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THE EFFECT OF LINOLEIC ACID AND BENZYL ALCOHOL ON THE ACTIVITY OF GLYCOSYLTRANSFERASES OF RAT LIVER GOLGI MEMBRANES AND SOME SOLUBLE GLYCOSYLTRANSFERASES

M.M. MITRANIC, J.M. BOGGS and M.A. MOSCARELLO *

Department of Biochemistry, Research Institute, The Hospital for Sick Children, Toronto, Ontario, M5G 1X8 (Canada)

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The effects of the membrane perturbing reagents linoleic acid and benzyl alcohol on the activities of four rat liver Golgi membrane enzymes, *N*-acetylglucosaminyl-, *N*-acetylgalactosaminyl-, galactosyl-, and sialytransferases and several soluble glycosyltransferases, bovine milk galactosyl- and *N*-acetylglucosaminyltransferases and porcine submaxillary *N*-acetylgalactosaminyltransferases have been studied. In rat liver Golgi membranes, linoleic acid inhibited the activities of *N*-acetylgalactosaminyl- and galactosyltransferases by 50% or greater, sialyltransferase by 10–15%, and *N*-acetylglucosaminyltransferase not at all. The isolated bovine milk *N*-acetylglucosaminyltransferase and porcine submaxillary *N*-acetylgalactosylaminyltranferase were not inhibited but bovine milk galactosyltransferase was inhibited by 95% or greater. The inhibition by linoleic acid on Golgi membrane galactosyltransferase appears to be a direct effect of the reagent on the enzyme. Incorporation of bovine milk galactosyltransferase into liposomes formed from saturated phospholipids, DMPC, DPPC, and DSPC (dimyristoyl-, dipalmitoyl-, and distearoylphosphatidylcholine) prevented inhibition of the enzyme activity suggesting that the lipid formed a barrier which did not allow linoleic acid access to the enzyme. The water soluble benzyl alcohol was more effective in inhibiting enzymes of the isolated rat liver Golgi complex. All four glycosyltransferases were inhibited, the *N*-acetylglucosaminyl- and *N*-acetylgalactosaminyltransferases by more than 95%. A higher concentration of benzyl alcohol was necessary to inhibit the galactosyltransferases than was required for the other Golgi enzymes. Benzyl alcohol also inhibited the isolated bovine milk *N*-acetylglucosaminyl- and galactosyltransferases 90% to 95%, respectively, but did not affect the isolated porcine submaxillary gland *N*-acetylgalactosaminyltransferase. Benzyl alcohol did not inhibit the milk galactosyltransferase incorporated into DMPC or DPPC liposomes but showed a complex effect on the activity of the enzyme incorporated into DSPC vesicles, a stimulation of activity at low concentrations followed by an inhibition. A lipid environment consisting of saturated lipids appears to present a barrier to inhibiting substances such as linoleic acid and benzyl alcohol, or lipid may stabilize the active conformation of the enzyme. The different effects of these reagents on four transferases of the Golgi complex suggest that the lipid environment around these enzymes may be different for each transferase.

* To whom all correspondence should be addressed.

Abbreviations: CMPneuraminic acid, cytidine monophosphoneuraminic acid; DMPC, dimyristoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; egg PC, egg phosphatidylcholine; EDTA, ethylenediaminetetraacetic acid; ESR, electron spin resonance; GalNAc, *N*-acetyl-D-galactosamine; GlcNAc, *N*-acetyl-D-glucosamine; Mes, 2(*N*-morpholino)ethanesulfonic acid; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); UDPgalactose, uridine diphosphogalactose; UDP-*N*-acetylgalactosamine, uridine diphospho-*N*-acetyl-D-galactosamine; UDP-*N*-acetylglucosamine, uridine diphospho-*N*-acetyl-D-glucosamine.

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Introduction

The topological distribution of enzymes in intracellular membranes has become a topic of active research interest [1]. While studies with the cytochrome b_5 system of the endoplasmic reticulum are well-advanced, studies with other membrane systems, such as the Golgi apparatus, have just begun. The multi-enzyme system of the Golgi complex consists of the membrane-bound transferases responsible for sequential addition of sugars to the polypeptide chain during the synthesis of glycoproteins. Neither the interaction of the enzymes with each other nor the specific requirements of the lipid environment are understood, although specific structural requirements must be necessary for optimal activity of membrane-bound enzymes [2–6].

The chemical and physical state of the lipid in the microenvironment of the enzyme has been shown to be important in regulating the activity of several membrane-bound enzymes [4,6–11]. On the other hand, the activity of $(Na^+ + K^+)$-ATPase from rat erythrocytes remained unchanged with changes in membrane fluidity [8].

In this communication, we report our studies on the effects of membrane environment on several glycosyltransferases of the Golgi complex isolated from rat liver. These include glycosyltransferases which transfer *N*-acetylglucosamine and *N*-acetylgalactosamine, galactose and sialic acid to the peptide chain and growing oligosaccharide. Each sugar is transferred by a specific glycosyltransferase.

