Effects of Subunit Mutation on the Rotational Dynamics of FcεRI, the High Affinity Receptor for IgE, in Transfected Cells†

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ABSTRACT: Erythrosin-labeled immunoglobulin E (IgE) and time-resolved phosphorescence anisotropy were used to monitor the rotational dynamics of transfected wild-type ($\alpha\beta\gamma_2$) and four mutant FcεRI receptors in the monomeric and dimeric state on P815 cells. Erythrosin-IgE bound to FcεRI on cells transfected with either β or γ subunits with truncated COOH-terminal cytoplasmic segments exhibit faster rotational motion than when bound to FcεRI on cells transfected with wild-type subunits. Deletion of the NH_2-terminal cytoplasmic segment of the β subunit or the COOH-terminal cytoplasmic segment of the α subunit does not cause any significant change in the anisotropy decay. Dimers of IgE−receptor complexes formed with anti-IgE monoclonal antibody B1E3 exhibit substantially slower anisotropy decays for all the receptor constructs used, including a receptor construct that only contains the ectodomain of the α subunit anchored to Chinese hamster ovary (CHO) cell membranes through a lipid tail. This loss of rotational motion of dimeric IgE−FcεRI complexes may be due to nonspecific entanglement or to specific interactions involving IgE or the extracellular portion of α. The results suggest that the β and γ subunits of the tetrameric $\alpha\beta\gamma_2$ receptor participate in interactions with other membrane components even in the absence of receptor aggregation. The loss of such interactions may be related to the functional impairments previously determined for these mutants.

FcεRI, the high affinity receptor for IgE, is present on mast cells and basophils (Siraganian, 1988) and was recently found to be present on human Langerhans cells (Bieber et al., 1992; Wang et al., 1992), eosinophils (Gounni et al., 1994), and monocytes (Maurer et al., 1994). This receptor mediates immediate hypersensitivity responses through antigen-dependent aggregation of IgE−FcεRI complexes. The structure and function of FcεRI on mast cells has been studied extensively [for reviews, see Ravetch and Kinet (1991) and Beaven and Metzger (1993)]. Because this receptor lacks sequence motifs suggestive of kinases or other enzymes, and because events like protein phosphorylation (including phosphorylation of the receptor itself) occur after receptor aggregation (Benhamou et al., 1990; Paolini & Kinet, 1991), interactions of the receptor with other cellular components are likely. On these and related receptors, molecular genetic and biochemical approaches have been used to define the critical sites on the receptor subunits that are responsible for such interactions, and they have identified the interaction of intracellular tyrosine kinases with FcεRI, both before and after receptor aggregation (Bolen & Eiseman, 1992a; Hutchcroft et al., 1992; Yamashita et al., 1995). Physical measurements that detect receptor mobility such as time-resolved phosphorescence anisotropy (TPA)[1] and fluorescence photobleaching recovery (FPR) can also reveal interactions with other membrane proteins and changes in these interactions that occur upon receptor activation. Furthermore, these physical measurements can detect weak or transient interactions that are disrupted by the detergents used to solubilize the receptors (Holowka & Baird, 1990).

Here, we have applied TPA to study the rotational motion of the transfected IgE receptor on P815 cells and CHO cells. Membrane proteins rotate with diffusion coefficients, D_R, in the range of 10^6-10^3 s^{-1}, and their translational motions have diffusion coefficients, D_T, in the range of $10^{-12}-10^{-8}$ cm^2 sec^{-1} (for reviews, see Jovin and Vaz (1989) and Edidin (1991)]. The unrestricted D_R is expected to be inversely proportional to the protein volume in the membrane (Saffman & Delbrück, 1975) and, as such, is sensitive to small changes in the state of aggregation and to the local dynamics of the membrane system. On the other hand, D_T is dependent logarithmically on the inverse of the radius of the cross-section of the protein transmembrane portion (Saffman & Delbrück, 1975) and therefore is sensitive only to much larger scale aggregation. Previously, FPR studies on transfected COS-7 cells showed that deletion of any of the five cytoplasmic tails of FcεRI does not significantly affect the measured lateral diffusion rate, whereas deletion of the COOH-terminal tail of the β subunit causes a partial reduction in the immobilization of the receptor after its aggregation (Mao et al., 1991) that correlated with a loss of

