A Nanosecond Fluorescence Depolarization Study on the Segmental Flexibility of Receptor-Bound Immunoglobulin E†

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ABSTRACT: Time-resolved fluorescence anisotropy measurements have been used to examine the segmental flexibility of anti-dansyl immunoglobulin E (IgE) bound to its high-affinity receptor on membrane vesicles from rat basophilic leukemia cells. Although IgE in this complex exhibits only a restricted angular range of segmental motion, much of this restricted motion occurs on a relatively rapid time scale. A fast component of motion with a rotational correlation time of 15–35 ns may correspond to the twisting of Fab segments about their major axis. Intermolecular cross-linking by a short bivalent ligand, *N,N'*-didansylcadaverine, results in complete loss of this segmental motion. Solubilization of monomeric IgE–receptor complexes using a zwitterionic detergent results in a time-dependent anisotropy decay that exhibits both a fast component and a slower component that is intermediate between the decay for soluble and membrane-bound forms of IgE at long times after excitation. These results are discussed in terms of a model in which binding of IgE to its membrane-bound receptor restricts not only its global rotation but also its slower modes of segmental flexibility as well, while allowing its Fab segments to undergo rapid reorientation within a limited angular range.

IgE[1] plays a central role in the allergic response by binding to high-affinity receptors ($Fc_\epsilon RI$) on mast cells and basophils and thereby sensitizing those cells to foreign antigens, or "allergens", that are recognized by the antibody combining sites of receptor-bound IgE. This antibody is composed of two heavy (ϵ) chains, each with one variable and four constant domains, in addition to two light chains that form a typical Y-shaped molecule similar to structure to the more prevalent IgG class of antibodies. A single IgE molecule binds to each $Fc_\epsilon RI$ receptor with high affinity ($K_a \geq 10^{10}$ M^{-1}; Kulczycki & Metzger, 1974), and cross-linking of IgE–receptor complexes by bivalent or multivalent antigens initiates a complex series of biochemical changes in the cell that lead to the release of histamine and other mediators during an exocytotic response (Metzger et al., 1986).

Spectroscopic studies have indicated that the average conformation of IgE is altered upon binding to $Fc_\epsilon RI$ (Holowka et al., 1985), such that the Fab segments project outward with the antibody combining sites at their tips ≥100 Å from the cell surface (Baird & Holowka, 1985), while the Fc segment is bent out of the IgE 2-fold axis of symmetry, and disulfide bonds in the hinge domain of the IgE molecule, $C_\epsilon 2$, are ≤50 Å from the cell surface (Baird & Holowka, 1988; see Figure 1). Recent studies that have employed genetic engineering of rodent IgE demonstrate that the C-terminal $C_\epsilon 4$ domains of the ϵ chains do not participate in the binding to $Fc_\epsilon RI$ (Baird et al., 1989). Studies of polypeptide fragments of human IgE exressed in *Escherichia coli* have indicated that a 76 amino acid sequence that overlaps the junction between the $C_\epsilon 2$ and $C_\epsilon 3$ domains is sufficient to mimic the binding of intact IgE (Helm et al., 1988).

The nature and extent of segmental flexibility in receptor-bound IgE is probably an important factor in determining the ability of bivalent and multivalent ligands to cross-link IgE–receptor complexes in an active aggregate. Recent studies have indicated that some forms of cross-linking are less efficient than others in mediating signal transduction (Kane et al., 1988; Ortega et al., 1988), so that different structural constraints may play a major role in determining effective receptor aggregation. A previous steady-state fluorescence polarization study that employed a monoclonal anti-5-(dimethylamino)naphthalene-1-sulfonyl (dansyl) IgE indicated that receptor-bound IgE retains at least some of the segmental flexibility that is observed with IgE in solution (Slattery et al., 1985). This method yields a single rotational correlation time that is a complex average of all of the modes of motion that the bound dansyl probe experiences.

In order to gain greater insight into the modes of motion responsible for fluorescence depolarization of the dansyl hapten bound in the antibody combining site, we have carried out time-resolved nanosecond depolarization experiments on anti-dansyl IgE bound to $Fc_\epsilon RI$ receptors before and after solubilization of plasma membranes derived from rat basophilic leukemia (RBL) cells. These studies indicate that segmental motion is fairly restricted for membrane-bound IgE, but there is a mode of motion with a correlation time of 15–35 ns that contributes substantially to the fluorescence depolarization observed. The significance of the likely modes of motion available to receptor-bound IgE for the function of that complex is discussed.

