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ROTATIONAL DYNAMICS OF IMMUNOGLOBULINS WITH FLUORESCENT HAPTENS ON A MEMBRANE SURFACE

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The rotational dynamics of rabbit immunoglobulin G with fluorescent lipid haptens on a membrane surface has been studied by nanosecond fluorescence emission anisotropic spectroscopy. It has been found that the rotational angles of the antibody are very restricted on the membrane, but that the rotation rate itself is not appreciably lower than that in solution, and is independent of the membrane fluidity.

There is considerable interest in the use of synthetic membranes to study the elementary molecular events involved in immune recognition and trigger [1–5]. Immunochemical studies with haptenated liposomes carrying specific antibodies provide strong evidence that the activations of both compliments and macrophages are dependent on the physical state of the membrane [6,7]. Thus, the lateral motions and distributions of membrane components are known to play significant roles in the immune responses of haptenated liposomes. Quite recently, the utility of fluorescent lipid haptens has been shown to be useful for such immunochemical and immunophysical studies of model membranes [8,9]. We have shown, for example, that the rate of binding reaction of an antibody molecule with haptens in model membranes is also dependent on the membrane fluidity [8,10].

In the present study, we attempted to investigate the rotational motions of immunoglobulins with haptens in membranes by nanosecond fluorescence depolarization. This technique has been used for rotational dynamics of a wide variety of immunoglobulins in solutions, but never for immunoglobulins on membranes [11–15].

A fluorescent lipid was prepared from *N*-iodoacetyl-*N'*-(5-sulfo-1-naphthyl)ethylenediamine (1,5-I-AEDANS) from Sigma by a method similar to that previously reported [2,8]. An anti-1,5-AEDANS immunoglobulin G was prepared from rabbit antisera by the use of keyhole limpet hemocyanin alkylated with iodo-1,5-AEDANS. This was purified by adding a solution of saturated ammonium sulfate to a final concentration of 40% of saturation. The precipitate was collected by centrifugation and was dialyzed against phosphate-buffered saline for a few days at 4°C. Then it was further purified by DEAE-cellulose ion exchange chromatography. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified IgG in nonreducing gels revealed only one major band of molecular weight 150000. Fab fragments of anti-1,5-AEDANS IgG were generated by papain digestion [16]. The molecular weight of the Fab fragment was approximately 50000. Two kinds of single vesicles of haptenated liposomes

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Abbreviations: 1,5-AEDANS, *N*-acetyl-*N'*-(5-sulfo-1-naphthyl)ethylenediamine.

were prepared from multilamellar liposomes by sonication [17]. One from a mixture of 1% 1,5-AEDANS-labelled phosphatidylethanolamine (1,5-AEDANS-PE) and 99% dipalmitoylphosphatidylcholine (DPPC) in phosphate-buffered saline, and the other from a mixture of 1% 1,5-AEDANS-PE and 99% dimyristoylphosphatidylcholine (DMPC) in phosphate-buffered saline. They were obtained through ultracentrifugation.

The nanosecond time-dependent fluorescence depolarization was observed by a single photon counting fluorometer as described previously [18].

Results and Discussions

In the fluorescence spectrum (excited at 340 nm) of a membrane-bound fluorescent hapten (1,5-AEDANS-PE) and free hapten (1,5-AEDANS-cysteine), the emission peaks were found at 490 nm in an aqueous solution [8,19]. The fluorescence quantum yield of these haptens increased markedly on binding to the anti-1,5-AEDANS antibody and the emission maximum of the haptens were shifted to shorter wavelength (460 nm). The fluorescence anisotropy of these haptens does not depend upon the excitation wavelength in the 360–400 nm region. Quantum yield and fluorescence anisotropy increase in a hyperbolic manner as a function of the antibody concentrations and they reach plateau at the high concentrations. IgG from nonimmunized control rabbits (Sigma) showed neither detectable fluorescence increase nor fluorescence anisotropy change. The affinity of an anti-1,5-AEDANS IgG for 1,5-AEDANS-cysteine was estimated to be 10^7 M^{-1}.