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[1] Abbreviations: TPA, time-resolved phosphorescence anisotropy decay; Er, erythrosin; IgE, immunoglobulin E; IgE−R, IgE−receptor complex; CHO, chinese hamster ovary cells; FPR, fluorescence photobleaching recovery; Ct, COOH-terminal truncation; Nt, NH_2-terminal truncation; BSS, buffered saline solution; ϕ, rotational correlation time; GPI, glycosylphosphatidylinositol.

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signaling (Alber et al., 1991). TPA measurements on RBL-2H3 cells showed that dimerization with monoclonal antibody B1E3 inhibited rotational motion of the endogenous FcεRI (Myers et al., 1992). In the present study we found different rates of rotational motion for different mutant receptors in the uncross-linked state, i.e., mobility differences that were not detectable in measurements of lateral diffusion on cells tranfected with the same mutants (Mao et al., 1991). In addition, we find that B1E3 can substantially reduce receptor rotational motion of all the receptor constructs used. Possible implications are discussed.

MATERIALS AND METHODS

Reagents. The IgE used in these studies was a murine monoclonal anti-2,4-dinitrophenyl antibody (Liu et al., 1980) that was purified as previously described (Holowka & Metzger, 1982). The purified monoclonal rat anti-murine IgE antibody B1E3 (Keegan et al., 1991) was a gift from Dr. D. Conrad (Medical College of Virginia). Erythrosin 5′-thiosemicarbazide (Er) was custom-synthesized by Molecular Probes Inc. and was used without further purification. Glucose oxidase (≈180,000 Sigma units/mg) was from Sigma Chemical Co., and argon was prepurified grade (O_2 < 5 ppm).

Cells. Mouse mastocytoma P815 cells stably transfected with wild-type and mutant IgE receptors (Miller et al., 1990; Alber et al., 1991) as well as the CHO cell line stably transfected with the ectodomain of human FcεRI α attached to a glycosylphosphatidylinositol lipid (α_H−GPI) have been previously described (Mao et al., 1992, 1993). Table 1 presents data on the cytoplasmic structures of FcεRI and its variants that are used in this study. P815 cells were cultured in flasks in selective medium containing RPMI 1640 with 25 mM HEPES, 4 mM glutamine, 17% (vol/vol) heat-inactivated fetal bovine serum (FBS, UBI Inc., Lake Placid, NY), 100 units/mL penicillin, 100 mg/mL streptomycin (GIBCO, Grand Island, NY), and 0.5 mg/mL geneticin (G418, GIBCO). CHO cells were grown adherent and cultured in Iscove's modified Dulbecco's medium (Sigma Chemical Co.) with 25 mM HEPES, 4 mM glutamine, 10% heat-inactivated FBS, 100 units/mL penicillin, and 100 mg/mLstreptomycin and 1 μM methotrexate (Sigma Chemical Co.). Both transfected CHO and P815 cells were grown free of G418 and methotrexate at 37 °C in a humidified atmosphere containing 5% CO_2 for at least a week prior to each experiment.

Preparation of Er−IgE. Purified IgE was derivatized with Er according to the procedure previously described (Myers et al., 1992) with minor modifications. Gel permeation chromatography of Er−IgE was performed on a Superose 6 column (Pharmacia) connected to a Waters HPLC system equipped with a 280-nm detector in order to isolate monomeric Er−IgE. The molar ratio of Er to IgE in the conjugates was determined as previously described (Myers et al. 1992) and was calculated to be 2.2:1 for the preparation used in these experiments.

