AGGLUTINATION OF GLYCOLIPID–PHOSPHOLIPID VESICLES BY CONCANAVALIN A

Evidence for steric modulation of lectin binding by phospholipid head groups

R. SUNDLER

Dept. Physiological Chemistry, University of Lund, P.O. Box 750, S 220 07 Lund 7, Sweden

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1. Introduction

Lectin binding to a glycolipid inserted in phospholipid vesicles seems to require that the carbohydrate group extends some distance from the hydrocarbon–water interface. Thus, vesicles containing a diglycosylceramide, but not a monoglycosylceramide, could be agglutinated by *Ricinis communis* agglutinin [1]. The same lectin could agglutinate glycolipid–phosphatidylcholine vesicles if the glycolipid contained a 13- or 22-member, but not a 4-member, spacer arm between the hydrophobic part and the carbohydrate group [2]. The interpretation favoured in [1,2] was that the lectin can only bind carbohydrate that extends beyond phospholipid head groups on the vesicle surface. If so, lectin binding to vesicles in which the lectin-binding group is properly located with respect to the hydrocarbon region might be sterically regulated by the (hydrated size) of surrounding phospholipid head groups.

Here, a synthetic glycolipid [3] containing a 4-member spacer arm (fig.1) has been inserted in vesicles differing in polar head group structure and con A has been used as the lectin. The results obtained lend support to the above idea and suggest that lectin–glycolipid interaction could provide a simple and sensitive probe for studies on the 'effective size' of lipid polar head groups and its modulation by counter-ions.

2. Experimental

2.1. *Materials*

Concanavalin A was obtained from Serva Biochemicals (Heidelberg). Pure phosphatidylcholine from soy bean (Epikuron 200) was obtained from Lucas Meyer (Hamburg). Phosphatidylethanolamine was isolated from egg yolk [4]. Phosphatidylinositol from soy bean was isolated from a crude preparation (Epikuron 510, Lucas Meyer, Hamburg) by chloroform extraction followed by column chromatography on CM-cellulose [5]. Phosphatidate and phosphatidylserine were prepared from soy bean phosphatidylcholine using phospholipase D (Boehringer, Mannheim) in the absence or presence of L-serine, respectively [5] and were isolated by chromatography on CM-cellulose. Phosphatidyl-[*Me*-^{3}H]choline was prepared as in [6] and was isolated by column chromatography on silicic acid. Octadecyl-maltobionamide was synthesized as in [3] and further purified by preparative thin-layer chromatography on silica gel G (chloroform–methanol–4 M ammonia, 65:35:5, by vol.).

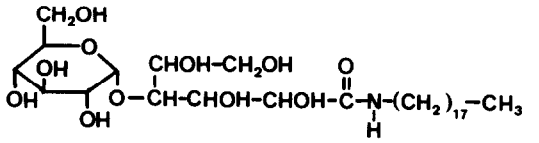

Fig.1. Structure of the synthetic glycolipid octadecyl-maltobionamide.

2.2. *Preparation of lipid vesicles*

Appropriate phospholipid(s) and OMBA (6 μmol total lipid) were mixed with 5 nmol [^{3}H]phosphatidylcholine in chloroform solution and the mixture evaporated to dryness. Small unilamellar vesicles were prepared by sonication in 2 ml 0.1 M NaCl–20 mM *N*-2-hydroxyethyl-piperazine-*N*^{1}-2-ethanesulfonate (pH 7.2) (buffer A) essentially as in [7].

Abbreviations: con A, concanavalin A; OMBA, octadecyl-maltobionamide

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2.3. *Assay of lectin-induced agglutination*

Sonicated vesicles (0.2 μmol lipid) were incubated for 45 min with con A and additions as indicated in 1 ml buffer A at 20°C. The assay mixture was then centrifuged for 15 min at 4500 rev./min in a fixed-angle rotor and the radioactivity of the supernate determined by liquid scintillation counting. Control experiments showed that extending the time of incubation to 3 h did not significantly change the results.

3. Results and discussion

In agreement with [8] we found that sonicated lipid vesicles containing OMBA and phosphatidylcholine did not agglutinate when incubated with con A (fig.2). However, replacement of up to half of the phosphatidylcholine with phosphatidylethanolamine gave vesicles which were increasingly sensitive to con A-induced agglutination (fig.2). As expected, mixtures of pure (egg) phosphatidylethanolamine and OMBA did not form stable vesicles. As in all other experiments in this report, agglutination was completely reversed by methyl-α-D-glucoside (5 mM) but in these zwitterionic vesicles agglutination was only slightly affected by Ca^{2+} (1 mM). Although con A must contain bound Mn^{2+} and Ca^{2+} to function as lectin [9] these ions are only slowly removed by EDTA at neutral pH.

