INTERACTIONS OF PLANT LECTINS WITH GLYCOLIPIDS IN LIPOSOMES

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SUMMARY: A panel of five plant lectins with different binding specificities was used to determine if plant lectins could bind specifically to membrane-associated glycolipids. Ricinis communis and wheat germ agglutinins both bound specifically to mixed brain gangliosides and globoside I from human erythrocytes. Wheat germ agglutinin also bound to ganglioside G_{M1} and human erythrocyte ceramide trihexoside, but not to ceramide dihexoside, mono-, or digalactosyl diglycerides. Concanavalin A bound to liposomes with or without glycolipid substituents, and this binding was partially inhibited by α-methyl mannoside. This study indicates that lectins can specifically recognize and bind to certain glycolipids in membranes.

Plant lectins interact with cells by binding to specific saccharide structures on the cell surface. Lectins have been used extensively both as probes of mammalian membrane structure and for studying lymphocyte mitogenesis and a variety of other membrane functions (reviewed in ref. 1). Although glycoproteins having lectin binding capacities have been isolated (1), it occurred to us that glycolipids, which are major cell surface components, might also function as specific lectin receptors. Indeed, Surolia et al. recently have demonstrated binding of Ricinis communis agglutinin to liposomes containing ganglioside G_{M1} (2). The present studies were undertaken to investigate specific binding of the lectins E- and L-phytohemagglutinin, concanavalin A, wheat germ agglutinin, and R. communis agglutinin to various glycolipids in liposomal model membranes.

MATERIALS AND METHODS

Glycolipids were obtained from the following sources: monogalactosyl diglyceride (β-galactosyl diacyl glycerol) and digalactosyl diglyceride (α-galactosyl-β-galactosyl diacyl glycerol) (Applied Science Laboratories, State College, PA); mixed beef brain gangliosides (Sigma Chemical Co., St. Louis, MO); G_{M1} ganglioside [β-galactosyl-β-N-acetyl galactosaminyl-(N-acetyl neuraminyl)-β-galactosyl-β-glucosyl ceramide] (Supelco, Inc., Bellefone, PA); ceramide dihexoside (β-galactosyl-β-glucosyl lignoceroyl ceramide) (Miles Laboratories, Inc., Kankakee, IL). Globoside I (β-N-acetyl galactosaminyl-α-galactosyl-β-

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galactosyl-β-glucosyl ceramide) and ceramide trihexoside (α-galactosyl-β-galactosyl-β-glucosyl ceramide) were prepared from human erythrocyte ghosts using methods described by Alving et al. (3). All lipids were checked routinely for purity by thin layer chromatography.

Phytohemagglutinin P (Difco Laboratories, Detroit, MI) was fractionated according to the method of Weber et al.(4) into leukoagglutinating and erythroagglutinating components. Ricinis communis agglutinin was prepared from ricin by affinity chromatography on ovomucoid sepharose (5). Crystalline concanavalin A was purchased from Miles Laboratories, and wheat germ agglutinin was purchased from Sigma Chemical Co.

Liposomes containing dimyristoyl phosphatidyl choline, cholesterol, and dicetyl phosphate in molar ratios of 2/1.5/0.22 were prepared as described previously (6). Glycolipids were incorporated into liposomes in the concentrations indicated in the individual tables and figure.

Studies of the binding of plant lectins to liposomes were carried out in 15 ml glass centrifuge tubes (Corex) presoaked with bovine serum albumin (5 mg/ml). Liposomes and lectins in the concentrations indicated in the individual tables and figure were incubated for 45 minutes at 25°C. After incubation, liposomes were washed twice with 5 ml of 0.9% NaCl-0.01 M $NaHCO_3$, and collected by centrifugation at 40,000 g for 15 minutes. Specific protein binding to liposomes was measured by methods described previously (7).

