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Reactivity of glycoconjugate in membrane system II: can a neutral glycolipid function as lectin receptor in the presence of gangliosides in plasma membrane?

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Abstract. To examine how surface potential controls the reactivity of glycoconjugates at cell surface, the interaction of galactose-specific lectins *e.g.* peanut agglutinin, *Ricinus cummunis* agglutinin with liposomes bearing asialo GM_1 were studied in the presence of varying amount of ganglioside mixture, GM_n. The presence of 5% GM_n causes complete slowing down of precipitin reaction and thereby make carbohydrate moiety of asialo GM_1 completely inaccessible *i.e.* 'cryptic'. In contrast the presence of 1–2% GM_n enhances the apparent rate and amplitude of the precipitin reaction as surface potential becomes more negative. The relevance of the findings has been discussed in relation to the expression and involvement of the cell-surface sialic acid residues during development and differentiation.

Keywords. Lectin receptor; ganglioside; accessibility; distribution in membrane.

Introduction

In recent years it has been shown from several laboratories that lectin can recognize complex carbohydrate sequence of glycolipids embedded in liposome and thereby give rise to precipitin reaction resembling cell agglutination (Grant and Peters, 1984). The formation of precipitin complex increases the turbidity of the solution in second-minute range and therefore can be studied quantitatively. In the presence of a specific sugar the precipitin complex dissociates (*i.e.* turbidity decreases) with time-constant characteristics of lectin-sugar interaction (Podder *et al*., 1974). The most interesting feature of the precipitin reaction is that the apparent rate and extent of reaction are controlled by several factors *viz.* (i) surface density of receptors *i.e.* mol per cent of glycolipid content (Surolia *et al*., 1975), (ii) sequence and length of the carbohydrate chain and an overall lipid composition of the vesicle i.e. membrane fluidity, head group interactions etc (Sundler, 1984). The mechanism leading to the required threshold concentration and the drastic enhancement of the initial rate with increase in the ratio of glycolipid to phospholipid is not yet understood. Besides, transmembrane assymetry, as in the case of GDla: DPPC system (Thomas and Podder, 1982), size-related topological distribution and surface potential of the vesicle undoubtedly influence the reactivity and accessibility of a given glycoconjugate in a binary system. Whatever be the mechanism it raises the question whether carbohydrate moiety of each glycolipid at the cell surface is available for reaction with lectins and/or carbohydrate specific antibody when a mixture of glycolipids are incorporated in liposome. If not, what controls the accessibility and reactivity of a particular

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Abbreviations used: WGA, Wheat germ agglutinin; PNA, peanut agglutinin; RCA_1, *Ricinus cummunis* agglutinin; EPC, egg phosphatidyl choline; NCAM, neuronal cell adhesion molecule; NgCAM, neuronal glial cell adhesion molecule; DML, dimenystayl phosphatidyl choline.

glycolipid in presence of others whose expression at cell surface are often developmentally regulated. This question is of fundamental importance in the assignment of any marker glycoconjugate to have an *in vivo* receptor function and act as antigenic determinant during development, differentiation and oncogenic transformation. For better understanding of the molecular basis of individual factors (*e.g.* fluidity, surface density, surface potential) that influence the kinetics and specificity, attempts have been made to develop suitable liposomal system for lectins like wheat germ agglutinin (WGA), peanut agglutinin (PNA), *Ricinus cummunis* agglutinin (RCA_1).

Recently we have investigated the formation of precipitin complexes between vesicles containing 5 mol per cent of asialo GM_1 and galactose specific lectins like ricin, RCA_1 and PNA. The rate and extent of lactose-induced dissociation are found to be very sensitive to the concentration of lactose and the type of lectin. The data obtained from quantitative analysis of sugar-induced dissociation rate of precipitin complex suggest that specificity of interaction between lectin and complex carbohydrate moiety of glycosphingolipid is at least two order magnitude higher than that of simple sugar. In the case of PNA it is even higher and comparable to high affinity binding site at the cell surface (Singh *et al*., 1986). Moreover the kinetics of formation and dissociation of precipitin complex is slower. In contrast the dissociation rate in case of ricin and RCA_1 is faster and only 36% of the total complex can be monitored under experimental set up. Because of its reported mitogenicity, kinetic property and uses in the study of lymphocyte maturation (Reisner *et al*., 1976), PNA-asialo GM_1 system is chosen for further study to examine the influence of other glycolipids on the reactivity of asialo GM_1 towards PNA. Hence we wish to report how ganglioside when incorporated in the same vesicle containing asialo GM_1 affect its reactivity towards galactose specific lectins. Data presented here show that at about 5 mol per cent of mixed gangliosides, asialo GM_1 is inaccessible to PNA and RCA_1 In contrast, in the presence of lesser mol per cent of GM_n, accessibility increases.

