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THE EFFECT OF TEMPERATURE ON THE GALACTOSYL- AND SIALYL-TRANSFERASES AND ON THE ULTRASTRUCTURE OF GOLGI MEMBRANES

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SUMMARY

The effect of temperature on the activity of galactosyl- and sialyltransferases of rat liver Golgi membranes and the galactosyltransferase of serum has been studied. Arrhenius plots for the three enzymes were different. Sharp breaks in the curves, indicative of phase transitions were observed for sialyltransferase (28 °C) of Golgi and galactosyltransferase (34 °C) of serum but not for galactosyltransferase of Golgi. The activation energy was greater above the break (above 28 °C) than below for sialyltransferase of Golgi. The activation energy was lower (above 34 °C) for galactosyltransferase of serum than below.

Electron microscopic freeze fracture replicas showed a patchy distribution of particles which increased as the temperature was raised accompanied by smooth areas. This was interpreted as representing lateral phase separation of the membrane components.

INTRODUCTION

Glycoproteins are synthesized in the Golgi complex of the cell by the sequential addition of monosaccharide units to the oligosaccharide covalently linked to the peptide chain. The enzymes responsible are the glycosyltransferases [1] which are an integral part of the membranes of the Golgi complex. In their passage through this membrane system, proteins are glycosylated and then secreted from the cell.

Membrane-bound enzymes of microorganisms and mitochondria of plant and animal cells have been studied particularly in relation to transport systems in *Escherichia coli* [2, 3], yeast mitochondria [4] and mammalian mitochondria [5]. Using physical techniques, these reports have indicated that discontinuities in Arrhenius plots of the membrane-bound enzymes are paralleled by a change in the physical state of the membrane lipids from liquid-crystalline to gel state [6]. In a study of the effects of phospholipids on the activity of purified $(Na^+ + K^+)$-stimulated adenosine triphosphatase, Kimelberg and Papahadjopoulos [7] conclude that the activity of the enzyme is related to the phase transitions in the phospholipid bilayers and biomembranes. The role of protein-lipid interactions is stressed.

The purpose of the present work has been to study the effect of temperature on the activities of two glycosyltransferases, galactosyl- and sialyltransferase, of purified rat liver Golgi membranes. These two enzymes form a part of the multiglycosyltransferase system for synthesis of plasma glycoproteins. It is interesting to observe that the temperature dependence of the activity is different for the two membrane-bound enzymes.

METHODS

Golgi fractions were isolated from fasted male Wistar rats according to the method of Sturgess et al. [8]. Each preparation was monitored for purity by electron microscopy and for galactosyltransferase activity [9]. Twelve Golgi preparations were pooled, sonicated, and stored at −10 °C in 200 μl aliquots to provide a uniform enzyme source. The protein concentration was determined by the method of Lowry et al. [10].

The Golgi galactosyltransferase activity was determined according to the method of Treloar et al. [9] using *N*-acetyl-D-glucosamine as acceptor. An initial rate during an incubation of 3 min was used unless otherwise indicated.

The sialyltransferase activity was assayed by a modification of the method of Schachter et al. [11]. The acceptor, desialylated orosomucoid, was generously donated by Dr. Harry Schachter of the Department of Biochemistry, University of Toronto. The reaction mixture contained 0.1 mg desialylated orosomucoid, 5 μl 0.5 M pipirazine-*N*,*N*′-bis (2-ethane sulfonate) monosodium monohydrate, pH 7.5, 5 μl 5% Triton X-100, 0.25 μM CMP-[^{14}C]*N*-acetylneuraminic acid (10^5 dpm) and 0.04 mg Golgi protein in a total volume of 40 μl. The incubation time of 4 min represented an initial rate.

The serum galactosyltransferase activity was assayed according to a modification of the method of Hudgin et al. [12]. The reaction mixture contained 0.25 mg *N*-acetylglucosamine as acceptor, 5 μl 0.2 M $MnCl_2$, 5 μl 0.5 M glycine buffer, pH 9.6, 5 μl 0.01 M UDP-[^{14}C]galactose (10^6 dpm) and approximately 1 mg serum protein in a total volume of 45 μl. A 10 min time of incubation represented an initial rate.

After incubation at the specific temperatures, all samples were subjected to high voltage electrophoresis on Whatman No. 3MM paper, in 0.1 % sodium tetraborate buffer at 3 kV (35–50 mA) for 90 min. All papers were scanned by ultraviolet light and a Packard radiochromatogram scanner. The origins were cut from the ionograms and counted in Aquasol in a Nuclear Chicago Mark I scintillation counter.

