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LECTIN-RECEPTOR INTERACTIONS IN LIPOSOMES

II. INTERACTION OF WHEAT GERM AGGLUTININ WITH PHOSPHATIDYLCHOLINE LIPOSOMES CONTAINING INCORPORATED MONOSIALOGANGLIOSIDE

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SUMMARY

Bovine brain gangliosides incorporated into phospholipid liposomes provide receptors for wheat germ agglutinin. Purified monosialogangliosides were mixed with egg phosphatidylcholine, and unilamellar liposomes were generated. Addition of wheat germ agglutinin induced the liposomes to fuse, and gel filtration analysis revealed that the lectin was incorporated into the fused liposomes. The fusion process was studied by following the changes in the 90° light scattering. Increasing the proportion of the monosialoganglioside in the liposomes was found to increase both the extent of the lectin-induced liposome fusion and the rate of the reaction; below a threshold of approx. 5 mol %, the process was extremely slow. The increase in light scattering could be prevented by the addition of the hapten inhibitor, *N*-acetyl-D-glucosamine (1 mM). Addition of the inhibitor, subsequent to the lectin, caused a partial decrease in light scattering due to the dissociation of unfused vesicle aggregates. Electron microscopic examination revealed that the ganglioside-containing liposomes were vesicles, 244±25 Å (S.D.) in diameter. Upon addition of wheat germ agglutinin, the vesicles appeared to fuse to form larger vesicles, corresponding to dimers and trimers of the initial vesicles. Inhibition studies with a variety of monosaccharides indicated that the sialic acid moieties present in the ganglioside acted as the lectin-receptor sites. This was confirmed by the observation that wheat germ agglutinin did not interact with phosphatidylcholine vesicles containing desialyated ganglioside.

INTRODUCTION

Plant lectins have become popular tools for probing membrane structure, following the recognition of the functional importance of surface heterosaccharides in cellular behavior [1–5]. The cell surface carbohydrates are contributed by glyco-

All sugars were of the D-configuration.

lipids and glycoproteins and it appears possible that certain lectins interact with the carbohydrate moieties irrespective of whether these are components of the membrane glycolipids or glycoproteins. Adair and Kornfeld [6] have shown that the isolated major sialoglycoprotein of erythrocyte membranes is a potent hapten inhibitor of wheat germ agglutinin, and have implicated the sialoglycoprotein as being a receptor for this lectin. In a previous publication, we described the formation of unilamellar phospholipid vesicles containing the major sialoglycoprotein of erythrocyte membranes [7]. The sialoglycoprotein appeared to be functionally incorporated into the vesicle membrane as judged by the vesicle interactions with wheat germ agglutinin, which were phenomenologically similar to the lectin-cell interactions [7]. Neuraminidase treatment of cells decreased their binding affinity for wheat germ agglutinin [6, 8] and abolished the lectin-induced cell agglutinability [9]. Similar enzyme treatment of phospholipid vesicles containing the isolated erythrocyte sialoglycoprotein inhibited their specific binding of wheat germ agglutinin and also the lectin-induced vesicle aggregation [7]. These studies indicated that sialic acid residues were part of the receptor sites for wheat germ agglutinin. Since cell surface sialic acids are components of glycolipids in addition to glycoproteins [10], we were interested in determining whether wheat germ agglutinin would interact with model membranes containing sialic acid residues attached to glycolipids. Accordingly, we have generated unilamellar phospholipid vesicles containing monosialogangliosides and in the present paper describe their interaction with wheat germ agglutinin.

MATERIALS AND METHODS

Purified bovine brain gangliosides were obtained from Applied Science Laboratories, and phosphatidylcholine was isolated from fresh egg yolks as previously described [11]. The lipids were examined by thin layer chromatography, and after hydrolysis were analyzed for sugar and inorganic phosphate using standard methods [12–16]. The gangliosides were desialyated by hydrolysis at 80 °C with 0.1 N H_2SO_4 for 90 min. Hexose and hexosamine were released by prolonged (> 3 h) hydrolysis of the glycolipid at 100 °C with 1 N HCl. Wheat germ agglutinin was purified by affinity chromatography on chitin as reported earlier [7, 17]. Protein was measured by the Lowry method [18]. *N*-palmitoyldihydro-lactocerebroside, *N*-acetylneuraminic acid (AcNeu), *N*-acetyl-D-glucosamine (GlcNAc), glucuronic acid, glucose, galactose, chitin and bovine serum albumin were from Sigma; *N*-acetyl-D-galactosamine (GalNAc) was purchased from Schwartz-Mann.