In a model system consisting of purified lipids and milk galactosyltransferase, it was shown that phosphatidylcholine had a marked stimulatory effect on the enzyme while phosphatidic acid was inhibitory [2], showing that the chemical nature of the lipid was important in regulating the activity of this 'soluble' galactosyltransferase. The importance of the physical state of the lipid was demonstrated by experiments employing DMPC. This lipid undergoes a gel to liquid crystalline phase transition at 23°C. However, the activity was much higher at 37°C, well above the transition temperature. Regression of the corpora lutea of prepubertal rats was related to phase changes of the lipids of microsomal membranes isolated from ovaries [12]. Using wide angle X-ray diffraction, these authors showed that regression of the corpora lutea was accompanied by the appearance of increasing amounts of gel-phase lipid, although membrane lipids are generally fluid at physiological temperature.

In the present studies, the structure of the Golgi membranes was perturbed by incorporating membrane-soluble reagents into isolated Golgi membranes. These included linoleic and palmitic acids and benzyl alcohol. Linoleic acid and benzyl alcohol fluidize the Golgi membrane while palmitic acid decreases the fluidity. The glycosyltransferases were found to be sensitive to the perturbing effects of linoleic acid and benzyl alcohol and much less sensitive to effects of palmitic acid.

Methods

Golgi fractions were isolated from livers of male Wistar rats according to the method of Sturgess et al. [13]. 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. [14].

Enzyme assays

Golgi galactosyltransferase activity, using *N*-acetylglucosamine as acceptor, was measured according to the method of Treloar et al. [15]. The incubation medium contained 1 μmol *N*-acetylglucosamine (GlcNAc), 0.05 μmol of UDPgalactose containing 10^5 dpm of UDP-[^{14}C]galactose, 3 μmol of $MnCl_2$, 5 μmol of Mes buffer, pH 5.7, 5 μl of 5% Triton X-100, 5–10 μl sonicated Golgi fraction (25–35 μg protein) and distilled H_2O to a total volume of 50 μl. The reaction was stopped by the addition of 5 μl of 0.25 M EDTA in 2% sodium tetraborate. The velocity of reaction was linear for up to 30 min incubation at 37°C.

Golgi sialytransferase activity was assayed as previously described [16]. The reaction mixture contained 0.1 mg desialylated orosomucoid (DSOM), 5 μl 0.5 M Pipes buffer, pH 7.5, 5 μl 5% Triton X-100, 0.25 μmol CMP-*N*-acetyl[^{14}C]neuraminic acid (10^5 dpm), 5–10 μl of sonicated

Golgi fraction (25–35 μg protein), and distilled H_2O to a total volume of 45 μl. The incubation time was 30 min at 37°C. The reaction was stopped by freezing.

Bovine milk galactosyltransferase was assayed by adapting the methods of Fitzgerald et al. [17] and Khatra et al. [18]. The reaction mixture contained 0.127 μg of the enzyme, 1 μmol GlcNAc, 0.05 μmol UDP[^{14}C]galactose (10^5 dpm), 1 μmol $MnCl_2$ 5 μmol Mes buffer, pH 7.5 and H_2O to a final volume of 45 μl. After incubating 30 min at 37°C, the reaction was stopped by the addition of 5 μl of 0.25 M EDTA in 2% sodium borate.

The products from the Golgi galactosyl and sialyltransferases and bovine milk galactosyltransferase assays were identified by high voltage electrophoresis on Whatman No. 3 mm paper in 1% sodium tetraborate buffer at 3 kV (34–50 mA) for 90 min. All papers were scanned by ultraviolet light and in a Packard radiochromatogram scanner to locate the radioactive areas. The origins were cut from the ionograms and counted in ACS in the Nuclear Chicago Mark I scintillation counter.

The Golgi *N*-acetylgalactosaminyltransferase was assayed using 0.1 mg of normal human basic protein isolated from myelin [19] as acceptor. The assay mixture contained 5 μmol of Mes buffer, pH 7.5, 3 μmol of $MnCl_2$, 0.055 μmol UDP *N*-[^{14}C]acetylgalactosamine, 5 μl of 5% Triton X-100, 5–10 μl Golgi fraction (25–35 μg protein) and distilled H_2O to a volume of 50 μl. Nonradioactive UDP-*N*-acetylgalactosamine was synthesized by the method of Carlson [20]. Following incubation time of 30 min at 37°C, the reaction was stopped by freezing.

The Golgi *N*-acetylglucosaminyltransferase activity was assayed using desialyldegalactosyldehexosaminylorosomucoid (DSDGDHxOM) as acceptor. The assay mixture contained 5 μmol of Mes buffer, pH 5.7, 3 μmol of $MnCl_2$, 5 μl 5% Triton X-100, 5 nmol UDP[^{14}C]*N*-acetylglucosamine containing 55 550 dpm, 200 μg desialyldegalactosyldehexosaminylorosomucoid (DSDGDHxOM), 5–10 μl of Golgi fraction (25–35 μg protein) and distilled H_2O to a volume of 50 μl. Following an incubation of 30 min at 37°C, the reaction was stopped by freezing or by adding 5 μl of 0.25 M EDTA in 2% sodium borate.

The products of the Golgi *N*-acetylgalactosaminyl and *N*-acetylglucosaminyltransferases were recovered on filter paper by an adaptation of the methods of Baxter et al. [21] and Durham et al. [22]. The assay mixtures were spotted onto filter paper strips (1 × 3 cm) and washed in 20% trichloroacetic acid for 20–30 min, two 20–30 min washes in 10% trichloroacetic acid, a wash in ethanol/ether (2 : 1, v/v) and a final wash in ether. When dry, the filter paper strips were counted in ACS in the Nuclear Chicago Mark I scintillation counter.