The results of the nanosecond time-dependent fluorescence depolarization are shown in Fig. 1. Their emission anisotropy curves could be fitted to sum of two exponential terms and a constant term:

$$A(t) = A_0[a_1 \exp(-t/\phi_1) + a_2 \exp(-t/\phi_2) + a_3] \quad (1)$$

where A_0 value was estimated as $A_0 = 0.357$, based on the fluorescence decay observed of 1,5-AEDANS-cysteine in glycerol at 0 °C. However, these fits are by no means unique. Similar but slightly less fitted $A(t)$ curves with fewer terms can be also produced. In contrast, the mean rotational

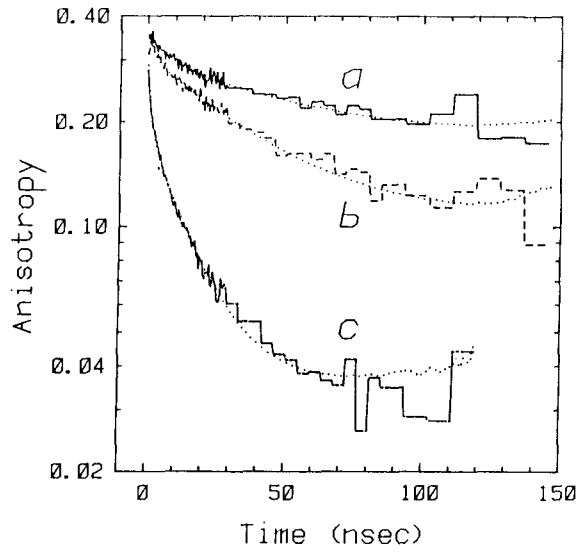

Fig. 1. Nanosecond emission anisotropy kinetics of 1,5-AEDANS-PE on a DPPC membrane at 35 °C (excited at 380 nm). (a) bound to IgG; (b) bound to Fab; (c) unbound. The dotted lines were calculated using the values shown in Table I.

correlation time $\langle\phi\rangle$, given by

$$\langle\phi\rangle = (a_1\phi_1 + a_2\phi_2)/(a_1 + a_2) \quad (2)$$

is well-defined. For IgG and Fab on the DPPC membrane, the mean rotational correlation time $\langle\phi\rangle$ were 42.9 and 31.8 ns, respectively. These results are summarized in Table I.

As is seen in Fig. 1, the decay of the emission anisotropy has an initial rapidly decreasing phase and then nearly constant one. The presence of the finite residual anisotropy, a_3, suggests that the rotations of the IgG and Fab on the DPPC membranes are restricted to a certain angle at least in the time range up to 200 ns. As the excess of IgG or Fab were added to the haptenated liposomes, one combining site of the IgG must be liganded here. The rotational motion of IgG is found to be more restricted on the membrane surface than that of Fab.

Similar experiment were made with the IgG or Fab bound lipid haptens on the DMPC membrane. The emission anisotropy curves of lipid hapten bound to the anti-1,5-AEDANS IgG or Fab on the DMPC membrane were almost equal to those on the DPPC membrane. This fact suggest that the rotational motions of IgG with

