SPECIFIC INTERACTION BETWEEN CONCANAVALIN A AND GLYCOLIPIDS INCORPORATED INTO PLANAR BILAYER MEMBRANES.

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Summary : Changes in conductance of glycerol monooleate planar membranes containing glucosylceramide, lactosylceramide or human brain gangliosides were measured after the addition of Concanavalin A into the aqueous phase. A 380 and 31-fold increase of conductance values was observed respectively with glucosylceramide and lactosylceramide, whereas gangliosides had no significant effect. Modifications of membrane conductance were suppressed by the addition of methyl α-D glucopyranoside, a Concanavalin A inhibitor. It is concluded that the lectin molecule interacts with specific glycolipids incorporated into the membrane model and that this interaction may be related to the position of the glucose molecule in the carbohydrate moeity of the lipids.

In recent years, significant progress has been made in the isolation of glycoproteins containing receptors for sugar specific plant proteins (lectins). Experiments using membrane models containing glycoproteins have contributed to elucidate this interaction (1, 2, 3). In biological membranes, similar hexoses were found in glycolipids and in glycoproteins and some glycolipids have been implicated as receptors for bacterial exotoxins (4, 5) or in agglutination process (6). The purpose of this communication is to report experiments which demonstrate a specific interaction between glycolipids incorporated into planar bilayer membranes and Concanavalin A, by measuring the modification of conductance after addition of the lectin molecule in the bathing solution. The demonstration of a peculiar affinity of glycolipids to Concanavalin A may be of interest sin-

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ce Concanavalin A specifically agglutinates tumor cells (7) and since several examples of changes in glycolipid patterns were reported in transformed cells (8).

Materials and Methods

GMO and α-D methyl glucopyranoside were purchased from Sigma Chemical Co. Glucosylceramide (N-palmitoyl-Dihydroglucocerebroside), lactosylceramide (N-palmitoyl-Dihydrolactocerebroside) and Concanavalin A (3 times crystallized) were Miles-Yeda products. Gangliosides (GM_1, GD_{1a}, GD_{1b}, GT_1) were isolated from human brain (9). N-decane, a reagent grade product was distilled under reduced pressure before use. Solutions were prepared by dissolving the glycolipids in a chloroform-methanol 2:1 mixture. After evaporation under nitrogen, lipids were redissolved in n-decane.

Bilayers were formed from lipids dissolved in decane (10) on a 1.3 mm diameter aperture in a teflon cell (11). The aqueous phase contained 150 mM NaCl + 10 mM KH_2PO_4 at pH = 7.4; the temperature was maintained at 36°C. The membrane conductance G_m was determined by measuring the current I_m as a function of an imposed potential difference V_m, with a Keithley electrometer (Model 602). The complete system was enclosed in a Faraday cage. The membrane formation was observed under reflected light with a low power microscope.

Results and Discussion

Results are summarized in Table I.

In absence of Concanavalin A, incorporation of glucosyl- and lactosylceramide in GMO bilayer membranes brings no change in membrane conductance whereas a fifteen-fold increase was observed with human gangliosides. Addition of Ca^{++} in the bathing solution increases the conductance of membranes containing 3 mole % of gangliosides. This change can be due to the formation of a Ca^{++}- ganglioside complex via the carboxylate groups of the n-acetylneuraminic acid (12).

In presence of Concanavalin A, the conductance of membranes containing glucosylceramide increases dramatically, the change was

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Abbreviations : GMO : glycerol monooleate
GM_1, GD_{1a}, GD_{1b}, GT_1 : four major brain gangliosides, according to Svennerholm's nomenclature.

Table 1 : Effect of Concanavalin A on the conductance of planar bilayer membranes containing glycolipids.