RESULTS

The results of studies investigating the binding of five different lectins to liposomes lacking glycolipid and liposomes containing mixed gangliosides or globoside I are shown in Table I. These data demonstrate that considerable differences are found among the various lectins with respect to their interactions with these glycolipids in liposomes. There was virtually no significant binding of E-PHA* or L-PHA to liposomes containing gangliosides or globoside I. By contrast RCA-I and WGA bound to both of these glycolipids. Con-A also bound to liposomes containing, or lacking, glycolipid (Table I). Separate experiments showed that the binding of con-A to glycolipid-free liposomes occurred equally well with liposomes containing or lacking cholesterol, thus suggesting that con-A bound to the phospholipid portion of the membrane. Because of the marked tendency of con-A for nonspecific adherence, an additional incubation with this lectin was performed in the presence of α-methyl mannose, a specific inhibitor

*Abbreviations used: L-PHA and E-PHA, leukocyte and erythrocyte agglutinating components of phytohemagglutinin P; RCA-I, Ricinis communis agglutinin; con-A concanavalin A; WGA, wheat germ agglutinin.

TABLE I. Binding of various lectins to liposomes.

Lectin	Protein bound (μg/μmole phospholipid) to liposomes containing: Mixed gangliosides	Globoside I	No glycolipid
E-phytohemagglutinin	0.6	0.3	0
L-phytohemagglutinin	0.6	2.1	0
Concanavalin A			
Alone	28.7	31.6	30.4
+ 0.1 M α-methyl mannoside	12.4	16.9	14.4
R. communis agglutinin	38.0	24.7	2.2
Wheat germ agglutinin	25.9	23.8	0

In glass tubes presoaked with bovine serum albumin, 300 μl of liposomes containing either 100 μg mixed beef brain gangliosides per μmole of liposomal phospholipid, 150 nmoles globoside I per μmole of liposomal phospholipid, or no added glycolipid, were mixed with 1 mg lectin in 2 ml bicarbonate-buffered saline. Pilot studies in which the concentration of lectin was varied indicated that these conditions were sufficient to saturate available lectin binding sites. For all lectins, parallel incubation mixtures without liposomes were carried out to correct for nonspecific binding to the glass tubes. Specific lectin binding was determined as described in the Methods.

of con-A binding (8). Residual binding in the presence of this saccharide is considered to be nonspecific. The data demonstrate that, by this criterion, specific binding of con-A did occur with liposomes lacking glycolipid substituents (Table I). Although there was also apparent specific con-A binding to liposomes containing mixed gangliosides or globoside I, our data are consistent with an interaction of the lectin with either the glycolipid or phospholipid portion of these liposomes.

The specificity of the lectin binding to glycolipids in liposomes was further examined in additional experiments employing WGA. Table II illustrates the ability of WGA to bind to liposomes which contain various galactosyl lipids. Ceramide trihexoside, globoside I, and G_{M1} ganglioside were very effective WGA receptors, but there was minimal WGA binding to monogalactosyl

TABLE II. Binding of wheat germ agglutinin to liposomes containing different glycolipids

Glycolipid	WGA bound (μg/μmole liposomal phospholipid)
No glycolipid	0
Monogalactosyl diglyceride	3.1
Digalactosyl diglyceride	2.8
Ceramide dihexoside	1.0
Ceramide trihexoside	40.1
G_{M1} ganglioside	87.6

Liposomes contained either 50 μg G_{M1} ganglioside or 150 nmoles of the other glycolipids per μmole liposomal phospholipid. Reaction mixtures consisted of 50 μl of liposomes and 600 μg WGA in 0.5 ml bicarbonate-buffered saline. Specific lectin binding was determined as described in the Methods.