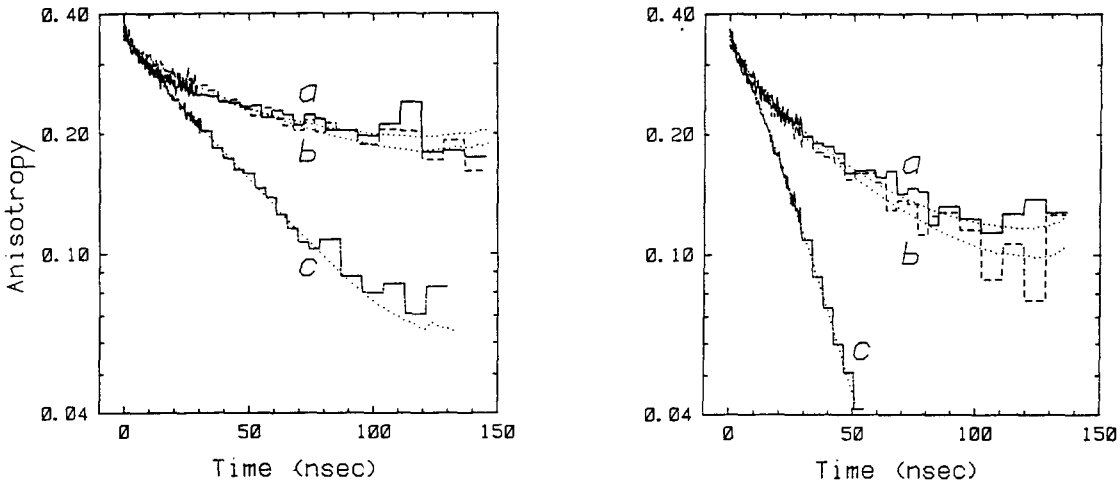

Fig. 2. Nanosecond emission anisotropy kinetics of 1,5-AEDANS-PE in a membrane and 1,5-AEDANS-cysteine (excited at 380 nm). (Left) (a) 1,5-AEDANS-PE in a DPPC membrane bound to IgG; (b) 1,5-AEDANS-PE in a DMPC membrane bound to IgG; (c) 1,5-AEDANS-cysteine bound to IgG. (Right) (a) 1,5-AEDANS-PE in a DPPC membrane bound to Fab; (b) 1,5-AEDANS-PE in a DMPC membrane bound to Fab; (c) 1,5-AEDANS-cysteine bound to Fab. The dotted lines were calculated using the values shown in Table I.

haptens in membrane are almost independent of the membrane fluidity.

For comparison, the nanosecond fluorescence and emission anisotropy kinetics were also examined of 1,5-AEDANS-cysteine bound to anti-1,5-AEDANS IgG or Fab in solution (Fig. 2). These results are well consistent with the previous ones carried out with both polyclonal and monoclonal antibodies [11–15]. The initial decay of the anisotropy of the hapten (in solution) bound to IgG was nearly equal rate with that of membrane hapten bound IgG (see the time range 0 to 10 ns in Fig. 2). This was the case also for the anisotropy of Fab. As the initial decay rate of the fluorescence anisotropy is proportional to the rotational rates of the fluorescent molecule [20], it may be concluded that the rotational rates of IgG are not greatly different irrespective whether it is in solu-

TABLE I

RESULTS OF FLUORESCENCE MEASUREMENTS (AT 35 °C)

Membrane or solution	Antibody	Lifetime (ns)	$\langle\phi\rangle$ (ns)	a_1	ϕ_1 (ns)	a_2	ϕ_2 (ns)	a_3	Rotational diffusion constant (D_w,ns^{-1})	Cone angle (θ_c)
DPPC-membrane	IgG	19.0	42.9	0.17	4.6	0.35	60.9	0.48	0.0027	38.9°
	Fab	19.1	31.8	0.17	1.9	0.55	41.3	0.28	0.0054	50.2°
	none	15.6	6.4	0.52	0.6	0.38	14.1	0.10	(0.036) [a]	(64.0°) [a]
DMPC membrane	IgG	19.8	59.7	0.12	6.0	0.50	72.0	0.38	0.0024	44.0°
	Fab	19.4	43.6	0.25	6.6	0.58	59.2	0.17	0.0047	57.7°
	none	16.1	4.9	0.52	0.6	0.46	9.8	0.02	(0.052) [a]	(76.2°) [a]
Solution	IgG	21.1	58.5	0.11	7.2	0.89	64.6		0.0028	180°
	Fab	21.1	21.3	1.0	21.3				0.0078	180°

[a] The transition moment of the free hapten on the membrane was assumed to wobble in a cone of semi-angle θ_c with a rotational rate of D_w.

tion or on the membrane surface, whereas the rotational angles of IgG are very restricted on the membrane.

In order to obtain further information of the dynamics of IgG on the membranes, we estimated the rotational angle and the rotation rate of antibody using the 'Wobbling in cone' model [20]. We assumed that the long axis of the Fab arm is confined with a cone, whose semi-angle is θ_c and fluctuates with this cone with a wobbling diffusion constant, D_w. When the transition moment of 1,5-AEDANS residue is parallel to the long axis of Fab arm, the D_w and θ_c are related with the observed $\langle\phi\rangle$ and a_3, as was previously shown [20], and are given in the last two columns of Table I. When the transition moment of 1,5-AEDANS residue is not parallel to the long axis of the Fab arm the value of θ_c are smaller than those in Table I [21].

In conclusion the rotational angles of the antibody are very restricted on the membrane but the rotation rate itself is not appreciably lower than that in solution.

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