Preparation of the phospholipid-ganglioside dispersions

Liposomes were generated according to the method of Batzri and Korn [19]. The major structural component of the membranes was phosphatidylcholine, which was intermixed with smaller molar proportions of the bovine brain gangliosides. Mixing was accomplished by adding a 3 % solution of phosphatidylcholine in ethanol to a 0.1 % solution of the purified gangliosides in warm ethanol. The solution was concentrated by evaporation under nitrogen until the phosphatidylcholine concentration in the ethanol approached 30 mM. This mixed lipid solution was then injected by means of a Hamilton 100 μl syringe into an acetate buffer (0.05 M sodium chloride-0.05 M sodium acetate, pH 5.0 at 4 °C) until the ethanol concentration in

the buffer reached 5 % (v/v). The liposome suspension was concentrated with an Amicon ultrafiltration apparatus using a XM 300 A membrane filter under an argon pressure of 10 lb · inch^{-2} and was immediately subjected to gel filtration on a Sepharose 4B column (1.6×40 cm), equilibrated with the acetate buffer, pH 5.0, at 4 °C. The absorbance of the column effluent at 240 nm was monitored by means of a Beckman (Acta III) spectrophotometer equipped with a flow cell. Samples of the column effluent were analyzed for ganglioside and phospholipid.

Electron microscopy

Samples of the phosphatidylcholine-ganglioside liposomes or mixtures of the liposomes with wheat germ agglutinin were sprayed on Formvar-coated copper grids, allowed to adhere for 1 minute, then negatively stained with 1 % uranyl formate for 2 min. After air drying, the grids were examined in a Philips 300 electron microscope operating at 80 kV. Latex spheres, 1090±27 Å (S.D.) in diameter, were added to some grids containing negatively stained liposomes for calibration [20].

Optical studies

Light scattering studies were carried out using a Perkin Elmer fluorescence spectrophotometer (Model MPF-3). The Rayleigh peak from unpolarized incident light at 500 nm was measured to monitor the 90° light scattering of the liposome suspensions. Temperature control was maintained at 25±0.5 °C with the aid of a circulating thermostat connected to the jacketed cell housing. All solutions were filtered through 0.45 μm Millipore filters just prior to the light scattering measurements. In titration studies, the wheat germ agglutinin solution (1 mg/ml) was added by means of a microliter syringe directly to the liposomes or buffer controls contained in quartz cuvettes. Hapten inhibition studies were usually carried out by addition of monosaccharides to the liposomes prior to titration with wheat germ agglutinin, but a limited number of studies were performed by addition of haptens following the titration with lectin.

RESULTS

Analysis of the lipids by thin layer chromatography on silica gel G using the solvent system, chloroform/methanol/2.5 N ammonia in water (60 : 40 : 9, by vol.), verified the purity of the phosphatidylcholine but indicated two closely migrating components in the ganglioside preparation, both of which contained AcNeu as detected with the resorcinol reagent [21]. Chemical analysis of the ganglioside mixture confirmed the absence of phospholipid, and yielded weight average molecular weights for the ganglioside of 1540±120 per AcNeu group, and 1400±70 per GalNAc group, respectively. Neutral sugar determinations indicated that the mixture contained 1 mole of glucose and approximately 1.5 mol galactose per 1500 molecular weight component. These values are consistent with an approximately equal proportion in the mixture of the monosialogangliosides GM1 and GM2 (according to Svennerholm's nomenclature, ref. 22). This analysis confirmed the one carried out by the supplier (Dr. Ramadan of Applied Science, personal communciation). The molar ratio of phospholipid to ganglioside used in the liposome preparations varied from 5 : 1 to 30 : 1, assuming mean molecular weights of 750 and 1500 for the phosphatidyl-

choline and ganglioside, respectively. Liposomes of purified phosphatidylcholine alone, and micelles of the purified gangliosides alone [23] were prepared for use as controls.

Gel-filtration studies

Molecular sieve chromatography of the phosphatidylcholine-ganglioside liposomes on Sepharose 4B yielded a separation into two distinct fractions, similar to that first reported by Huang [24]. Electron microscopic examination of negatively stained samples of the column effluent revealed that fraction I contained multilamellar liposomes, while fraction II contained a relatively uniform size population of small vesicles. Chemical analysis of the multilamellar liposomes and the fraction II vesicles gave similar molar ratios of phospholipid to AcNeu, which were also equal to the ratios determined in the initial ethanol solutions used in the liposome preparation. These results indicated that the ethanol injection procedure provided homogeneous molecular mixing of the ganglioside and phosphatidylcholine in the nascent liposomes and also precluded the formation of significant quantities of ganglioside micelles. A typical gel filtration separation is illustrated in Fig. 1a. Determinations of organic phosphate in the column effluent revealed that only 10 % of the total phospholipid was eluted in fraction I.