Materials and methods

Egg yolk lecithin was isolated using the method of Litman (1973) and total mixed ganglioside by the procedure of Folch *et al*. (1957). Asialo GM_1 was prepared from mixed ganglioside by acid hydrolysis with O' l NH_2SO_4 at 80°C for 1 h. The purity of the gangliosides were checked by thin-layer chromatography methods using resorcinol and orcinol reagents for mixed gangliosides and asialo GM_1 , respectively. RCA_1 was isolated from the locally available seeds using DEAE-Sephadex followed by G-100 column chromatography (Douglas *et al*., 1978). The activity was detected by guar gum and asialo GM_1 vesicles by measuring the increase in turbidity at 340 nm.

PNA was isolated from local seeds using Sepharose 6B affinity chromatography (Terao *et al*., 1975). Figure 1 shows the elution profile of 60% $(NH_4)_2SO_4$ precipitated proteins from peanut seeds. PNA was detected by liposomal assay using vesicles containing 5 mol per cent asialo GM_1 in the absence and presence of galactose. Agglutination reaction of mouse thymocytes gives the hemagglutinating activity of PNA as 2000 HU/mg of protein approximately. The assay thus described, avoids the complicated procedure of treating the erythrocytes with neuraminidase for activity assay of PNA. Purity of both the proteins were checked by sodium dodecyl sulphate gel electrophoresis (Laemmli *et al*., 1970).

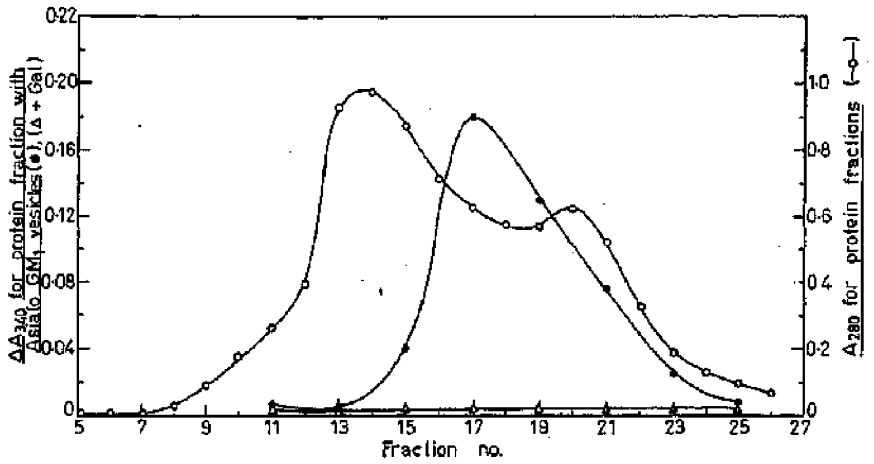

Figure 1. Elution profile of 60% $(NH_4)\ 2SO_4$ fraction of peanut seeds (*Arachis hypogaea*) on Sepharose 6B column (2 × 45 cm). Protein loaded 30'31 mg, fraction volume 7 ml, flow rate 7 ml/h.

Egg phosphatidyl choline (EPC) vesicles containing various types of gangliosides were prepared by sonication for 20 min till it becomes visually clear. Its size and stability were characterized by measuring the absorbance as well as the initial velocity of precipitin reaction with lectins.

Results

Although co-solubilisation of lecithin and mixture of glycolipid would lead to the formation of liposomes having carbohydrate moiety of glycoconjugate exposed at the outer surface during sonication, the reactivity of exposed carbohydrate moiety towards lectins may change with time due to the vesicle instability resulting from the formation of either large unilamellar or multilammellar vesicle upon fusion of sonicated small vesicles. It could also result from the slow transmembrane flip-flop motion of incorporated glycoconjugate. To circumvent the problem, sonicated vesicles were annealed for a longer period of time (24–48 h) before use. During this course it was observed that long period of annealing was necessary to obtain precipitin reaction of constant amplitude. It may be mentioned that the annealing time is sensitive to duration of sonication, nature of lecithin as well as mol per cent of glycoconjugate. It also depends on the time history of annealing and storage condition. The details of these findings will be published elsewhere. Figure 2 shows the precipitin reaction of PNA with liposome containing 5 mol per cent asialo GM_1 in the presence of various amounts of mixed gangliosides in the same liposome. It is clear that the addition of GM_n though reduced initial reaction of precipitin reaction presumably due to electrostatic repulsion, the amplitude at 40 min for vesicles containing 1% and 2% GM_n is about 1·3 and 1·1 times increases respectively, than that of asialo GM_1 vesicle alone. From the absortion/mMPO_4 value shown in table 1, it is seen that the size of the lecithin vesicle is dependent on the amount of GM_n content in the vesicle. To what extent the observed difference in amplitude of precipitin reaction can be related to the difference in size and topological distribution

