X-100 on the Transfer of Mannose, Glucose and *N*-Acetylglucosamine Phosphate to Dolichol Monophosphate by Preparations of Rough and Smooth Endoplasmic Reticulum and of Mitochondria of Rat Liver

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Triton X-100 and exogenous dolichol monophosphate have been used to investigate the nature of enzymes responsible for the transfer of mannose, glucose and *N*-acetylglucosamine phosphate from nucleotide donors to dolichol monophosphate in vesicles derived from rough and smooth endoplasmic reticulum and mitochondria. Mitochondria were shown to contain the highest specific activities of these enzymes. The responses of the glycosyltransferases to increasing concentrations of Triton X-100 and the effect on these responses of exogenous dolichol monophosphate suggest that the enzymes for mannose and glucose transfer are less hydrophobic, and therefore less intrinsic, in the membrane than the enzyme for *N*-acetylglucosamine phosphate transfer. In smooth vesicles the results are consistent with mannosyl- and glucosyl-transferases being located at both inner and outer faces of the membrane. In rough vesicles and in mitochondria mannosyl- and glucosyl-transferases were confirmed at the outer face. There is, however, only one site of *N*-acetylglucosamine phosphate transfer, this being more hydrophobically located in the membrane than the other sites of glycosyl transfer. Mitochondrial enzyme activity closely resembled that of rough endoplasmic reticulum in response to Triton X-100 and exogenous dolichol monophosphate, and is probably associated with the outer membrane.

The stimulatory effect of detergents on the formation of Dol-*P* or Dol-P_2 sugars from NDP-sugars in several mammalian microsomal systems has been recognized since the first reports of such activity. However, not all investigations have supported this observation.

Production of mannolipid by a rabbit liver preparation (Caccam *et al.*, 1969) was doubled in the presence of low concentrations of Zonyl A. Similar results were obtained by using Triton X-100 in rat and rabbit liver systems (Tetas *et al.*, 1970) and in rat brain microsomal fractions (Wolfe *et al.*, 1974). Sodium deoxycholate and Triton X-100 were found to double the yield of glucolipid from rat liver microsomal fractions (Behrens & Leloir, 1970). The subsequent transfer of glucose to Dol-P_2-oligosaccharides and thence to protein was also stimulated by sodium deoxycholate, but over a narrow concentration range (Parodi *et al.*, 1972). Triton X-100 was not effective in the same system. The production of Dol-*P*-NAcGlc by a yeast membrane preparation was stimulated by low amounts of Triton X-100, but substantially inhibited by high concentrations (Reuvers *et al.*, 1977). However, the synthesis of Dol-P_2-*NN'*-diacetylchitobiose from UDP-NAcGlc and Dol-P_2-NAcGlc was not prevented by high concentrations of the detergent. The formation of lipid-linked oligosaccharides by an oviduct system (Chen & Lennarz, 1976) increased when detergent was omitted from the system. A similar result with yeast membranes has been reported (Lehle & Tanner, 1978) and was apparently due to the prevention of further transfer of oligosaccharide to protein.

Solubilization of the enzymes resonsible for the production of Dol-P_2-NAcGlc, Dol-P_2-*N'N*-diacetylchitobiose and Dol-*P*-Man (Heifetz & Elbein, 1977*a*) and for the transfer of the first mannose residue to Dol-*N'N*-diacetylchitobiose (Heifetz & Elbein, 1977*b*) has been achieved by using Nonidet P-40 and Triton X-100.

More recently, Nillson *et al.* (1978) have observed differential effects of Triton X-100 on the *N*-acetyl-

Abbreviations used: Dol-*P*, dolichol (mono)phosphate; Dol-P_2, dolichol diphosphate; Dol-P_2-NAcGlc, dolichol diphosphate *N*-acetylglucosamine; Dol-*P*-Man, dolichol phosphate mannose; Dol-*P*-Glc, dolichol phosphate glucose.