The ratios of phospholipid to ganglioside in fractions I and II were 7.84: 1, and 8.07: 1, respectively, compared to 8.0: 1 in the initial liposome suspension. In order to study the interaction of wheat germ agglutinin with the liposomes, fraction II liposomes from this separation (fraction numbers 20–28, Fig. 1a) were reconcentrated by ultrafiltration, and the resulting suspension was divided into two equal portions. An equal volume of 0.1 % wheat germ agglutinin was added to one portion, while buffer was added to the other as control. The reconcentrated control liposomes were resubjected to gel filtration, and produced the elution profile shown in Fig. 1b. The negligible proportion of void volume material in the liposome suspension indicated that there was an extremely low rate of spontaneous aggregation or fusion of the fraction II vesicles. (Overnight aging of similar control liposome suspensions did not cause a significant increase in the amount of aggregated material.) Upon addition of the lectin, however, the liposome suspensions became turbid compared to the control. The mixture was incubated at 4 °C for 1 h, then applied to the same Sepharose 4B column. The resulting elution profile is illustrated in Fig. 1c. Samples of the column effluent were hydrolyzed and assayed for inorganic phosphate using the Bartlett method [16]. This revealed that liposomes were present in the first and second peaks (Fig. 1c). A Lowry analysis [18] on the column effluent indicated that protein was present in all three peaks, the last corresponding to the free lectin. A control column run with the lectin alone (ultraviolet absorption profile not shown) confirmed that the lectin did not aggregate under the experimental conditions, but was eluted only under the last peak position shown for the lectin-liposome mixture in Fig. 1c.

The average ratios of protein to phosphatidylcholine measured in the first and second liposome peaks (Fig. 1c) were 166 μg protein/μmol phospholipid, and 72 μg protein/μmol phospholipid, respectively. These values correspond to molar ratios of phospholipid to lectin of approx. 216 and 500: 1, assuming a molecular weight of 36000 for wheat germ agglutinin [25, 26]. Since the molar ratio of phospholipid to

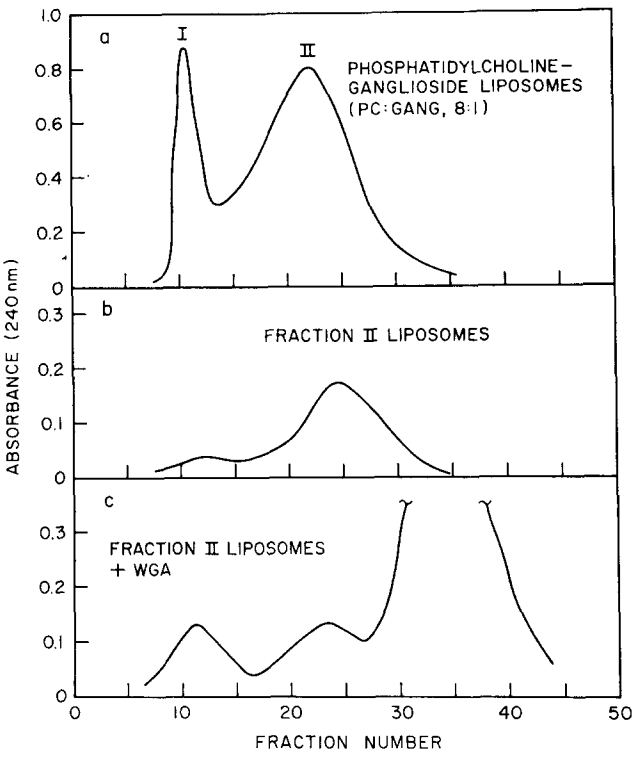

Fig. 1. Gel filtration of phosphatidylcholine-ganglioside liposome (8 : 1, molar ratio) on Sepharose 4b: a, elution profile of liposomes; b, elution profile of rechromatographed fraction II liposomes from 1a; c, elution profile of fraction II liposomes after incubation with 0.05 % wheat germ agglutinin for 60 min. Buffer system: 0.05 M sodium chloride/0.05 M sodium acetate, pH 5.0 at 4 °C.

ganglioside in the fraction II liposomes was determined separately to be 8.07: 1, the corresponding molar ratios of ganglioside to the lectin in the two fractions were 27 and 62: 1, respectively. These data indicate that there was more lectin associated with the receptors in the fused liposomes than in the free liposomes.