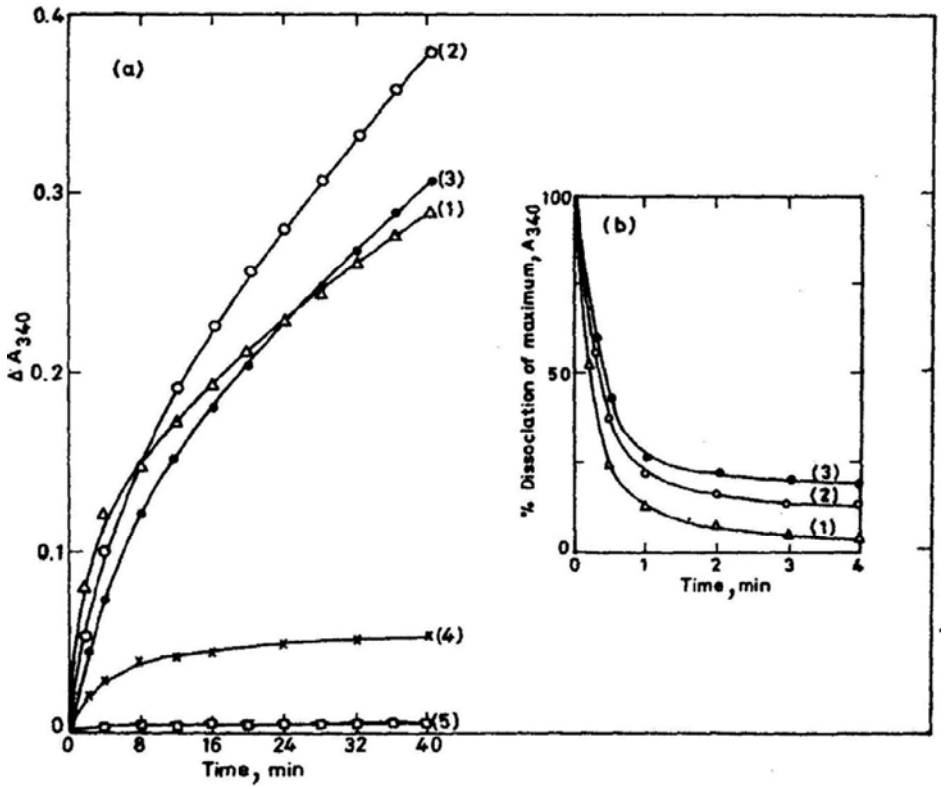

Figure 2. Kinetics of precipitin reaction of liposomes bearing asialo GM_1 (5 mol per cent) with various amounts of gangliosides wmyh PNA (a) and its dissociation (b) in the presence of 23'33 mM galactose.

Per cent mol content of GM_n: (1) without GM_n; (2) 1; (3) 2; (4) 3; (5) 2 per cent GM_n, alone; also 5 per cent GM_n.

need to be ascertained. In figure 3, the effect of salt on the rate of precipitin reaction is shown. From such kinetic data, the interaction specificity of carbohydrate lectin interaction was calculated as described earlier (Singh *et al.*, 1986). These are summarised in table 1 and table 2. Some of the preliminary data obtained for free $RCA_1$and liposome bound RCA_1 are also included in the table 1. In the case of liposome bound RCA_1 the presence of GM_n in the same liposome reduced the affinity constant K_a, by about 10 times while that of free RCA_1 reduced marginally. This is not observed in the case of PNA where the affinity value remains almost same. It is not known whether this difference could be ascribed to the difference in pI values of PNA (5·96) and RCA_1 (7·1). It may be emphasised here that the calculated value is found to be insensitive to the observed variation of the amplitude of precipitin reaction which is critically dependent on the time history of the sample as well as nature and composition of the vesicles.

Discussion

From the results presented here it appears that ganglioside GM_n embedded in the liposome containing asialo GM_1 can modulate the rate and extent of precipitin

Table 1. Characteristics of precipitin reaction of liposomes containing various types of gangliosides with galactose specific lectins.

