Biochimica et Biophysica Acta, 685 (1982) 177–181
Elsevier Biomedical Press

BBA 71045

IMMUNOGOBULIN-LIPID INTERACTION

A MODEL MEMBRANE STUDY

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(Received June 10th, 1981)

Key words: Immunoglobulin; Lipid monolayer; Planar lipid bilayer; Hydrophobic interaction

We recently presented evidence (Vandenbranden, M., De Coen, J.L., Jeener, R., Kanarek, L. and Ruysschaert, J.M. (1981) Mol. Immunol. 8, 621–631) for the existence of two conformational rabbit serum IgG immunoglobulin isomers. In the present report, their capacity to interact with lipid is investigated in model membranes. (1) One isomer, IgG(H), behaves like several intrinsic membrane proteins: it induces a large surface pressure increase when injected under a lipid monolayer in the close packed state and increases by 20-fold the conductance of a planar bilayer. The other isomer, IgG(S) doesn't interact significantly with the lipids. (2) IgG(H) marked a preference for monolayers made of lipids with a negatively charged polar headgroup and bearing unsaturations in their acyl chains. Penetration is stronger with lipid monolayer in the fluid state than in the rigid state. (3) As previously shown (Vandenbranden, M., De Coen, J.L., Jeener, R., Kanarek, L. and Ruysschaert, J.M. (1981) Mol. Immunol. 18, 621–631), circular dichroïsm spectra and antigen precipitation tests don't allow to detect any difference in the overall secondary conformation and antigen recognition properties of the two isomers. (4) Papaïn cleavage of the hinge region suppresses the hydrophobic properties of IgG towards lipid monolayers. (5) The hypothesis of a binding of the hinge region with the lipid bilayer is discussed.

Introduction

Numerous reports have dealt with the mode of binding of immunoglobulins to membranes [1–10]. Immunoglobulin (Ig) molecules can indeed exist in two structures: one secreted form, largely soluble in water and one hydrophobic form inserted into the lymphocyte membrane. Based on indirect data, it has often been assumed that membrane IgG and IgM possess an additional hydrophobic segment allowing their insertion in the membrane. It will remain speculative to localize such a segment as long as the complete amino acid sequence of secreted and membrane Ig of the same origin has not been determined. Another way to explain the Ig behaviour is to suppose that the membrane and the secreted form, independently of their own amino acid sequence, could correspond to two different conformational states: one compatible with the aqueous medium and the other with the hydrophobic environment. We recently presented evidence for the existence of two conformational IgG isomers [11]. It is the purpose of this paper to study in model membranes (lipid monolayers, planar lipid bilayers) the capability of these two isomers to interact with the lipid matrix. Lipid monolayers were spread at the air/water interface and IgG injected into the aqueous phase. The IgG penetration was evaluated by the change in surface pressure at constant area. The lipid-IgG isomers interaction was analyzed in terms of lipid composition and lipid charge. Conductance measure-

ments in planar lipid bilayers were performed to establish the capacity of the IgG isomer to be inserted into the lipid hydrophobic core.

Materials and Methods

DL-α-Dipalmitoylphosphatidylcholine (DPPC), DL-α-dimyristoylphosphatidylcholine (DMPC), phosphatidylcholine from egg yolk (PC), DL-α-dipalmitoylphosphatidylethanolamine (DPPE), cardiolipin from bovine heart (CL), cholesterol and glycerol monoleate were purchased from Sigma Co. (St. Louis, MO). Phosphatidylserine (PS) was a Koch-Light Laboratories product. Other reagents were of analytical grade. Water was redistilled over permanganate.

IgG were isolated from rabbit serum by precipitation with ammonium sulfate (45% sat.). In order to eliminate traces of serum albumin, gel filtration through a Sephadex G-200 column (2 m high, 2.5 cm diameter) equilibrated with phosphate-buffered saline (pH 7.5) was performed followed by affinity chromatography on a DEAE-cellulose column, eluted with KH_2PO_4/K_2HPO_4 (pH 8; 0.02 M phosphate).

Monolayers. Lipids dissolved in chloroform were spread on a subphase containing usually 100 ml of 0.1 M citric acid/0.2 M Na_2HPO_4 buffer at pH 7 and thermostatically controlled at 29°C. The surface was limited by a teflon ring (13 cm diameter). The surface pressure was recorded by the Wilhelmy plate technique, using a Cahn RG Electrobalance.