The same glycolipid was also incorporated in vesicles of phosphatidylinositol, phosphatidylserine or phosphatidate (fig.3). In the absence of divalent ions,

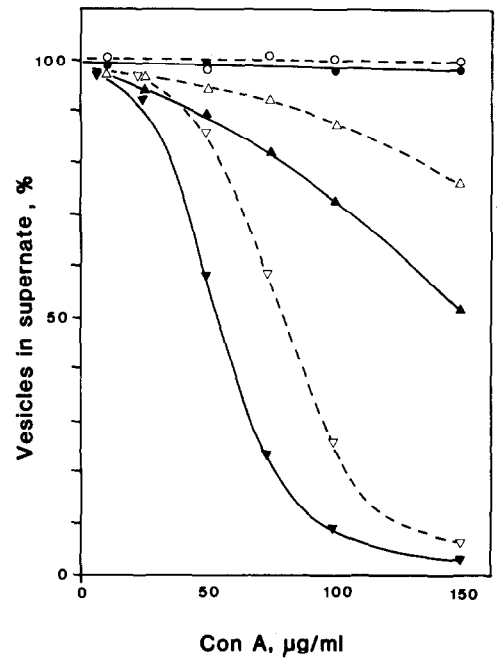

Fig.2. Con A-induced agglutination of neutral glycolipid–phospholipid vesicles. Sonicated lipid vesicles containing 20 mol% OMBA and 80 mol% of either phosphatidylcholine (○) or phosphatidylcholine–phosphatidylethanolamine (3:1) (△,▲) or (1:1) (▽,▼) were incubated with con A in buffer containing 1 mM EDTA (open symbols) or 1 mM Ca^{2+} (filled symbols). Agglutinated vesicles were separated by centrifugation and radiolabeled vesicles remaining in the supernate were determined.

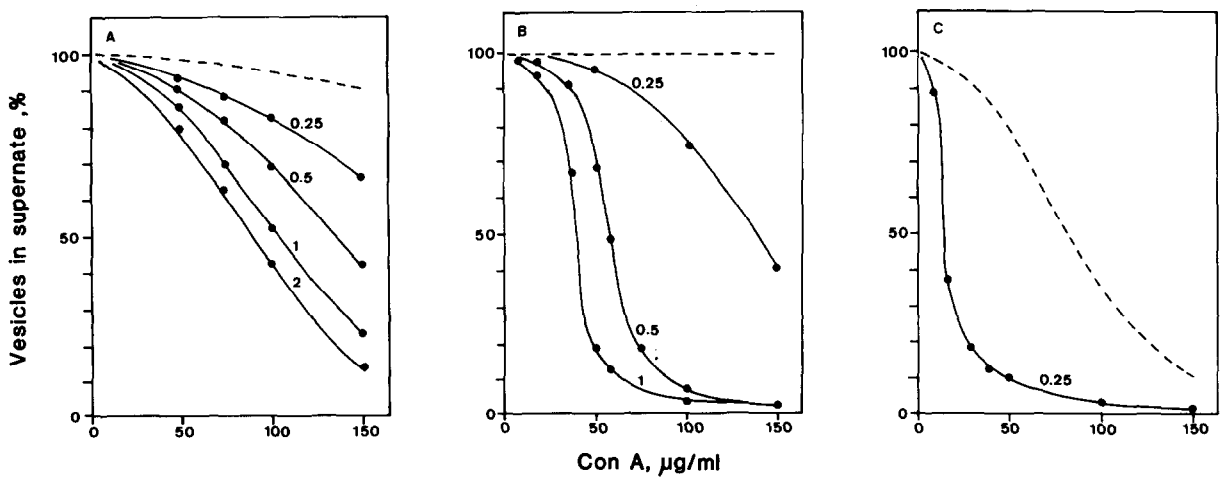

Fig.3. Con A-induced agglutination of anionic glycolipid–phospholipid vesicles. Sonicated vesicles containing 20 mol% OMBA and 80 mol% phosphatidylinositol (A), phosphatidylserine (B) or phosphatidic acid (C) were incubated with con A in buffer containing 1 mM EDTA (– – –) or various concentrations of Ca^{2+} (mM, ——) as indicated.

con A-induced significant agglutination only in glycolipid–phosphatidate vesicles. Therefore, it is unlikely that the lack of agglutination of phosphatidylserine and phosphatidylinositol vesicles was due to electrostatic repulsion between vesicles or between con A and vesicles. Indeed, the charge density of OMBA–phosphatidate vesicles at a bulk pH of 7.2 should be higher than that of the other anionic vesicles studied [10].

