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LECTIN-RECEPTOR INTERACTIONS IN LIPOSOMES

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SUMMARY

The major sialoglycoprotein of mammalian erythrocytes has been incorporated into phosphatidylcholine membranes to generate a model system, glycoprotein-liposomes. Electron microscopic examination revealed these structures to be vesicles, approximately 300 Å in diameter. An aqueous compartment inside the glycoprotein-liposomes has been identified by trapped volume studies with [^{14}C]sucrose. These glycoprotein-liposomes were found to interact with the lectins, wheat germ agglutinin, and phytohemagglutinin, to form aggregates of mainly unfused vesicles. The aggregation process has been studied by electron microscopy, 90° light scattering, and differential ultracentrifugation analysis. Hapten inhibitors of the lectins were found to inhibit the lectin-induced aggregation of the glycoprotein-liposomes. Binding of ^{125}I-labeled wheat germ agglutinin to glycoprotein-liposomes was studied by differential ultracentrifugation. Hapten inhibitors of wheat germ agglutinin were also found to inhibit the binding of ^{125}I-labeled wheat germ agglutinin to the glycoprotein-liposomes. The characteristics of the lectin interactions with glycoprotein-liposomes appeared to be phenomenologically similar to lectin-cell interactions.

INTRODUCTION

Membrane reconstitution, using purified membrane-derived proteins and lipids, appears to offer a suitable approach to the elucidation of the molecular organization of biomembranes [1]. Considerable effort has been devoted to the characterization of the physical properties of the unmodified phospholipid lamellar systems, including the planar bilayer [2], multilamellar liposomes [3] and homogeneous vesicles [4]. In recent years, significant progress has also been made in the isolation and characterization of certain membrane proteins [5–7]. One of the best studied integral membrane proteins has been the major sialoglycoprotein of human erythrocytes, which has been isolated and partially characterized [8–10] and found to contain receptors for several sugar specific plant proteins (lectins) [11], MN blood group substances [10] and influenza virus [10]. Interaction of the isolated sialoglycoprotein with model phospholipid membranes was first reported by Tosteson

et al. [12]. These workers incorporated the isolated sialoglycoprotein into planar lipid bilayer membranes, and found that the reconstituted membranes responded to a challenge of concanavalin A by an increase in the membrane conductance [12]. Segrest et al. [13] have shown that the hydrophobic tryptic peptide derived from the major sialoglycoprotein of human erythrocytes reassociated with multilamellar phospholipid membranes and led to the formation of freeze-etch intramembranous particles. More recently, Grant and McConnell [15] have incorporated the entire sialoglycoprotein into multilamellar liposomes. Freeze-fracture electron microscopy also revealed that the reincorporated sialoglycoprotein had a tendency to cluster into groups of particles. The sialoglycoprotein-liposomes could be agglutinated by influenza virus.

We have prepared phospholipid membranes in the form of closed vesicles, approximately 300 Å in diameter, containing incorporated sialoglycoprotein. These glycoprotein-liposomes are considerably smaller than the multilamellar structures studied by other groups [13, 14] and therefore offer the advantage of a more homogeneous system for interaction studies [4]. We have previously reported that the characteristics of interactions of wheat germ agglutinin with the glycoprotein-liposomes are reminiscent of cell agglutination reactions with lectins [15]. In the present study, we detail the interactions of both wheat germ agglutinin and phytohemagglutinin with the model system and discuss the results in relationship to lectin-cell interactions.

MATERIALS AND METHODS

Phosphatidylcholine was purified as previously described [16]. Wheat germ agglutinin was purified by two methods [17, 18]. The wheat germ agglutinin preparations were examined for purity by sodium dodecyl sulfate-acrylamide gel electrophoresis, and also assayed for neutral sugar and amino sugars by standard methods [19–21]. Phytohemagglutinin-P from *Phaseolus vulgaris* was purchased from Difco (Detroit, Mich.) and used without purification. Chitin, *N*-acetylneuraminic acid (AcNeu), *N*-acetyl-D-glucosamine (GlcNac), *N*-acetyl-D-galactosamine (GalNac), galactose, glucuronic acid and crystalline bovine serum albumin were all obtained from Sigma (St. Louis, Mo.). Neuraminidase (protease-free) was from Behring Diagnostics (Somerville, N.J.). All chemicals used in this project were reagent grade, and the water was double distilled from an all glass apparatus. Fresh dog blood was collected in acid-citrate-dextrose anticoagulant solution, and the erythrocytes were separated by low speed centrifugation. Hemoglobin-free erythrocyte ghosts were prepared by the Dodge procedure [22]. The major sialoglycoprotein was extracted from the membranes using 0.3 M lithium diiodosalicylate as described by Marchesi and Andrews [23], and examined for purity on sodium dodecyl sulfate-acrylamide gels [19]. Sialic acid content was determined by the method of Warren [24].