Bilayers	$CaCl_2$ (mM/l)	Concanavalin A (µg/ml)	Inhibitor (µg/ml)	G_m 10^{-9} Mho cm^{-2}	N^r of experiments
GMO	0	0	0	35 ± 6	8
	1	0	0	30 ± 7	16
	1	250	0	239 ± 55	1
	1	250	48	85 ± 14	5
	1	0	48	58 ± 13	4
GMO-glucosylceramide Molar ratio 97/3	0	0	0	31 ± 5	4
	1	0	0	73 ± 9	4
	1	250	0	27700 ± 5900	5
	1	250	48	120 ± 18	5
	1	0	48	142 ± 32	8
GMO-lactosylceramide Molar ratio 97/3	0	0	0	35 ± 6	4
	1	0	0	75 ± 14	6
	1	250	0	2350 ± 500	3
	1	250	48	120 ± 225	5
	1	0	48	150 ± 21	8
GMO-gangliosides Molar ratio 97/3	0	0	0	480 ± 125	12
	1	0	0	1378 ± 320	7
	1	250	0	14250 ± 2890	5
	1	250	48	360 ± 54	4
	1	0	48	370 ± 70	8

slighter with lactosylceramide whereas no modification, compared with GMO bilayer conductance, was observed with gangliosides. To rule out the possibility of non specific binding, experiments were also carried out in the presence of α-D methylglucopyranoside, a Concanavalin A inhibitor. Fig. 1 shows that the inhibitor completely suppressed the lectin effect.

The results reported in this paper strongly suggest a specific interaction between glycolipids and Concanavalin A, depending on the position of glucose (13), a specific Concanavalin A receptor, in the carbohydrate moeity. The most striking change in

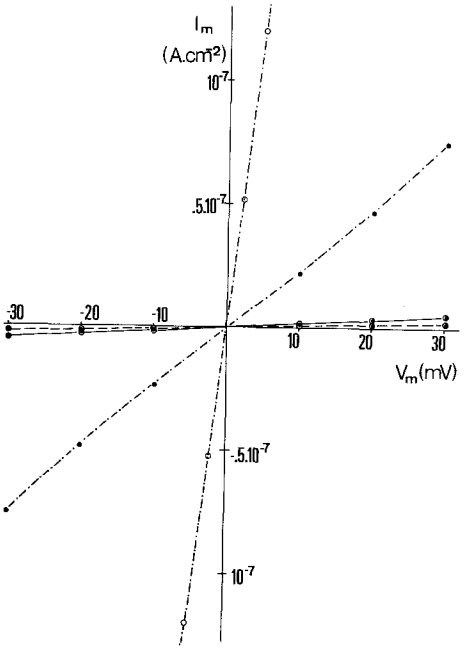

Fig. 1 : Current (I_m) - Voltage (V_m) characteristics of GMO bilayers containing glycolipids (o glucosylceramide, • lactosylceramide) in the absence of Concanavalin A (– – –), in the presence of Concanavalin A (–•–) and in presence of both inhibitor and Concanavalin(——). The bathing solution contained 250 µg per ml Concanavalin A in 150 mM NaCl + 10 mM KH_2PO_4 at pH = 7.4, 36°C. Inhibitor concentration was 48 µg per ml.
An ohmic relationship was observed in all cases up to about 40 mV and the results were identical for reverse polarity.

membrane conductance was obtained with glycolipids which possess a terminal glucose (glucosylceramide). It decreased if other sugars were attached to glucose (lactosylceramide, ganglioside). To the best of our knowledge, glycolipids - lectin interaction in planar bilayers has not been reported. The magnitude of the effect on the bilayer conductance indicated however that the interaction is not confined to the lipid hydrophilic surface but involved modifications inside the hydrophobic part of the bilayer structure. The mechanism of this modification requires further investigations.

These data provide at least a partial explanation for agglutination of transformed cells by Concanavalin A. Indeed, in our experiments, the lectin molecule reacts with glucose in terminal position (glucosylceramide) or placed in a short carbohydrate chain (lactosylceramide). Precisely, the change in the glycolipid pattern in transformed cells consists in a shortening of the carbohydrate moeity.

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