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glucosamine transferase to dolichol pyrophosphate in rough and smooth endoplasmic reticulum and Golgi membranes. The present paper extends their work and presents a rationalization of the observations.

Materials and Methods

Materials

Dol-*P* and purified baker's yeast RNA were obtained from Sigma. GDP-D-[U-^{14}C]mannose (179mCi/mmol), UDP-D-[U-^{14}C]glucose (312mCi/mmol) and UDP-D-*N*-acetyl[U-^{14}C]glucosamine (300mCi/mmol) were purchased from The Radiochemical Centre. Chloroform and methanol were redistilled before use, all other solvents and chemicals were of analytical grade.

Methods

Preparation of purified endoplasmic reticulum. Purified rough- and smooth-endoplasmic-reticulum fractions were prepared at 4°C by a modification of the method of Caccam *et al.* (1969). Male Wistar rats (250–300g) were killed by cranial fracture, and their livers excised. Approx. 20g of liver was homogenized with a Potter–Elvehjem apparatus in 2.3vol. of ice-cold 0.05M-Tris/acetate buffer (pH7.4)/0.25M-sucrose and centrifuged in an MSE 8×50ml angle rotor at 20000*g* for 10min. To the supernatant was added 1.5M-CsCl to give a final concentration of 0.015M. A portion (1vol.) of this was layered over 2vol. of 1.3M-sucrose in 0.015M-CsCl and centrifuged in an MSE 6×38 swing-out rotor at 115300*g* for 2¼h. The pellet of rough endoplasmic reticulum was resuspended in 0.05M-Tris/HCl, pH7.2, to a final volume of 0.3ml/g of liver used. The top layer and the opaque interface were carefully removed and diluted with an equal volume of 0.05M-Tris/HCl, pH7.2, and recentrifuged at 115300*g* for 2¼h in the swing-out rotor. The pellet of smooth endoplasmic reticulum obtained was resuspended in 0.05M-Tris/HCl, pH7.2, to a final volume of 0.1ml/g of liver used.

Preparation of mitochondria. Mitochondria were prepared by using a modification of the method of Weinbach (1960).

Weighed livers were minced in a Polytron homogenizer (model X-1020, setting 4 for 10s) in 3vol. of 0.05M-Tris/acetate buffer, pH7.4, containing 0.25M-sucrose and centrifuged in an MSE 8×50 angle rotor at 500*g* for 10min. The supernatant was carefully removed and re-centrifuged in the same rotor at 4000*g* for 10min. The mitochondrial pellet was resuspended in homogenizing buffer and the 4000*g* centrifugation repeated. The pellet of washed mitochondria was resuspended in homogenizing buffer to a final volume of 0.3ml/g of liver used.

Chemical and enzymic assessments of subcellular fractions. Protein. Protein from the liver fraction was precipitated with ice-cold 10% (w/v) trichloroacetic acid and delipidated with chloroform/methanol/hydrochloric acid (500:500:3, by vol.). A modified biuret method (Gornall *et al.*, 1949) was used to estimate protein content. A 0.5ml sample was used, with a final volume of 3ml, containing 0.17M-NaOH. The colour developed by the biuret reagent was read at 555nm against a reagent blank, and the protein content determined by reference to a standard curve constructed by using bovine serum albumin.

Lipid. Lipid, estimated as phosphate, was extracted by using chloroform/methanol/hydrochloric acid (as above) and determined by using a modification of the method of Bartlett (1959). The sample was digested with 0.7ml of $HClO_4$ (72%, w/v) at 180°C. After cooling, 4ml of water, 0.2ml of ammonium molybdate (5%, w/v) and 0.2ml of 1-amino-2-naphthol-4-sulphonic acid reagent were added, and, after incubation at 90°C for 15min, the A_{830} was read against a reagent blank. The phosphorus content was determined by reference to a standard curve constructed with KH_2PO_4.