Lectin	Type of vesicle	A/mMPO$_4$ of vesicle at 560 nmx 10^2	conc. of asialo GM$_1$ (μM)	v_i (% of amplitude at 1 min/30 min)	Dissociation with 23·33 mM Gal. (%)	$t_{\frac{1}{2}}$ (s)	k_d (s^{-1})	K_a^{**} (M^{-1})
	*EPC vesicle with 5 mol per cent of asialo GM$_1$							
	without GM$_n$	1·29	20	9·7	96·5	14·4	$2·18 \times 10^{-3}$	$1·7 \times 10^7$
PNA	1% GM$_n$	1·27	20	9·35	87·86	19·2	$1·64 \times 10^{-3}$	$2·25 \times 10^7$
2·5 μM	2% GM$_n$	1·38	20	6·6	88·26	19·2	$1·64 \times 10^{-3}$	$2·25 \times 10^7$
	3% GM$_n$	0·9	20	2·5	92·33	19·2	$1·64 \times 10^{-3}$	$2·25 \times 10^7$
	2% GM$_n$ alone	0·71	20	no reaction	—	—	—	—
	†EPC vesicle with 5 mol per cent of asialo GM$_1$			v_i (% of amplitude at 1 min/20 min)	(with 121 μM Gal)			
	without GM$_n$	—	20	21·4	53·53	12·7	0·37	$1·2 \times 10^6$
RCA$_1^*$	1% GM$_1$	2·69	20	16·0	71·19	7·26	0·65	$0·7 \times 10^6$
2·2 μM	2% GM$_n$	1·8	20	15·79	80·50	7·26	0·65	$0·7 \times 10^6$
	3% GM$_n$	1·52	20	very slow reaction	—	—	—	—
	2% GM$_n$ alone	1·03	20	no reaction	—	—	—	—
RCA$_1$	5% asialo GM$_1$							
2·1 μM	in DML vesicle	0·124	12·4	71	(11·5 mM) 97·3	24	$2·0 \times 10^{-3}$	$2·2 \times 10^8$
1·8 μM	2% GM$_n$ + 5% asialo GM$_1$ in DML vesicle	0·155	20	38	(2·4 mM) 93	17	$1·4 \times 10^{-2}$	$3·2 \times 10^7$

*Vesicle used after 3 days of storage.
†On the 5th day.
*Conjugated RCA$_1$ in DML vesicle.
**K_a calculated using K_f for lectin-galactose system.

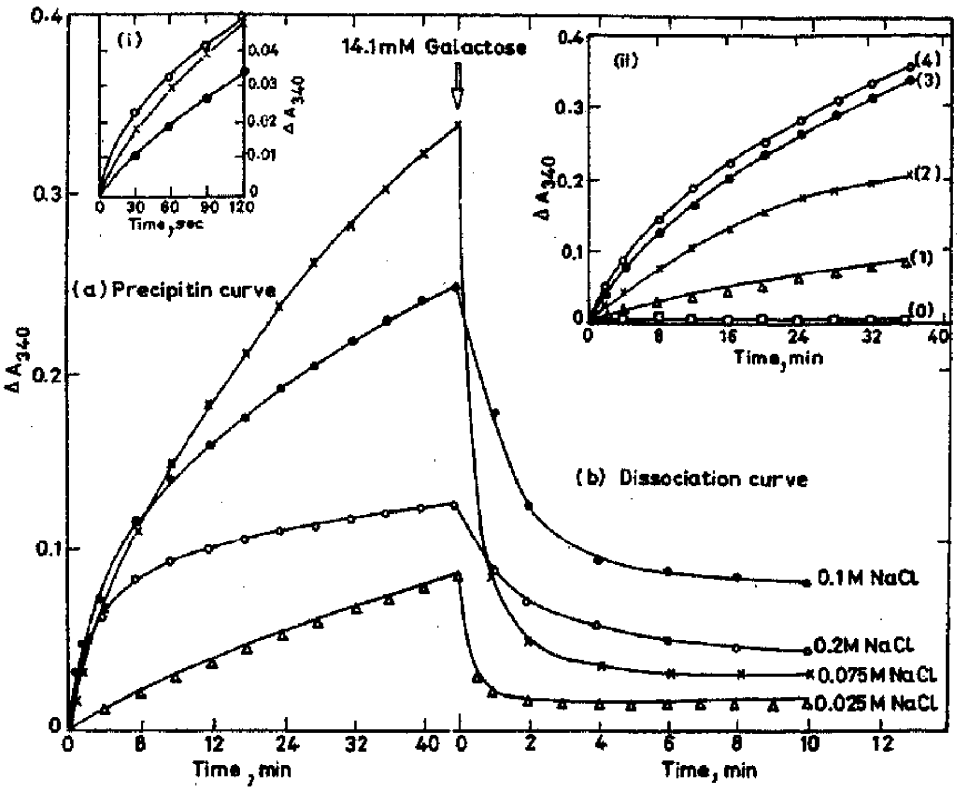

Figure 3. Precipitin reaction of vesicles containing 5 mol per cent of asialo GM_1 and 2 mol per cent of GM_n with PNA with varying amounts of salt (NaCl) and their subsequent dissociation in the presence of 14'1 mM galactose. Concentration of PNA (2'5 μM) and of asialo GM_1 (20μM).