Electron spin resonance (ESR) measurements

The fatty acids and benzyl alcohol were incorporated into Golgi membranes to be used for spin labelling in the same way as for determination of the effect on enzyme activity except that 400 μg protein were used in a total volume of 0.5 ml to which $0.25 \cdot 10^{-4}$ to $3.75 \cdot 10^{-4}$ M of fatty acid or benzyl alcohol were added. After sonication and incubation at 37°C for 5 min, the samples were transferred to test tubes in which a chloroform solution of the fatty acid spin label, 5-doxylstearate, had been evaporated. 20 μl of 0.25 M $K_3Fe(CN)_6$ were added to the Golgi suspensions to prevent reduction of the spin label.

Spectra were obtained at 37°C on a Varian E-4 spectrometer equipped with a Varian temperature control accessory. Microwave power was 10 mW. The order parameter of the spin label was used as a relative measure of the effects of linoleic and palmitic acid and benzyl alcohol on membrane fluidity and was calculated from the anisotropic hyperfine splittings $T_{\parallel}$ and $T_{\perp}$ [23,24].

Materials

The radioactive nucleotide sugars were purchased from New England Nuclear Corporation. The specific activities of these compounds were as follows: UDP[^{14}C]galactose (302 mCi/mmol), UDP *N*-[^{14}C]acetylgalactosamine (47.2 mCi/mmol), UDP *N*-[^{14}C]acetylglucosamine (290 mCi/mmol) and CMP[^{14}C]sialic acid, (1.6 mCi/mmol). The acceptors desialylated orosomucoid (DSOM) and desialyldegalactosyldehexosaminylorosomucoid (DSDGDHxOM) and the bovine colostrum *N*-acetylglucosaminyltransferase I were gifts from Dr. H. Schachter of the Research

Institute, The Hospital for Sick Children, Toronto, Ontario, Canada.

Results

Effect of linoleic, palmitic acids and benzyl alcohol on the order parameter, S, of 5-doxylstearate in Golgi membranes

The physical state of the lipid in the Golgi membranes can be assessed by studying the order parameter after incorporation of a spin labelled fatty acid as described in Methods. The results are shown in Table I. The order parameter in the absence of any of the perturbing agents was 0.660 for Golgi membranes. Addition of linoleate to a concentration of $2.14 \cdot 10^{-4}$ M decreased the order parameter to 0.578 indicating a marked increase in fluidity. The addition of palmitate did not affect the order parameter. Benzyl alcohol decreased the order parameter to 0.535 from 0.575 for the control membranes at $50 \cdot 10^{-3}$ M. The order parameter varied for different preparations of Golgi membranes as can be seen by the zero values of 0.660 and 0.575 for different preparations.

Effect of linoleic acid on Golgi N-acetylglucosaminyl-, N-acetylgalactosaminyl-, galactosyl- and sialyltransferases

The effect of linoleic acid on the activities of the four glycosyltransferases is shown in Fig. 1 A, B, C, and D for *N*-acetylglucosaminyl-, *N*-acetylgalactosaminyl-, galactosyl- and sialyltransferases, respectively. Increasing concentrations of linoleic acid had no effect on the *N*-acetylglucosaminyltransferase (Fig. 1A). *N*-Acetylgalactosaminyltransferase (Fig. 1B) was moderately inhibited. Inhibition of the galactosyltransferase (Fig. 1C) was observed at higher concentrations, i.e., above $1.5 \cdot 10^{-4}$ M, above which concentration the enzyme was inactivated. Sialyltransferase was slightly inhibited only (Fig. 1D).

TABLE I

EFFECT OF LINOLEATE, PALMITATE AND BENZYL ALCOHOL ON THE ORDER PARAMETER, *S*, OF 5-DOXYLSTEARATE IN GOLGI MEMBRANES

	$T_{\parallel}$	$T_{\perp}$	S
Fatty acid (M)($\times 10^4$)			
0	27.07	9.91	0.660
Linoleate			
0.71	26.10	10.30	0.608
2.14	25.40	10.37	0.578
Palmitate			
0.78	26.90	9.91	0.654
Benzyl alcohol (M)($\times 10^3$)			
0	25.26	10.30	0.575
1	25.25	10.28	0.576
10	25.18	10.28	0.573
30	24.80	10.39	0.554
50	24.25	10.53	0.535

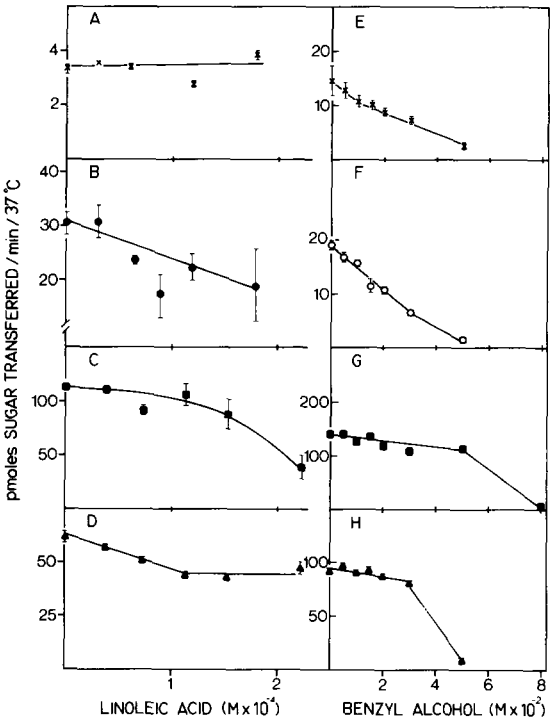

Fig. 1. The effect of linoleic acid (A–D) and benzyl alcohol (E–H) on rat liver Golgi membrane enzymes; *N*-acetylglucosaminyltransferase (X-X), *n*-acetylgalactosaminyltransferase (●——●), galactosyltransferase (■——■), and sialyltransferase (▲——▲).