Preparation of the phospholipid dispersions

Liposomes were generated according to the method of Batzri and Korn [25]. The sialoglycoprotein was incorporated into the liposomes by injecting the ethanol solution of phospholipid into an aqueous 0.1 % (w/w) solution of the sialoglycoprotein, usually in a total volume less than 1 ml. The aqueous solutions contained

0.05 M NaCl and 0.05 M sodium acetate titrated to pH 5.0 at 25 °C. The sialoglycoprotein-liposome suspension was diluted in the same buffer to 50 ml, then free sialoglycoprotein was removed by repeated ultrafiltration of the suspension using fresh buffer with an Amicon (Lexington, Mass.) XM-100A membrane filter under an argon pressure of 10 lb/inch^2. Treatment of the glycoprotein-liposomes with neuraminidase to remove surface sialic acid groups was effected by incubating the suspension overnight with catalytic concentrations of the protease-free enzyme in the acetate buffer at pH 5.0 and 25 °C.

Electron microscopy

Samples of the glycoprotein-liposomes or mixtures of glycoprotein-liposomes and purified wheat germ agglutinin were placed on Formvar-carbon coated copper grids, allowed to adhere for 1 min, then negatively stained with an equal volume of 2 % solution of potassium phosphotungstate at pH 5, and the excess solution removed. After air-drying, the grids were examined in a Philips 300 electron microscope operatting at 60 kV. Latex spheres, 1090$\pm$27 Å (S.D.) in diameter were used for calibration [26].

Optical studies

Fluorescence measurements and light scattering studies were performed using a Perkin Elmer fluorescence spectrophotometer (Model MPF 3). The Rayleigh peak from unpolarized incident light at 400 nm was measured to monitor the 90° light scattering of the suspensions. The fluorescence emission spectrum of the suspensions was recorded for excitation at 280 nm. Temperature control was maintained with the aid of a circulating thermostat connected to the jacketed cell housing. The buffer solutions and liposome suspensions were filtered through 0.45 μm Millipore filters just prior to the optical measurements. In the titration studies, the lectin solutions were added directly to the liposomes and buffer controls contained in the quartz cuvettes by means of a microliter syringe. The sugar haptens were usually added in a similar manner prior to the lectin addition. The ultraviolet spectra of these solutions were recorded using a Cary 17 spectrophotometer.

Binding studies

The lectin-liposome mixtures prepared for the optical studies were also examined by differential ultracentrifugation analysis. The 250 nm absorption was monitored before and after centrifugation of the mixtures at 100 000 $\times g$ for 60 min. Similar ultracentrifugation studies were carried out with ^{125}I-labeled wheat germ agglutinin (specific activity: $1.6 \cdot 10^{11}$ cpm/g). ^{125}I-labeled wheat germ agglutinin was prepared essentially according to Hunter [27]. The ^{125}I-labeled wheat germ agglutinin was separated from free $^{125}I^-$ and carrier I^- by gel filtration on Sephadex G25 in 0.05 M sodium chloride/0.05 M sodium acetate at pH 3.8. The liposomes employed in the binding studies with ^{121}I-labeled wheat germ agglutinin were concentrated by ultrafiltration until they became colloidally unstable. These modified liposomes could be separated from solution by ultracentrifugation at 100 000 $\times g$ for 30 min. The preparation, therefore, provided a convenient matrix for the binding studies. Aliquots of these liposomes were added to the acetate buffer or buffer containing the hapten inhibitors; ^{125}I-labeled wheat germ agglutinin was then added and was allowed to

TABLE I

EFFECT OF INHIBITORS ON LECTIN-INDUCED AGGLUTINATION OF DOG ERYTHROCYTES

Assay system: serial dilutions of the respective lectins in 0.1 ml of phosphate buffered saline were incubated with 0.05 ml of 1 % freshly washed dog erythrocytes in phosphate-buffered saline at 25 °C for 15 min to obtain half maximal agglutination titers. To obtain inhibition titers, serial dilutions of the haptens were preincubated for 10 min with the half maximal dose of the respective lectins, then cells were added.

Lectin	Minimum concentration of hapten necessary for inhibition of half maximal agglutination					
	GlcNac (mM)	GalNac (mM)	Galactose (mM)	AcNeu (mM)	Glucuronic Acid (mM)	Sialoglycoprotein (μg/ml)
Wheat germ agglutinin	20	> 50	> 50	50	> 50	10
Phytohemagglutinin-P	> 50	5	20	> 50	> 50	7

interact for 15 min. Binding was determined by differential centrifugation of the mixtures, followed by counting the pellets for radioactivity. In one set of experiments, an excess of cold wheat germ agglutinin was added following the incubation with ^{125}I-labeled wheat germ agglutinin, but prior to centrifugation of the suspensions.

RESULTS

Purified wheat germ agglutinin prepared by repeated ion-exchange chromatography on DEAE-cellulose and SP-Sephadex [17] was found to be free of neutral and amino sugars. The ultraviolet spectrum of the fraction I isolectin was similar to that reported by Rice and Etzler [28]. Comparable results were obtained with the wheat germ agglutinin preparation purified by affinity chromatography on chitin [18]. The wheat germ agglutinin appeared as a single band on sodium dodecyl sulfate-acrylamide gels stained with Coomassie Brilliant Blue. The isolated sialoglycoprotein from dog erythrocytes also gave a single band on sodium dodecyl sulfate-acrylamide gels. No attempt was made to separate this material into its protein components [29]. The ultraviolet absorption spectrum of a 1 % solution of the sialoglycoprotein revealed no shoulder around 323 nm, indicating that there was no lithium diiodosalicylate contamination ($< 10^{-6}$ M). The isolated sialoglycoprotein was found to be a potent inhibitor of the agglutination of dog erythrocytes by both wheat germ agglutinin and phytohemagglutinin-P. Hydrolysis of the sialoglycoprotein in 0.1 M HCl at 80 °C for 1 h markedly lowered its efficacy as an inhibitor of agglutination of dog erythrocytes by wheat germ agglutinin. The effects of the monosaccharide haptens and the free sialoglycoprotein on the inhibition of agglutination of dog erythrocytes by wheat germ agglutinin and phytohemagglutinin-P are listed in Table I.