Materials and Methods

IgE and RBL Cell Membranes. Monoclonal anti-dansyl IgE from hybridoma cell line 27-74 purified by affinity chromatography as previously described (Dangl et al., 1988)

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[1] Abbreviations: IgE, immunoglobulin E; dansyl-Lys, N^ϵ-[[5-(dimethylamino)naphthalen-1-yl]sulfonyl]-L-lysine; RBL, rat basophilic leukemia; CHAPS, 3-[[(3,12-dihydroxy-5β-cholan-24-amido)propyl]dimethylammonio]-1-propanesulfonate.

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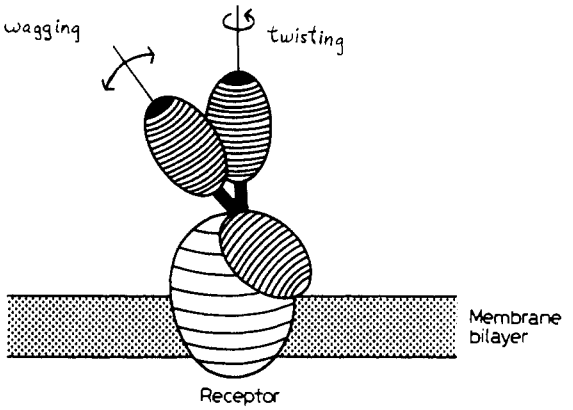

FIGURE 1: Hypothetical model for the IgE–receptor complex associated with the plasma membrane illustrating two major modes of segmental motion for the Fab regions [adapted from Holowka et al. (1985)].

and generously provided by Dr. V. T. Oi (Becton Dickinson Monoclonal Antibody Center, Mountain View, CA). This antibody was further purified by chromatography on a Du Pont Zorbax GF-450 gel permeation column to remove small amounts of higher molecular weight aggregates or proteolytic fragments (Slattery et al., 1985). Plasma membrane vesicles were induced to form and pinch off from adherent RBL cells as previously described (Slattery et al., 1985). Their properties and those of the receptor for IgE associated with them have been extensively characterized (Holowka & Baird, 1983; Holowka & Baird, 1984). Receptors on these vesicles were occupied by anti-dansyl IgE as previously described (Slattery et al., 1985). Control samples containing receptor occupied by anti-dinitrophenyl IgE, which does not bind dansyl-L-lysine (dansyl-Lys), were prepared in parallel to those containing specifically bound anti-dansyl IgE.

The number of anti-dansyl combining sites in each preparation was determined by titrating the membrane vesicles containing bound anti-dansyl IgE with dansyl-Lys, while monitoring the fluorescence of bound dansyl-Lys at 500 nm (Reidler et al., 1982) by using an SLM 8000 steady-state fluorimeter. The K_d for dansyl-Lys binding to membrane-bound IgE was found to be indistinguishable from that previously reported for IgE in solution (17 nM; Oi et al., 1984). Samples for anisotropy measurements typically had ~60–80% of their combining sites occupied with dansyl-Lys (~50 nM bound hapten); the concentration of dansyl-Lys in the control sample was adjusted to be equal to the concentration of unbound dansyl-Lys in the sample with receptor-bound anti-dansyl. Specifically bound dansyl-Lys typically contributed 40–50% of the total fluorescence signal at 500 nm, while unbound dansyl-Lys contributed ~5%.

Solubilized IgE–receptor complexes were prepared by incubating membrane samples containing dansyl-Lys with 10 mM 3-[[(3,12-dihydroxy-5β-cholan-24-amido)propyl]dimethylammonio]-1-propanesulfonate (CHAPS) for 10 min at 4 °C and then removing any insoluble residue by centrifugation for 10 min at 50000*g* in a Beckman airfuge (Palo Alto, CA). In these samples, the background signal was greater than that for membranes in the absence of detergent, and specifically bound dansyl-Lys contributed ~25% of the total fluorescence intensity, while unbound dansyl-Lys contributed ~10% of this total intensity. At least 50% of the membrane-bound anti-dansyl binding activity was recovered in the CHAPS-solubilized extracts.