Effect of benzyl alcohol on Golgi N-acetylglucosaminyl-, N-acetylgalactosaminyl-, galactosyl- and sialyltranferase

The effect of benzyl alcohol on the four transferases is shown in Fig. 1E–H. Both the *N*-acetylglucosaminyl- and *N*-acetylgalactosaminyltransferases were inhibited markedly by

the benzyl alcohol (Fig. 1 E and F). At a concentration of $5 \cdot 10^{-2}$ M benzyl alcohol, less than 20% of the initial enzyme activity remained. At this concentration, a significant fluidizing effect on the membranes was observed (Table I). However, even at a concentration of $1 \cdot 10^{-2}$ M, where there was little or no effect on the order parameter, a significant decrease in the activity of the enzymes was found. The loss of activity of both these transferases was almost linear from the control values.

The effects of benzyl alcohol on the activities of the galactosyl- and sialyltransferases (Fig. 1 G and H) were different from the other two transferases. In the case of the galactosyltransferase, very little inhibition of activity was observed up to $5 \cdot 10^{-2}$ M benzyl alcohol. At higher concentrations, a sharp decrease of activity was observed so that at $8 \cdot 10^{-2}$ M only about 4% of the initial enzyme activity remained. The effect of benzyl alcohol on the sialyltransferase activity was similar. A sharp decrease of activity was observed at $3 \cdot 10^{-2}$ M benzyl alcohol, and total loss of activity at $5 \cdot 10^{-2}$ M.

Although benzyl alcohol is known to fluidize membranes, a change in membrane fluidity was not detected at low concentrations (below $1 \cdot 10^{-2}$ M) by the spin label method. Changes in fluidity at these low concentrations may be detected by some other method. The change in the activities of some of the glycosyltransferases implies some perturbation of the membrane structure and/or enzyme structure has occurred.

Effect of palmitic acid on the activities of Golgi N-acetylglucosaminyl-, N-acetylgalactosaminyl-, galactosyl- and sialyltransferases

The effect of palmitate on the four glycosyltransferases of rat liver Golgi membranes is shown in Fig. 2 A, B, C, and D. None of the transferases were affected to a significant extent by the presence of palmitate in the membrane although moderate activation of the *N*-acetylglucosaminyltransferase and slight inhibition of the galactosyltransferase was observed.

Effect of linoleic acid and benzyl alcohol on some soluble glycosyltransferases

Milk galactosyltransferase is a commercially available enzyme. The effect of linoleic acid and

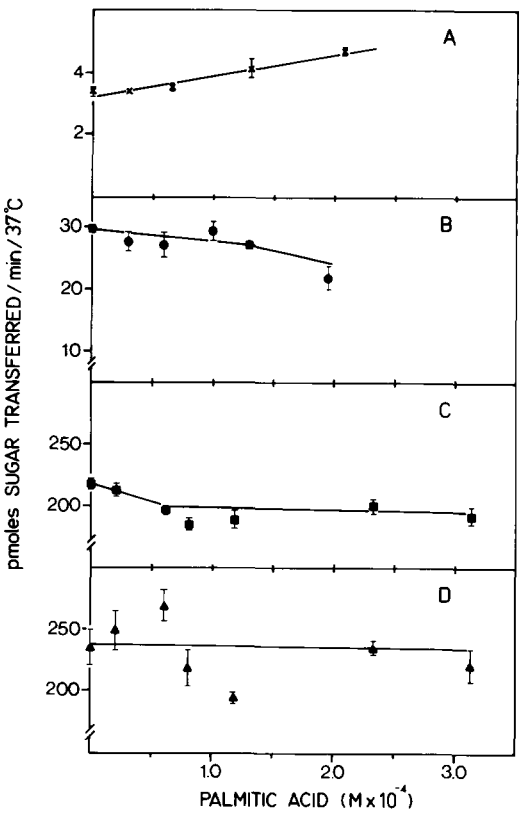

Fig. 2. The effect of palmitic acid on rat liver Golgi glycosyltransferases; *N*-acetylglucosaminyltransferase (X-X), *N*-acetylgalactosaminyltransferase (●——●), galactosyltransferase (■——■), and sialyltransferase (▲——▲).

benzyl alcohol on the activity of this enzyme is shown in Fig. 3A and D. Both reagents abolished the activity of this enzyme in solution. In fact, the Golgi membrane-bound galactosyltransferase was somewhat less sensitive than the soluble milk enzyme (Fig. 1 C and G).

N-Acetylgalactosaminyltransferase was isolated from porcine submaxillary gland [25]. The effects of linoleic acid and benzyl alcohol on the soluble enzyme is shown in Fig. 3 B and E. Neither linoleic acid nor benzyl alcohol affected the activity of this enzyme. In contrast, both of these agents inhibited the activity of the liver Golgi membrane enzyme markedly (Fig. 1 B and F).