Electron microscopy

The results of the electron microscopic studies are illustrated in Figs 1 and 2. The glycoprotein-liposomes were observed to be vesicular structures, somewhat

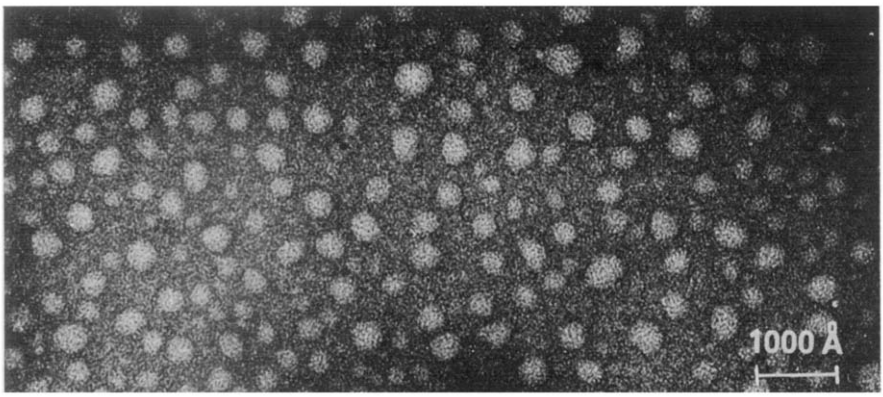

Fig. 1. Electron micrograph of glycoprotein-liposomes negatively stained with 1 % potassium phosphotungstate (× 105 000).

larger in size than the pure phosphatidylcholine vesicles prepared by the ethanol injection technique [25] or phospholipid vesicles generated by ultrasonication [4, 26]. The size homogeneity of the glycoprotein-liposomes is indicated in Fig. 1. The mean diameter of the vesicles was 300±15 Å (±2 S.E.), but the particle size ranged from 200 to 500 Å. There was a notable absence of multilamellar material in these preparations. No attempt was made to separate the glycoprotein-liposomes on the basis of size, e.g. by gel filtration on Sepharose 4B [4]. Addition of the purified wheat germ agglutinin to the glycoprotein-liposomes caused the aggregation of the small vesicles into larger particles. A typical preparation is shown in Fig. 2. The aggregates appeared to be composed largely of unfused vesicles (see insert); however, occasional evidence of fused liposomes was also found. The size of the aggregates increased with the concentration of wheat germ agglutinin added to the glycoprotein-liposome mixture, albeit the correlation was difficult to quantify. Addition of GlcNac to the mixture of glycoprotein-liposomes and wheat germ agglutinin significantly reduced the size of the aggregates. These aggregates were not observed in the preparations of phosphatidylcholine liposomes not containing sialoglycoprotein.

Physical characteristics of the glycoprotein-liposomes

Since the electron microscopic examination of the glycoprotein-liposomes indicated that the structures were similar in appearance to phospholipid vesicles, it was important to determine whether they enclosed an internal aqueous compartment. For this purpose, the liposomes were generated in the acetate buffer solution containing 20 mM sucrose and 20 μCi/ml [^{14}C]sucrose (New England Nuclear). Free [^{14}C]sucrose was removed by gel filtration through a Sephadex G25 column at 4 °C, and the glycoprotein-liposomes appearing in the void volume were counted for radioactivity to determine the trapped volume of aqueous solution containing the [^{14}C]sucrose. These measurements revealed that the internal volume of the glycoprotein-liposomes was approximately 20 % larger than the internal volume of the phosphatidylcholine liposome controls not containing the sialoglycoprotein. This result was consistent with the slightly larger size of the glycoprotein-liposomes observed with the electron microscope. The nature of the incorporation of the sialoglycoprotein

