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Lectins facilitate calcium-induced fusion of phospholipid vesicles containing glycosphingolipids

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Ca^{2+}-induced fusion of phospholipid vesicles containing globoside (GL-4) or disialoganglioside (G_{Dla}) is several-fold slower than the fusion of the pure phospholipid vesicles. Lectins specific for these glycosphingolipids, soybean agglutinin and wheat germ agglutinin, respectively, enhance the rate of fusion when added to the vesicle suspension before the introduction of Ca^{2+}. The enhancement depends on the lectin concentration and the time of preincubation with the lectin. We propose that lectins facilitate membrane fusion by inducing intermembrane contact, which is the first step in the overall process of membrane fusion, or by laterally phase separating the inhibitory glycolipids.

Membrane fusion *Glycolipid* *Lectin* *Calcium* *Liposome*

1. INTRODUCTION

Glycosphingolipids are thought to be involved in cell–cell interactions and recognition, cell growth regulation, oncogenesis, and to act as differentiation markers, cell surface antigens and stabilizers of cell membranes [1]. The physicochemical properties of purified glycosphingolipids [2,3] and their mixtures with phospholipids [4–7] have been studied. Plant lectins have been shown to interact with and agglutinate phospholipid vesicles containing various glycosphingolipids [8–12] or synthetic glycolipids [14,15]. The agglutination or aggregation of phospholipid vesicles is the first step in the process of vesicle fusion induced by divalent cations; this step is followed by membrane destabilization and the fusion reaction per se [16].

The phospholipid requirements for divalent cation-induced membrane fusion have been studied extensively [16–18]. Various Ca^{2+}-binding proteins have been demonstrated to control the fusion of phospholipid vesicles [18,19]. The presence of glycosphingolipids in phospholipid membranes is expected to alter the fusion susceptibility of these membranes. It is likely that lectins will further modulate membrane fusion by cross-linking two membranes, by destabilizing them, by inducing lateral phase separation of the glycolipids, or by a combination of these effects. Here we have investigated these possibilities, utilizing a membrane fusion assay for the intermixing of aqueous contents of phospholipid/glycolipid vesicles and studying the effects of lectins on the fusion of these vesicles induced by divalent cations. We have found that glycolipids inhibit the Ca^{2+}-induced fusion of phospholipid vesicles and that the addition of lectins specific for the glycolipids enhances the rate of fusion. Some of our results have been reported elsewhere in preliminary form [20].

2. MATERIALS AND METHODS

Phosphatidylserine (PS) was prepared as in [21] and also purchased from Avanti Polar Lipids (Birmingham, AL). Phosphatidate (PA) and phosphatidylethanolamine (PE), prepared by transphosphatidylation of egg phosphatidylcholine, were

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obtained from Avanti. Globoside (GL-4) and disialoganglioside (G_{Dla}) were from Supelco (Bellefonte, PA). Soybean agglutinin (SBA), wheat germ agglutinin (WGA) and *Sophora japonica* agglutinin (SJA) were from Vector Laboratories (Burlingame, CA). *Helix pomatia* agglutinin (HPA), *Codium fragile* agglutinin (CFA), *N*-acetylgalactosamine (galNAc) and *N*-acetylneuraminic acid (NANA) were obtained from Sigma (St. Louis, MO). *Ricinus communis* agglutinin II (RCA_{60}) was from Miles-Yeda (Rehovot, Israel). The source and purity of all other chemicals have been described [17,22].

Vesicles were prepared by reverse-phase evaporation and subsequent extrusion through polycarbonate membranes of 0.1 μm pore size [17,22]. The vesicles contained either of the following: (i) 2.5 mM $TbCl_3$, 50 mM sodium citrate; (ii) 50 mM sodium dipicolinate (DPA), 20 mM NaCl; (iii) 50 mM carboxyfluorescein (CF), all buffered to pH 7.4 with 2 mM histidine and 2 mM tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Tes). In some experiments, nitrilotriacetic acid was substituted for the citrate and 5 mM Tes used as the buffer.

