RICINUS COMMUNIS TOXIN INTERACTS SPECIFICALLY WITH GM_1 GANGLIOSIDE INCORPORATED INTO PLANAR LIPID BILAYERS

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1. Introduction

A wide variety of toxins is capable of entering cells by a receptor-mediated transport process. Having gained entry these proteins are directed to specific cellular compartments where they exert their pathological function [1]. *Ricinus* toxin is amongst the most toxic substances known [2,3]. It consists of 2 polypeptide chains A and B: A is an enzyme capable of inactivating protein synthesis and B is responsible for the binding of the toxin to receptors on the cell surface. There is good evidence that the B chain interacts with membrane carbohydrate residues containing non-reducing terminal galactose residues [4]. In the cell membrane, these carbohydrates are associated to glycoproteins or to glycolipids. Most of the membrane receptor sites are usually supposed to be associated to a glycoprotein structure and it has been recently suggested that glycolipids could be putative receptors for glycoprotein hormones [5], bacterial toxins [6–8] and viruses [9].

Significant progress has been obtained in the comprehension of ganglioside-protein interactions with model membranes which allowed the duplication of the recognition properties of natural cell membranes [10–15]. This approach was used in this report. We present evidence of a specific interaction between the *Ricinus* toxin and GM_1 ganglioside incorporated in a planar lipid bilayer. The association constants determined in model membranes were in excellent agreement with those found for the receptor–ricin association constants in cell culture. The recognition process is inhibited by the same saccharides in the two systems.

2. Material and methods

Ricinus toxin (RCA60) and agglutinin (RCA120), glucocerebroside (*N*-palmitoyldihydroglucocerebroside) and lactocerebroside (*N*-palmitoyldihydrolactocerebroside) were Miles Yeda products. Glycerol monooleate (GMO), *N*-acetylneuraminic acid were Sigma Chemical Co products. GT_1 ganglioside (*N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl-*N*-acetylneuraminyl)-galactosylglucosylceramide), GD_{1a} ganglioside (*N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide) and GM_1 ganglioside (galactosyl-*N*-acetylgalactosaminyl-(*N*-acetylneuraminyl)-galactosylglucosylceramide) were Supelco products. Lipids were checked for purity by thin-layer chromatography.

Lactose, glucose and galactose were 'pro analysi' products from Union Chimique Belge (Bruxelles, Belgique). *n*-Decane, a reagent grade product, was redistilled before use [16]. The mixtures GMO-gangliosides were dissolved in a chloroform/methanol/decane (30/5/65) mixture. Bilayers were formed on a 1.3 mm diameter aperture in a Teflon cell separating two aqueous phases (2.5 cm^3 each). The aqueous phase contained 0.15 M NaCl/0.01 M Tris-HCl, pH 7.3; the temperature was maintained at 20°C. The membrane conductance G_m was determined by measuring the specific current I_m/cm^2 as a function of imposed potential differences V_m, with a 602 Keithley electrometer. The complete system was enclosed in a Faraday cage. Membrane formation was observed under reflected light with a low-power microscope. Toxin and agglutinin were added in each chamber.

Table 1
Effect of *Ricinus* toxin (RCA60) and *Ricinus* agglutinin (RCA120) on the conductance of planar membranes containing gangliosides

Bilayers	Conductance (10^{-7} S/cm^2)			
	Without RCA60	With RCA60[a]	Without RCA120	With RCA120
GMO	0.20 (6)[c]	0.20 (5)	0.20 (6)	0.20 (6)
GMO-GM_1[b]	1.1 (7)	16 (16)	1.2 (6)	8 (12)
GMO-GD_{1a}	0.50 (6)	0.60 (7)	0.50 (6)	0.60 (8)
GMO-GT_1	3.2 (6)	5 (9)	3.3 (6)	3.6 (8)
GMO-glucocerebroside	0.35 (6)	0.40 (7)	0.35 (6)	0.35 (8)
GMO-lactocerebroside	0.40 (6)	0.40 (7)	0.40 (6)	0.40 (7)