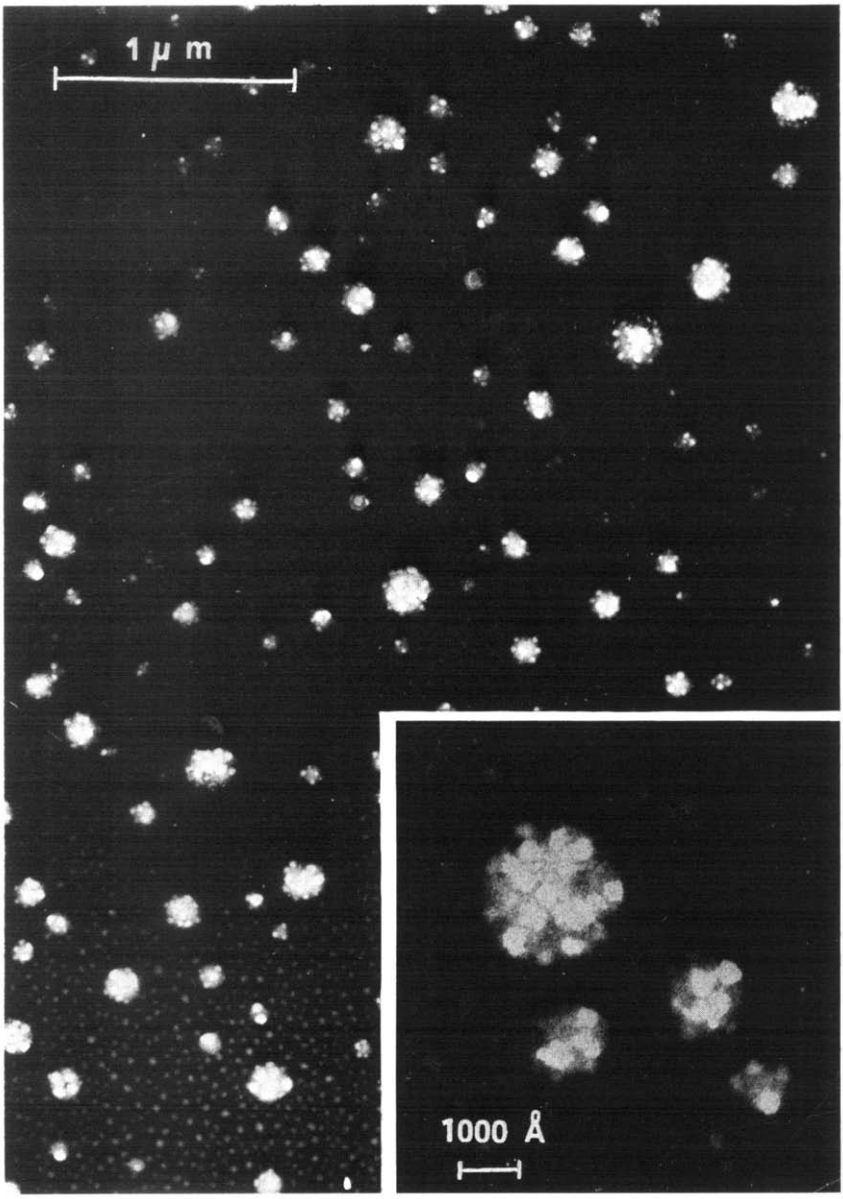

Fig. 2. Electron micrograph of glycoprotein-liposomes after 15 min incubation at 25 °C, pH 5, with 125 μg/ml wheat germ agglutinin. The preparation was negatively stained with 1 % potassium phosphotungstate (× 31 000). Insert at higher magnification (× 75 000).

into the glycoprotein-liposomes leading to the observed results will be discussed later in this report.

The permeabilities of the glycoprotein-liposomes and liposome controls to the trapped [^{14}C]sucrose were measured by the dialysis technique of Papahadjopoulos et al. [30]. The efflux rates were found to be 0.67 and 0.26 %/h for the glycoprotein-

liposomes and control liposomes, respectively. These values, measured at 30 °C, express the rates of leakage of [^{14}C]-sucrose as percentages of the total trapped solute inside the liposomes.

Optical studies

The flucrescence at 348 nm due to the wheat germ agglutinin was linearly proportional to the concentration of this lectin, and was not affected by the presence of glycoprotein-liposomes or control liposomes. A similar result was obtained for phytohemagglutinin-P fluorescence in the systems. However, additions of the wheat germ agglutinin caused an increased light scattering of the glycoprotein-liposomes with respect to that observed for either the buffer solution alone or the purified phosphatidylcholine liposomes not containing the sialoglycoprotein (Fig. 3). In the latter two solutions, the change in light scattering was directly proportional to the amount of wheat germ agglutinin added, and was equal in both systems. This result confirmed that wheat germ agglutinin does not cause the aggregation of the control liposomes. The changes in the light scattering of the buffer or liposome control were subtracted from the values for the glycoprotein-liposomes to obtain the differential light scattering as a function of the wheat germ agglutinin concentration. The differential light scattering curve for the glycoprotein-liposomes displayed pseudo-saturation at wheat germ agglutinin concentrations of approximately 15 μg/ml (Fig. 4) and was sigmoidal at lower lectin concentrations. The maximal increases in the differential light scattering upon the addition of the wheat germ agglutinin were approximately 3–4 times the initial light scattering of the glycoprotein-liposomes. Doubling of the light scattering would be expected, a priori, if the glycoprotein-liposomes formed dimers [31]. The observed increases probably reflected the association of the glycoprotein-liposomes into larger aggregates. The pseudo-saturation of the differential light scattering curve at higher wheat germ agglutinin concentrations may reflect optical interference occurring in the larger soluble aggregates [32]. A similar titration

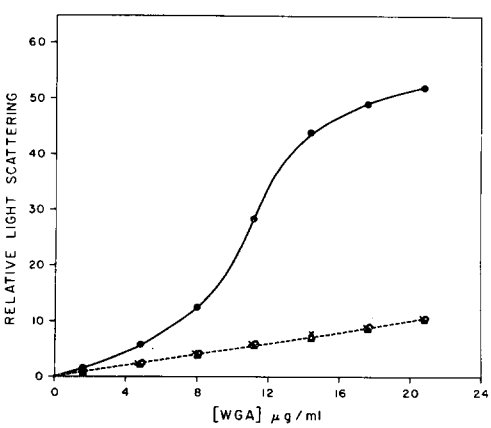

Fig. 3. Changes in the 90 ° light scattering on addition of wheat germ agglutinin solutions of : ●, glycoprotein-liposomes, pH 5; ■, control liposomes, pH 5; ×, pH 5 buffer (0.05 M NaCl/0.05M sodium acetate); also, ○, glycoprotein-liposomes at pH 2.5. [WGA] denotes wheat germ agglutinin concentration.