Inset (i). Expanded scale of initial rate of precipitin reaction.

Inset (ii). Precipitin reaction of vesicles, with different concentration of NaCl. (0) without salt; (l) 25mM; (2) 50 mM; (3) 65 mM; (4) 75mM.

reaction even when present to the extent 1–3 mol per cent of total lipid. At 5 mol per cent of GM_n. The complete slowing down of rate of the precipitin reaction was observed. Similar modulation by ganglioside have been reported by Alving *et al.* (1980), in the case of binding of antibodies to liposomal phospholipids as well as neutral glycolipids *viz*, globosides, galactocerebrosides, Forsman antigens etc. Endo *et al.* (1982). reported the inhibition of antigenic activity of liposomal Forsman antigen by glycophorin which contains several saccharides terminated by NANA and bind to WGA. The observed inhibition by sialylglycoconjugates is attributed to either carbohydrate-carbohydrate interaction in glycoconjugates and/or head group interaction between carbohydrate moiety of ganglioside and phospholipids.

It is known that even though sialic acid residues of embedded ganglioside are at a significant distance from the surface, the electrostatic potential with lecithin-ganglioside liposome becomes more negative as the mol content of GM_n increases. With the increase in monovalent salt concentration the negative value decreases. When this fact is taken into account the data presented here suggest that the liposomal

Table 2. Effect of salt on the precipitin reaction of liposomes bearing different gangliosides with PNA.

Lectin	Characteristics of vesicle	Salt conc. (mM NaCl)	v_i (% of amplitude at 2 min/40 min)	Dissociation with 14·11 mM gal. (%)	$t_{\frac{1}{2}}$ (s)	k_d (s^{-1})	K_a (M^{-1})
PNA (2·5 μM)	1. EPC vesicle with 5 mol per cent asialo GM_1 and 2 mol per cent GMn.	0	no reaction	—	—	—	—
		25	8·7	90	16·8	3×10^{-3}	$1·2 \times 10^{7}$
		35	7·14	97	21·8	$2·4 \times 10^{-3}$	$1·5 \times 10^{7}$
	2. A/mMPO$_4$ at 560 nm $= 1·38 \times 10^{-2}$	50	8·98	—	—	—	—
		65	10·64	—	—	—	—
		75	12·25	94·8	33·6	$1·54 \times 10^{-3}$	$2·4 \times 10^{7}$
	3. Conc. of asialo GM_1 (20 μM)	100	17·5	64·9	48·48	$1·07 \times 10^{-3}$	$3·4 \times 10^{7}$
		200	31·4	62·5	67·2	$0·77 \times 10^{-3}$	$4·8 \times 10^{7}$

surface potential and topological distribution of embedded glycoconjugates are far more important in controlling the rate and extent of the precipitin reaction. This view can be further tested by measuring the rate of precipitin reaction when other negatively charged phospholipids *i.e*. phosphatidyl glycerol and phosphatidyl serine are present in the liposome instead of GM_n. Whatever be the mechanism of the observed modulation of the reaction between lectin and carbohydrate moiety of asialo GM_1 the data presented here allows us to draw the following conclusion. The individual ganglioside though function as specific receptor will always influence other receptor-ligand interactions at the cell surface by modulating surface charge density and head group interaction resulting in the changed conformation of exposed carbohydrate moiety and its topological distribution. At higher content of charged gangliosides it can completely slow down the rate of reaction as reported here and thereby make the exposed carbohydrate at the outer surface completely inaccessible. In other words, exposed carbohydrate would appear cryptic. This secondary influence of sialic acid residue on the accessibility of other surface carbohydrates is distinct from that resulting from masking due to incorporation of sialic acid residue at the terminal galactose residue by sialyltransferase action (Toporowicz and Reisner, 1986). Thus, there need not be any direct relation between changes in sialic acid content and expression of functional receptors in differentiation and other carbohydrate mediated phenomena particularly those are developmentally regulated *via*. adhesion molecules like NCAM and NgCAM (Edelman, 1985).

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