[a] Protein concentration 10^{-7} mol/l
[b] GMO-glycolipid molar ratio 98/2
[c] Number of experiments

3. Results and discussion

Conductance changes of GMO planar membranes containing GM_1, GD_{1a}, GT_1, glucocerebroside and lactocerebroside were measured after addition of the toxin RCA60 to the two compartments containing the aqueous phase. A 14-fold increase of conductance was obtained when GM_1 was incorporated in the lipid bilayer. No significant effect was observed with GT_1, GD_{1a}, gluco- and lactocerebroside (table 1). The conductance changes observed allow one to suppose a penetration of the plant toxin into the membrane inducing a destabilization of the lipid layer. A penetration due to the hydrophobicity of the ricin can be ruled out because no conductance change was observed with a pure GMO bilayer. Our results indicate no significant binding for lactocerebroside (table 1). It must be supposed that in the GMO lipid environment the terminal galactose of lactocerebroside is not recognized by the toxin. Two different explanations could be proposed. First, the terminal galactose is not accessible for the toxin and the presence of 2 or 3 additional saccharides in the glycolipid hydrophilic region could place the galactose in a terminal position favorable to maximal recognition. Second, the galactose is not the only saccharide involved in the recognition site and other residues are essential to allow the ganglioside-toxin interaction.

The GM_1-toxin interaction observed on model membranes is in agreement with cell culture data. The ability of ricin to inhibit protein synthesis in fibroblasts is strongly reduced when lactose or galactose are present in the culture medium. Moreover the ability of toxin to bind to saccharide columns in which galactose residues are present has been utilized for toxin preparation [17].

If the ganglioside-ricin interaction is specific, it should be possible to reverse it by addition in the aqueous phase, of the saccharides present in the hydrophilic moiety of the ganglioside.

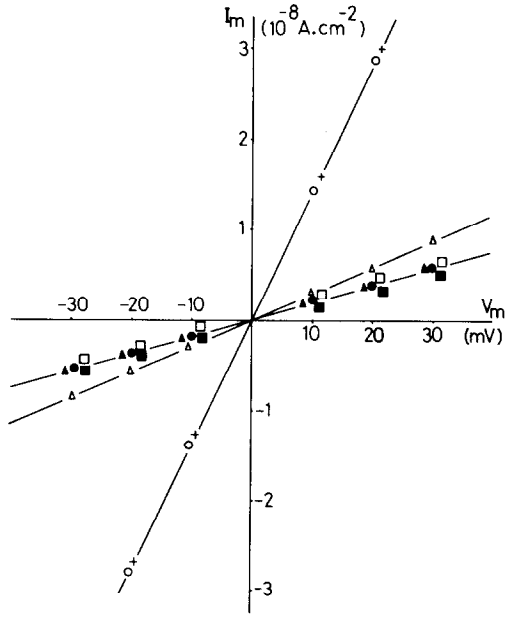

Fig.1. Current-voltage characteristics of GMO bilayers containing GM_1 ganglioside (molar ratio 98/2) in the absence (▲) and presence of toxin (○) and in the presence of inhibitors: +, glucose; ●, *N*-acetylgalactosamine; □, *N*-acetylneuraminic acid; △, lactose and ■, galactose. Toxin RCA60 concentration 10^{-7} mol/l. Inhibitor concentration 10^{-6} mol/l.