RNA. RNA was separated from DNA and protein by the method of Schneider (1957), and assayed by using orcinol reagent (Mejbaum, 1939). Purified yeast RNA was used for the construction of a standard curve.

Enzyme assays. Acid phosphatase activity was assayed as described by Schachter *et al.* (1970) as a marker for lysosomal contamination of membrane fractions.

Mitochondrial integrity was assessed by measurement of succinate–cytochrome *c* reductase activity as described by Devartanian & Veeger (1964). Microsomal contamination of mitochondrial fractions was assessed by activity of glucose 6-phosphatase, determined by the method of de Duve *et al.* (1955).

Incubation of membrane fractions. Fresh or stored (0°C) tissue fractions (0.15ml) were incubated with (*a*) 0.14nmol (0.025μCi) of GDP-[U-^{14}C]mannose, or (*b*) 0.4nmol (0.125μCi) of UDP-*N*-acetyl[U-^{14}C]-glucosamine or (*c*) 0.42nmol (0.125μCi) of UDP-[U-^{14}C]glucose in a total volume of 225μl containing 8.8mM-$MnCl_2$, 2.2mM-EDTA and 26.4mM-Tris/HCl, pH7.1. Triton X-100 and Dol-*P* were included as indicated in the Results section. When required, Dol-*P* was added to empty tubes in chloroform/methanol (2:1, v/v) and evaporated to dryness under N_2. If Triton X-100 was also to be included, it was added to the dried Dol-*P* with thorough vortex-mixing. Normal procedure was then to add the rest of the incubation components, except the labelled NDP-sugar, and preincubate for 5min, after which time the NDP-sugar was added and the timed incu-

bation begun. Length of incubations are indicated in the Results section, and were performed at 37°C with shaking.

Lipid extraction of incubated tissue fractions

Incubations were stopped after the required time with 2ml of chloroform/methanol (2:1, v/v) and 2ml of water. After thorough vortex-mixing the layers were separated by bench centrifugation, and the chloroform phase removed with a Pasteur pipette and retained. The remaining aqueous and protein layers were washed twice with 2ml of chloroform/methanol (2:1, v/v), by using vortex-mixing and centrifugation, the chloroform phases being pooled with the initial extract. The bulked extract was washed with 2ml of water, the layers being separated by centrifugation, the lower layer being retained as the 'CM fraction'.

Chromatography. Samples of labelled lipid extracts were chromatographed on precoated silica-gel plates (DC-Plastikfolien Kieselgel 60 F254; 0.25mm thick; Merck) in chloroform/methanol/water (60:25:4, by vol.). After being run, the plates were tested for radioactivity with a Panax RTLS-1A scanner and, for more definitive results, were radioautographed by placing in contact with X-ray film (Kodirex, Kodak) for 2–3 weeks. Development of the films was performed by using PQ Universal developer (Ilford) and fixed with F-40 fixer (Ilford).

DEAE-cellulose column chromatography was performed on labelled lipid extracts by using Whatman DE-52 microgranular pre-swollen cellulose (W. and R. Balston, Maidstone, Kent, U.K.). The DEAE-cellulose was converted into the acetate form by standing it overnight in acetic acid, which was subsequently removed by filtration under vacuum on a Buchner funnel and flask. The pad of cellulose was washed free of acetic acid with methanol, and then washed with chloroform/methanol (2:1, v/v). The cellulose was packed into 0.6cm columns in chloroform/methanol (2:1, v/v) and, after application of the sample, was eluted as described by Palamarczyk & Hemming (1975). This involved a stepwise elution with 20ml of chloroform/methanol (2:1, v/v) followed by 20ml of methanol, then 30ml of 0.01M-ammonium acetate in chloroform/methanol (2:1, v/v) and finally 30ml of 0.05M-ammonium acetate in chloroform/methanol (2:1, v/v). The ammonium acetate was subsequently removed from the eluted fractions by washing with Folch upper phase, consisting of chloroform/methanol/water (3:48:47, by vol.) (Folch *et al.*, 1957).