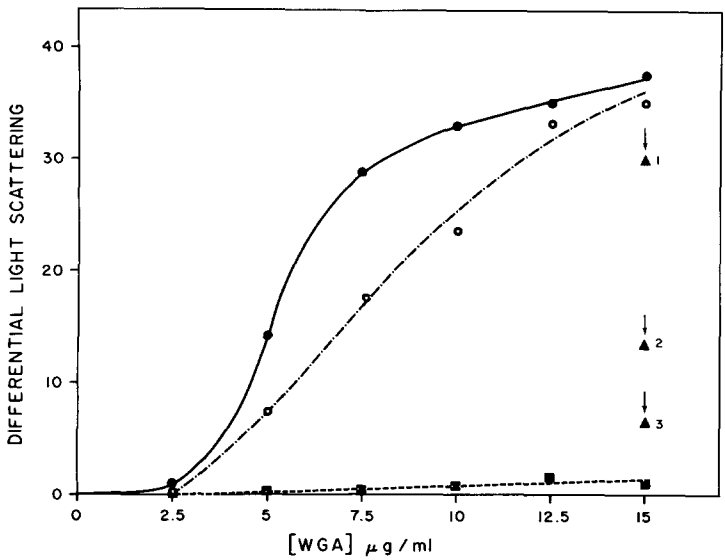

Fig. 4. Effect of GlcNac on the differential light scattering (90 °) of glycoprotein-liposomes at pH 5 ●, no GlcNac present. GlcNac added prior to titration with wheat germ agglutinin: ○, 10 mM GlcNac; ■, 50 mM GlcNac. GlcNac added after addition of 15 μg/ml wheat germ agglutinin: ▲1, 15 mM GlcNac; ▲2, 30 mM GlcNac; ▲3, 50 mM GlcNac.

was carried out at pH 5.0, 25 °C, using the glycoprotein-liposomes and bovine serum albumin. In this system, the differential light scattering of the suspension was close to zero at molar concentrations of the albumin similar to those producing maximal effects with wheat germ agglutinin, namely approx. 10^{-6} M.

The light scattering of the mixtures of wheat germ agglutinin and glycoprotein-liposomes did not vary appreciably with time following the initial rapid increase. No temperature dependence was observed between 4 and 25 °C. There was also little

TABLE II

EFFECT OF INHIBITORS ON GLYCOPROTEIN-LIPOSOME LIGHT SCATTERING IN THE PRESENCE OF WHEAT GERM AGGLUTININ

System	Concentration	% of control*
GlcNac	8 mM	91
	40 mM	8
GalNac	8 mM	97
	40 mM	46
AcNeu	7 mM	92
Neuraminidase	0.1 unit/ml-16 h	28
Sialoglycoprotein	40 μg/ml	2

* Each solution contained 15 μg/ml wheat germ agglutinin. The glycoprotein-liposomes contained 2.66 μg sialoglycoprotein combined with 16.6 μg phosphatidylcholine per ml in 0.05 M NaCl/0.05 M sodium acetate at pH 5.0, 25 °C. This weight ratio corresponds to a molar ratio of approx. 1 : 250, sialoglycoprotein : phosphatidylcholine, assuming molecular weights of 30 000 and 750, respectively.

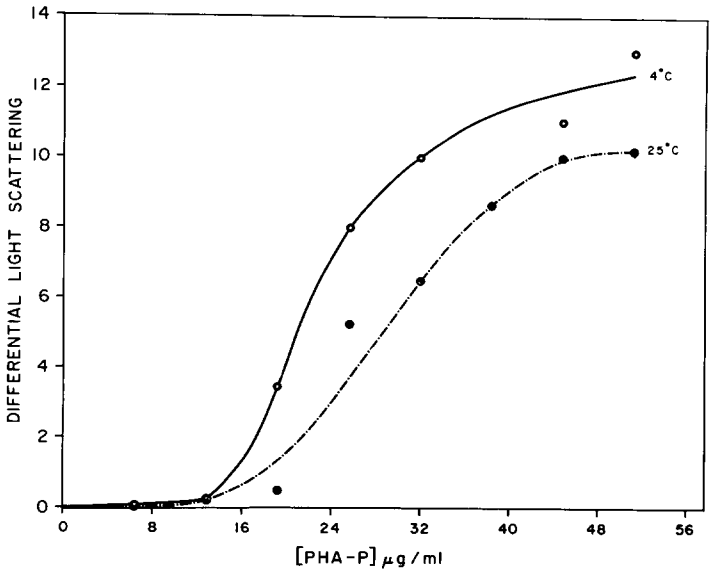

Fig. 5. Differential light scattering (90 °) of glycoprotein-liposomes on addition of phytohemagglutinin-P (PHA-P).