Time-dependent anisotropy decay measurements were carried out by using a picosecond pulsed dye laser system described previously (Reidler et al., 1982), with a 780-nm

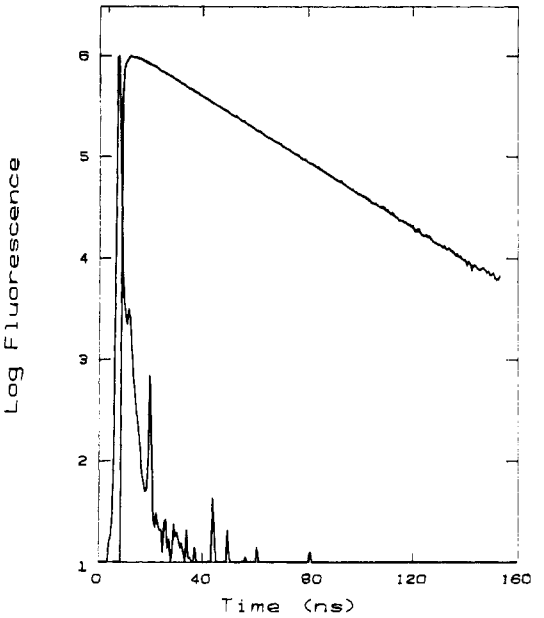

FIGURE 2: Fluorescence lifetime decay for dansyllysine bound in the antibody combining sites of membrane-bound IgE corrected for background signal by using eq 2 as described in the text. The straight line through the data points represents a single exponential decay with a fluorescence lifetime of 27 ns. The irregular curve at the left indicates the lamp profile.

output frequency doubled to 340 nm for sample excitation, with <30-ps full width full-maximal pulses and with an instrument response time of <1 ns at 0.8 MHz. Emitted light was filtered with a wide-band interference filter (40 nm bandwidth) centered at 500 nm (Ditric Corp., Hudson, MA) and detected with a single photon counting apparatus as previously described (Wensel et al., 1988). Samples (200 μL) were thermostated at 20 °C, and data were typically collected for 120 min for each sample, with emission polarizers electronically switched between vertical and horizontal positions every 30 s. It was determined that the anisotropy decay curves of these membrane suspensions remained constant for at least 60 min without mixing, so samples were mixed every 60 min to prevent potential artifacts due to settling of membrane vesicles.

Decay curves for both types of emitted light were collected from control samples (those without specifically bound anti-dansyl IgE) for the same time interval as from the anti-dansyl samples, and these data sets were used to correct for background fluorescence and scattered light according to the relationship:

$$A(t) = \frac{[I(t)_{\parallel} - I'(t)_{\parallel}] - [I(t)_{\perp} - I'(t)_{\perp}]}{[I(t)_{\parallel} - I'(t)_{\parallel}] + 2[I(t)_{\perp} - I'(t)_{\perp}]} \quad (1)$$

where $A(t)$ is the anisotropy at time t, $I(t)_{\parallel}$ and $I'(t)_{\parallel}$ are the vertically polarized emission intensities for anti-dansyl and control samples, respectively, and $I(t)_{\perp}$ and $I'(t)_{\perp}$ are the horizontally polarized emission intensities for these samples. Prior to calculation of the anisotropy decay curves, small corrections for possible drift in the pulse rate between anti-dansyl sample and control data collection or for slight differences in the concentration of membrane vesicles and/or free dansyl-Lys were made by scaling the control data set by ≤10% and fitting the total fluorescence decay, $F(t)$, such that

$$F(t) = [I(t)_{\parallel} - I'(t)_{\parallel}] + 2[I(t)_{\perp} - I'(t)_{\perp}] \quad (2)$$

yielded the best fit to a single exponential decay with a lifetime $\tau = 27$ ns (Reidler et al., 1982; Slattery et al., 1985). Figure 2 shows the quality of this fit for membrane-bound anti-dansyl