Counting of extracts for radioactivity. Radioactivity was determined by using Packard Tri-Carb liquid-scintillation spectrometers, models 3375 and 3255. The lipid samples were dried under N_2 and to them were added 10ml of xylene/phosphor scintillation 'cocktail' {5g of PPO (2,5-diphenyloxazole) and 0.01g of POPOP [1,4-bis-(5-phenyloxazol-2-yl)-benzene] in 1 litre of xylene}.

Results

Enzymic and chemical analyses of fractions

Acid phosphatase specific activities in smooth- and rough-endoplasmic-reticulum fractions were found to be between 5 and 10%, and 10 and 15% of the specific acitivity in total homogenate respectively. Modified assays for this enzyme demonstrated that the specific activities were not altered when detergent concentrations were used that corresponded to the Triton X-100-to-phospholipid ratios used in the subsequent glycosyl transfer assays. Glucose 6-phosphatase activity in mitochondrial preparations was 5% of that in total microsomal fractions. The protein-to-phospholipid ratio in rough endoplasmic reticulum was slightly higher than in smooth endoplasmic reticulum or mitochondria, but in all cases it approximated to 3. The RNA-to-phospholipid ratio was 4.5-fold higher in rough endoplasmic reticulum than in smooth (0.33 and 0.07 respectively).

Time-dependence of Dol-P-[^{14}C]monosaccharide production

Fig. 1 shows the time-dependence of Dol-*P*-monosaccharide production in (*a*) rough and (*b*) smooth endoplasmic reticulum and (*c*) mitochondria. In rough endoplasmic reticulum, Dol-*P*-[^{14}C]Man synthesis is greatest between 5 and 10min, reaching a measured maximum of 2.1pmol per mg of protein. Highest smooth-endoplasmic-reticulum activity was 70% of this value Dol-*P*-[^{14}C]Glc formation gave similar profiles and again there was more activity in rough endoplasmic reticulum. Dol-P_2-NAc[^{14}C]Glc production was almost constant for 30min at 0.28 pmol per mg of protein in rough endoplasmic reticulum, but again in smooth endoplasmic reticulum there was less activity, with a maximum of 0.2pmol incorporated per mg of protein after 5min.

Profiles for Dol-*P*-[^{14}C]monosaccharide formation in mitochondria were similar to those gained for microsomal fractions. The striking feature of these results is the higher specific activity of the enzymes in mitochondria, approximately double that in rough endoplasmic reticulum with regard to Dol-*P*-[^{14}C]Glc and Dol-P_2-NAc[^{14}C]Glc formation and 50% higher with regard to Dol-*P*-[^{14}C]Man formation. In view of the results presented in Fig. 1, all subsequent assays of transferase activities reported in the present paper were over a 5min period.

Product identification was confirmed by using t.l.c. and DEAE-cellulose column chromatography

as described in the Materials and Methods section. Dol-*P*-[^{14}C]Man was identified as the product of incubations with GDP-[^{14}C]mannose by its elution from a column of DEAE-cellulose acetate by 0.01 M-ammoniun acetate in chloroform/methanol (2:1, v/v) and by its identical mobility with the authentic compound in the t.l.c. system (R_F 0.34). The production of Dol-*P*-[^{14}C]Glc from UDP-[^{14}C]glucose was confirmed in a similar manner. The formation of Dol-P_2-NAc[^{14}C]Glc from UDP-*N*-acetyl[^{14}C]-glucosamine was shown by its elution from a DEAE-cellulose acetate column by 0.05 M-ammonium acetate in chloroform/methanol (2:1, v/v) and by its mobility on t.l.c. All three products released the appropriate [^{14}C]monosaccharide on mild treatment with acid, but were stable to mild alkali treatment (see, e.g., Palamarczyk & Hemming, 1975).