The effects of linoleic acid and benzyl alcohol on the soluble milk *N*-acetylglucosaminyltransferase are shown in Fig. 3 C and F. Linoleic acid had little effect on the enzyme while benzyl alcohol

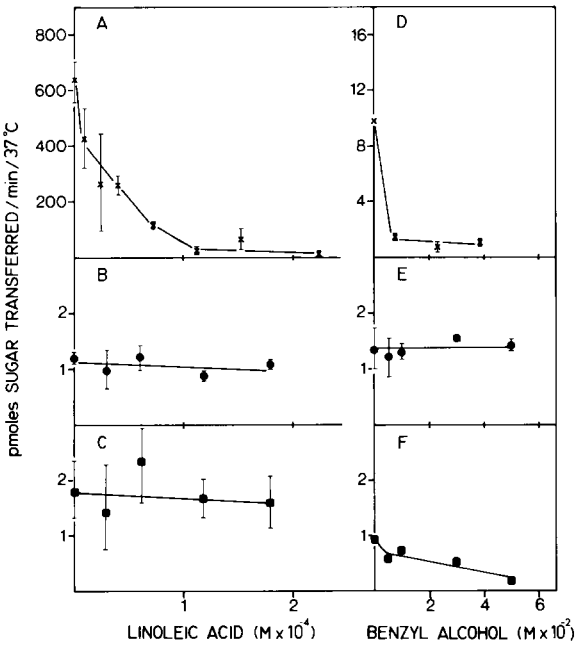

Fig. 3. The effect of linoleic acid (A–C) and benzyl alcohol (D–F) on some isolated (soluble) glycosyltransferases; bovine milk galactosyltransferases (X-X), porcine submaxillary gland *N*-acetylgalactosaminyltransferase (●———●), and bovine milk *N*-acetylglucosaminyltransferase (■———■).

inhibited the enzyme moderately even at very low concentrations. The effect of these two agents on this soluble enzyme is similar to that observed with the Golgi membrane *N*-acetylglucosaminyltransferase (Fig. 1 A and E).

Studies with lipid vesicles

Milk galactosyltransferase was incorporated into lipid vesicles as described earlier [2]. Incorporation of the enzyme into egg phosphatidylcholine (PC) resulted in a marked stimulation of enzymatic activity [2]. This stimulation was observed with the synthetic PC analogs DMPC and DPPC, also.

The effect of linoleic acid and benzyl alcohol on milk galactosyltransferase incorporated into lipid vesicles is shown in Fig. 4 A and B. In Fig. 4A, the incorporation of linoleic acid into egg PC liposomes containing the transferase resulted in a loss of activity to about 50% of the control value. Incorporation of benzyl alcohol into similar vesicles resulted in a sharp decrease of activity with increasing amounts of benzyl alcohol. Thus, these

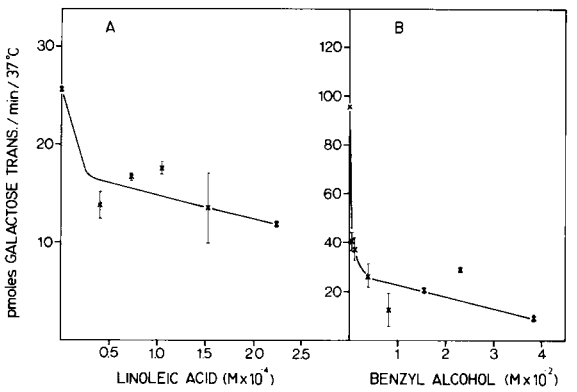

Fig. 4. The effect of linoleic acid (A) and benzyl alcohol (B) on the activities on bovine milk galactosyltransferase incorporated into egg phosphatidylcholine liposomes.

agents rapidly inhibited this enzyme both in solution and in lipid vesicles, while the galactosyltransferase of Golgi membranes was not affected by these agents in low concentrations (Fig. 1 C and G). The inhibition of the enzyme in egg PC vesicles was similar to the inhibition of the enzyme in the absence of lipid (Fig. 3 A and D). Since one of the two fatty acids of egg PC is unsaturated, the highly fluid lipid in the vicinity of the enzyme was not able to prevent permeation of the agents to the enzyme causing inhibition.

When the liposomes were prepared from di-

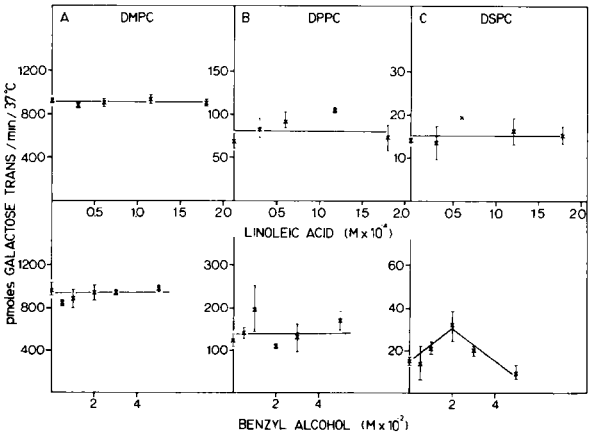

Fig. 5. The effect of linoleic acid and benzyl alcohol on bovine milk galactosyltransferase incorporated into liposomes prepared from saturated phosphatidylcholines: A, dimyristoylphosphatidylcholine; B, dipalmitoylphosphatidylcholine; C, distearoylphosphatidylcholine.

oleoylphosphatidylcholine (DOPC) in which both fatty acids are unsaturated, greater inhibition of the enzyme occurred in the presence of linoleic acid and benzyl alcohol (data not shown), than observed in egg PC vesicles.