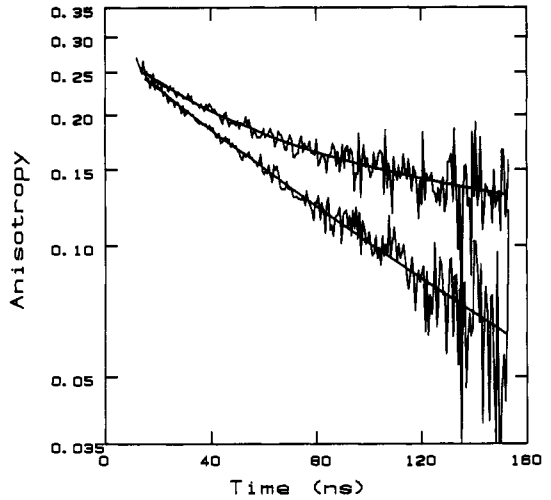

FIGURE 3: Fluorescence anisotropy decay for dansyllysine bound in the antibody combining sites of membrane-bound IgE (upper curve) or soluble IgE in the presence of membranes preoccupied with irrelevant IgE (lower curve). The smooth curves through the data represent the best fits to eq 3 as described in the text. The concentration of IgE in these samples was 30 nM.

IgE. For membrane-containing samples, the background correction made little difference in the shape of the anisotropy curves at long times (>50 ns), and small changes in the scaling factor used (≤2%) did not cause significant changes in the shape of the anisotropy decay curves even at shorter times. Application of this correction procedure for well-matched samples and controls resulted in consistently reproducible anisotropy decay data among several different preparations for each of the experiments reported.

Anisotropy decay curves were analyzed by a Marquardt nonlinear least-squares fitting routine using a two-exponential function:

$$A(t) = \alpha_s \exp(-t/\phi_s) + \alpha_l \exp(-t/\phi_l) \quad (3)$$

where α_s and α_l are the preexponential weighting factors for the shorter rotational correlation time, ϕ_s, and the longer rotational correlation time, ϕ_l, respectively. For some purposes, ϕ_s was held constant at a predetermined value and α_s, α_l, and ϕ_l were determined from the least-squares analysis.

Results

Figure 3 shows the time-dependent anisotropy decay for dansyl-Lys bound in the antibody combining sites of membrane-associated monoclonal anti-dansyl IgE (upper curve), together with that for anti-dansyl IgE in solution obtained in the presence of RBL cell membrane vesicles that were preblocked with an irrelevant IgE (lower curve). This lower curve is very similar to that described previously for this anti-dansyl IgE in solution at much higher concentrations and in the absence of membranes (Oi et al., 1984). This indicates that potential artifacts due to improper correction of contributions from unbound dansyl and the highly light scattering membrane suspensions do not significantly influence the shape of the anisotropy decay curve. A slightly lower value for initial anisotropy is often noted in the presence of membranes due to scatter-dependent depolarization of the emitted fluorescence, but this has been shown not to affect the values of the rotational correlation times obtained (Slattery et al., 1985).

The anisotropy decay for anti-dansyl IgE bound to its high-affinity receptor on RBL cell membrane vesicles (upper curve, Figure 3) shows much more curvature in its logarithmic decay curve than does that for the same IgE in solution (lower curve, Figure 3), and the anisotropy of membrane-bound anti-dansyl does not decay below a value of ~0.12 even at t = 150 ns. These results indicate that the dansyl hapten bound to membrane-associated IgE–receptor complexes exhibits much more restricted angular depolarization than that bound to IgE in solution. At least some of this difference is probably due to the anticipated large increase in rotational correlation time for global tumbling of membrane-bound IgE (see Discussion). Smooth curves through the data points in Figure 3 represent best fits to two-exponential decay curves (see Materials and Methods), and the parameters for these fits are summarized in Table I. The shorter correlation time from these fits, ϕ_s, is similar but slightly larger for IgE in solution (48 ns) than that for IgE bound to its receptor in membranes (34 ns), and these correlation times have a similar normalized preexponential weighting factor, ~0.3. The longer correlation time, ϕ_l is markedly greater for membrane-bound IgE (438 ns) than for IgE in solution (125 ns), and the weighted arithmetic average correlation time ($\langle\phi\rangle$ = 309 ns, Table I) reflects this large difference in ϕ_l.