Electron microscopy

The appearance in the electron microscope of negatively stained preparations of the fraction II vesicles containing a 10: 1 molar ratio of phosphatidylcholine to ganglioside was similar to control fraction II vesicles not containing the ganglioside [19, 24]. The mean diameter of the vesicles was found to be 244±25 Å (S.D.). Fig. 2 illustrates the time-dependent increase in particle size that occurred when wheat germ agglutinin was added to a homogeneous sample of small vesicles. The sample taken for Fig. 2a was negatively stained within one minute of mixing the vesicles and lectin, was air dried and examined in the electron microscope shortly thereafter. Fig. 2b is from the same initial mixture, but the sample was processed for electron microscopy 30 min after addition of the lectin. Fig. 2c illustrates the same liposome sample after 60 min incubation with the lectin. After prolonged incubation of the lectin/liposome mixture (> 2 h), large aggregates of the fused vesicles were observed, and there were relatively few of the initial 244 Å diameter vesicles remaining in the

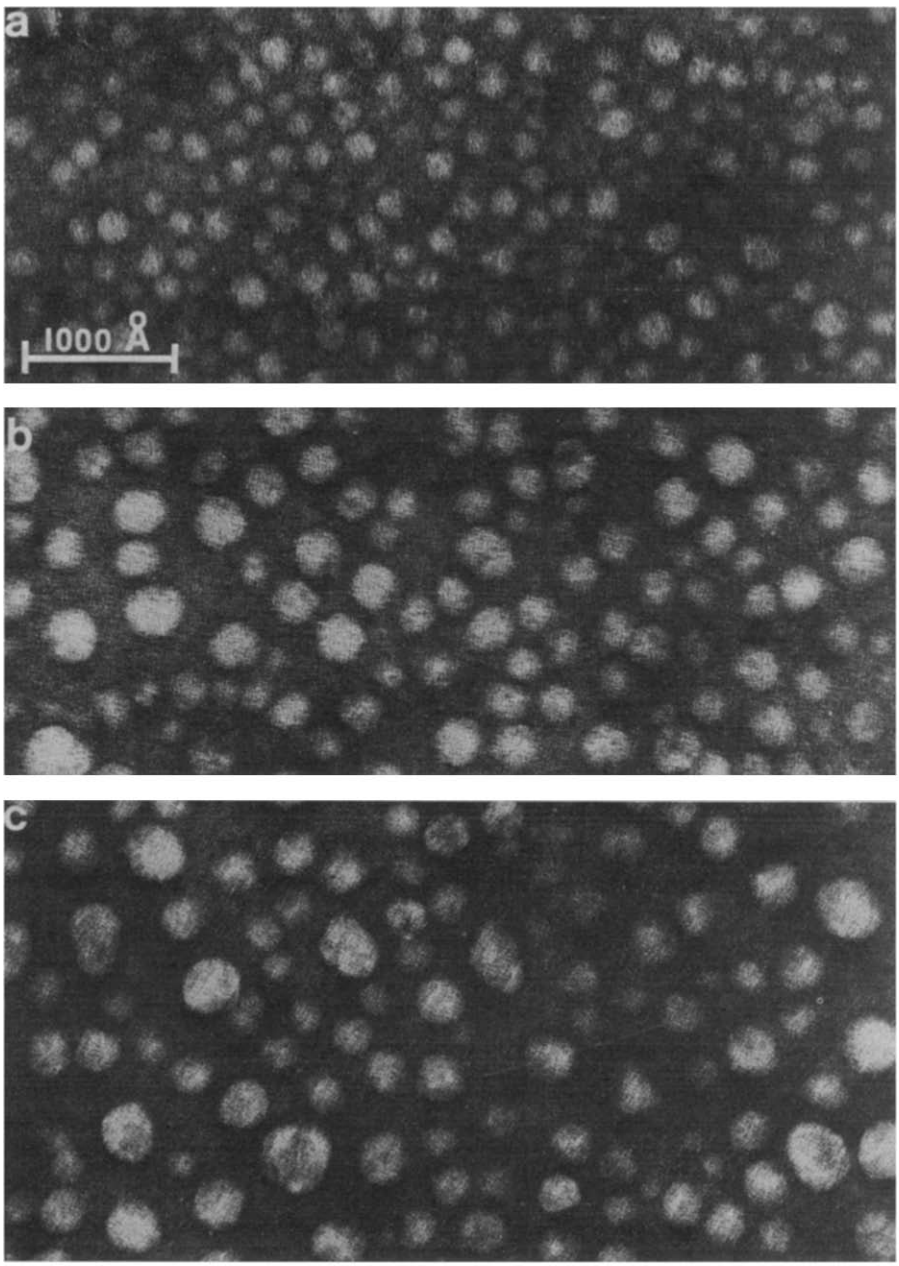

Fig. 2. Electron micrographs of phosphatidylcholine-ganglioside (10 : 1 molar ratio) vesicles in the presence of wheat germ agglutinin (100 μg/ml), stained with 1 % uranyl formate ($\times$173 000). The time of incubation of the lectin with the vesicles varied as follows: (a) 1 min, (b) 30 min, and (c) 60 min. Vesicle size measurements taken from these samples are listed in Table I.