Fusion of the vesicles was followed by measuring the fluorescence generated when Tb^{3+} and dipicolinic acid (encapsulated in two separate vesicle populations) intermix to form a chelation complex [17,22]. The fluorescence intensity reflects the percentage of Tb vesicles which have fused with dipicolinate vesicles, 100% fluorescence being determined by lysing an equivalent amount of Tb vesicles in the presence of excess free dipicolinate. Fluorescence (F) measurements were made in an SLM-4000 fluorometer. Tb- and DPA-containing vesicles were mixed in a 1:1 ratio (0.025 μmol lipid/ml each) in 1 ml of 100 mM NaCl, buffered to 7.4 with either 2 mM histidine, 2 mM Tes or 5 mM Tes and containing 0.1 mM EDTA. Temperature was maintained at 25°C.

Release of vesicle contents was determined by the relief of self-quenching of CF fluorescence [17,22]; 100% fluorescence was determined by lysing the vesicles with 0.1% Triton X-100.

Agglutination (aggregation) of the vesicles was followed by turbidity measurements at 450 nm in a Beckman Model 34 spectrophotometer [13], or 90° light scattering at the same wavelength in the fluorometer [22,23].

3. RESULTS AND DISCUSSION

The time course of fusion of PS/GL-4 (95:5) vesicles in the presence of 3 mM Ca^{2+} is shown in fig.1. The initial rate of fusion was about 4-times slower than the fusion of pure PS vesicles (fig.1, table 1). Similarly, the incorporation of GL-4 or G_{Dla} into PE/PA vesicles reduced drastically the initial rate of fusion (table 1). The presence of the glycolipid in the membrane may have prevented the close approach of the vesicles and the formation of an intermembrane Ca^{2+}–phospholipid complex, which would be necessary for fusion [23], presumably due to the bulky and strongly hydrated oligosaccharide head group.

The preincubation of PS/GL-4 vesicles with 50 μg/ml SBA caused an increase in the initial rate of fusion from 4 to 13% max. F/min (fig.1). Although Ca^{2+} addition alone did not cause any leakage of the aqueous contents of the vesicles, Ca^{2+} addition in the presence of the lectin induced considerable leakage after a lag period. However, no release occurred during the early fusion events. Introduction of the same amount of SBA, after fusion was initiated by Ca^{2+} alone, did not alter the rate of fusion. Other lectins, such as WGA and

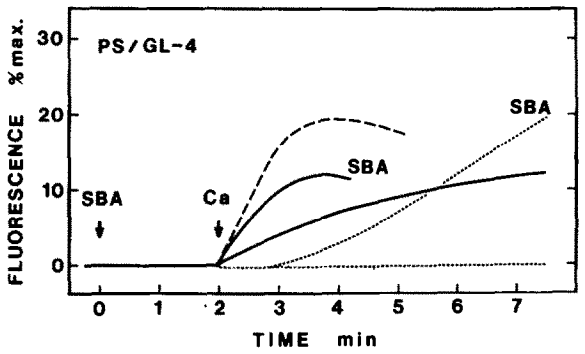

Fig.1. The effect of SBA on the fusion of PS/GL-4 (95:5) vesicles induced by Ca^{2+}; 50 μg/ml SBA was added to the vesicle suspension at $t = 0$, and 3 mM Ca^{2+} was introduced at t = 2 min (——, marked SBA). (——) Time course of fusion of the vesicles in the absence of lectin. For comparison, the fusion of pure PS vesicles in the presence of 3 mM Ca^{2+} is shown by (– – –). Release of CF from PS/GL-4 vesicles in the presence (· · ·, marked SBA) and absence (· · ·) of lectin; 100% max. F for the Tb/DPA reaction (fusion) and dequenching of CF (release) was determined as in section 2.

Table 1

The effect of GL-4 and G_{Dla} on the initial rate of fusion of liposomes in the presence of 3 mM Ca^{2+}

Liposome composition	Initial rate of fusion (% max. $F \cdot min^{-1}$)
PS	17
PS/GL-4 (95:5)	4
PE/PA (75:25)[a]	15
PE/PA/GL-4 (70:20:8)	0.8
PE/PA/G_{Dla} (75:20:5)[b]	0

[a] Data from [19]

[b] No fusion was observed with these vesicles even in the presence of 10 mM Ca^{2+}

