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The interaction of an anti-lipid antibody (TEPC 15) with a model biomembrane system (monolayer)

Maria A. Urbaneja, Gerardo D. Fidelio, Jack A. Lucy and Dennis Chapman

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine (University of London), London (U.K.)

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The interaction which occurs between an anti-lipid antibody (TEPC 15) and two phospholipids, phosphatidylcholine and phosphatidylethanolamine, when they are arranged in a lipid monolayer system has been studied. It is shown that the antibody is stabilised under the influence of a high lateral pressure when it is mixed with a lipid monolayer and that the behaviour of the antibody depends upon the lipid used. Measurements of the surface pressure and surface potential parameters of the lipid monolayers indicate that the antibody interacts differently with phosphatidylcholine compared with phosphatidylethanolamine. The antibody also exhibits a different interaction when it is pretreated with phosphorylcholine prior to being spread with a phosphatidylcholine monolayer. The interaction of the antibody with phosphatidylcholine-cholesterol monolayers has also been studied.

The important roles of lipids in immunological phenomena have recently become of interest [1]. Certain phospholipids have been shown to display immunological activity and may elicit antibody formation, probably directed against their polar head groups [2–4]. Indeed, a high titer of anti-lipid antibodies has been reported in some autoimmune diseases, such as multiple sclerosis and systemic lupus erythematosus [5,6]. It has also been observed that a single injection of liposomes, containing lipid A derived from endotoxin, results in anti-liposome antibody production in nearly every animal tested [2,7], while Richards and Alving [8,9] have provided evidence that naturally occurring antibodies against membrane phospholipids are widespread in normal human sera. Naturally-occurring anti-lipid antibodies also exhibits a variety of specificities against different phospholipids. One possible mechanism for the induction and/or pathogenesis of these antibodies involves alterations in the phospholipid architecture of the cell [8,9].

TEPC 15 is an immunoglobulin A that precipitates with *Pneumococcus* C polysacharide, an antigen that has choline as constituent. The specificity of this antibody has been tested by immunodiffusion, and it has been demonstrated that it reacts with lipids carrying phosphorylcholine groups [10]. Phosphatidylcholine and sphingomyelin occur predominantly on the outer surface of many cell membranes [11] and both these lipid classes have the same phosphorylcholine polar group.

We report here some studies of the interactions of this TEPC 15 antibody against two types of phospholipid, i.e. phosphatidylcholine (PC) and phosphatidylethanolamine (PE). We are concerned with the question as to whether the antibody can interact with these lipids in a monolayer form. To this end, monolayers of these phos-

Correspondence: D. Chapman, Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine (University of London), Rowland Hill Street, London NW3 2PF, U.K.

pholipids were studied at the air/water interface, and measurements were made of their surface pressure and surface potential.

The details of the monolayer equipment used have been given previously [12,13]. The phospholipids, from egg yolk, cholesterol, phosphorylcholine and NaCl were from Sigma Chemical Co., Poole, Dorset, U.K. The purity of the phospholipids was checked by HPTLC. Phosphatidylcholine was further purified according to the method of Singleton [14]. The phospholipids were dissolved in chloroform/methanol (2 : 1, v/v). All the solvents were Aristar grade BDH, Poole, Dorset. U.K.

TEPC 15, BALB/c mouse IgA Myeloma Protein (pristane induced), was purchased from Bionetics, Lab. Products, Charleston, U.S.A. The antibody was dissolved in Tris-saline buffer (pH 8.1). The purity of the antibody was checked by SDS-polyacrylamide gel electrophoresis.

The range of concentration of protein spread varied from 0 to $2.2 \cdot 10^{-8}$ M depending upon the molar ratio required.

The mixed protein-lipid monolayers were prepared by spreading small drops of lipid and protein solutions at the aqueous surface, and they were analyzed by comparing the mixed force-area and surface potential-area curves with the isotherms for the corresponding pure lipid. All the experiments were carried out at $22 \pm 1°C$ on a subphase of unbuffered 145 mM NaCl, adjusting the pH around 8.1. The reproducibility of the parameters in triplicate experiments was as follows: molecular areas ± 0.03 nm^2/molecule, surface potential ± 5 mV and surface pressure ± 1 mN $\cdot$ m^{-1}.

When required, TEPC 15 was pretreated with an excess of phosphorylcholine, at a molar ratio antibody-phosphorylcholine 1 : 10.

