STIMULATION OF LIVER MICROSOMAL SIALYLTRANSFERASE ACTIVITY BY LYSOLECITHIN

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1. Introduction

Although sialic acids on glycoproteins have been implicated in numerous important biological processes including virus binding, protection against proteolytic attack, cell aggregation, potassium transport, and determination of the plasma survival time of cells and glycoproteins [1], little is known about the factors that control the activity of the sialyltransferases involved in the synthesis of these glycoproteins. The sialyltransferase activity of rat liver microsomes is membrane associated and markedly stimulated by the nonionic detergent Triton X-100 [2]. We have observed that lysolecithin, which is a normal membrane component [3] and a naturally occurring detergent [4], stimulates rat liver microsomal sialyltransferase activity up to 6.5-fold without solubilizing the enzyme activity. Higher levels of lysolecithin are inhibitory. The stimulatory activity of lysolecithin appears to result from the detergent properties of the entire molecule rather than a specific structural component of the molecule. Sialyltransferases involved in the synthesis of many glycoprotein carbohydrate moieties act immediately after galactosyltransferases [5], which are also stimulated by lysolecithin in rat kidney [6,7] and liver microsomes [8,9]. These results support the concept that the detergent properties of lysolecithin may provide the physical basis for a novel role by this lipid as a membrane transducer in the coordinate regulation of groups of functionally related membrane-associated enzymes.

2. Materials and methods

All phospholipids were obtained from Sigma Chemical Co., except stearoyl lysolecithin, which was prepared from distearoyl phosphatidylcholine (Sigma) by treatment with phospholipase A_2 (*Crotalus adamanteus* venom, Worthington) according to the method of Wells and Hanahan [10]. Desialized fetuin (DSF) was prepared by mild acid hydrolysis of fetuin (Sigma) according to the method of Spiro [11]. Cytidine 5′-monophosphate [4-^{14}C] sialic acid (9 mC/mmole) was obtained from New England Nuclear. Triton X-100 was obtained from Bass Chemical Co.

Rat liver microsomal enzyme preparations were prepared from female Sprague-Dawley (Holtzman) as described previously [2]. Protein was determined by the method of Lowry et al. [12] using bovine serum albumin as standard. The standard sialyltransferase assay conditions were as previously described [2] except that the assay mixture contained 5.6 nmol of CMP-[^{14}C] sialic acid, 0.1 mg of DSF and 0.5 mg of enzyme protein in 0.125 ml of 40 mM Tris citrate, pH 7.0. The amount of sialic acid enzymatically transferred to DSF was calculated as the phosphotungstic acid precipitable radioactivity minus that obtained with parallel incubation mixtures lacking DSF. In all calculations egg yolk lysolecithin was assigned a mol. wt. of 495.6 (that of palmitoyl lysolecithin) and Triton X-100, a mol. wt. of 602.8 (that of nonaoxyethylated octylphenol).

3. Results and discussion

As shown in fig.1, egg yolk lysolecithin stimulates the transfer of sialic acid from CMP-sialic acid to DSF by rat liver microsomes by a factor of up to 6.5 with maximum stimulation in the concentration range 2 to 8 mM. The physiological concentration of lysolecithin in rat liver is approx. 3 mM assuming its uniform distribution in the cell [3]. Several lines of evidence suggest that the stimulatory activity of lysolecithin is probably derived from its detergent properties which result from the presence in the same molecule of both a hydrophilic region (the *sn*-glycero-3-phosphoryl-choline moiety) and a hydrophobic region (the fatty acid ester moiety) with the appropriate balance of hydrophilicity and hydrophobicity [4]. Triton X-100 stimulates sialyltransferase activity to a similar level and over a broader concentration range (2 to 50 mM). An approximately additive stimulation of transferase activity was observed with a mixture of equal weights of lysolecithin and Triton X-100 in the range of suboptimal detergent concentrations.

Examination of the series of lipids tested for sialyltransferase stimulating activity (table 1) indicates that the stimulatory activity observed with egg yolk lysolecithin is not caused solely by the esterified fatty acid moiety, the choline moiety (which are present in lecithin), nor the L-α-lysophosphatidyl moiety (which is present in lysophosphatidylethanolamine). Lecithin and lysophosphatidylethanolamine probably are not

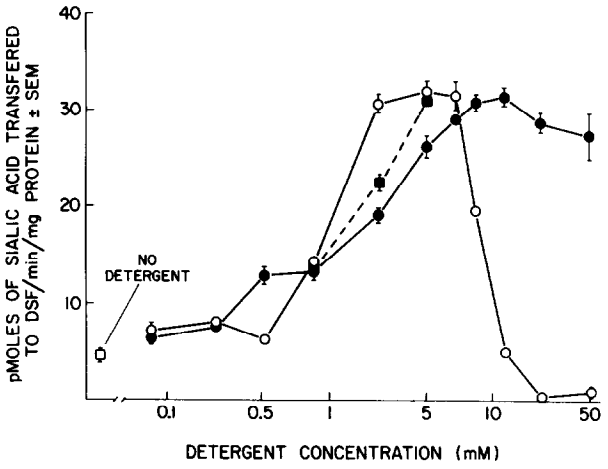

Fig.1. The effect of varying the concentration of lysolecithin (-○-), Triton X-100 (-●-), and a mixture of equal weights of lysolecithin and Triton X-100 (–■–) on the transfer of sialic acid from CMP-sialic acid to desialyzed fetuin.