Effect of Triton X-100

Fig. 2 shows the effects of increasing concentrations of Triton X-100 on the enzyme activities in (*a*) rough and (*b*) smooth endoplasmic reticulum, and (*c*) mitochondria. Although Dol-*P*-[^{14}C]Man production was inhibited completely by a Triton X-100-to-phospholipid ratio of 0.45 in rough endoplasmic reticulum, in the smooth-membrane fraction there was only 45% inhibition. Only a very low dose of

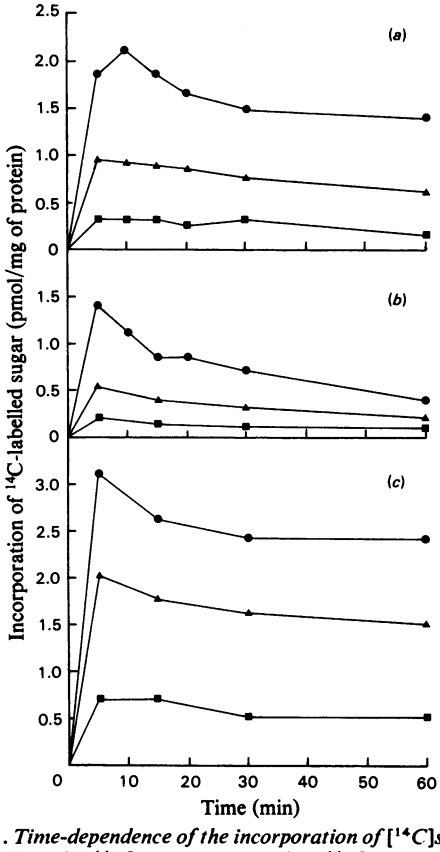

Fig. 1. *Time-dependence of the incorporation of* [^{14}C]*sugars from NDP-*[*U*-^{14}C]*sugars into Dol-P-*[^{14}C]*sugars in* (*a*) *rough endoplasmic reticulum,* (*b*) *smooth endoplasmic reticulum and* (*c*) *mitochondria*
●, GDP-[U-^{14}C]mannose; ▲, UDP-[U-^{14}C]glucose; ■, UDP-*N*-acetyl[U-^{14}C]glucosamine. Incubation details are given in the Materials and Methods section. Points represent mean values from five determinations. The deviation from the mean was ±7.5%.

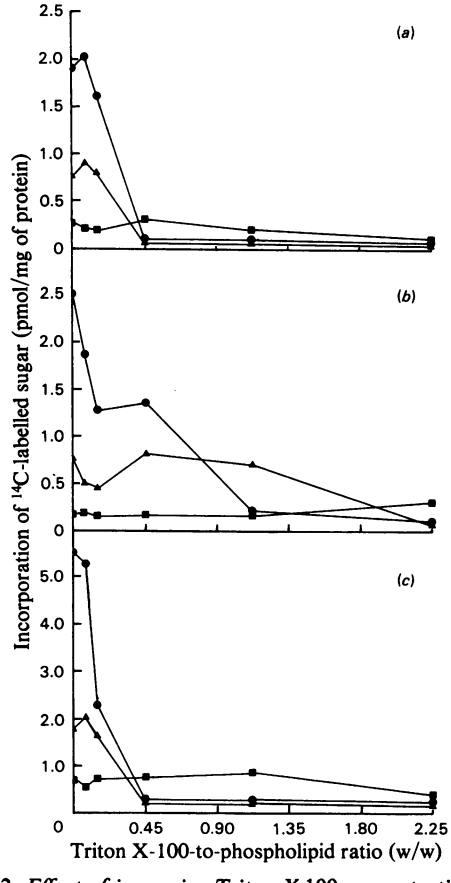

Fig. 2. *Effect of increasing Triton X-100 concentration on the transfer of* [^{14}C]*sugars from NDP-*[*U*-^{14}C]*sugars into Dol-P-*[^{14}C]*sugars in* (*a*) *rough endoplasmic reticulum,* (*b*) *smooth endoplasmic reticulum and* (*c*) *mitochondria*
●, GDP-[U-^{14}C]mannose; ▲, UDP-[U-^{14}C]glucose; ■, UDP-*N*-acetyl[U-^{14}C]glucosamine. Incubations (5 min) were as described in the Materials and Methods section. Points represent means from four to six determinations. The deviation from the mean was ±5%.