TABLE I

SIZE INCREASE OF PHOSPHATIDYLCHOLINE-GANGLIOSIDE VESICLES UPON THE ADDITION OF WHEAT GERM AGGLUTININ

System	Time of incubation (min)	Mean diameter of vesicles (Å)	Calculated diameters of fused vesicles (Å)
Vesicles alone	0	244±25 (S.D.)	(Monomer: 244)
Vesicles+wheat	1	252±30	
germ agglutinin	30	310±35	Dimer: 328
(100 μg/ml)	60	365±36	
	60*	(427±44)	Trimer: 393

* After 60 min incubation, a significant number of vesicles, approx. 427 Å in diameter appeared in the mixture. These are listed separately since the value is significantly larger than the mean for the total sample.

mixture. The mean diameters of the vesicles in the mixture at 1 min, 30 min, and 60 min are listed in Table I. It is possible to explain these observations on the basis of fusion of initial vesicles to form larger structures. Table I lists the diameters predicted for a dimer and trimer generated by the fusion of the initial 244 Å diameter vesicles. After 30 min incubation, there was clear evidence that dimers had formed and after 60 min there was a significant number of particles in the mixture that corresponded to the size of fused trimers.

Optical studies

Addition of wheat germ agglutinin to fraction II phosphatidylcholine-ganglioside vesicles, in general, caused an increase in the 90° light scattering. Differential light scattering titration curves are shown in Fig. 3. The extent of interaction decreased as the molar ratio of phosphatidylcholine to ganglioside increased and no interaction was observed with a molar ratio of 30: 1. Purified phosphatidylcholine vesicles, as previously noted [7], did not interact with the lectin. There was also no significant change of light scattering when the lectin was added to the ganglioside molecules, which had been diluted to the same final ganglioside concentration used in the light scattering studies with the phosphatidylcholine-ganglioside vesicles, this value being below the critical micelle concentration of 0.02 % ganglioside [23]. The rate of increase of light scattering was also dependent upon the composition of the vesicles, decreasing with increasing molar ratios of phosphatidylcholine to ganglioside, as indicated in Fig. 4.

Addition of certain monosaccharides to the vesicles prior to the addition of the lectin caused an inhibition of the increase in light scattering. Inhibition studies were usually carried out by complete titration of the vesicles with the lectin (see Fig. 5), the monosaccharide being present in the vesicle suspension and buffer controls. Table II lists the inhibition values observed for each monosaccharide tested. GlcNAc was found to be the most potent inhibitor, producing almost 60 % inhibition at 0.1 mM. When GlcNAc was added subsequent to complete titration of the vesicles with the lectin, it was found to be considerably less potent than when

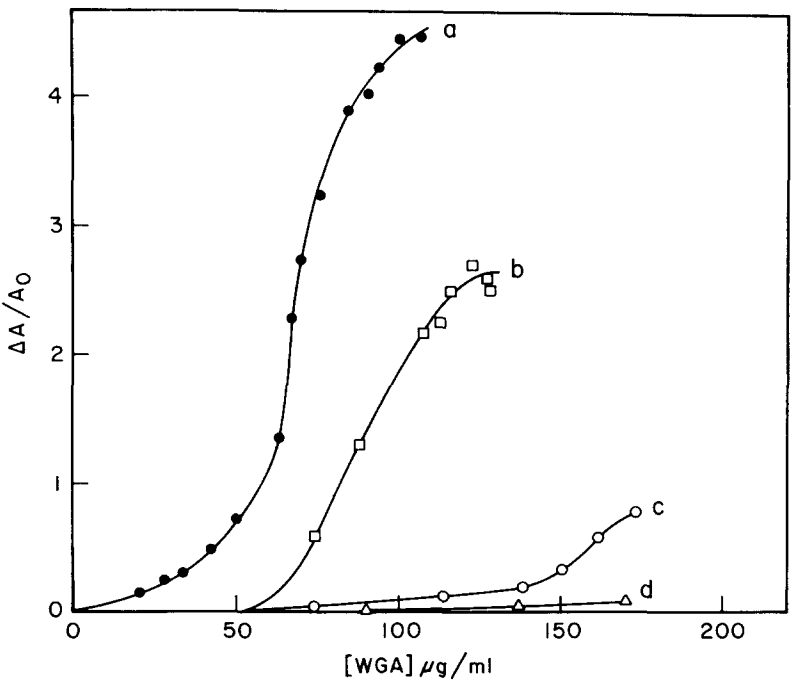

Fig. 3. Differential light scattering of phosphatidylcholine-ganglioside vesicles upon the addition of wheat germ agglutinin. The curves were generated as follows: At each lectin concentration, the 90° light scattering of the sample was corrected for the increase in the relative light scattering of the sodium acetate (pH 5.0) buffer controls, then the corrected relative increase was divided by the initial light scattering of the liposome suspension. Each reading was recorded 5 min after addition of the lectin. [WGA] represents the concentration of wheat germ agglutinin in the reaction mixtures. The concentration of phospholipid in each cuvette was approx. 0.1 mg/ml, while the molar ratios of phosphatidylcholine to ganglioside for each preparation were: (a) 5 : 1; (b) 8 : 1; (c) 20 : 1; and (d) 30 : 1.