Table I: IgE–Receptor Complexes: Summary of Fits for the Anisotropy Decay Data[a]

sample	α_s'	ϕ_s (ns)	α_l'	ϕ_l (ns)	$\langle\phi\rangle$	χ^2/N
IgE in solution	0.30	48	0.70	125	102	1.9
IgE–receptor complexes on membranes	0.32	34	0.68	438	309	2.1
solubilized IgE–receptor complexes	0.31	18	0.69	173	125	2.0

[a] Fits are shown in Figures 3 and 5, and the fitting procedure is described under Materials and Methods. α_s' and α_l' are the normalized preexponential weighting factors [$\alpha_s' = \alpha_s/(\alpha_s + \alpha_l)$ and $\alpha_l' = \alpha_l/(\alpha_s + \alpha_l)$], $\langle\phi\rangle$ is the arithmetic average rotational correlation time ($\langle\phi\rangle = \alpha_s'\phi_s + \alpha_l'\phi_l$), and χ^2/N is a relative indicator of the goodness of fit [$\chi^2 = \sum[A(t)\,F(t) - B(t)]^2[[I(t)_\parallel - I'(t)_\parallel] + 4\,[I(t)_\perp - I'(t)_\perp]]$, where $B(t)$ is the best fit function of the raw data $A(t)\,F(t)$ and N is the total number of data points].

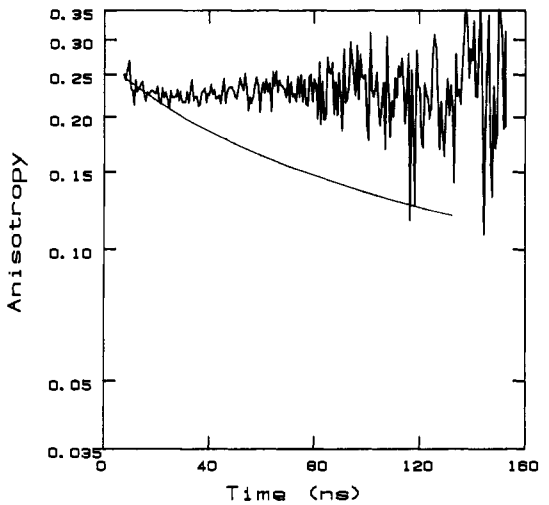

FIGURE 4: Fluorescence anisotropy decay is shown for didansylcadaverine bound in the antibody combining sites of membrane-bound IgE. The lower smooth curve shows the normalized best fit of the data for dansyllysine bound to membrane-associated IgE from Figure 3 (A = 0.27 at t = 7.5 ns).

Figure 4 shows the anisotropy decay curve for membrane-bound IgE–receptor complexes that have been cross-linked by the bivalent ligand N,N'-didansylcadaverine. Titration of this ligand into a sample of anti-dansyl IgE on RBL cell membranes showed nearly stoichiometric binding of both dansyl groups at the concentration of ligand used in the experiment, indicating that cross-linking of IgE–receptor complexes must be occurring very efficiently. The short length of this bivalent ligand (~10 Å between dansyl groups) makes intramolecular bridging between combining sites on the same IgE molecule

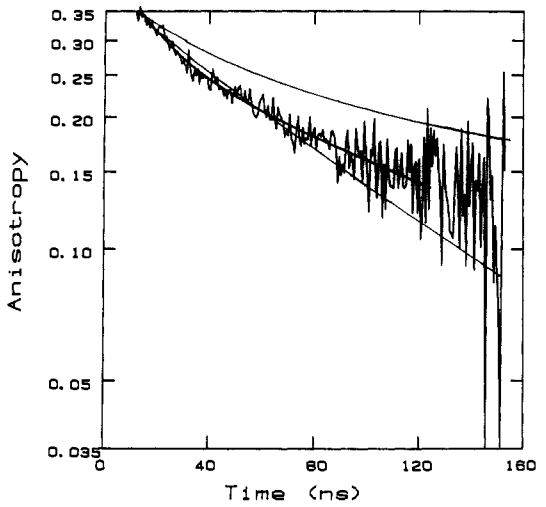

FIGURE 5: Fluorescence anisotropy decay for dansyllysine bound to IgE–receptor complexes solubilized from membrane vesicles with 10 mM CHAPS. The smooth curve through the data is the best fit to eq 3, and the upper and lower smooth curves are normalized best fits for membrane-bound and soluble IgE from Figure 3 (A = 0.25 at t = 12.9 ns).

very unlikely (Kane et al., 1988), and gel permeation chromatography of 1:1 complexes between this bivalent ligand and anti-dansyl IgG that has the same combining sites (Oi et al., 1984) indicates that the formation of didansylcadaverine-mediated cyclic dimers of these antibodies occurs very readily (data not shown). The data in Figure 4 show that the limited anisotropy decay seen for the monovalent dansyl ligand bound to membrane-associated IgE (smooth curve, Figure 4) is completely abrogated by the use of the bivalent dansyl ligand, indicating that the segmental motion of IgE giving rise to this dansyl fluorescence anisotropy decay is virtually eliminated when the Fab segments are involved in cross-links.