Liposomes were prepared from several saturated lipids as well, including dimyristoyl-, dipalmitoyl-, and distearoylphosphatidylcholine with transition temperatures of 23, 41 and 56°C, respectively. Therefore, at the temperature of the assay (37°C) DMPC is totally in the liquid crystalline state, DPPC has gone through a pretransition and is approaching its melting temperature, while DSPC is totally gel phase lipid.

The effects of linoleic acid and benzyl alcohol on milk galactosyltransferase incorporated into these saturated, synthetic lipids are shown in Fig. 5. In DMPC vesicles, neither linoleic acid nor benzyl alcohol affected the enzyme activity (Fig. 5A). When DPPC or DSPC were used to prepare the liposomes, no inhibition by either linoleic acid or benzyl alcohol was observed (Fig. 5 B and C). Therefore, gel phase lipid as present in DPPC and DSPC liposomes, or the more highly ordered liquid crystalline phase of DMPC relative to unsaturated PC, prevents lipid soluble molecules such as linoleic acid and benzyl alcohol from interacting with galactosyltranferase, thereby preventing loss of activity. A complex behaviour was observed when benzyl alcohol was added to DSPC vesicles, an initial stimulation followed by inhibition. However, neither stimulation nor inhibition were marked. Further experimentation is underway to explain this apparent complex behaviour.

Discussion

The concept of the fluid-mosaic membrane has been accepted as a viable working model for biological membranes. The importance of this concept is demonstrated in the numerous publications applying the principles embodied in this model to almost every cellular function [1,26,27]. The importance of protein-lipid interactions within the membrane has been emphasized in such processes as passive ion diffusion [38], active transport (β-glucoside) [29,30], and cell growth [31] with the maintenance of optimal membrane fluidity by 'homeoviscous adaptation' [32], and in the modification of protein structure and/or position resulting from changes in the membrane fluidity [28].

The activity of membrane-bound enzymes can be modified by changes in fluidity of the membrane [1,28,31,32]. Kimmelberg et al. [7] studied rat kidney Mg^{2+} and $(Na^+ + K^+)$-ATPase and found that a decrease in membrane fluidity resulting from the addition of cholesterol or saturated fatty acyl chain lipids, inhibited the activity of the enzyme while an increase in fluidity, due to a lower melting lipid, stimulated the enzyme.

In contrast, some, but not all, Golgi glycosyltransferases studied were inhibited by the fluidizing agents, linoleic acid and benzyl alcohol, while the nonperturbing agent, palmitic acid, had no effect on the activity. Since the inhibitory effect of benzyl alcohol on the sialyltransferase was observed at nonfluidizing concentrations (although patches of fluid lipid could exist and not be detected by our methods), it must be questioned whether these perturbing agents affect the enzyme activity through a fluidizing effect only. Many other membrane processes, most notably nerve conduction, are affected by anesthetics and perturbing agents at concentrations below their fluidizing concentration. A fluidizing effect of benzyl alcohol at concentrations of $10 \cdot 10^{-3}$ M and below cannot be demonstrated by ESR [24,33], ^{2}H-NMR [28] or X-ray diffraction [35] although concentrations below $2 \cdot 10^{-3}$ M do decrease the lipid phase transition temperature, indicating perturbation of the gel phase lipid [35]. An increase in membrane thickness by about 25% has been reported in phosphatidylcholine bilayers at benzyl alcohol concentrations of $7.5 \cdot 10^{-3}$ M [36].

Fluidizing agents which act at hydrophobic sites should be able to act directly at hydrophobic sites of proteins, also, possibly causing conformational changes. In fact, a direct effect of linoleic acid or benzyl alcohol on the enzymes has been demonstrated. A summary of the effects of these two agents on the Golgi membrane-bound glycosyltransferase and soluble glycosyltransferases from bovine milk and porcine submaxillary gland are found in Table II.

Linoleic acid has no effect on the Golgi membrane *N*-acetylglucosaminyltransferase, a significant effect on *N*-acetylgalactosaminyltransferase and sialytransferase, but a marked effect on the

TABLE II

THE EFFECT OF LINOLEIC ACID, BENZYL ALCOHOL AND PALMITIC ACID ON THE ACTIVITIES OF SEVERAL GOLGI MEMBRANE AND SOLUBLE GLYCOSYLTRANSFERASES

Perturbing agent	Enzyme source	Effect on transferase			
		GlcNAc	GalNAc	Galactosyl	Sialyl
Linoleic acid	Rat liver Golgi membrane	None	50% inhibition	> 50% inhibition	10–15% inhibition
Benzyl alcohol	Rat liver Golgi membrane	95% inhibition [a]	95% inhibition [a]	Complete inhibition above $5 \cdot 10^{-2}$ M	Slow inhibition up to $2 \cdot 10^{-2}$ M then rapid
Palmitic acid	Rat liver Golgi membrane	Slight activation	None	Slight inhibition	
Linoleic acid	Bovine milk	None		> 95% inhibition	
	Porcine submaxillary gland		None		
Benzyl alcohol	Bovine milk	90% inhibition [a]		> 95% inhibition [a]	
	Porcine submaxillary gland		None		

[a] Effect observed at non fluidizing concentrations as detected by ESR method.

activity of the galactosyltransferase above $1 \cdot 10^{-4}$ M (Fig. 1). Marked inhibition of the soluble milk galactosyltransferase was observed but neither the bovine milk *N*-acetylgalactosaminyltransferase nor the porcine submaxillary *N*-acetylgalactosaminyltransferases were inhibited (Fig. 3) and may lack hydrophobic binding sites for this unsaturated fatty acid.