SJA, were not effective in enhancing the rate of fusion. Since SJA specifically binds galNAc as does SBA, it appears that the effect of SBA is not merely the result of binding to the terminal sugar residue of the glycolipid. The addition of 200 μM galNAc to the vesicle suspension before the introduction of SBA completely inhibited the enhancement of fusion, indicating that the interaction of the SBA with the terminal galNAc is necessary for its effect. The Ca^{2+}-induced fusion of PS/GL-4 vesicles was not affected by galNAc. However, the addition of galNAc after SBA did not prevent the effect of SBA on fusion. This observation suggests that after SBA binds to the GL-4 it interacts irreversibly with the membrane. The observation that 10 μg/ml SBA alone causes fusion of PA/PE/GL-4 (70:20:8) vesicles at an initial rate comparable to that induced by 3 mM Ca^{2+} (0.6 *vs* 0.8% max. $F \cdot min^{-1}$, respectively) also supports this suggestion. In the latter liposome system, addition of 3 mM Ca^{2+} after preincubation with SBA resulted in a 4-fold enhancement of the fusion rate compared to Ca^{2+} alone.

Lectin specificity was also observed in the agglutination of PA/PE/GL-4 vesicles. SBA caused a 16-fold higher increase in turbidity compared to SJA at 10 μg/ml. No agglutination was induced by HPA, CFA or RCA_{60} at these lectin concentrations.

The effect of SBA on the initial rate of fusion of PS/GL-4 vesicles increased with the time of preincubation with lectin before the introduction of Ca^{2+} (fig.2) and with the lectin concentration

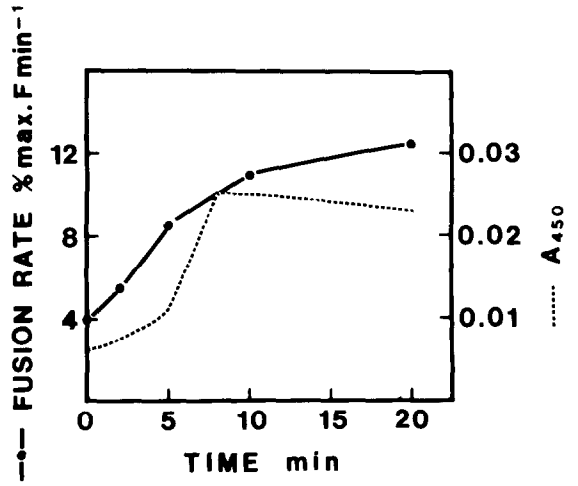

Fig.2. The initial rate of fusion of PS/GL-4 (95:5) vesicles as a function of the time of preincubation with 5 μg/ml SB (●—●, left-hand scale). Fusion was initiated by the addition of 3 mM Ca^{2+} (final concentration). The initial rate is given as the % max. F/min. (···) Absorbance of the vesicle suspension in the presence of lectin before the addition of Ca^{2+} (right-hand scale).

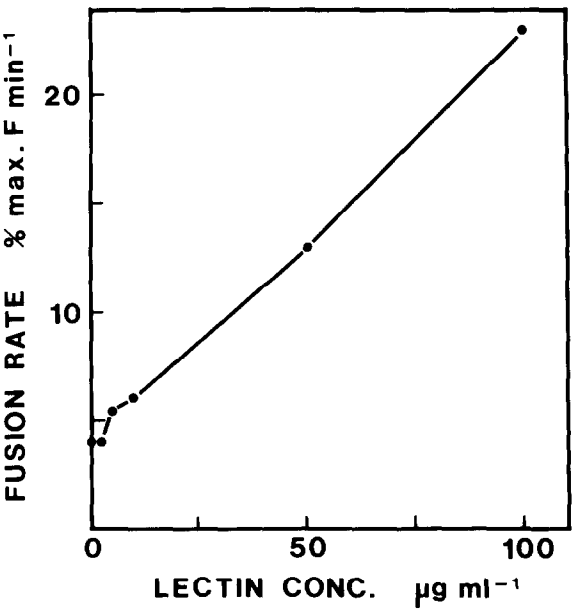

Fig.3. The initial rate of fusion of PS/GL-4 vesicles as a function of the SBA concentration. The vesicles were preincubated with the lectin for 2 min prior to the addition of 3 mM Ca^{2+}. The initial rate is given as the % max. F/min.

(fig.3). The preincubation with lectin caused the agglutination (aggregation) of the vesicles, as determined by turbidity (fig.2, dotted line) or 90° light scattering measurements (not shown). The lectin concentration dependence of fusion exhibited a threshold between 2.5 and 5 μg/ml lectin (fig.3). At this concentration range, less than 2% of the GL-4 would be expected to interact with a lectin molecule assuming that all the lectins were bound.