In order to investigate the contribution of membrane association to the restricted segmental motion seen for IgE–receptor complexes in Figure 3, the membranes were solubilized in the zwitterionic detergent CHAPS, and the anisotropy of the solubilized IgE–receptor complexes was measured. The data in Figure 5 indicate that the initial anisotropy decay is at least as fast as that seen with IgE in solution, and at longer times the decay is intermediate between the curves for soluble IgE and membrane-associated IgE–receptor complexes. There is more uncertainty in the earliest part of the decay curve for solubilized IgE–receptor complexes than for membrane-bound complexes due to the potential contribution of detergent-associated dansyl-Lys that exhibits more substantial background fluorescence with a short fluorescence lifetime in the control sample (data not shown), but the distinct curvature of this anisotropy decay over the measurable range was reproducibly observed in experiments from several different preparations.

The best fit to the data for CHAPS-solubilized IgE–receptor complexes is shown as the smooth curve through the data in Figure 5, and the parameters are listed in Table I. In this case, the shorter correlation time (18 ns) is less than that for either IgE in solution or IgE bound to receptors on membranes, but the normalized preexponential weighting factors (0.3) is similar for all three. As expected from a visual inspection of the data, the longer correlation time for solubilized IgE–receptors (173 ns) is intermediate between that for the other two samples, as is the average correlation time, $\langle\phi\rangle$ = 125 ns. The value for ϕ_l is somewhat less than 240 ns, the value expected for the global tumbling of the $M_r \simeq 280$K IgE–receptor complex (Neuman et al., 1976) that is associated with a $M_r \simeq 6$K micelle of CHAPS (Hjemeland et al., 1983), suggesting that some segmental motion may contribute to this component of the anisotropy decay as well. These data indicate that at least some of the restriction of segmental motion observed for membrane-bound IgE is due to the association of IgE–receptor complexes with the intact membranes.

Discussion

The time-dependent anisotropy studies described in this report are the first that have been carried out on an antibody molecule bound to a mammalian cell surface receptor. Using the laser excitation of the dansyl probe bound in the antibody combining sites of receptor-bound IgE, we have been able to obtain satisfactory anisotropy decay data with ~2-h collection time from highly light scattering membrane suspensions in which the probe concentration is ≤50 nM. These experiments take advantage of the high-affinity interaction between IgE and $Fc_\epsilon RI$ receptors (Kulczycki & Metzger, 1974) and the ~30-fold enhancement of dansyl fluorescence when it is bound to the antibody combining sites (Reidler et al., 1982). Under the conditions of these experiments, free dansyl-Lys interacts very little with the plasma membrane vesicles in the absence of IgE, so that corrections for fluorescence due to dansyl-Lys not bound to the antibody combining sites are very small. Dansyl-Lys does exhibit more significant fluorescence in the presence of micellar detergents, but the control samples that lack receptor-bound IgE provide an adequate means of correcting for this background signal.

The high value of anisotropy observed for membrane-bound IgE even after 150 ns may indicate that the segmental flexibility of this IgE is more restricted that that of IgE in solution. Although interpretation of the immunoglobulin anisotropy decay curves in terms of particlar modes of motion is not straightforward, an assessment of the significance of the differences between solution and membrane-bound IgE can be made by analyzing the results in terms of two different models for probe reorientation. In the first model (Yguerabide, 1972), the probe is considered to be bound to a rotating sphere (representing the Fab segment) which is restricted to a range of angles so that at very long times the decay curve approaches a constant (nonzero) value of the anisotropy. The data are consistent with this model, as the curve appears to be flattening out at times longer than 100 ns. However, the limited lifetime of the probe (27 ns) prevents reliable analysis of data collected after 150 ns. Preliminary data with a longer lived pyrene derivative of IgE (Slattery et al., 1985) suggest that the anisotropy does in fact approach a limiting value at times longer than 200 ns, rather than decaying to zero (D. Holowka, unpublished results). For the dansyl-Lys probe, if it is assumed that the value of A at 150 ns (0.12) is the limiting value, then the average angular excursion can be estimated to be 36°, which is $\sim^2/_3$ of the value for a fully depolarized molecule.[2]