When spread directly at the air/water interface, the maximum increment of surface pressure brought about by the compression of TEPC 15 alone is less than 2 mN $\cdot$ m^{-1}. This indicates that the pure anti-lipid antibody does not form a stable monolayer. On the other hand, surface potential measurements show that the antibody has a dipolar component which contributes to the surface potential with a value of the order of 200 mV.

When it is spread in a monolayer with phospholipids, the TEPC 15 protein is stabilised at the surface, i.e. it can support a high lateral pressure. By comparison with the pure phospholipid monolayers, the mixed films exhibit an expansion in area. Such an expansion is observed with both types of lipid investigated (Fig. 1). However, the magnitude of the effect depends upon the lipid used. The expansion in area for the PC monolayers reaches a maximum at a molar ratio antibody-phosphatidylcholine of 1 : 80, and no additional expansion is obtained when more antibody is included in the mixed film (Fig. 1). This maximum is not observed when the antibody is added to PE monolayers (Fig. 1). Pretreatment of the antibody with a phosphorylcholine hapten enables interactions of the antibody with phospholipid monolayers to be investigated when the binding sites on the antibody are occupied. Pretreated antibody similarly expands phospholipid monolayers, but a saturation point is not reached when it is added to PC monolayers (Fig. 1).

By comparison with a pure phospholipid monolayer the TEPC 15-PC films have a negative dipolar component (Fig. 2). The interaction of the antibody with this lipid has thus led to a suppres-

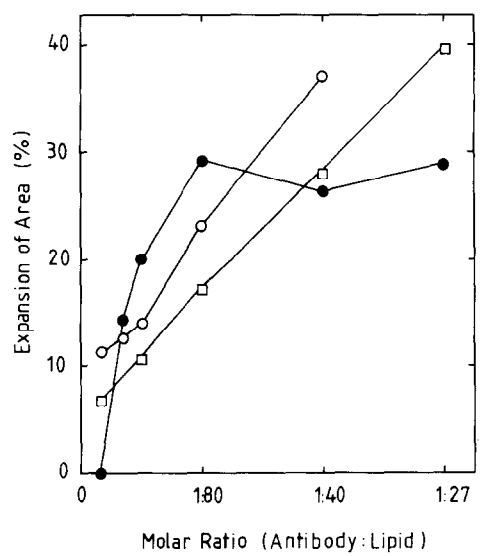

Fig. 1. Interaction of the anti-lipid antibody TEPC 15 with phospholipids. Percentage of expansion in lipid molecular area after adding TEPC 15 is to (●) PC, (○) PE, or (□) TEPC 15 pretreated with phosphorylcholine to PC monolayers. The expansion was measured at 20 mN $\cdot$ m^{-1}. The molecular area of the pure phospholipid was taken as 100%.

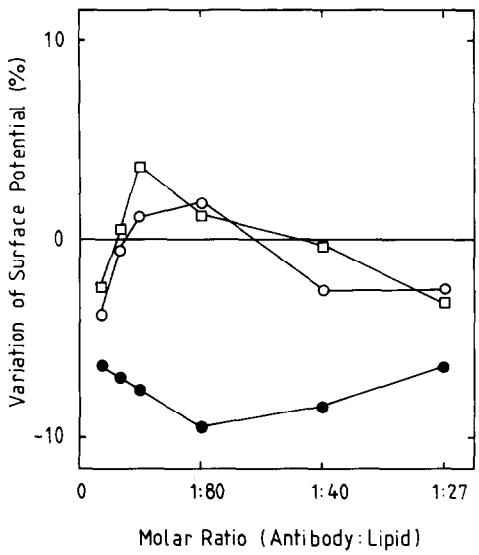

Fig. 2. Influence of the antibody upon the interfacial potential. Percentage variation of surface potential after adding TEPC 15 to (●) PC, (○) PE or (□) TEPC 15 pretreated with phosphorylcholine to PC monolayers. The variation was measured at 20 $mN \cdot m^{-1}$. The surface potential of the pure lipid was taken as 100%.

sion of the dipolar component which we have observed with the pure protein. By contrast the surface potential measurements of TEPC 15-PE films and phosphatidylcholine films mixed with TEPC 15 that has been pretreated with phosphorylcholine give little or no dipolar component (Fig. 2).