The activation energies were calculated by the formula

$$E_a = \frac{4.56 \times T_1 T_2 (\log k_2 - \log k_1)}{T_2 - T_1}$$

where E_a represents the Arrhenius activation energy, k_1 and k_2 are rate constants at absolute temperatures T_1 and T_2.

The UDP-[^{14}C]galactose (specific activity = 274 μCi/mol) was purchased from New England Nuclear Co. The CMP-[^{14}C]-*N*-acetylneuraminic acid was a gift from Dr. Harry Schachter. The piperazine-*N*,*N*′-bis (2-ethane sulfonate) monosodium, monohydrate was purchased from Calbiochem Corp. All other chemicals were purchased from Nutritional Biochemicals Corp.

Electron microscopy

Ultrastructural changes in the Golgi complex at different temperatures (0, 20, 33 and 43 °C) were investigated in ultrathin sections, by negative staining and by freeze-fracture techniques.

For thin sectioning, membranes were fixed for 30 min with 2 % glutaraldehyde in 0.1 M sodium phosphate buffer, which had been incubated at the same temperature as the membranes. After rinsing in 0.1 M sodium phosphate buffer, the membranes were postfixed in 1 % osmium tetroxide in veronal acetate buffer, pH 7.4, dehydrated in graded ethanol solutions and then embedded in Spurr low viscosity epoxy resin. The ultrathin sections were cut on a Porter-Blum MT-2 Ultramicrotome using a diamond knife. Section contrast was enhanced with uranyl acetate and lead citrate.

For negative staining, the Golgi membranes without fixation were dropped gently onto the surface of 1.5 % potassium phosphotungstate, pH 7.0. Carbon-coated grids were placed on the surface, excess stain was removed and the grids were allowed to dry in air.

For freeze fracture, the membranes were fixed in 2 % glutaraldehyde for 3 min by centrifugation through a sucrose gradient. The membranes were then incubated for 2 h in 30 % glycerol as cryoprotectant. Specimens were placed in gold cups, frozen quickly in liquid Freon 22 and then stored in liquid N_2. Fractures were performed at −115 °C at $2 \cdot 10^6$ Torr, shadowed with platinum-carbon and replicated with carbon using a Balzers BA 360 freeze-etch apparatus.

All specimens were examined at 60 kV in a Philips EM 200 electron microscope.

RESULTS

In the Arrhenius plot, the logarithm of the activity is plotted against the reciprocal of the absolute temperature. Such a plot of the galactosyltransferase activity of the Golgi-rich fraction is shown in Fig. 1. The results form a straight line from which the energy of activation was calculated to be 20 397 cal. At 43 °C, (not shown) there was an abrupt change in the slope with rapid loss of activity which represented thermal denaturation of the enzyme.

The Arrhenius plot for sialyltransferase was discontinuous (Fig. 2) showing a break at 28 °C. The activation energy below 28 °C was 4548 and 13 302 cal above 28 °C. As with the galactosyltransferase thermal denaturation was observed above 43 °C.

The Arrhenius plot for the serum galactosyltransferase is shown in Fig. 3. A discontinuity was observed at 34 °C. The activation energy below 34 °C was 19 118 cal and 5 674 cal above.

Electron microscopy

In ultrathin sections, the characteristic appearance of the Golgi complex with stacked cisternae, networks of fine tubulus, small vesicles and larger secretory vesicles was preserved at 0–33 °C. At 43 °C, swelling was observed among the cisternae, tubules and vesicles and some cisternae were ruptured.

In negatively stained preparations, the Golgi complex was similar at 0–33 °C, and characterized by flattened, plate-like structures each with a central cisterna

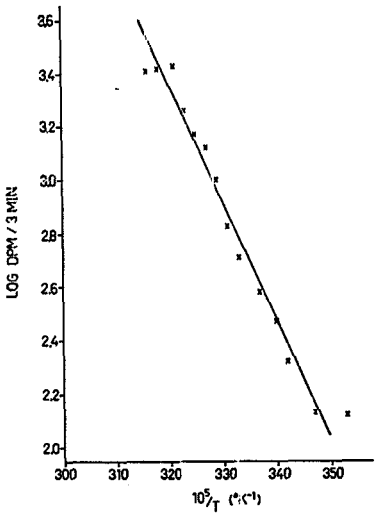

Fig. 1. Arrhenius plot of galactosyltransferase activity (log dpm/3 min) of the Golgi rich fraction against the reciprocal of absolute temperature ($°K^{-1}$)