added prior to the lectin. The degree of reversal observed under those conditions was dependent upon the GlcNAc concentration, but complete reversal was not achieved even at high concentration, 30 mM (Table III). After interaction had occurred between the vesicles and the lectin, it was not possible to lower the differential light scattering, $\Delta A/A_0$, below approximately 1.1, which corresponded to a doubling of the initial light scattering of the mixture.

The efficacies of the other monosaccharides as hapten inhibitors in this reaction were: AcNeu > GalNAc > galactose > glucose. Glucuronic acid was tested to ascertain whether the effect of AcNeu was simply attributable to its charge. This proved not to be the case (Table II). However, it was found that lowering the pH of the medium below pH 2.5 abolished the lectin-receptor interaction, indicating that there was some contribution to the reaction from ionized carboxylic groups.

In order to determine the specificity of the lectin-glycolipid interaction, ancillary experiments were carried out with phosphatidylcholine vesicles containing either desialyated ganglioside or lactocerebroside. Both glycolipids were incorporated into the vesicles with a molar ratio, 5: 1, phosphatidylcholine: glycolipid, using the

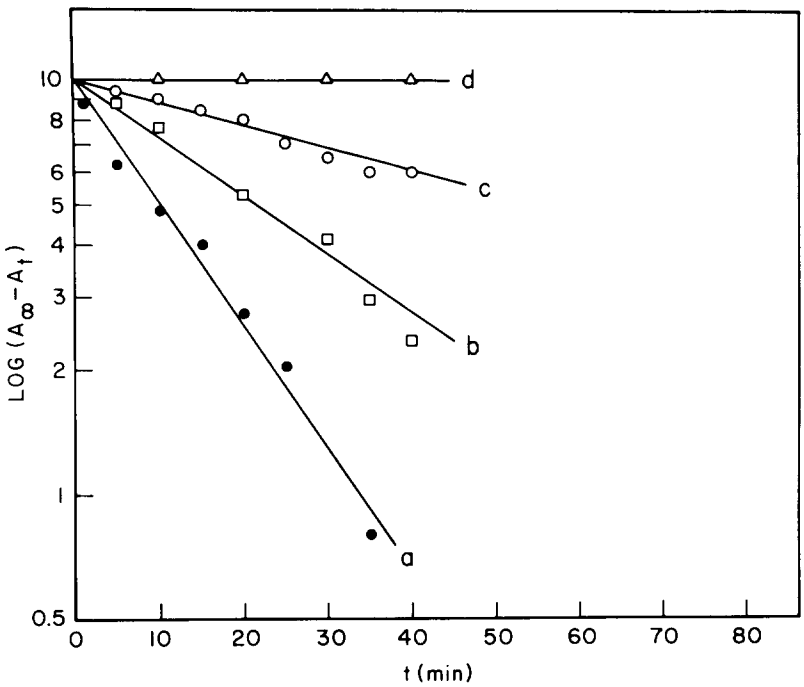

Fig. 4. Kinetics of vesicle-fusion in the presence of wheat germ agglutinin. The phospholipid concentrations and ganglioside concentrations of the samples were identical to those used in the titration studies, see Key in Fig. 3. At $t = 0$, the lectin was added to each sample in the following concentrations: (a) 100 μg/ml; (b) 150 μg/ml; (c) 175 μg/ml; and (d) 200 μg/ml. A_∞ represents the corrected 90° light scattering at pseudo-saturation (cf Fig. 3), while A_t represents the corrected light scattering at each measurement from the time of mixing. Temperature of the study was 25 °C.

same ethanol injection method used for the ganglioside. Addition of wheat germ agglutinin (400 μg/ml) to each of these glycolipid-vesicles preparations did not cause a significant increase in light scattering in acetate buffer at pH 5.0, whereas addition of less lectin (100 μg/ml) caused a maximal change with the ganglioside-containing vesicles (Fig. 3). It was also confirmed that addition of a non-specific protein, namely bovine serum albumin (100 μg/ml) did not cause fusion or aggregation of the ganglioside-containing vesicles in acetate buffer at pH 5.0.