Benzyl alcohol, which also fluidizes membranes, has different effects. Both *N*-acetylglucosaminyl and *N*-acetylgalactosaminyltransferases of Golgi membranes are inhibited by about 95% by benzyl alcohol (Fig. 1). The inhibition of these transferases begins at low concentrations of the alcohol and increases progressively. The effect begins well below fluidizing concentrations of the alcohol (as

TABLE III

HILL PLOTS SHOWING THE EFFECTS OF LINOLEIC ACID AND BENZYL ALCOHOL ON ENZYME ACTIVITIES

Log $(v/(V_0 - v))$ is plotted against log of inhibitor. Slope of the line is the Hill coefficient.

Tissue source	Enzyme	Hill coefficient	
		Linoleic acid	Benzyl alcohol
Rat liver Golgi membranes	Galactosyltransferase	0.74	1.6
	Sialyltransferase	1.3	3.6
	N-Acetylglucosaminyltransferase	–	1.6
	N-Acetylgalactosaminyltransferase	0.6	1.9
Bovine milk			
Soluble	Galactosyltransferase	2.1	0.72
	N-Acetylglucosaminyltransferase	–	–
Lipid vesicles	Galactosylstransferase	0.99	0.79
Porcine submaxillary gland			
Soluble	*N*-Acetylgalactosaminyltransferase	–	0.92

detected by ESR), implying that fluidity per se does not account for the inhibition. The purified *N*-acetylglucosaminyltransferase from bovine milk was inhibited markedly by the alcohol as was the milk galactosyltransferase but the porcine submaxillary *N*-acetylgalactosaminyltransferase was not (Fig. 3). The sialyltransferase of Golgi membranes is inhibited slightly up to $3 \cdot 10^{-2}$ M benzyl alcohol and then extensively similar to the galactosyltransferase (Fig. 1). These latter two transferases are contrasted with the GlcNAc and GalNAc transferases which were inhibited at low concentrations, implying that their microenvironments are more permeable to benzyl alcohol than those of the galactosyl- and sialyltransferases of Golgi membranes.

The soluble milk galactosyl- and *N*-acetylglucosaminyltransferase were inhibited by benzyl alcohol implying that the inhibition in this case was the result of a direct effect of benzyl alcohol on the enzymes. However, the porcine submaxillary gland *N*-acetylgalactosaminyltransferase was not inhibited by benzyl alcohol (Fig. 3), whereas the Golgi enzyme was extensively inhibited. It is tempting to conclude that the latter effect is due to the membrane environment of the enzyme which maintains the enzyme in a conformation susceptible to benzyl alcohol inhibition.

The nature and physical state of lipid surrounding the enzyme plays an important role in maintaining enzyme activity by causing either a conformational change in the enzyme or acting as a barrier to inhibiting substances. Linoleic acid was shown to almost totally inhibit the activity of the soluble milk galactosyltransferase in solution (Fig. 3A). However, in lipid vesicles consisting of phosphatidylcholine with various saturated fatty acyl chains ranging from C14 to C18 as in DMPC, DPPC and DSPC, no inhibition of enzyme activity was observed. These saturated lipids in the microenvironment of the enzyme provided an effective barrier to linoleic acid so that it was unable to inhibit the enzyme while the unsaturated egg PC and DOPC did not.

Benzyl alcohol also inhibits the soluble milk galactosyltransferase (Fig. 3D). At a concentration of $1 \cdot 10^{-2}$ M, almost total inhibition of the enzyme was observed. When the enzyme was incorporated into lipids with saturated fatty acyl chains, DMPC, DPPC, and DSPC, the enzyme activity was affected only in DSPC vesicles, in which activation was followed by inhibition (Fig. 5), although the effect was not great. Displacement of the 'boundary' or 'annular' lipid as suggested by Gordon et al. [37] may explain the observation. Benzyl alcohol had no effect on the enzyme activity in DPPC vesicles. Marked inhibition of the enzyme in DOPC (both fatty acyl chains are unsaturated) and egg PC vesicles implies that these lipids present no barrier to inhibiting substances.

Analysis of the data by Hill plots gives information about the possible cooperative nature of membrane phenomena or protein structure. These are shown in Table III. A slope greater than 1.0 when log $(v/(V_0 - v))$ was plotted against log linoleic acid or benzyl alcohol concentration implies 'cooperativity' (v = velocity of reaction at a particular concentration of inhibitor, V_0 = velocity in the absence of inhibitor).

The Hill coefficient for the Golgi enzymes in the presence of linoleic acid gave values near or less than 1.0 (sialyltransferase was 1.3). On the other hand, Hill coefficients for benzyl alcohol were greater than 1.0 in all cases, implying some cooperative membrane phenomena with this inhibitor.

