Selected positions of acyl chains are affected differently by antibody binding which results in decreased membrane fluidity

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We have studied the interaction between monoclonal anti-trinitrophenyl antibodies (IgG1 and IgG2a) and haptenated phospholipid vesicles using stopped-flow fluorometry. Conformational changes of the antibodies were induced very rapidly (within 0.1 s) after binding to lipid haptens (TNP-Cap-DPPE) on the membrane surfaces. Conversely, after that, the bound antibody molecules decreased the degree of molecular motion at different depths in the bilayer, ranging from the polar head group to the terminal methyl groups of the fatty acyl chains. Such an effect reaches all places of the bilayer within 40 s at 25°C.

Membrane fluidity *Stopped-flow* *Liposome* *Antibody* *Lipid hapten* *Excimer fluorescence*

1. INTRODUCTION

In recent years, biophysical and biochemical systems have provided us with a great deal of information concerning properties of lipid bilayers. ESR, NMR and fluorescence polarization studies, for example, have been used to analyze dynamic motions (lateral diffusion and rotational motion) in lipid bilayers [1–4]. These methods have also been used to describe that a fluidity gradient exists along the acyl chain at different depths within the bilayers [1–4]. Such kinds of changes in membrane fluidity are considered to be very important for the process of recognition among cells of the immune system. To study such a problem, lipid vesicles containing specific antigens or lipid haptens have been introduced and a number of interesting works have been done in this field [5–9]. However, molecular properties of such membranes, after binding to antibodies or cells, have not been well defined yet [10–12].

Our aim is to describe our analysis of the change in membrane fluidity after binding to antibody molecules, using stopped-flow fluorometry. Monoclonal antibodies (IgG1 and IgG2a) were found to have rapidly induced conformational changes after binding to lipid haptens on the membrane surfaces. Conversely, after that, the bound antibody molecule decreased the degree of molecular motion in the bilayer, depending on selected positions along the acyl chains.

2. MATERIALS AND METHODS

Monoclonal antibodies (IgG1 and IgG2a) for a trinitrophenyl residue (TNP) were obtained from Dr M. Ueda (Kyoto University). Monoclonal antibodies were prepared as described by Köhler and Milstein [13]. A lipid hapten (TNP-Cap-DPPE) was obtained from Dr T. Yasuda (University of

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Abbreviations: DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PBS, phosphate-buffered saline; TNP-Cap-DPPE, trinitrophenylaminocaproylphosphatidylethanolamine; SUV, single unilamellar vesicle; pyrene-DPPEA, *N*-(1-pyrenesulfonyl)dipalmitoyl-L-α-phosphatidylethanolamine

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Tokyo). DMPC and DPPC were purchased from Sigma (St. Louis, MO). Three pyrene derivatives (1-pyrenedodecanoic acid, 1-pyrenedecanoic acid, 1-pyrenebutanoic acid) were purchased from Molecular Probe (Oregon). Pyrene-DPPEA was also from Molecular Probe.

SUVs were prepared by the injection of an ethanolic solution of a mixture of phospholipids (DMPC or DPPC) (99%) and a TNP lipid hapten (1%) into a PBS solution at 70°C [14,15]. Other SUVs for fluidity measurements were prepared from a mixture of a phospholipid (94.5%), a pyrene derivative (5%) and a TNP lipid hapten (0.5%) by the same method. SUVs prepared were checked by their trapped umbelliferone phosphate [16].

Fluorescence spectra were observed with a Hitachi model 650-10S fluorescence spectrophotometer. Stopped-flow fluorescence measurements were taken with a Union Giken RA-401 stopped-flow spectrophotometer in combination with an RA-450 microcomputer [17]. We used a Hoya Y46 cut filter (which allows only emitted light with wavelengths longer than 460 nm to enter the detector) in fluorescence measurements. The dead time of this instrument is 0.5 ms.

3. RESULTS

3.1. *Antibody binding to the lipid hapten on the membrane*

Fig.1 shows the stopped-flow fluorometric traces when IgG1 or IgG2a was mixed with a lipid hapten (TNP-Cap-DPPE) in the DMPC membrane. Antibody binding to the TNP haptens on the membrane surface reduced the tryptophan fluorescence intensity of these molecules. This is due to the fluorescence energy transfer from tryptophan residues of antibodies to the bound TNP residues. The rate constants of these reactions were almost independent of the concentration of antibodies (3.5×10^{-7}–2.8×10^{-6} M). This indicates that the reactions observed here were due to the conformational changes of antibody molecules after binding to the haptens, and not to diffusion-controlled encounter complex formation. The rate constants of the conformational changes on the membrane were very similar (halflife 20 ms). Nonspecific rabbit IgGs did not cause any such fluorescence changes on TNP-labeled SUVs as shown in fig.1c.