Planar lipid bilayers. Glycerolmonoleate was dissolved in redistilled *n*-decane. Bilayers were formed on a 1.3-mm diameter aperture in a teflon wall separating two aqueous compartments (2.5 cm^3 each). The aqueous phase contained 0.15 NaCl + 0.01 M Tris-HCl at pH 7. Measurements were made at 20°C. Potential differences V_m (mV) were imposed by two Ag/AgCl platinum electrodes. Specific current I_m (A/cm^2) was measured using a Keithley electrometer. The membrane formation was observed under reflected light with a low power microscope. IgG was added in the two compartments. For further details see Refs. 12 and 13.

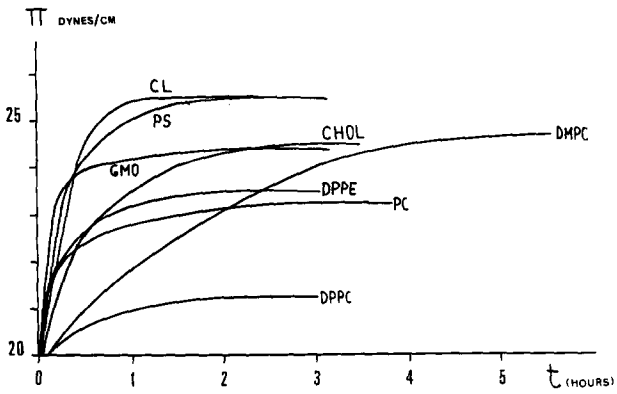
Papain digestion. Digestion was made according to Porter [14]. A solution of 1 mg/ml IgG (pH 7 buffer, 0.1 M citric acid + 0.2 M Na_2HPO_4), 10 μg/ml papain, 0.002 M EDTA and 0.01 M cysteine was incubated at 37°C for two hours.

IgG(H) preparation. The IgG isolated from the rabbit serum and originally at pH 7 (IgG(S)), was dialyzed against a buffer at pH 2 (a small quantity of concentrated HCl was added to a 0.1 M citric acid + 0.2 M Na_2HPO_4 buffer at pH 3 until it reaches pH 2). After 12 h dialysis, the IgG were dialyzed back to pH 7 during 24 h (0.1 M citric acid + 0.2 M Na_2HPO_4, pH 7). All the dialysis were performed at 4°C. CD spectra, antigen precipitation curves and molecular weight estimation by SDS-polyacrylamide gel electrophoresis were performed to test the absence of IgG degradation during the pH treatment.

Results

In our experiments, the lipid monolayer was spread at an initial pressure of 20 dyn/cm. In these conditions, the high surface pressure increase can be attributed to protein penetration into the lipid monolayer and not to a spontaneous protein adsorption into some lipid-free sites at the interface. Indeed, at 20 dyn/cm, it is reasonable to admit that lipids used exist almost exclusively in the close packed state.

Fig. 1 shows that only IgG(H) induces a large change in surface pressure with all the lipids used. This surface pressure increase at constant area, if not directly correlated with the amount of protein penetrating the monolayer, indicates however to what extent a protein can overcome the lateral pressure between the lipids in order to insert itself beween them. Such a surface pressure increase has been observed with membrane proteins such as mellitin [15], glycophorin [16], the basic protein of myelin [17] and spectrin [18] which are able to insert hydrophobic segments between the lipid hydrocarbon chains. As shown in Fig. 1, IgG(H) penetrates preferentially unsaturated lipids bearing a negatively charged polar headgroup (cardiolipin, PS). Both the final surface pressure value and the initial penetration rate were maximal. For lipids with the same polar headgroup but with different degree of unsaturation in their hydrocarbon chains the initial rate of penetration at 29°C is slower with the saturated ones (DMPC,



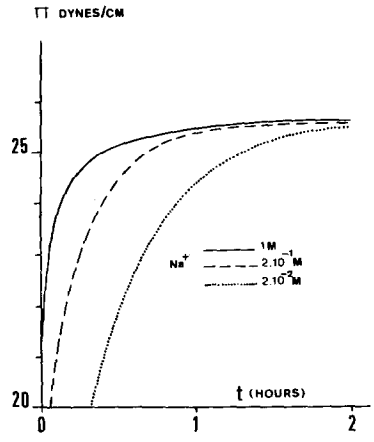


Fig. 1. Records of surface pressure versus time for lipid monolayer after IgG(H) (1 mg) injection into the subphase (100 ml 0.1 M citric acid + 0.2 M Na_2HPO_4, pH 7.0). No significative pressure increase was observed with IgG(S) under the same conditions. Temperature: 29°C. The initial surface pressure was in all the cases 20 dyn/cm. CHOL, cholesterol; CL, cardiolipin; GMO, glycerolmonoleate.