Triton X-100, giving a Triton X-100-to-phospholipid ratio of 0.015, was not inhibitory to the enzyme in rough endoplasmic reticulum. In contrast, in smooth endoplasmic reticulum the enzyme activity was stable between Triton X100-to-phospholipid ratios of 0.15–0.45. Mito-chondrial Dol-*P*-[^{14}C]Man synthesis closely resembled that of rough endoplasmic reticulum in response to detergent.

Dol-*P*-[^{14}C]Glc production in rough endoplasmic reticulum was affected by Triton X-100 in a very similar manner to Dol-*P*-[^{14}C]Man formation in this fraction. However, in smooth endoplasmic reticulum at Triton X-100-to-phospholipid ratios of between 0.45 and 1.125, comparatively high Dol-*P*-[^{14}C]Glc production remained, whereas Dol-*P*-[^{14}C]Man production decreased considerably. Again, mitochondrial Dol-*P*-[^{14}C]Glc production responded similarly to rough endoplasmic reticulum with increased Triton X-100 concentrations.

Dol-P_2-NAc[^{14}C]Glc formation was inhibited slightly by detergent concentrations giving a Triton X-100-to-phospholipid ratio between 1.125 and 2.25 in rough endoplasmic reticulum, and between 0.075 and 1.125 in smooth endoplasmic reticulum. In the smooth-membrane fraction the activity of the enzyme producing Dol-P_2-NAc[^{14}C]Glc was restored and enhanced at a Triton X-100-to-phospholipid ratio of 2.25. Mitochondrial Dol-P_2-NAc[^{14}C]Glc synthesis was affected by detergent in a similar manner to that in rough endoplasmic reticulum.

Effect of exogenous Dol-P

The effects of exogenous Dol-*P* (15nmol) on enzyme activities in rough and smooth endoplasmic reticulum at Triton X-100-to-phospholipid ratios of 0.15 and 1.125 are shown in Fig. 3. Dol-*P* did not restore enzyme activity with regard to Dol-*P*-[^{14}C]-Man and Dol-*P*-[^{14}C]Glc formation in rough endoplasmic reticulum at a Triton X-100-to-phospholipid ratio of 1.125, nor for Dol-*P*-[^{14}C]Glc formation in smooth endoplasmic reticulum. However, a marked enhancement of activity was seen for these two enzymes in rough and smooth endoplasmic reticulum with the lower dose of detergent and for Dol-*P*-[^{14}C]Man formation by using the higher dose of Triton X-100 in smooth endoplasmic reticulum.

Dol-*P* had little effect on Dol-P_2-NAc[^{14}C]Glc formation at a Triton X-100-to-phospholipid ratio of 0.15 in either rough or smooth endoplasmic reticulum. However, with the higher dose of detergent a marked stimulation was apparent in both fractions.

The effect of dolichol monophosphate on the mitochondrial enzymes with the two doses of detergent is shown in Fig. 4. At a Triton X-100-to-phospholipid ratio of 0.15, exogenous Dol-*P* stimulated the production of Dol-*P*-[^{14}C]Man and Dol-*P*-[^{14}C]Glc, but had little effect on Dol-P_2-NAc[^{14}C]Glc