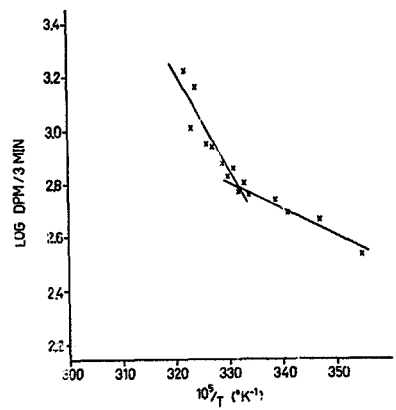

Fig. 2. Arrhenius plot of sialyltransferase activity (log dpm/3 min) of the Golgi rich fraction against the reciprocal of absolute temperature ($°K^{-1}$)

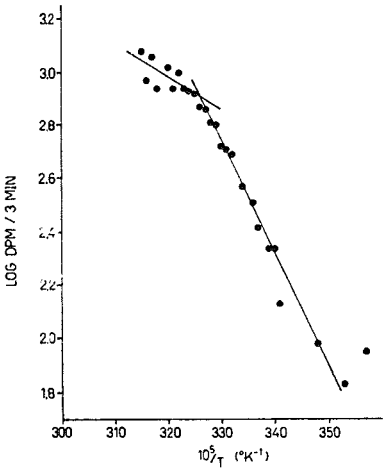

Fig. 3. Arrhenius plot of serum galactosyltransferase activity (log dpm/3 min) against the reciprocal of absolute temperature (°K^{-1})

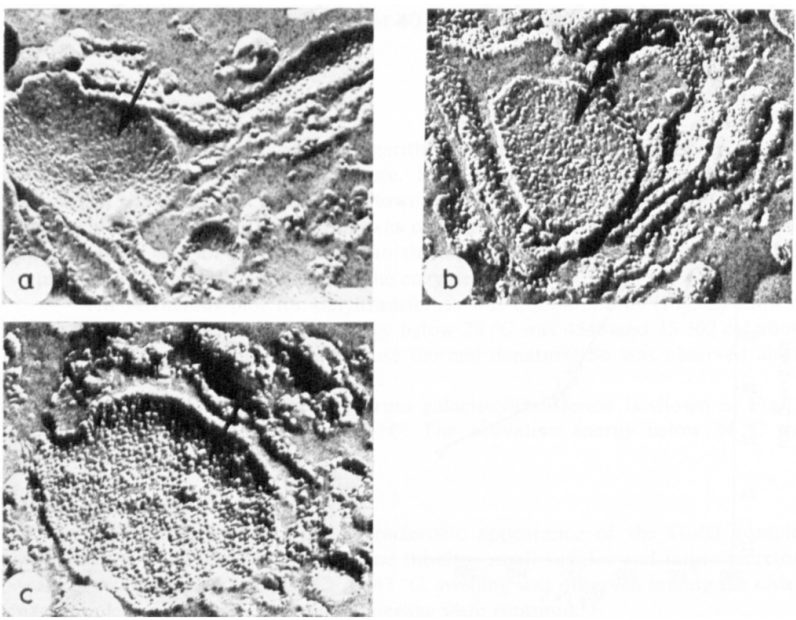

Fig. 4. Freeze fracture replicas of Golgi membranes after incubation at different temperatures (a) 0 °C, (b) 20 °C, (c) 33 °C. The intramembrane particles of the cisternae (arrows) are more prominent with increasing temperature. Magnification 82 000×.

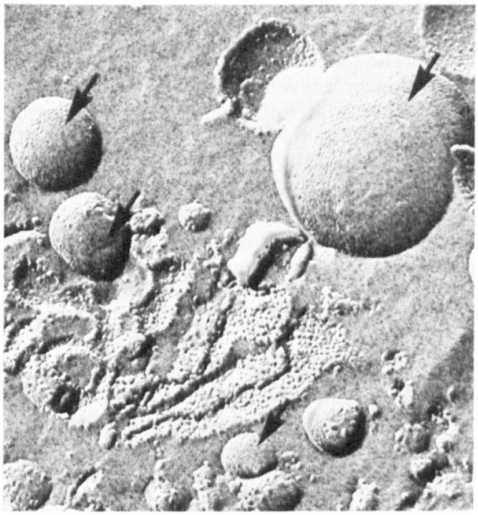

Fig. 5. Freeze fracture replica of Golgi complex, after incubation at 33 °C showing the increased number of smooth fracture faces (arrows) in Golgi rich fraction. Magnification 81 000×.