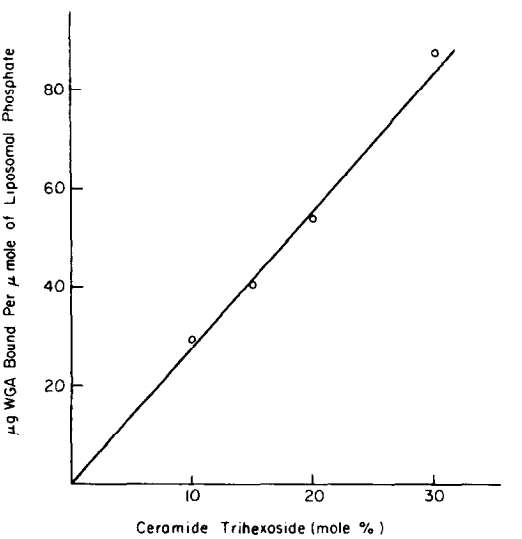

Figure 1. Wheat germ agglutinin binding to liposomes containing varying concentrations of ceramide trihexoside. Reaction mixtures consisted of 50 μl of liposomes containing 10-30 mole % ceramide trihexoside and 600 μg WGA in 0.5 ml bicarbonate-buffered saline. The mole % of ceramide trihexoside was calculated with reference to liposomal phospholipid. Specific lectin binding was determined as described in the Methods.

TABLE III. Effect of monosaccharides on wheat germ agglutinin binding to glycolipids.

Glycolipid	Monosaccharide inhibitor	Percent inhibition
ceramide trihexoside	N-acetyl glucosamine	77.4
	N-acetyl galactosamine	66.9
	galactose	27.6
globoside I	N-acetyl glucosamine	71.3
	N-acetyl galactosamine	38.0
	galactose	16.2
mixed gangliosides	N-acetyl glucosamine	0
	N-acetyl galactosamine	0
	galactose	2.4
	N-acetyl neuraminic acid	54.2

Liposomes contained either 150 nmoles of ceramide trihexoside or globoside I or 100 µg of mixed gangliosides per µmole liposomal phospholipid. Reaction mixtures consisted of 50 µl of liposomes and 600 µg WGA in 0.5 ml bicarbonate-buffered saline. Monosaccharide inhibitor was present in a concentration of 0.1 M. Inhibition was measured as a decrease of lectin binding as described in the Methods.

diglyceride, digalactosyl diglyceride, and ceramide dihexoside (Table II). The glycolipid-dependence of WGA binding to the liposomes was examined, as shown in Fig. 1. In this experiment a saturating concentration of WGA was maintained while the percentage of ceramide trihexoside in the liposomes was varied. Under these conditions the quantity of WGA bound to the liposomes was directly related to their content of ceramide trihexoside.

Inhibition of WGA binding to glycolipids in the presence of various saccharides is shown in Table III. Binding to ceramide trihexoside and globoside I was inhibited by N-acetyl glucosamine, and to lesser extents by N-acetyl galactosamine and galactose. These sugars did not inhibit WGA binding to mixed gangliosides, but this binding was inhibited by N-acetyl neuraminic acid.

DISCUSSION

The experiments described in this report indicate that certain glycolipids, when incorporated into liposomes, can serve as lectin receptors. Our data therefore confirm and extend the observations of Surolia *et al.* who have

investigated in detail the interaction of RCA-I with liposomes containing purified G_{M1} ganglioside (2). The interaction of RCA-I with ganglioside- and globoside-bearing liposomes is consistent with the known specificity of this lectin for galactose and N-acetyl galactosamine residues (8).