variation with pH over the range pH 7.5–3.8. Below pH 3.0, however, the differential increase in the light scattering of the glycoprotein-liposomes dropped sharply, with no effect observable at pH 2.5 (Fig. 3). The light scattering was also found to be lowered by the prior addition of GlcNac to the glycoprotein-liposomes. Fig. 4 illustrates the inhibitory effect of this sugar hapten. It was also found that a partial reversal of the light scattering changes in the suspensions could be effected by the subsequent addition of GlcNac (Fig. 4). Table II lists the effects of GlcNac and other sugar haptens. GalNac had a smaller inhibitory effect than GlcNac. This agreed well with the finding that GlcNac is the more potent monosaccharide inhibitor of cell agglutination by wheat germ agglutinin (Table I). AcNeu also partially inhibited the reaction. Glycoprotein-liposomes which had been pretreated with neuraminidase displayed markedly lower reactivity with wheat germ agglutinin. Addition of the free sialoglycoprotein to the glycoprotein-liposomes proved to be a most effective method of inhibiting the light scattering changes with wheat germ agglutinin (Table II).

Similar light scattering changes were observed when phytohemagglutinin-P was added to the glycoprotein-liposomes (Fig. 5). With this lectin, GalNac was found to be a more efficient hapten inhibitor than GlcNac, congruent with its inhibition characteristics for the cell agglutination reaction (Table I). The sigmoidal character of the difference curves in both the wheat germ agglutinin and phytohemagglutinin-P titrations implied cooperativity in the glycoprotein-liposome aggregation process, possibly involving lectin-lectin interactions.

Differential ultracentrifugation studies

Incorporation of the membrane sialoglycoprotein into the phospholipid liposomes did not reduce their colloidal stability; no significant decrease in the solution

TABLE III

FORMATION OF PRECIPITABLE COMPLEXES ON ADDITION OF WHEAT GERM AGGLUTININ TO GLYCOPROTEIN-LIPOSOMES

System (wheat germ agglutinin concentration: 115 μg/ml)	Decrease in absorption following centrifugation at 100 000 × *g* for 1 h (% decrease $A_{250\ nm}$ ±S.E.)	Precipitable complex (% of control)
Glycoprotein-liposomes I**		
pH 5.0	46.8±7.0 (8)*	–
Effect of pH		
Glycoprotein-liposomes II**		
pH 5.0	65.6±2.7 (12)	100
pH 7.2	65.8±0.6 (2)	100
pH 5.8	66.4±1.2 (2)	101
pH 2.7	0.9±0.9 (2)	1.4
Effect of inhibitors at pH 5		
Glycoprotein-liposomes II		
GlcNac (45 mM)	18.3±5.2 (10)	28
GalNac (45 mM)	48.1±5.3 (5)	73
AcNeu (10 mM)	50.3±5.2 (8)	77
GlcNac (45 mM)+AcNeu (10 mM)	25.3±1.8 (4)	39
Glucuronic acid (10 mM)	61.1±3.4 (7)	93
Neuraminidase (10 units/ml, 16 h)	12.4±1.9 (4)	19

* Number of experiments.

** Composition of glycoprotein-liposomes: I, 18.5 μg sialoglycoprotein combined with 220 μg phosphatidylcholine per ml; II, 32 μg sialoglycoprotein combined with 110 μg phosphatidylcholine per ml.

absorption was observed after centrifugation of the glycoprotein-liposomes at 100 000 ×*g* for 1 h. Addition of soluble wheat germ agglutinin to the glycoprotein-liposomes caused a larger increase in the solution turbidity compared with control liposomes. This result was consistent with the light scattering data. Ultracentrifugation of the lectin-liposome mixtures resulted in the formation of a pellet only in the case of the glycoprotein-liposomes. Formation of the precipitable complex was monitored by the reduction in the solution absorption at 250 nm following the ultracentrifugation. The concentrations of wheat germ agglutinin and glycoprotein-liposomes used in the centrifugation experiments were approximately ten-fold higher than employed for the light scattering studies. The results are listed in Table III. In preliminary studies, it was found that the amount of precipitable complex formed under the standard ultracentrifugation conditions increased with the wheat germ agglutinin concentration, but saturation was attained at lectin concentrations above 100 μg/ml. The extent of aggregation also increased as the amount of sialoglycoprotein in the glycoprotein-liposomes was increased (glycoprotein-liposomes I, II, Table III). It was confirmed that no formation of a precipitable complex occurred when bovine serum albumin (100 μg/ml) was added to the glycoprotein-liposomes under the same experimental conditions. The influence of pH on the formation of precipitable lectin-liposome complex was consistent with the light scattering data (Fig. 3). It is relevant that Privat et al.

TABLE IV

FORMATION OF PRECIPITABLE COMPLEXES ON ADDITION OF PHYTOHEMAGGLUTININ-P TO GLYCOPROTEIN-LIPOSOMES

System (phytohemagglutinin-P concentration: 230 μg/ml)	Decrease in absorption following centrifugation at 100 000 × *g* for 1 h (% decrease $A_{250\ nm}$ ±S.E.)	Precipitable complex (% of control)
Glycoprotein-liposomes II (For composition, see Table III) pH 5.0	63.5±1.4 (9)*	100
Glycoprotein-liposomes II, pH 5		
GlcNac (45 mM)	57.0±3.4 (4)	90
GalNac (45 mM)	48.7±5.3 (10)	77
Galactose (45 mM)	55.1±2.3 (12)	87
AcNeu (10 mM)	65.0±1.2 (4)	102
Glucuronic acid (10 mM)	62.2±2.6 (4)	98

* Number of experiments.