The addition of Ca^{2+}, which only weakly affected the zwitterionic vesicles, dramatically enhanced con A-induced agglutination of all the anionic vesicles (fig.3). The effect of Ca^{2+} showed quantitative differences among the 3 anionic lipids decreasing in the order phosphatidate, phosphatidylserine and phosphatidylinositol. Ca^{2+} alone causes aggregation of anionic lipid vesicles at concentrations higher than those used here [11,12] but significant association of Ca^{2+} with phosphatidylserine occurs already at lower concentrations [12,13]. Calcium causes screening of the negative surface charge but, in the case of phosphatidylserine, this effect is insufficient to explain the drastic increase in transition temperature, the fusion and collapse of vesicles and the virtually complete dehydration that follows exposure to sufficiently high [Ca^{2+}] [12]. The dehydration of the phosphoserine group, caused by Ca^{2+} binding, is likely to be of central importance for the fusion induced by this ion [14]. Ca^{2+}-induced fusion of phosphatidate vesicles and the lack of fusion of phosphatidylinositol vesicles [15] also emphasize the importance of Ca^{2+}-induced modulation of the hydrated size of lipid polar head groups for vesicle fusion.

This study shows that con A-binding to the glycolipid OMBA is highly sensitive to the structure of surrounding phospholipid head groups. Among zwitterionic phospholipids phosphatidylethanolamine, but not phosphatidylcholine, enhanced lectin-induced agglutination. The head group of the former may be smaller and less hydrated than that of the latter phospholipid [16]. Among the anionic phospholipids studied phosphatidate did not prevent lectin-induced agglutination in the absence of divalent cations. In contrast, phosphatidylinositol vesicles required relatively high concentrations of lectin for agglutination even in the presence of near aggregating concentrations of Ca^{2+}. The similarity between the effects of polar head groups on con A-binding on the one hand and on Ca^{2+}-induced vesicle fusion on the other [15,17], suggest that they may be exerted by related mechanisms. Steric hindrance of con A-binding, varying with the bulkiness and degree of hydration of the phospholipid head groups, seems to be a reasonable explanation for the effects observed here.

Acknowledgements

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References

[1] Curatolo, W., Yan, A. O., Small, D. and Sears, B. (1978) Biochemistry 17, 5740–5744.
[2] Slama, J. S. and Rando, R. R. (1980) Biochemistry 19, 4595–4600.
[3] Williams, T. J., Plessas, N. R., Goldstein, I. J. and Lönngren, J. (1979) Arch. Biochem. Biophys. 195, 141–151.
[4] Litman, B. J. (1973) Biochemistry 12, 2545–2554.
[5] Comfurius, P. and Zwaal, R. F. A. (1977) Biochim. Biophys. Acta 488, 36–42.
[6] Stoffel, W., Le Kim, D. and Tschung, T. S. (1971) Z. Physiol. Chem. 352, 1058–1064.
[7] Sundler, R., Alberts, A. W. and Vagelos, P. R. (1978) J. Biol. Chem. 253, 4175–4179.
[8] Hampton, R. Y., Holz, R. W. and Goldstein, I. J. (1980) J. Biol. Chem. 255, 6766–6771.
[9] Goldstein, I. J. and Hayes, C. E. (1978) Adv. Carbohydr. Chem. Biochem. 35, 128–340.
[10] Träuble, H. and Eibl, H. (1975) in: Functional Linkage in Biomolecular Systems (Schmitt, F. O. et al. eds) pp. 59–101, Raven Press, New York.
[11] Papahadjopoulos, D., Vail, W. J., Pangborn, W. A. and Poste, G. (1976) Biochem. Biophys. Acta 448, 265–283.
[12] Portis, A., Newton, C., Pangborn, W. and Papahadjopoulos, D. (1979) Biochemistry 18, 780–790.
[13] Kurland, R. J., Hammoud, M., Nir, S. and Papahadjopoulos, D. (1979) Biochem. Biophys. Res. Commun. 88, 927–932.
[14] Wilschut, J., Düzgünes, N. and Papahadjopoulos, D. (1981) Biochemistry 20, 3126–3133.
[15] Sundler, R. and Papahadjopoulos, D. (1981) Biochim. Biophys. Acta 649, 743–750.
[16] Hauser, H., Pascher, I., Pearson, R. H. and Sundell, S. (1981) Biochim. Biophys. Acta 650, 21–51.
[17] Sundler, R., Düzgünes, N. and Papahadjopoulos, D. (1981) Biochim. Biophys. Acta 649, 751–758.