Our studies indicate that WGA bound to ceramide trihexoside and globoside I from human erythrocytes. Hakomori et al. have described an apparent interaction between WGA and a tumor-specific glycolipid containing galactose, glucose, fucose, and N-acetyl glucosamine (9). It is noteworthy that none of the glycolipids in our study contained N-acetyl glucosamine, which is generally considered to be the specific determinant of WGA binding (8). Our data demonstrate that WGA binding to certain glycolipids can be inhibited by galactose and N-acetyl galactosamine, in addition to N-acetyl glucosamine. These experiments thus clearly establish that (a) the binding of WGA to liposomes was mediated by lectin-carbohydrate interactions and (b) WGA recognized carbohydrate determinants other than N-acetyl glucosamine. It has also been reported that WGA showed high affinity binding to N-acetyl neuraminic acid, although this sugar was not effective as a hapten inhibitor of WGA-induced agglutination of transformed cells (10). Such interaction with N-acetyl neuraminic acid may in fact account both for the binding, and the saccharide-inhibition of binding, of WGA to liposomes containing ganglioside. It is of interest that a WGA receptor glycoprotein isolated by affinity chromatography from human erythrocytes was rich in galactose and N-acetyl galactosamine as well as N-acetyl neuraminic acid and N-acetyl glucosamine (5), and each of these sugars can inhibit WGA binding to various glycolipids in liposomes.

The observation that con-A could bind to glycolipid-free liposomes partly confirms some of the work of Bosch and McConnell in which con-A induced fusion of pure dipalmitoyl phosphatidyl choline liposomes (11). The interaction of con-A with glycolipid-free liposomes in our experiments, and in theirs, was partially inhibited by α-methyl mannose. This binding of con-A to glycolipid-free liposomes might be due to hydrophobic interactions analogous to the hydro-

phobic effects that occurred following con-A binding to planar bilayer membranes (12).

Ceramide trihexoside, globoside I and gangliosides are major surface components of a variety of cell types (13). The present studies confirm the ability of these compounds to serve as lectin receptors and therefore implicate cell surface glycolipids as likely participants in the important yet poorly understood phenomena of lectin-induced cell agglutination and mitogenesis, and other membrane-associated phenomena (1). By virtue of its simplified membrane structure and flexible composition, the liposome should serve as a useful model system for further investigating the interactions between lectins and their glycolipid receptors. After this work was completed two additional papers also reported binding of lectins to glycolipids in planar bilayer membranes (12), or to liposomes prepared from total lipids of erythrocyte membranes (14).

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REFERENCES:

1. Sharon, N., and Lis, H. (1975) in Methods in Membrane Biology (E. D. Korn, ed.), pp. 147-200, Plenum Press, New York.
2. Surolia, A., Bachhawat, B. K., and Podder, S. K. (1975) Nature, 257, 802-804.
3. Alving, C. R., Joseph, K. C., and Wistar, R. (1974) Biochemistry, 13, 4818-4824.
4. Weber, T., Nordman, C. T., and Grasbeck, R. (1967) Scand. J. Haematol., (Suppl. 4), 77-81.
5. Adair, W. L., and Kornfeld, S. (1974) J. Biol. Chem., 249, 4696-4704.
6. Kinsky, S. C., Haxby, J. A., Zopf, D. A., Alving, C. R., and Kinsky, C. B. (1969) Biochemistry, 8, 4149-4158.
7. Alving, C. R., and Kinsky, S. C. (1971) Immunochemistry, 8, 325-343.
8. Lis, H., and Sharon, N. (1973) Ann. Rev. Biochem., 42, 541-574.
9. Hakomori, S., Koscielak, J., Block, K. J., and Jeanloz, R. W. (1967) J. Immunol., 98, 31-38.
10. Greenaway, P. J., and Levin, D. (1973) Nature New Biol., 241, 191-192.
11. Bosch, J. V. D., and McConnell, H. M. (1975) Proc. Nat. Acad. Sci. U. S. A., 72, 4409-4413.
12. Deleers, M., Poss, A., and Ruysschaert, J. (1976) Biochem. Biophys. Res. Comm., 72, 709-713.
13. Nigam, V. N., and Cantero, A. (1973) Adv. Cancer Res., 17, 1-80.
14. Rendi, R., Kuettner, C. A., and Gordon, J. A. (1976) Biochem. Biophys. Res. Comm., 72, 1071-1076.