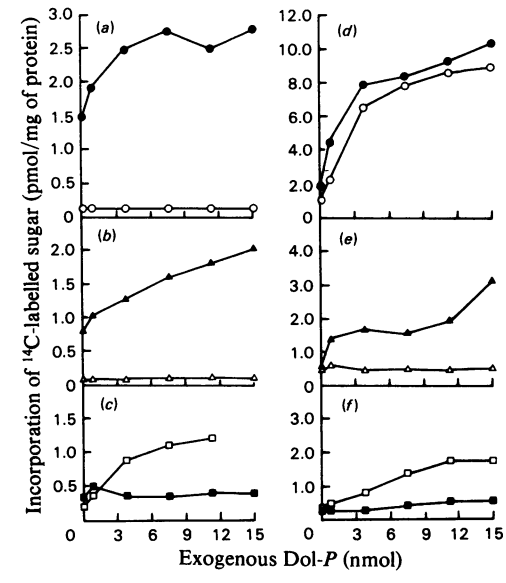

Fig. 3. *Effect of exogenous dolichol monophosphate on the transfer of* [^{14}C]*sugars from NDP-*[*U-*^{14}C]*sugars into Dol-P-*[^{14}C]*sugars in* (*a–c*) *rough and* (*d–f*) *smooth endoplasmic reticulum at Triton X-*100*-to-phospholipid ratios of* 0.15 (*closed symbols*) *and* 1.125 (*open symbols*)
●, GDP-[U-^{14}C]mannose; ▲, UDP-[U-^{14}C]glucose; ■, UDP-*N*-acetyl[U-^{14}C]glucosamine. Incubations (5min) were as described in the Materials and Methods section. Points represent means from four determinations. The deviation from the mean was ±5%.

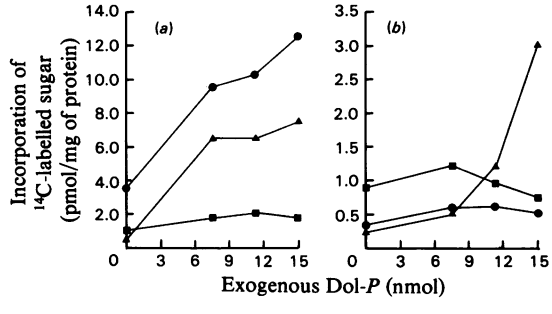

Fig. 4. *Effect of exogenous Dol-P on the transfer of* [^{14}C]-*sugars from NDP-*[*U-*^{14}C]*sugars into Dol-P-*[^{14}C]*sugars in mitochondria at Triton X-*100*-to-phospholipid ratios of* (*a*) 0.15 *and* (*b*) 1.125
●, GDP-[U-^{14}C]mannose; ▲, UDP-[U-^{14}C]glucose; ■, UDP-*N*-acetyl[U-^{14}C]glucosamine. Incubations (5min) were as described in the Materials and Methods section. Points represent means from four determinations. The deviation from the mean was ±8%.

synthesis. With the higher dose of detergent, only Dol-*P*-[^{14}C]Glc production was stimulated, there being little overall effect of Dol-*P* on the other two enzyme activities.

Discussion

The action of Triton X-100 on membranes has been dealt with in a comprehensive review by Helenius & Simons (1975). At concentrations of the detergent below those causing lysis, slight disruption of the lamellar structure of the bilayer occurs, allowing easier passage of small molecules across the membrane (stage 1). As the amount of detergent is increased to that causing lysis, the permeability barrier to macromolecules is lost, but the basic structure of the membrane is retained (stage 2). Higher concentrations of detergent cause lamellar–micellar phase transition and thus solubilization into mixed micelles of detergent, lipid and protein (stage 3). At very high concentrations of detergent, protein and lipid are separated, micelles of either detergent and protein or detergent and lipid being formed (stage 4).

The Triton X-100-to-phospholipid ratio is an adequate parameter for the assessment of the extent of detergent–membrane interaction. Accumulated data (Helenius & Simons, 1975) would indicate that stage 1 prevails up to a ratio of 0.06, stage 2 at a ratio of up to 1.0, stage 3 between ratios of 1.0 and 2.8, and stage 4 at a ratio in the order of 6.6.