Table 1
Effect of various lipids on rat liver microsomal sialyltransferase activity

Lipid added	Final concentration (mg/ml)	Pmoles of sialic acid transferred to DSF/min/mg/protein ± SEM	Activation relative to control
Control	–	5.34 ± 0.14	1.00
Lysolecithin (egg yolk)	0.5	8.65 ± 0.09	1.62
	5.0	15.43 ± 0.27	2.89
Lysolecithin (soybean)	0.5	12.07 ± 1.36	2.26
	5.0	24.08 ± 1.32	4.51
Lysolecithin (bovine liver)	0.5	7.90 ± 0.24	1.48
	5.0	22.21 ± 0.95	4.16
Palmitoyl lysolecithin	0.5	10.02 ± 0.31	1.88
	5.0	1.17 ± 0.04	0.22
Stearoyl lysolecithin	0.5	7.17 ± 0.16	1.34
	5.0	8.78 ± 0.38	1.64
Lecithin (egg yolk)	0.5	5.35 ± 0.05	1.00
	5.0	5.52 ± 0.26	1.03
Lysophosphatidyl-ethanolamine (egg yolk)	0.5	4.90 ± 0.22	0.92
	5.0	4.95 ± 0.18	0.93

stimulatory because they do not have the optimal balance of hydrophilicity and hydrophobicity. The inhibition of sialyltransferase activity by 5 mg/ml palmitoyl lysolecithin suggests that its range of stimulatory concentrations is lower than the range for stearoyl lysolecithin, which has a slightly different balance of hydrophobicity and hydrophilicity.

Lysolecithin probably does not stimulate sialyltransferase activity by acting as a lipid intermediate. Extraction of enzyme assay mixtures using the method of Waechter et al. [13] yielded two lipid fractions each in the presence and absence of added lysolecithin. Less than 1% of the radioactivity in these fractions could be transferred to acid precipitable, chloroform:methanol:water (20:10:1, by vol) insoluble material in the standard assay conditions.

The stimulatory effect of lysolecithin was observed at all concentrations of the CMP-sialic acid donor and of the glycoprotein acceptor that were examined. Normal bisubstrate kinetics were observed when the concentration of CMP-sialic acid was varied, but not when the concentration of DSF was varied. The apparent K_M for CMP-sialic acid was 78 μM in the absence of lysolecithin and it was essentially the same in the presence of 2 mM lysolecithin. The V_{max} in the absence of lysolecithin was 44.7 pmol of sialic acid transferred to DSF/min/mg enzyme protein, and this value was increased to 83.8 pmol of sialic acid transferred to DSF/min/mg enzyme protein in the presence of 2 mM lysolecithin.

One conceivable mechanism by which lysolecithin could stimulate sialyltransferase activity is by solubilizing the enzyme, thereby facilitating the interaction of the enzyme with the macromolecular acceptor. The results presented in table 2 indicate that solubilization of sialyltransferases is not necessary for stimulation of activity. Although some enzyme activity was observed in both the soluble (i.e. not sedimented by 100 000 *g* for 1 h) and particulate fractions after treatment with 2 mM lysolecithin, the degree of stimulation was much greater in the particulate fraction (5.2-fold) than in the soluble fraction (1.1-fold).

Low concentrations of surfactants such as lysolecithin affect most membrane-associated enzyme activities in one direction or the other [4]. For example, it has recently been shown in this laboratory that similar concentrations of lysolecithin stimulate the activity of guanylate cyclase and inhibit adenylate cyclase in mouse fibroblast microsomes [14]. The amount of lysolecithin in cell membranes is determined by a phosphoglyceride deacylation–reacylation cycle [15] in which membrane-associated phospholipase A_2 enzymes act on lecithin in the membrane to produce lysolecithin which, in turn, can be converted back to lecithin by membrane-associated acyl CoA:lysophosphoglyceride acyltransferases. Perturbation of

Table 2
Effect of 2 mM lysolecithin and 2 mM Triton X-100 on the sedimentation of microsomal sialyltransferases at 100 000 *g*

Treatment agent[a]	Percent of total enzyme activity in the soluble fraction	Specific activity of enzyme preparations ± SEM (pmoles of sialic acid transferred to DSF/min/mg/protein	
		Soluble	Particulate
Water	28.5	8.85 ± 0.35	6.21 ± 0.25
Lysolecithin	17.5	9.88 ± 0.26	32.1 ± 0.4
Triton X-100	32.1	24.0 ± 0.9	37.8 ± 0.6

[a]Aliquots (2 ml) of rat liver microsomal enzyme preparation (5 mg protein/ml) were diluted with 0.08 ml of water, 50 mM lysolecithin, or 50 mM Triton. The mixtures were incubated 10 min at 37°C and centrifuged at 100 000 *g* for 1 h at 0°C. The supernatants (soluble fractions) were analyzed directly and the pellets were resuspended in 2.08 ml of 10^{-3} M Tris citrate buffer, pH 6.2 (particulate fraction). Both detergents solubilized approx. 20% of the protein.

this cycle by altering the activity of either type of enzyme (for example, by raising the intracellular concentration of Ca^{2+}, which activates microsomal phospholipases [15]) would alter the level of lysolecithin in the membrane. Lysolecithin generated at one part of the cell could function as a membrane transducer by diffusing rapidly through the lipid proteins of the cellular membranes to modify the activity of membrane-associated enzymes in other parts of the cell, as well as possibly altering general properties of the membrane such as fluidity and permeability. The possible involvement of the phosphoglyceride deacylation–reacylation cycle in the control of the biosynthesis of some species of phosphoglycerides has been considered [15]. However, the observations that lysolecithin stimulates two enzymes involved in consecutive steps of glycoprotein synthesis and that it modifies the activities of enzymes involved in the synthesis of two regulatory cyclic nucleotides support the suggestion that lysolecithin may play a wider role as a membrane transducer for the coordinate activation or deactivation of groups of functionally related membrane-associated enzymes involved in other cellular processes.

Acknowledgements

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