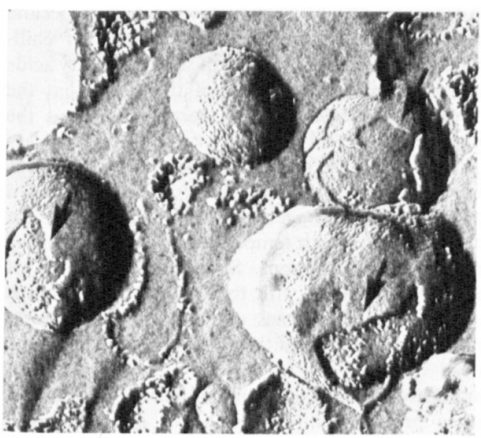

Fig. 6. Freeze fracture replica of Golgi complex after incubation at 43 °C, showing the typical "patchy" appearance of fracture faces. Magnification 89 000×.

surrounded by a network of fine tubules and vesicles. At 43 °C, the cisternae were vacuolated and disrupted.

In freeze-fracture replicas, there was no significant difference in distrubition of intramembrane particles on either concave or convex fracture faces at different temperatures. However, particles on the convcae faces of the cisternae became more prominent with increasing temperatures from 0–33 °C (Fig. 4). At higher temperatures, 33–43 °C, the proportion of smooth fracture faces increased (Fig. 5). In addition, the proportion of "patchy" surfaces increased, where areas of smooth membrane faces were observed over the particulate faces (Fig. 6). These patchy surfaces, observed on 3–4% of vesicles at 0–20 °C, were present on 20–30% of vesicles at 33–43 °C. Their distribution was mainly on convex fracture faces and were particularly numerous among larger vesicles.

DISCUSSION

It is recognized that the fluidity of the biological membrane is related to the lipid composition. An increase in the length of the fatty acid chain and the degree of saturation of lipid increases the transition temperature, above which, the membrane changes from a crystalline to a liquid-crystalline state [13]. In addition, the presence of cholesterol appears to decrease the fluidity of the membrane [5, 7]. All of these factors have a direct bearing on the activity of protein components in the membrane.

Raison et al. [14] have demonstrated that the physical disruption of the mitochondrial membrane by sonication, freezing and thawing or hypotonic swelling produces no changes in the temperature-dependent activity of respiratory enzymes. However, detergent-induced alterations in the lipid-phase result in changes in activation energies of the membrane-bound enzymes. Measuring the temperature-dependency of the respiratory enzymes of homeothermic animals and chill-sensitive plants, Raison et al. [15] have found phase-transition temperatures of 23 and 12 °C, respectively, which correspond to the temperatures at which injury from exposure occurs. By contrast, no such phase transitions occur in poikilothermic animals or chill-resistant plants. This latter group contains a higher proportion of saturated fatty acids in the membrane compared with the first group. It is evident therefore, that the activity of the membrane-bound enzymes reflects the lipid composition and the physical state of the membrane components.

In the present study, freeze fracture of Golgi complex demonstrate alterations in the membrane structure at higher temperatures. Evidence from the increased proportion of smooth faces and of patchy faces, suggest that the membrane or the plane of cleavage through the membrane varies with temperature possibly resulting from changes in lipid-lipid or lipid-protein interactions. Since the appearance of membranes by freeze-fracture replication correlates with the fluidity of membrane lipids [16], the altered behaviour of Golgi membranes at different temperatures may reflect the physical state of the lipids. It is likely that this influences the membrane proteins and their functional activity.

Both galactosyl- and sialyltransferases are membrane-bound enzymes of the Golgi complex. The sialyltransferase presumably functions after the galactosyltransferase since sialic acid is a terminal sugar. The discontinuity in the Arrhenius plot for the sialyl- and not the galactosyltransferase implies that the two enzymes have

different lipid environments. The Arrhenius plot for the galactosyltransferase shows no phase transitions suggesting that this enzyme is in a more liquid environment than the sialyltransferase.

The importance of the lipid environment may be in maintaining the correct conformation of the membrane-bound enzyme [7]. In the case of glycosyltransferases, the lipid environment of the enzymes has a further important role in providing access of lipid intermediates to the enzymes in the transferase reactions. The dolichols, a group of polyprenols with from 17 to 22 isoprene units have been shown to be important in glycoprotein biosynthesis in rat liver [1]. Access to the appropriate transferase would be facilitated by a liquid-crystalline lipid environment around the enzyme.

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