Preparation of Cells for Phosphorescence Measurements. Suspensions of the P815 cells were centrifuged at 200*g* for 10 min and resuspended in their original medium to a final concentration of 1 × 10^7 cells/mL. CHO cells were harvested by exposure to 0.05% trypsin/0.02% EDTA (GIBCO BRL, Grand Island, NY), washed, and resuspended in original medium to a final concentration of 1 × 10^7 cells/mL. Two 2 mL aliquots of the cells at this concentration, designated "sample" and "blank", were used for each experiment. Unlabeled IgE (26 μg/mL) was added to the "blank" cells, and both aliquots were incubated at 37 °C for 15 min; then 1.3 μg/mL Er−IgE was added to both aliquots, and incubation was continued for 1 h at 37 °C. Both samples were washed by centrifugation and resuspension twice in buffered saline solution (BSS) containing 135 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5.6 mM glucose, and 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4. The samples containing cells at 1 × 10^7 cells/mL were deoxygenated in their measuring cuvettes at 25 °C by a combination of 800 units of glucose oxidase (Englander et al., 1987) and gentle flow of humidified argon over the suspension as previously described (Myers et al., 1992). Both phosphorescence intensity and lifetime are decreased by collisional quenching with O_2, and we determined that 20 min of deoxygenation is sufficient to ensure no further change in phosphorescence decay during subsequent measurement of the anisotropy decay. It is particularly important to be sure that deoxygenation is complete for the L-format measurment that we used, since $I_{||}$ and $I_{\perp}$ are not collected at the same time (see below). The viability of the cells was monitored by Trypan Blue exclusion and was always >95% after the anisotropy measurements, which were carried out at 25 °C.

TPA Measurements. The principles of the instrumentation and methods for determination of TPA decay have been previously described (Myers, 1990; Myers et al., 1992). Phosphorescence emission was induced with 532-nm excitation light from a frequency-doubled Nd:YAG laser (Quanta-Ray DCR-2A) that was polarized with a Glan-Thompson prism. The stirred, thermostated sample chamber held a 1 cm × 1 cm path length fused silica cuvette for all the measurements. The cuvettes were irradiated at a frequency of 10 Hz, and the polarized phosphorescence emission was measured at 90° to the incident light through a train of optical filters: 2 mm of saturated potassium dichromate in H_2O, a Schott KV-550 barrier filter, and a Schott RG-645 barrier filter (Pecht et al., 1991). A 45-mm diameter sheet polarizer (Melles-Griot) in rotating mounts was used after the filters to select the polarized emission components. A commercially gated photomultiplier (Thorn-EMI 9816) was used for detection, and the amplified signal was digitized at 3.125 MHz with a 25-MHz 8-bit A/D board (Sonotek STR-8000) in an IBM-AT computer. Signal-averaging was accomplished with software as previously described (Myers, 1990). In L-format, collection of vertically polarized emission was followed by collection of horizontally polarized emission. Records of 10 000 decay curves were accumulated in 1000 s for each emission polarization component, and the cumulative laser power was measured and recorded simultaneously to correct for any long-term drift in the laser output. Following the collection of anisotropy data for monomeric Er−IgE−receptor complexes, B1E3 was added at a final concentration of 30 nM (except for α_H−GPI, where 100 nM was added), and cells were incubated for 10 min under argon to allow binding and removal of oxygen from the B1E3-containing samples.

Correction for the autoluminescence background of the cells and any phosphorescence decay due to nonspecifically bound Er−IgE was done by subtracting vertically and horizontally polarized decay curves of the "blank" cells from the appropriate decay curves for the "sample" cells. The

anisotropy decay $r(t)$ was calculated as

$$r(t) = d(t)/S(t) \quad (1)$$

in which $d(t)$ and $S(t)$ are determined from the corrected parallel and perpendicular polarized phosphorescence intensity values, $I_{||}(t)$ and $I_{\perp}(t)$, respectively