Alternatively, the data can be considered as representing two principal modes of rotation, each contributing one of two components (ϕ_s and ϕ_l) in a biexponential fit as displayed in Figure 3. This model is particularly useful for comparing the present results to those obtained with immunoglobulins in solution, which have commonly been analyzed by using this model. For IgE in solution the longer component (ϕ_l = 125 ns) is shorter than the value expected for an "equivalent sphere" with the same sedimentation coefficient as IgE ($\phi \simeq$

[2] The average angle $\phi(t)$ through which a sphere rotates can be calculated from (Yguerabide, 1972)

$$\phi(t) = \cos^{-1}\,[1/3[1 + 2A(t)/A_0]]^{1/2}$$

where A_0 is the initial anisotropy (0.25) and A(150 ns) = 0.12.

155 ns; Slattery et al., 1985). Thus, there may be some internal modes of motion contributing to this correlation time in addition to the contribution due to global tumbling. The value of the shorter correlation time (ϕ_s = 48 ns) is intermediate between that expected for the fastest mode of motion that a tethered Fab could experience (i.e., twisting about its major axis) and that expected for slower wagging motion about the point of attachment to the Fc (Figure 1). Both twisting of the Fab about its major axis as well as possible "elbow bending" between the variable and constant domains of the Fab have been predicted to have correlation times of ~15–25 ns for unhindered rotations (Hanson et al., 1981). Wagging or "scissoring" motion of an Fab attached to the Fc by a "universal joint" is predicted to have a correlation time of 50–90 ns (Wegener et al., 1980; Hanson et al., 1981).

Previous studies had indicated that IgE in solution has more limited segmental flexibility than IgG subclasses (Oi et al., 1984; Slattery et al., 1985), and this has been correlated with the inability of IgE to fix complement (Oi et al., 1984) and rationalized as a consequence of its lack of a true hinge region separating the Fab and Fc segments (Dorrington & Bennich, 1978). A recent structural model for the Fc region of IgE suggests very limited opportunities for angular rotation between $C_\epsilon2$ and $C_\epsilon3$ (Padlan & Davies, 1986), but the proximity of potential contact residues between $C_\epsilon1$ and $C_\epsilon2$ is not known, and previous studies on immunoglobulin M, which has a domain analogous to $C_\epsilon2$ in place of an extended hinge region, indicate that substantial segmental flexibility is possible with such a structure (Holowka & Cathou, 1976). Our results obtained with IgE in solution do not permit us to determine which modes of motion are contributing to the overall depolarization decay. For membrane-bound IgE, however, it is clear that a fast mode of motion is contributing significantly to this decay. The best fit value for ϕ_s for this sample, 34 ns, is too short to be entirely accounted for by wagging motion of the Fab, and best fits obtained by fixing ϕ_s at 50–60 ns (the smallest values of ϕ compatible with this motion) are visibly inadequate (D. Holowka, unpublished observations).

Further insight is gained from examination of the values of ϕ_s and ϕ_l for solubilized IgE–receptor complexes. In this case, ϕ_s = 18 ns is clearly short enough to be accounted for by twisting of the Fab segment about its major axis and/or by elbow-bending motion at the V–C junction. Although there is substantial evidence that this latter motion can occur (Wrigley et al., 1983; Davies et al., 1988), it is unclear whether it is unhindered enough to occur on a nanosecond time scale, and no evidence for fast elbow bending has been found in nanosecond experiments on Fab fragments (Reidler et al., 1982). The normalized preexponential weighting factor for ϕ_s of solubilized IgE–receptor complexes (α_s = 0.31, Table I) is consistent with a fast mode of motion contributing very substantially to the anisotropy decay. Since the value of ϕ_l for these complexes (173 ns) is less than that expected for global tumbling (~250 ns; Slattery et al., 1985), some contribution from slower modes of segmental motion, such as wagging of the Fab segments (Figure 1), could contribute to the anisotropy decay in this situation. As with membrane-associated IgE–receptor complexes, fixing ϕ_s at 50–60 ns results in an inadequate fit of the data.