The Triton X-100 concentrations used in the present experiments gave Triton X-100-to-phospholipid ratios of between 0.0 and 2.25. The majority of intrinsic proteins in a membrane have been isolated without loss of biological activity by using Triton X-100 (Tzagoloff & Penefsky, 1971). Only at delipidating concentrations of this detergent is activity lost (Walter & Hasselbach, 1973).

Also relevant to the interpretation of the effects of Triton X-100 reported in the present paper is the observation (Dallner *et al.*, 1975), based on results of partial digestion of membranes with trypsin, that mannose transfer to Dol-*P* is mediated by enzymes found on both the outer and inner surface of the bilayer of vesicles derived from endoplasmic reticulum.

From the preceding discussion it is apparent that the most likely explanation for the observations presented in Fig. 2 is that transferase activities expressed at Triton X-100-to-phospholipid ratios below 0.3 are due primarily to enzymes located on the outer face of the vesicles. Changes seen as the detergent concentration is increased to that causing solubulization are therefore due to enzymes at the inner face of the vesicles. Vesicles prepared in the described manner are not subject to inversion of the membrane components (Nillson & Dallner, 1977).

It follows that the stimulation of mannosyl- and glucosyl-transferase activities in vesicles derived from rough endoplasmic reticulum at a Triton X-100-to-phospholipid ratio of 0.15 is due to changes at the outer face. Conversely, the stimulation of activity in vesicles derived from smooth endoplasmic reticulum at a ratio of 0.45 is due to changes at the inner face. The changes involved are, possibly, due to an increase in the availability of substrate.

The decreases in activity at Triton X-100-to-phospholipid ratios of between 0.0 and 0.3 are best explained by assuming a decrease in activity of the enzymes in the outer face of vesicles. That this could be due to separation of enzymes from endogenous pools of Dol-*P* is supported by the stimulatory effect of exogenous Dol-*P*, at a low Triton X-100-to-phospholipid ratio, in all membranes studied (Figs. 3 and 4).

Thus these results are consistent with the presence of mannosyl- and glucosyl-transferase activity in smooth vesicles at both the inner and the outer faces of the membrane. They also confirm the presence of these enzymes at the outer face of rough vesicles and mitochondria. Although the results do not reflect the presence of these enzymes at the inner face of either rough vesicles or mitochondrial outer membrane, such a location is still possible. Other factors, such as ribosomal binding, may alter the susceptibility of these membranes to detergent and thus mask the effects of the detergent.

High Triton X-100-to-phospholipid ratios cause inhibition of glucosyltransferase in all membranes studied and mannosyltransferase in rough vesicles and mitochondria. The nature of this inhibition is not attributable to separation of Dol-*P* from the enzymes, since exogenous acceptor does not restore activity. Other factors are therefore involved. However, this does seem to be the main explanation for loss of mannosyltransferase activity in smooth vesicles at high Triton X-100-to-phospholipid ratios, since exogenous Dol-*P* restores enzyme activity to similar values at both high and low Triton X-100 concentrations (Fig. 3).

The slight response of the activity of *N*-acetylglucosamine phosphate transferase to increasing Triton X-100 concentrations is consistent with a very hydrophobic enzyme that is in very close association with lipid. The relative insensitivity of this enzyme in all membrane preparations to exogenous Dol-*P* at low Triton X-100-to-phospholipid ratios supports this view. Only at high Triton X-100-to-phospholipid ratios is stimulation of activity apparent in all membrane preparations in the presence of exogenous dolichol monophosphate. This is also true in smooth vesicles as regards endogenous activity. It appears that the strong binding to lipid protects this enzyme from the inactivation caused by high Triton X-100-to-phospholipid ratios to which the more hydrophilic

mannosyl- and glucosyl-transferases are susceptible. It follows that the *N*-acetylglucosamine phosphate transferase is probably more deeply embedded in the lipid matrix of the membranes than are the other transferases.

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