DISCUSSION

Since wheat germ agglutinin did not interact with phosphatidylcholine vesicles containing either desialyated ganglioside or lactocerebroside, and because AcNeu inhibited the lectin interaction with the ganglioside-containing vesicles, it would appear that the sialic acid moieties on the gangliosides were the probable sites for lectin interaction. Equilibrium dialysis measurements have confirmed that the lectin binds AcNeu and have also shown that the AcNeu could be displaced from the lectin binding sites by low concentrations of GlcNAc [27]. On this basis, it was not surprising that GlcNAc was the most potent inhibitor tested (Table II) despite the fact that this monosaccharide was not present in the ganglioside molecules. It is prob-

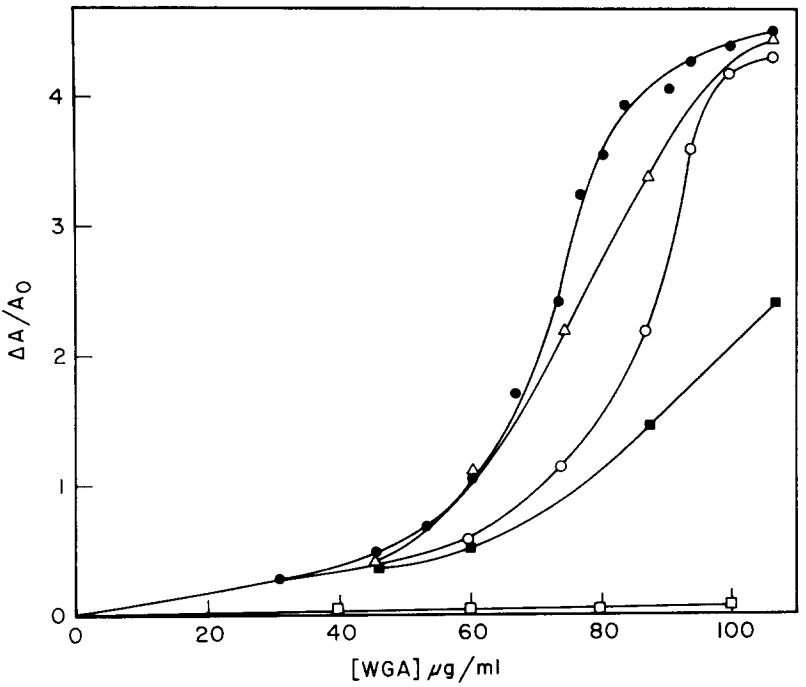

Fig. 5. Effect of monosaccharide inhibitors on the differential light scattering of phosphatidylcholine-ganglioside vesicles (5 : 1 molar ratio). Each inhibitor was added to the liposome suspension prior to the addition of the lectin as follows: ●, no inhibitor present; ○, 0.1 mM GlcNAc; □, 1 mM GlcNAc; ■, 1 mM AcNeu; △, 1 mM GalNAc.

TABLE II

EFFECT OF INHIBITORS ON VESICLE LIGHT SCATTERING IN THE PRESENCE OF WHEAT GERM AGGLUTININ

Monosaccharide	Concentration (mM)	Inhibition of* light scattering (% of control)
GlcNAc	1	100
	0.1	58±2 (S.D.)
AcNeu	10	100
	1	60±5
GalNAc	10	91±5
	1	8±2
Galactose	10	59±4
	1	0
Glucose	10	41±2
	1	0
Glucuronic acid	10	0

* These values correspond to the degree of inhibition of the differential light scattering measured at the lectin concentration which caused half maximal increase in the control vesicle suspension in the absence of the inhibitor.

TABLE III

LOWERING OF THE LIGHT SCATTERING OF VESICLE-LECTIN MIXTURES BY MONOSACCHARIDES

Monosaccharide	Concentration (mM)	$\Delta A/A_0$	Decrease* (% of control)
None		4.5	
GlcNAc	1	3.8	15±5 (S.D.)
	2.5	2.7	39±6
	5	1.5	66±6
	30	>1.1	<75
GalNAc	5	3.7	17

* The values listed represent the mean decrease in the differential light scattering of vesicle-lectin mixtures observed upon addition of the monosaccharide. In all cases, the initial mixture contained phosphatidylcholine ganglioside vesicles (5 : 1 molar ratio) in the presence of 100 μg/ml wheat germ agglutinin having interacted to give a differential light scattering, $\Delta A/A_0$, of 4.5 (cf. Fig. 5).

able that the effects of GalNAc, galactose and glucose at 10mM are nonspecific, since each of these sugars was essentially inactive at 1 mM.