[33] have recently reported that the binding of GlcNac to wheat germ agglutinin decreased at low pH, with no binding apparent below pH 2.8. The observed decrease in the lectin-induced aggregation of glycoprotein-liposomes may, therefore, be due in part to a decreased affinity between the wheat germ agglutinin and the receptor at pH 2.8. The dissociation of the lectin into subunits at low pH probably contributed to this phenomenon [34, 35].

Addition of the sugar haptens, GlcNac, GalNac and AcNeu inhibited the formation of the precipitable complexes (Table III). The magnitude of the inhibition effects was comparable to that observed using the light-scattering technique (Table II). Glucuronic acid was employed to determine whether the effect of AcNeu was attributable to its charge. The data in Table III indicate that this was not the case, but that AcNeu has a specific interaction with wheat germ agglutinin. Greenaway and Levine [36] have reported that wheat germ agglutinin binds AcNeu and that, at low hapten concentrations, wheat germ agglutinin has a higher affinity for binding AcNeu than GlcNac. The inhibition study with both AcNeu and GlcNac (Table III) confirmed that there was competition for binding to wheat germ agglutinin by the AcNeu and GlcNac, with AcNeu having the higher binding affinity, albeit lower inhibition of the lectin-induced aggregation of the glycoprotein-liposomes. Neuraminidase treatment of the glycoprotein-liposomes markedly lowered the complex formation (cf. Table II).

A limited number of experiments was carried out using the same differential ultracentrifugation method with glycoprotein-liposomes and phytohemagglutinin-P (Table IV). Precipitable complexes were also observed with this lectin. GalNac was found to be the best hapten inhibitor in this system.

Binding studies

The differential ultracentrifugation experiments with ^{125}I-labeled wheat germ agglutinin and the colloidally unstable liposomes (see Methods) revealed that significantly larger quantities of ^{125}I-labeled wheat germ agglutinin were bound to the

TABLE V

SPECIFIC BINDING OF ^{125}I-LABELED WHEAT GERM AGGLUTININ TO GLYCOPROTEIN-LIPOSOMES

System	^{125}I-labeled wheat germ agglutinin bound (cpm±S.D.)	% of Control*
Effect of pH		
Glycoprotein-liposomes, pH 2.5	0	0
Glycoprotein-liposomes, pH 5.0	81 200±3 000	100
Effect of Inhibitors at pH 5		
Unlabeled wheat germ agglutinin (100 molar excess)	34 800±4 000	43
GlcNac (50 mM)	23 500±2 500	29
Neuraminidase (10 units/ml, 16 h)	20 400±3 000	25
Sialoglycoprotein (40 μg/ml)	10 100±4 500	12.5

* Each solution contained 15 μg/ml ^{125}I-labeled wheat germ agglutinin. The glycoprotein-liposomes contained 60 μg sialoglycoprotein combined with 400 μg phosphatidylcholine in a total volume of 0.40 ml of 0.05 M NaCl/0.05 M sodium acetate at pH 5.0, 25 °C.

glycoprotein-liposomes than to liposome controls. The nonspecific binding could be reduced by washing both types of liposome pellets with the appropriate cold buffer solutions. The binding data reported in Table V have been corrected for the nonspecific binding by subtraction of the radioactivity associated with corresponding liposome controls. The specific binding of ^{125}I-labeled wheat germ agglutinin to the glycoprotein-liposomes could be partly reversed by the addition of a large excess of cold lectin. The incomplete dissociation may be due to secondary factors, such as hydrophobic interactions between the ^{125}I-labeled wheat germ agglutinin and glycoprotein-liposomes. No specific binding of ^{125}I-labeled wheat germ agglutinin to the glycoprotein-liposomes was observed at pH 2.5 (Table V). The inhibitory effects of GlcNac, neuraminidase treatment and free sialoglycoprotein were similar to the corresponding effects on the lectin-induced aggregation of glycoprotein liposomes (Tables II and III).

DISCUSSION

The lectin-induced formation of aggregates of unfused glycoprotein-liposomes is reminiscent of cell agglutination by lectins [11, 37, 38]. Furthermore, the effects of GlcNac and GalNac on the lectin reactions with glycoprotein-liposomes are closely parallel to the hapten inhibition of cell agglutination by the lectins. The decrease in the light scattering caused by the subsequent addition of GlcNac to the mixtures (Figure IV) was correlated with a reduction in the size of the aggregates observed using the electron microscope. This partial reversal of the glycoprotein-liposome aggregation is analogous to the dissociation of lectin-agglutinated dog erythrocytes when GlcNac was added. Burger and Goldberg [39] have reported that treatment of mouse L-1210 cells with neuraminidase abolished their agglutinability by wheat germ agglutinin. Neuraminidase treatment of the glycoprotein-liposomes resulted in a similar inhibition of their aggregation by wheat germ agglutinin. The data in Tables