How do the data for solubilized and membrane-bound IgE–receptor complexes compare? If ϕ_s for membrane-bound IgE is fixed at a value of 20 ns (similar to that for solubilized IgE–receptor complexes), then a good fit of the data is obtained by visual and statistical criteria (χ^2/N = 2.5) with ϕ_l = 283 ns and α_s' = 0.22. This suggests that, as with solubilized IgE–receptor complexes, fast twisting motion of the Fab segments of membrane-bound complexes probably contributes significantly to the anisotropy decay. Since rotational motion of the entire IgE–receptor complex is expected to be >1000 ns under these conditions (Slattery et al., 1985), the value of ϕ_l obtained implies that there is (are) some slower mode(s) of segmental motion in addition to the faster modes of twisting and/or elbow bending that contribute to the overall depolarization decay. The value of α_s' in this analysis is smaller than that for solubilized IgE–receptors (α_s' = 0.31; Table I, row 3), and this might suggest that there is a greater range of angular freedom for Fab twisting motion in solubilized IgE–receptor complexes, but the quality of the data does not permit a definitive interpretation to be made.

As implied in the foregoing discussion, the potential complexity of the decay components for IgE in solution does not permit us to determine unambiguously whether the modes of segmental motion that contribute to depolarization in this case are substantially different from those for IgE bound to its receptor. When ϕ_s for soluble IgE is fixed at 20 ns, then a good fit is obtained with ϕ_l = 114 ns and α_s' = 0.15. Since this value of ϕ_l is substantially less than that expected for global tumbling, some other mode(s) of segmental motion in addition to those represented by ϕ_s = 20 ns is (are) implied (Hanson et al., 1981). This conclusion is qualitatively consistent with that for receptor-bound IgE, but whether all the modes of segmental motion in these two cases are similar in rate and extent remains unclear. Further insight into this question may be gained from the detailed analysis of IgE cross-linking by bivalent antigens that is currently ongoing (Erickson et al., 1990). In particular, determining the efficiency with which IgE forms cyclic dimers with these bivalent ligands when bound to receptors vs free in solution may contribute to our understanding of this question. Preliminary results indicate that cyclic dimers in solution with didansylcadaverine are energetically less favorable with anti-dansyl IgE than those with the corresponding IgG subclasses (D. Holowka, unpublished results).

In summary, the results described here indicate that segmental flexibility for membrane-bound IgE is restricted in angular range. This may be due in part to the loss of segmental motion at the $C_\epsilon2$–$C_\epsilon3$ junction where interaction with the receptor occurs, but additional experiments will be necessary to answer this question. Other modes of segmental motion are at least partially preserved, and in particular, there is evidence for a significant contribution from a fast mode of motion (ϕ < 40 ns) that is most likely to represent twisting of the Fab about its long axis. Our results serve to illustrate the advantage of nanosecond depolarization data over steady-state methods: the correlation times obtained by this latter method are more heavily weighted toward the faster modes of motion that the probe experiences, and substantial differences in the anisotropy decay curves for soluble and membrane-bound IgE described here are not readily detectable when steady-state analysis is carried out (Slattery et al., 1985).

Our results also reveal an advantage in the study of antibody segemental motion when the Fc region is anchored to a large particle: faster modes of motion are easier to resolve from the global tumbling of the membrane vesicles than from that of a soluble antibody molecule. An analogous situation was previously analyzed by Hanson et al. (1985) for complexes of IgG with protein A. Although the fast twisting and/or elbow-bending motions of the Fab segments detected in the present case have only a limited angular range, they appear to be sufficient to permit cross-linking of IgE–receptor com-

plexes by antigens that have some degrees of orientational freedom [Figure 4 and Erickson et al. (1986)]. The constraints on segmental motion that are apparent from the current studies have important implications regarding the structural requirements for cross-linking of IgE–receptor complexes that leads to a productive biological response. They imply, for example, that IgE–receptor complexes cross-linked by long, rigid bivalent antigens are unlikely to permit receptor–receptor contact during active signal transduction (Kane et al., 1988). Further studies will be aimed at exploring the relationships between structural dynamics and transmembrane signaling in this system in greater detail.

Added in Proof

We have recently obtained evidence from phosphorescence anisotropy measurements that IgE receptors have some segmental flexibility of their own at the extracellular side of the membrane (J. Myers, D. Holowka, and B. Baird, manuscript in preparation), and this may contribute to the values for ϕ_1 that are reported in the present study.

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