Fig. 2. Effect of the salt concentration (NaCl) on the interaction between IgG(H) and a monolayer of cardiolipin. Initial surface pressure is 20 dyn/cm. Temperature: 29°C.

DPPC) than with unsaturated (egg PC). The final surface pressure and the initial penetration rate were higher at 29°C for DMPC than for DPPC. At 29°C, DMPC is above its main phase transition temperature ($T_c = 23$°C) and DPPC exists in the rigid state ($T_c = 41$°C)

Thus, in the case of two lipid species, differing only by the length of their hydrocarbon chains and their transition temperature, the penetration proceeds much easier in the fluidest one.

To try to precise the relative contribution of the hydrophobic and electrostatic forces in the lipid-Ig interaction, the role of the ionic strength in the penetration process was investigated (Fig. 2). Low ionic strength would lower the shielding of the negative net charge of the polar headgroup (the two phosphates of cardiolipin) and increase charge repulsion between IgG molecule (which bear a slight negative net charge at pH 7) and the lipid polar headgroup. This repulsion could delay the IgG adsorption underneath the polar headgroup which is required prior to penetration into the lipid monolayer. This process could explain the slow Π increase, preceeded by a lag time, observed at low salt concentration. The electrostatic repulsion doen't however modify the final π value. This means that hydrophobic forces mainly, are involved in the final stabilization of the protein in the lipid monolayer.

Interaction of Ig with planar lipid bilayers offers

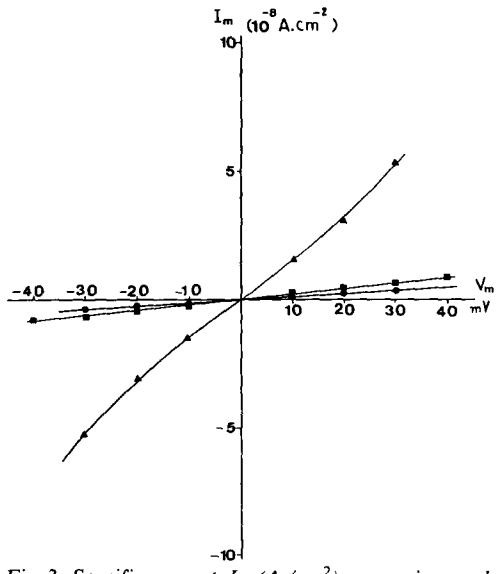


Fig. 3. Specific current I_m (A/cm^2) versus imposed potential difference V_m (mV) for a glycerolmonoleate planar bilayer. (■) IgG(S) at 0.14 mg/ml, (▲) IgG(H) at 0.14 mg/ml, (●) no protein added.

another way to confirm the capacity of IgG(H) to interact with the lipid. Addition of IgG(H) to each side of a planar lipid bilayer caused a 20-fold conductance increase whereas a 2-fold increase was observed with the IgG(S) form (Fig. 3). This conductance increase can be attributed to protein penetration into the planar lipid bilayer as observed with other proteins bearing hydrophobic sites [19], or with proteins that acquire an hydrophobic site after interaction with a receptor included in the bilayer [12,13]. It could be possible that the hydrophobic properties of IgG result from a drastic and irreversible unfolding of its globular domains during the momentary passage at pH 2 which is needed to obtain the IgG(H) form. Such a modification would bring to the surface hydrophobic amino acid segments which were normally burried inside the 'native' molecule. As shown elsewhere [11] circular dichroïsm spectrum of IgG at pH 2 is strongly modified. After return at pH 7, the CD spectrum is extremely similar to that of the 'native' form [11]. This would not be observed if the spatial structure of the IgG globular domains was irreversibly modified by the pH 2 treatment. It seems thus reasonable to admit that the hydrophobic properties of IgG(H) result from a very limited and localized conformational change of the molecule. This conformational modification is in any case, not sufficient to alter the Ig capacity to recognize its specific antigen [11]. Also the molecular weight determined by SDS-polyacrylamide gel electrophoresis shows that there is no detectable enzymatic degradation during the preparation of IgG(H) (data not shown). In order to localize the IgG molecule segment containing the hydrophobic site, monolayer measurements were performed with

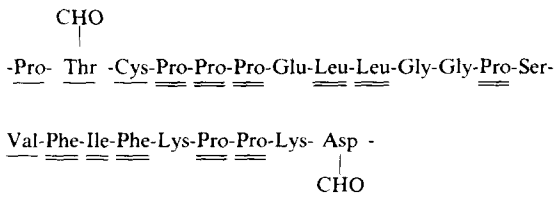


Fig. 4. Amino acid sequence of the hinge region of IgG. The hydrophobic amino acids are underlined once or twice according to the free energy of transfer of their side chain from an aqueous to an apolar medium. —, <2 kcal/mol, =, > 2 kcal/mol.