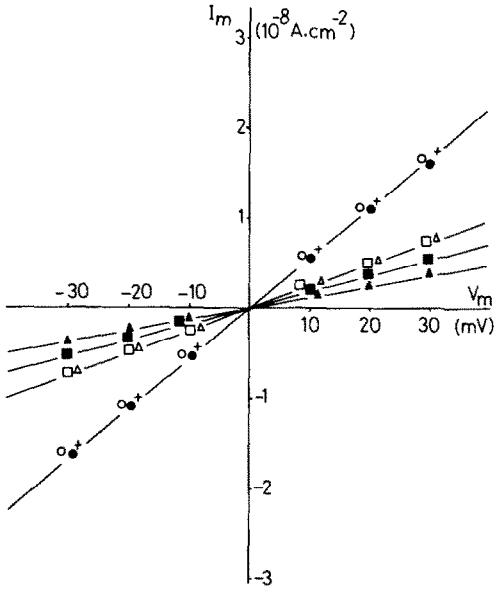

Fig.2. Current–voltage characteristics of GMO bilayers containing GM_1 ganglioside (molar ratio 98/2) in the absence (▲) and presence of agglutinin (○) and in presence of inhibitors: +, glucose; •, *N*-acetylgalactosamine; □, *N*-acetylneuraminic acid; △, lactose and ■, galactose. Agglutinin RCA120 concentration 10^{-7} mol/l. Inhibitor concentration 10^{-6} mol/l.

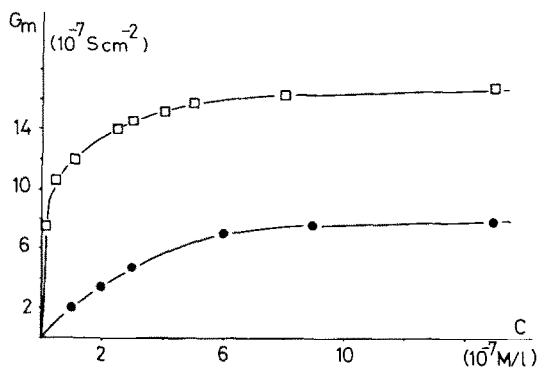

Fig.3. Dependence of the membrane conductance (G_m) of GMO-GM_1 ganglioside (molar ratio 98/2) bilayers on the concentration of toxin (□) and of agglutinin (•).

The inhibitory capacity of glucose, galactose, lactose, *N*-acetylneuraminic acid and *N*-acetylgalactosamine was tested independently for the toxin (fig.1). The recognition process is highly specific. Indeed, if an identical experimental approach is used for hemagglutinin RCA120 extracted from *Ricinus communis*, but consisting of 2 A chains and 2 B chains, the same specificity for GM_1 is observed (fig.2). However, if lactose, galactose and *N*-acetylneuraminic acid inhibit the ganglioside-toxin and the ganglioside-agglutinin interactions, *N*-acetylgalactosamine is a specific inhibitor for the toxin. These inhibitory effects on model membranes are similar to those found on cell cultures, indeed *N*-acetylgalactosamine inhibits toxin fixation but not agglutinin fixation [18]. This point could suggest that the toxin recognizes GM_1 with a higher specificity than the agglutinin. This higher specificity is illustrated by the high association constant between the toxin and GM_1 as compared to the agglutinin-GM_1 association constant. Fig.3 shows the dependence of membrane conductance on the ricin concentration in the bathing solution. From a classical saturating relation between membrane conductance and ricin concentration, it was possible from the change of the ricin concentration needed to produce half maximal conductance to calculate an association constant [9]. The values of these association constants for the agglutinin and the toxin were 4.2×10^6 M^{-1} and 10^8 M^{-1}, respectively. These values obtained in a model system were in agreement with the association constants between the ricin and the membrane surface receptor of HeLa cells (4.2×10^8 M^{-1} for the toxin) [19], of human erythrocyte ghosts (6×10^7 M^{-1} for the agglutinin) [20], or of human lymphocytes (6×10^6 M^{-1} for the agglutinin) [21].

Finally, our results demonstrate clearly a specific interaction between GM_1 ganglioside and the *Ricinus* toxin. The experimental approach used here avoids the risk of unspecific interaction between ligand and receptor. However, it remains that a definitive demonstration of GM_1 as a receptor would suppose that cells lacking GM_1 [8] could be made toxin responsive by incorporating exogenous GM_1 into their membrane. This possibility is under investigation.

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