The soluble enzymes, either from milk or from porcine submaxillary gland yielded Hill coefficients greater than 1.0 in only one case (galactosyltransferase was 2.1). There was no evidence of cooperative phenomena with any of the other enzymes with either inhibitor and no evidence of cooperative phenomena was observed when milk galactosyltransferase was incorporated into lipid vesicles.

For the enzymes of the Golgi complex, linoleic acid acts in a noncooperative fashion probably directly on the enzymes whereas the effect of benzyl alcohol may involve a membrane cooperative phenomena as well. An effect of membrane environment secondarily affecting the tertiary structure of the enzyme as shown for rat liver plasma membrane adenylate cyclase [38] is also possible for the benzyl alcohol effect on Golgi membranes.

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References

1 DePierre, J.W. and Ernster, L. (1977) Annu. Rev. Biochem. 46, 201–262
2 Mitranic, M.M. and Moscarello, M.A. (1980) Can. J. Biochem. 58, 809–814
3 Mitranic, M.M., Sturgess, J.M. and Moscarello, M.A. (1979) Can. J. Biochem. 57, 1008–1013
4 Stier, A. (1976) Biochem. Pharmacol. 25, 109–113
5 Dehlinger, P.J., Jost, P.C. and Griffith, O.H. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 2280–2284
6 Hesketh, T.R., Smith, G.A., Houslay, M.D., McGill, K.A., Birdsall, N.J.M., Metcalf, J.C. and Warren, G.B. (1976) Biochemistry 15, 4145–4151
7 Kimmelberg, H.K. and Papahadjopoulos, D. (1974) J. Biol. Chem. 249, 1071–1080
8 Farias, R.N., Bloj, B., Morero, R.D., Sineriz, F. and Trucco, R.E. (1975) Biochim. Biophys. Acta 415, 231–235
9 Dipple, I. and Houslay, M.D. (1978) Biochem. J. 174, 179–190
10 Moore, B.M., Lentz, B.K. and Meissner, G. (1978) Biochemistry 17, 5248–5255
11 Silvius, J.R. and McElhaney, R.N. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1255–2159
12 Buhr, M.M., Carlson, J.C. and Thompson, J.E. (1979) Endocrinology 105, 1330–1335
13 Sturgess, J.M., Katona, E. and Moscarello, M.A. (1973) J. Membrane Biol. 12, 367–384
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
15 Treloar, M., Sturgess, J.M. and Moscarello, M.A. (1974) J. Biol. Chem. 249, 6628–6632
16 Mitranic, M., Sturgess, J.M. and Moscarello, M.A. (1976) Biochim. Biophys. Acta 443, 190–197
17 Fitzgerald, D.K., Colvin, B., Mawal, R. and Ebner, K. (1970) Anal. Biochem. 36, 43–61
18 Khatra, S.E., Herries, D.G. and Brew, K. (1974) Eur. J. Biochem. 44, 537–560
19 Lowden, J.A,, Moscarello, M.A. and Morecki, R. (1966) Can. J. Biochem. 44, 567–577
20 Carlson, D.M., Swanson, A.L. and Roseman, S. (1964) Biochemistry 3, 402–405
21 Baxter, A. and Durham, J.P. (1979) Anal. Biochem. 98, 95–101
22 Durham, J.P., Gilles, P., Baxter, A. and Lopez-Solis, R.O. (1979) Clin. Chim. Acta 95, 425–432
23 Seelig, J. (1970) J. Am. Chem. Soc. 92, 3881–3887
24 Boggs, J.M., Yoong, T. and Hsia, J.C. (1976) Mol. Pharmacol. 12, 127–135
25 Hagopian, A. and Eylar, E.H. (1969) Arch. Biochem. Biophys. 129, 515–524
26 Coleman, R. (1973) Biochim. Biophys. Acta 300, 1–30
27 Gennis, R.B. and Jonas, A. (1977) Annu. Rev. Biophys. Bioeng. 6, 195–238
28 Papahadjopoulos, D., Jacobson, K., Nir, S. and Isac, T. (1973) Biochim. Biophys. Acta 311, 330–348
29 Linden, C.D., Wright, K.L., McConnell, H.M. and Fox, C.F. (1973) Proc. Natl. Acad. Sci. U.S.A. 70, 2271–2275
30 Read, B.D. and McElhaney, R.N. (1976) Biochim. Biophys. Acta 419, 331–341
31 McElhaney, R.N. and Souza, K.A. (1976) Biochim. Biophys. Acta 443, 348–359
32 Senisky, M. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 522–525
33 Borochov, H. and Shinitzky, M. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 4526–4530
34 Turner, G.L. and Oldfield, E. (1979) Nature 277, 669–670
35 Ebihara, L., Hall, J.E., MacDonald, R.C., McIntosh, T.J. and Simon, S.A. (1979) Biophys. J. 28, 185–196
36 Ashcroft, R.G., Coster, H.G.L. and Smith, J.R. (1977) Nature 269, 819–820
37 Gordon, L.M., Sauerheiber, R.D., Esgate, J.A., Dipple, I., Marchmont, R.J. and Houslay, M.D. (1980) J. Biol. Chem. 255, 4519–4527
38 Needham, L., Whitton, A.D. and Houslay, M.D. (1982) FEBS Lett. 40, 85–88