The lectin-induced increase in light scattering of the phospholipid-ganglioside vesicles is similar to that previously observed with phospholipid vesicles containing sialoglycoprotein [7]. Electron microscopic examination of the vesicle-lectin mixtures confirmed that aggregation occurred in both systems. However, the vesicle aggregation induced by the lectin-sialoglycoprotein interaction did not appear to involve a significant degree of vesicle fusion, unlike the results observed with the glycolipid vesicles (Fig. 2). In both systems, the aggregation reactions could be specifically inhibited by the prior addition of low concentrations of the wheat germ agglutinin hapten inhibitor, GlcNAc. Also, the addition of the monosaccharide subsequent to the formation of the vesicle aggregates caused their dissociation, albeit to a limited extent in the case of the phosphatidylcholine-ganglioside vesicles. The vesicle fusion that occurred concomitantly with the lectin-ganglioside interaction obviously would prevent complete dissociation of the aggregates to the original vesicles upon addition of GlcNAc. The light scattering data (Table III) indicated that the limiting size of the dissociated particles was approximately twice that of the initial vesicles, consistent with the preponderance of larger vesicles seen in the electron micrographs (Fig. 2b and 2c).

The fusion of negatively charged lipid vesicles induced by addition of Ca^{2+} has been described by Papahadjopoulos et al. [28]. Extensive fusion occurred between phosphatidylserine vesicles incubated in the presence of $CaCl_2$ (> 1 mM). However, only limited fusion occurred between neutral phosphatidylcholine vesicles or phosphatidylcholine vesicles containing less than 20 % phosphatidylserine, in the presence of $CaCl_2$ (> 4 mM) and albumin (100 μg/ml) [28]. Direct measurements confirmed that $CaCl_2$ (5 mM) did not cause the fusion of the glycolipid-vesicles used in the present studies, which contained less than 16 mol % of monosialoganglioside in the phosphatidylcholine matrix.

The rate of the Ca^{2+}-induced aggregation of negatively charged phospholipid

vesicles has been measured by Lansman and Haynes [29], who found that the concentration-dependent time constant for vesicle dimerization was between 0.4–2 s under the conditions of study. Using a kinetic analysis, these authors calculated the apparent rate constants for dimer formation to be approximately two orders of magnitude lower than the rate constant for vesicle collision [29], and therefore concluded that the rate limiting step was a process occurring at the membrane surface. This conclusion would seem to apply equally to the lectin-induced vesicle aggregation described here, since the rate constants for aggregation, calculated from the data in Fig. 4 and employing equation 7 of ref. 29, were approximately five orders of magnitude lower than the rate constant for the vesicle collision. Lansman and Haynes also reported that diluting the negatively charged phospholipid with 50 % phosphatidylcholine decreased the extent of the Ca^{2+}-induced vesicle dimerization and also decreased the reaction rate [29]. An analogous decrease in the extent of the lectin-induced vesicle aggregation (Fig. 3) and decrease in the reaction rate (Fig. 4) occurred as the lectin receptor was diluted with increasing proportions of phosphatidylcholine. The threshold concentration of ganglioside, below which lectin-induced aggregations occurred very slowly, was approximately 5 mol %. This corresponds to roughly 150 ganglioside molecules assuming that there are approximately 3000 phosphatidylcholine molecules per vesicle [24]. These data suggest that many receptor molecules are involved in the formation of a stable vesicle dimer. Direct analysis of fused liposomes (Fig. 1c) revealed that the molar ratio of ganglioside to lectin was 27 : 1 for the liposome preparation containing 11 mole % ganglioside. This corresponds to about 12 lectin molecules associated with each original vesicle. That the aggregation mechanism is highly cooperative with respect to the lectin is indicated by the sigmoidal shape of the titration curves (A Hill coefficient of approximately 5 was calculated from the titration curve, without inhibitor, in Fig. 5).

It is conceivable that the rate determining step in the lectin-induced fusion is the formation in the vesicle membrane of "patches" containing the critical surface concentration of ganglioside molecules necessary to stabilize the lectin interactions between the vesicles. It is proposed that vesicle fusion occurs after stabilization of the collisional encounter complex by the bridging of lectin molecules between these patches on the apposed membranes. Polymerization of the stable vesicle dimers into larger aggregates would be in part determined by the availability on the exposed membrane surfaces of patches of receptors necessary to serve as anchors for further lectin bridging. Such a scheme would at least qualitatively explain the reactions reported in this paper. In a very recent article, Surolia et al. [30] have described the interaction of a galactose-specific lectin from *Ricinus communis* with liposomes containing gangliosides, and have concluded that the incorporated gangliosides were recognized through the non-reducing terminal galactose moiety. Both studies demonstrate the potential importance of glycolipids as receptors for carbohydrate-binding proteins on cell surfaces.

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