The data of Figs. 1 and 2 indicate that the antibody adopts different arrangements in the monolayer, depending upon the phospholipid used and whether the antibody has been pretreated with its hapten. We suggest that the Fab fragments of the antibody, which contain the binding sites, interact with the phosphoryl polar group of the phospholipid when TEPC 15 is added to PC monolayers. This interaction produces an expansion in area and also decreases the dipolar component of the monolayer. However, when the antibody is added to PE monolayers or the pretreated TEPC 15 is added to PC monolayers, the Fab fragments will face the aqueous solution, producing an expansion in area but not affecting the dipolar component of the lipid monolayer.

The expansion of molecular area observed with TEPC 15-PC monolayers was surface pressure dependent, being greater at lower surface pressure than at higher surface pressure (Fig. 3). This effect is not found when TEPC 15 interacts with phosphatidylethanolamine or when the pretreated antibody interacts with the phosphatidylcholine monolayer (Fig. 3). These results indicate that with TEPC 15-PC films, the protein might be squeezed out as the film is compressed. This event could take place without a protein collapse process occurring. No discontinuities are observed in the isotherms at the lateral pressures below the collapse pressure of the mixed protein-lipid films.

The interaction of the anti-lipid antibody with PC-cholesterol monolayers was also studied (Fig. 4). These results show that the interaction of TEPC 15 with PC-cholesterol films are similar to the behaviour of TEPC 15-PE or TEPC 15-PC after antibody pretreatment.

Recently it has been shown [15,16] that the interaction of an anti-lipid antibody, i.e. an anticoagulant lupus erythematosus antibody, with

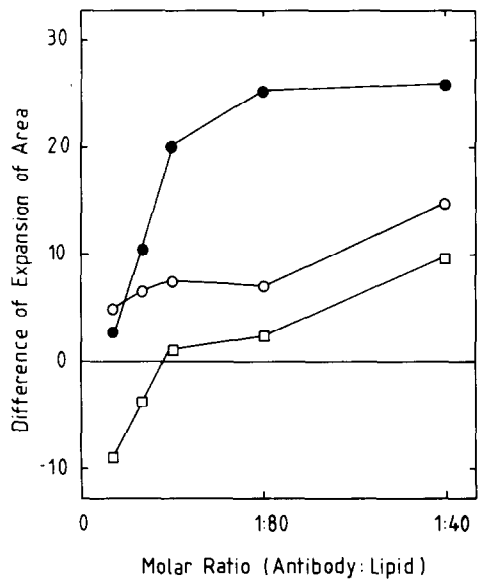

Fig. 3. Influence of the lateral lipid packing upon the antibody–lipid interaction. Difference in the percentage of expansion in molecular area between the lift off area and the limiting molecular area for (●) TEPC 15-PC (○) TEPC 15-PE and (□) TEPC 15 pretreated-PC mixed monolayers. The limiting molecular area is defined as the area occupied by the lipid molecule at the maximum lateral pressure that the monolayer arrangement can support (collapse pressure).

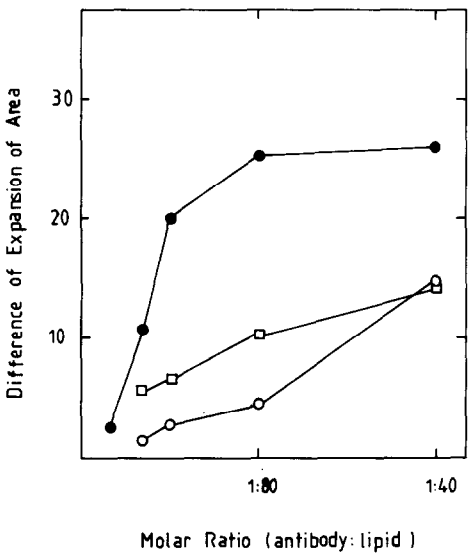

Fig. 4. Influence of cholesterol in the anti-lipid antibody–lipid interaction. Difference in the percentage expansion in area between the lift off area and the limiting molecular area for (●) PEPC 15-PC, (○) TEPC 15-PC-cholesterol (3:1) and (□) TEPC 15-PC-cholesterol (1:3) mixed monolayers.

lipids requires the phospholipid to be in a hexagonal form. The work of Niedick [10] has also indicated that a micellar form of PC-cholesterol-amphiphile enhances the precipitation of the anti-phosphorylcholine antibody. The present studies show that the TEPC 15 antibody can interact with phosphatidylcholine when it is also present in a monolayer form.

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