A similar enhancement of fusion was found in the case of PA/PE/G_{Dla} (20:75:5) vesicles preincubated with WGA. In the absence of lectin, these vesicles did not undergo any fusion when 10 mM Ca^{2+} was added to the medium. However, when preincubated with 50 μg/ml lectin, the initial rate of fusion increased to approx. 5% max. $F \cdot min^{-1}$. When 50 μM NANA was present before the addition of WGA the rate of fusion in the presence of 10 mM Ca^{2+} was reduced to 2.8% max. $F \cdot min^{-1}$. Although this concentration of NANA is far in excess over the concentration of sialic acid associated with G_{Dla}, the inhibition was not complete. Authors in [10] noted that WGA binding to various glycolipids could be inhibited by a number of monosaccharides, indicating the lack of absolute specificity of this lectin. These authors also reported a 54% inhibition of WGA binding to liposomes containing mixed gangliosides in the presence of NANA. Thus, the free monosaccharide does not inhibit completely the interaction of WGA with gangliosides.

Finally, we investigated whether lectins affect the fusion of glycolipid-containing liposomes with pure phospholipid liposomes. Liposomes composed of PA/PE/G_{Dla} and containing Tb were able to fuse with PA/PE (25:75) vesicles containing DPA in the presence of >5 mM Ca^{2+} (table 2), indicating that the phospholipid segments of the two liposome types can come sufficiently close to one another despite the presence of the bulky head group of the glycolipid. The addition of WGA greatly facilitated the fusion of these vesicles (table 2). Since WGA is not expected to interact with the pure phospholipid vesicles [10] and hence not cross-link these vesicles with the glycolipid-containing vesicles, this observation suggests that the lectin may be acting by laterally phase-separating the inhibitory glycolipids in the plane of the membrane, allowing the phospholipids of the

Table 2

The effect of WGA on the fusion of PA/PE/G_{Dla} vesicles with PA/PE vesicles at various Ca^{2+} concentrations[a]

$[Ca^{2+}]$ (mM)	Initial rate of fusion (% max. $F \cdot min^{-1}$)	
	− WGA	+ WGA
2	0	3.6
5	0.5	19
10	9.4	45

[a] 25 μM PA/PE/G_{Dla} (20:75:5) vesicles containing Tb^{3+} were mixed with 25 μM PA/PE (25:75) vesicles containing sodium dipicolinate. The WGA concentration was 50 μg/ml

two membranes to establish contact. It should be noted that fusion of the Tb-containing glycolipid/phospholipid liposomes among themselves is very slow under the conditions of the experiment and would not contribute significantly to increase the amount of Tb available for complexation with DPA upon fusion of a DPA-containing pure phospholipid vesicles.

Our results demonstrate that lectins specific for the receptor glycosphingolipids embedded in phospholipid vesicles can enhance the rate of fusion of these vesicles induced by Ca^{2+}. The enhancement depends on the time of preincubation with the lectin, and the lectin concentration, and can be inhibited by the haptenic monosaccharide. The establishment of intermembrane contact via lectin-mediated cross-linking of receptors on two vesicles appears to facilitate membrane fusion when a sufficient concentration of Ca^{2+} is present. Aggregation and close approach of vesicles is the first step in the process of membrane fusion, which then proceeds by the destabilization and merging of the bilayers [16]. These steps may have different dependencies on the ionic environment and specific molecules such as proteins. Thus, certain ionic conditions insufficient to induce aggregation in the absence of lectins may be sufficient to cause fusion once the membranes are brought into contact by means of the lectins. Recently authors in [24] have shown that PE/PA vesicles containing a synthetic glycolipid can undergo fusion at lower Ca^{2+} concentrations in the presence of lectin than

in its absence. Since lectins are present in many animal tissues [25], it is not unlikely that they are involved in intra- and intercellular membrane adhesion and fusion phenomena. Authors in [26] suggested that a membrane-bound lectin is involved in the fusion of L_6 myoblasts; authors in [27] however, found evidence to the contrary in chick embryo myoblasts. Authors in [28] reported that a lectin from chick embryo skeletal muscle is inhibitory to the fusion of myoblasts, but have suggested that it may mediate cell–cell contact. It remains to be investigated whether the recognition of intracellular membranes before their fusion with each other is mediated by membrane-bound lectin-like molecules.

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