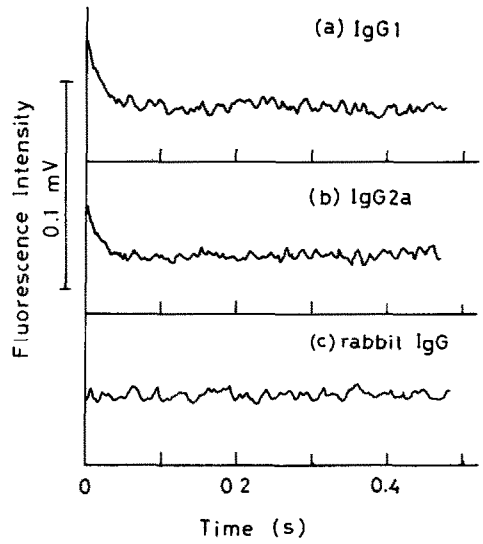

Fig.1. Stopped-flow fluorometric traces indicating conformational changes of IgG1 and IgG2a after binding to haptens in the membranes, at 25°C; excited at 280 nm. Emitted light with wavelengths longer than 300 nm was observed using a Hoya Y30 cut filter. (a) IgG1 (8.8×10^{-7} M) in PBS was mixed with DMPC SUVs (4.6×10^{-6} M), containing 1% TNP-Cap-DPPE, in PBS. (b) IgG2a (1.4×10^{-6} M) in PBS was mixed with DMPC SUVs (5×10^{-6} M), containing 1% TNP-Cap-DPPE, in PBS. (c) Nonspecific rabbit IgG (2.0×10^{-6} M) was mixed with DMPC SUVs (8.3×10^{-6} M), containing 1% TNP-Cap-DPPE, in PBS.

3.2. *Dependence of membrane fluidity on acyl chain length*

Fig.2 shows the fluorescence spectra of pyrene-loaded 'fluid' DMPC SUVs (containing 0.5% TNP-Cap-DPPE) in which the lateral diffusion coefficients of lipids are in the range of 10^{-7}–10^{-8} cm^2/s [18]. The spectra for the 3 species of pyrene derivatives, which form excimers in fluid biological membranes, gave monomer fluorescence peaks at 382 and 400 nm and a broad excimer fluorescence peak at 480 nm [19,20]. The intensity of excimer fluorescence in DMPC SUVs is in the following order: 1-pyrenedodecanoic acid > 1-pyrenedecanoic acid > 1-pyrenebutanoic acid. This difference is a direct reflection of the degree of molecular motion occurring at different depths in the bilayer, ranging from the polar head group

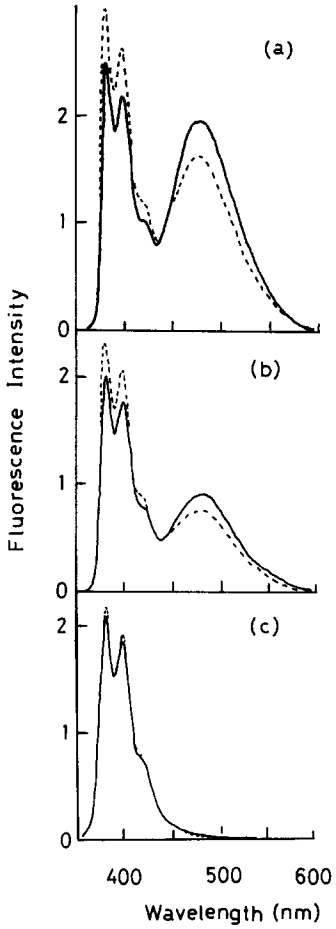

Fig.2. Fluorescence spectra of pyrene-loaded SUVs at 25°C. SUVs contained DMPC (94.5%), a pyrene derivative (5%), and TNP-Cap-DPPE (6×10^{-8} M, 0.5%). (——) Without antibodies, (– – –) with IgG2a. (a) SUV with 1-pyrenedodecanoic acid. (b) SUV with 1-pyrenedecanoic acid. (c) SUV with 1-pyrenebutanoic acid.

to the terminal methyl groups of the fatty acyl chains [1–4]. Similar results were also obtained using 'solid' DPPC SUVs, labeled with the 3 different pyrene derivatives, in which the lateral diffusion coefficients are of the order of 10^{-10} cm^2/s or less [18]. Thus, the excimer fluorescence described here is mostly due to the rapid flexing of acyl chains attached to the glycerol backbone of the phospholipid molecule or the rapid rotational diffusion about the long axis of the phospholipid, not due to the lateral movement of the phospholipid.