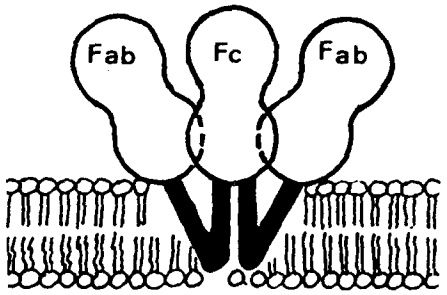


Fig. 5. Schematic view of the binding of the Ig(H) to the lipid bilayer.

the IgG Fab and Fc fragments. If IgG(S) cleaved by papaïn is submitted to the pH treatment, the so treated Fab and Fc fragments don't induce a surface pressure increase (data not shown) when injected under a DPPC monolayer (initial surface pressure: 20 dyn/cm). It seems thus that the enzymatic cleavage destroys a site of IgG which is able to acquire hydrophobic properties after the pH treatment. Since papaïn cleaves specifically the hinge region [14], the idea that the hydrophobic properties could be located in or near the hinge region appears attractive. A local effect on the hinge region seems more probable than a long range conformational effect acting on another part of the molecule. Indeed, neither Fab nor Fc have retained the hydrophobic properties of the entire molecule. This idea agrees with the fact that the hinge region contains a high proportion of hydrophobic amino acids (Fig. 4). Conformational prediction has revealed that an U-shaped form is highly probable [11]. This would facilitate the insertion of the hinge region in approximatively half the thickness of the lipid bilayer, the Fab and Fc being exposed to the bulk (Fig. 5).

Discussion

As we have previously suggested [11], our results indicate that IgG can exist under two main forms: IgG(H) which shows hydrophobic properties and IgG(S) which doesn't interact with lipids. CD control measurements indicate that the hydrophobic properties of IgG(H) are not the consequence of nonspecific denaturation extending through all the globular domain but is the consequence of local and limited conformational change.

As demonstrated by papaïn cleavage, the hydrophobic site is probably located in or near the hinge region. This region contains a high proportion of hydrophobic amino acids and can adopt an U-shaped form in solution as predicted by the conformational analysis. This form would facilitate the insertion of the hinge region into the lipid layer, the Fab and Fc being exposed to the bulk. The fact that IgG can be inserted by its hinge region in a lipid membrane would have serious implications in immunology. What kind of factor could induce the transconformation of IgG in the 'hydrophobic' form or vice versa? The presence of numerous proline in the hinge region could have some connection with the conversion IgG(S) to IgG(H). Indeed, it has been shown that the peptide bond adjacent to proline can isomerize either '*cis*' or in '*trans*' form [20]. It is interesting here to note that the proline isomeric state is strongly dependent on the polarity of the medium [20,21] and that the passage from one to the other is catalyzed by acid in the case of polyproline. Moreover a short peptide (*t*-butyloxycarbonylprolylleucylvalylmethyl ester) adopts two conformations according to its proline isomeric state, one of these conformations allowing its insertion into a model membrane [22]. It is therefore tempting to make the hypothesis that the hinge region conformational state depends on the isomerization of its proline. In a previous work [11], we have evaluated the activation energy of passage from IgG(H) to IgG(S). This energy was approx. 12 kcal/mol, which is not incompatible with the 20 kcal/mol generally reported for the *cis-trans* isomerization of proline peptide bond. This isomerization could induce a IgG(S) $\rightleftharpoons$ IgG(H) transconformation during the eventual contact between the hinge region and the highly non polar medium of lipid hydrocarbon chains or during passage through some acidic compartments of the cell.

Acknowledgements

We wish to thank R. Jeener, J. Urbain, L. Kanarek and J.L. De Coen for their fruitful collaboration. One of us (M.V.) thanks I.R.S.I.A. (Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture) for financial assistance.

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