II and III show that AcNeu inhibits the interaction of wheat germ agglutinin with the glycoprotein-liposomes. These observations support the conclusions made by other workers that AcNeu residues are a component of the lectin-receptor site [11, 36]. It was demonstrated in this work that the free sialoglycoprotein is a potent inhibitor of both the cell agglutination and glycoprotein-liposome aggregation reactions with wheat germ agglutinin. The characteristics of ^{125}I-labeled wheat germ agglutinin binding to the glycoprotein-liposomes share close similarities with wheat germ agglutinin binding to cell surfaces. Adair and Kornfeld [11] found that the best hapten inhibitor of the binding of this lectin to erythrocyte ghosts was GlcNac and the binding could be inhibited by the isolated sialoglycoprotein. However, neuraminidase treatment of the sialoglycoprotein resulted in a marked decrease of its inhibitory capacity (cf. Table V). Similar binding characteristics have been reported by Cuatrecasas [40] using ^{125}I-labeled wheat germ agglutinin and isolated fat cells from rats, and Nicholson et al. [41] who studied a fibroblast system.

The binding data listed in Table V were obtained at a ^{125}I-labeled wheat germ agglutinin concentration of 15 μg/ml in order to compare the results directly to the light scattering data (Table II). At this lectin concentration, the receptor sites in the glycoprotein-liposomes were found to be below their saturation binding of wheat germ agglutinin. These studies were also carried out at higher concentrations of ^{125}I-labeled wheat germ agglutinin up to 50 μg/ml and, over this concentration range, linearity of the amount of the bound to free lectin was observed. This result is interesting since the concentration of wheat germ agglutinin which caused significant cell agglutination was also below that required to saturate the cell surface binding sites for the lectin [11]. Furthermore, the wheat germ agglutinin concentrations required for significant particle aggregation were similar in both systems.

The studies with phytohemagglutinin-P demonstrate that the lectin-induced aggregation of glycoprotein-liposomes is not limited to a single lectin, wheat germ agglutinin. The interactions with phytohemagglutinin-P could be inhibited by GalNac, which is the best monosaccharide inhibitor of the cell agglutination reaction [37]. It is interesting to note that galactose also acts as an inhibitor (Table IV). Kornfeld and Kornfeld have shown that removal of galactose in the purified phytohemagglutinin receptor site destroys the affinity of the receptor for this lectin [42].

The interactions of both wheat germ agglutinin and phytohemagglutinin with glycoprotein-liposomes are phenomenologically similar to the interactions of these lectins with erythrocytes. This strongly suggests that the orientation of some of the sialoglycoprotein molecules in the glycoprotein-liposomes is topologically similar to that in the native membranes. Segrest et al. [43] have argued that the major sialoglycoprotein of human erythrocytes spans the plane of the membrane, with the receptor domain exposed to the external milieu of the cell, the hydrophobic domain extending across the lipophilic core of the membrane, and the C-terminal domain exposed to the cytoplasmic interior of the cell. In the model system, it is evident from the data that some receptor sites are exposed to the external aqueous phase. It is probable that a significant number of the sialoglycoprotein molecules are also oriented with their receptor domain facing the interior aqueous compartment of the glycoprotein-liposomes. At present we have no experimental information concerning the interaction of the hydrophobic region of the sialoglycoprotein with the phospholipid matrix. However, the method of generating the glycoprotein-liposomes might be conducive to

hydrophobic interaction between the sialoglycoprotein and the phospholipid molecules. In contrast to the present method, we have found that simply adding the sialoglycoprotein to preformed phosphatidylcholine vesicles does not generate glycoprotein-liposomes. The stoichiometry of sialoglycoprotein to phospholipid in the membranes of the glycoprotein-liposomes has not been directly measured. Nevertheless, it is possible to estimate that a maximum of approx. 12 sialoglycoprotein molecules may be incorporated per glycoprotein-liposome, on the basis of the molar ratios of sialoglycoprotein to phospholipid in the solutions used to generate these structures (e.g. Table II), tentatively assuming that there are approximately 3000 phosphatidylcholine molecules per glycoprotein-liposome, and that all the sialoglycoprotein becomes directly associated with the phospholipid matrix.

Inbar et al. [44] have reported that the agglutinability of rat lymphocytes by phytohemagglutinin was temperature dependent; this lectin was considerably more effective as an agglutinin at 25 °C than at 4 °C. In contrast, agglutination by wheat germ agglutinin was not temperature dependent. There was no significant temperature dependence for the light scattering changes of the glycoprotein-liposomes with wheat germ agglutinin, but phytohemagglutinin-P was found to have a slightly bigger effect at 4 °C, compared to 25 °C (Figure 5). There appeared to be no aggregation of the phytohemagglutinin-P molecule caused by increasing the temperature from 4 to 25 °C. The temperature sensitivity of the lymphocyte agglutination by phytohemagglutinin must therefore be attributed to other factors, occurring at the membrane level. Horowitz et al. [45] have recently studied the effects of membrane fluidity on the lectin-induced agglutinability of 3T3 and SV 101 3T3 cells. The glycoprotein-liposome system described here offers an ideal membrane model with which to vary the lipid composition and thus control the membrane fluidity. Studies are currently being conducted on the influence of the lipid composition on the lectin-receptor interaction using this liposome system. Glycolipids are also being used in the continued investigation in an attempt to more closely simulate the native cell surface.

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