3.3. *Effect of antibody binding on membrane fluidity*

Fig.2 also illustrates the effect of the specific interaction of IgG2a with the TNP lipid hapten in membranes. The binding of IgG2a to the TNP residue caused a decrease in excimer fluorescence and an increase in monomer fluorescence of each pyrene derivative in membranes. These results reflect a decrease in membrane fluidity due to antibody binding. A control experiment with a nonspecific rabbit IgG showed no change in pyrene fluorescence. In the SUV sample labeled with 1-pyrenebutanoic acid, there was very little change in monomer and excimer fluorescence after antibody binding because the motion of this chromophore was very restricted even without an antibody. Similar results were obtained with DPPC SUVs, although the increase in monomer fluorescence and the decrease in excimer fluorescence were not as great as those seen with DMPC SUVs. Then, IgG1 binding to SUVs gave results similar to those when IgG2a was used (not shown).

3.4. *Kinetics of the change of membrane fluidity*

The kinetics of the change of membrane fluidity as a result of the antibody-TNP interaction on DMPC SUVs were next examined by stopped-flow fluorometry. DMPC vesicles, which contained 0.5% TNP-Cap-DPPE and 5% of a single pyrene derivative, were mixed with IgG2a. As can be seen in fig.3, there was a time-dependent decrease in excimer fluorescence intensity in SUVs with 1-pyrenedodecanoic acid and 1-pyrenedecanoic acid. Again, a control experiment with a nonspecific rabbit IgG showed no change in fluorescence intensity.

The changes in excimer fluorescence were mostly first-order processes. The IgG2a binding reactions (halflives) are 5 s for 1-pyrenedodecanoic acid and 1 s for 1-pyrenedecanoic acid at 25°C. These facts demonstrate that antibody binding reduces fluidity first in the outer parts of membranes and then such an effect is translated into the interior of the bilayers depending on acyl chain length. This was confirmed by the experiments using SUVs containing pyrene-DPPEA. In these SUVs the pyrene moiety, which lies close to the polar head group and may be located on the surface of the bilayer, had a considerable excimer fluorescence and their

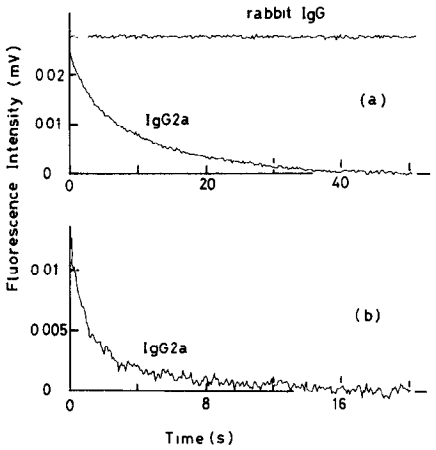

Fig.3. Stopped-flow fluorometric traces indicating the changes of membrane fluidity after antibody binding to SUVs at 25°C. SUVs contained DMPC (94.5%), a pyrene derivative (5%), and TNP-Cap-DPPE (6×10^{-8} M, 0.5%); excited at 340 nm. Emitted light with wavelengths longer than 460 nm was observed using a Hoya Y46 cut filter. (a) IgG2a (2.4×10^{-7} M) in PBS was mixed with SUV with 1-pyrenedodecanoic acid (6×10^{-7} M) in PBS. Nonspecific rabbit IgG showed no fluorescence change. (b) IgG2a (2.4×10^{-7} M) in PBS was mixed with SUV with 1-pyrenedecanoic acid (6×10^{-7} M) in PBS.

excimer fluorescence intensity was reduced too rapidly to be observed.

4. DISCUSSION

The present results demonstrate that the conformational changes of antibodies (IgG1 and IgG2a) occurred very rapidly (0.1 s) after binding to lipid haptens on the membrane surfaces. Conversely, after that, the bound antibody molecules decreased membrane fluidity dependent on the position of the acyl chain length. The effect of antibody binding is observed more quickly at the surface of the membranes and then goes through the terminus of hydrocarbon chains. After 40 s the effect reaches all places of bilayer membranes at 25°C.

It would be interesting to apply these findings to immunochemical systems on the membranes and determine whether antigen-antibody and ligand-receptor interactions cause a physical change in the cell membranes and whether such a physical change in the membrane can be correlated with a subsequent immune response.

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