$$S(t) = I_{||}(t) + ghI_{\perp}(t) \quad (2)$$

and

$$d(t) = I_{||}(t) - gI_{\perp}(t) \quad (3)$$

in which g accounts for the difference in detector sensitivity for $I_{||}(t)$ and $I_{\perp}(t)$ and is defined as the ratio of the parallel to the perpendicular emission intensities when the excitation polarizer is in the horizontal position. It was determined as previously described (Myers, 1990) to be 1.01 for the L-format collection configuration used here. The aperture correction, h, has a value of 1.75 in our system (Myers et al., 1992). For most experiments, the anisotropy decays for the data described were fit between 15 μs and 180 to 200 μs with a single-exponential decay plus residual anisotropy using a weighted nonlinear least-squares fitting routine (Myers et al., 1992):

$$r(t) = a + b\exp(-t/\phi) \quad (4)$$

in which ϕ is the rotational correlation time, a is the residual anisotropy (r_∞), and b is the pre-exponential term, such that $a + b$ yields the initial anisotropy (r_0) for the best fit to the data. The data in Figure 1 were fit with a two-exponential equation of the form

$$a + b\exp(-t/\phi_1) + c\exp(-t/\phi_2) \quad (5)$$

in which ϕ_1 and ϕ_2 represent the shorter and longer rotational correlation times, respectively.

RESULTS

TPA for Er−IgE bound to wild-type FcεRI transfected into P815 cells is shown in Figure 1, lower curve. A two-exponential analysis (eq 5) was initially performed to fit the anisotropy decay that was collected from 10 to 350 μs, and this is shown as the smooth continuous line through the data points. The initial anisotropy (r_0), with a value of about 0.11, decays to a lower, positive residual anisotropy (r_∞) value of 0.05. Two rotational correlation times are derived from the fit. $\phi_1 = 3$ μs is the major component of decay during the first 15 μs. The presence of a decay component with $\phi <$ 10 μs is a common feature shared by all the transfected receptors on P815 cells (see below) and is indictive of some slow segmental motion of the complex. The slower component of phosphorescence anisotropy decay, $\phi_2 = 63$ μs, is similar to but somewhat larger than the values previously reported for monomeric Er−IgE−receptor complexes on RBL cells at this temperature (Myers et al., 1992). Because it is unlikely that the fast component of decay represents the rotation of IgE−R in the membrane (see Discussion), and because it is insignificant after the first 15 μs, we analyzed the data from 15 to 200 μs to obtain a single-exponential fit that could be compared for all of the constructs examined. Figure 2, panel A, shows that the same data as those shown in Figure 1 are well-fit over this more limited time range by a single-exponential expression to yield

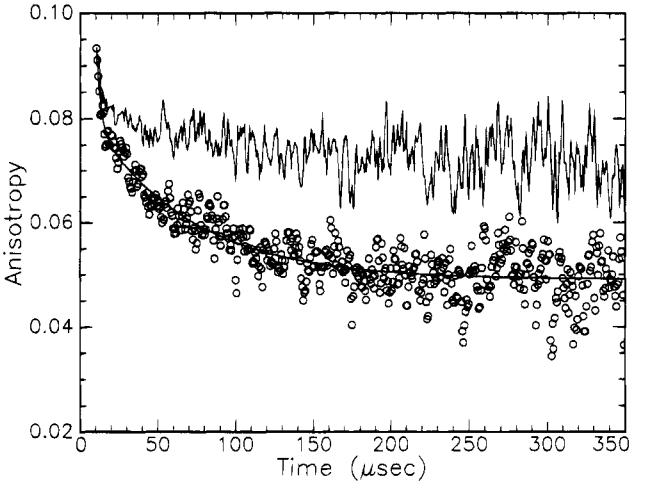

FIGURE 1: Representative phosphorescence anisotropy decay of Er−IgE bound to wild-type receptor on P815 cells. The lower curve shows anisotropy decay before cross-linking with B1E3, and the upper curve shows anisotropy decay after cross-linking with 30 nM B1E3 at 25 °C. The lower curve was analyzed with a two-exponential equation (see eq 5; Materials and Methods), and the best fit parameters ($a = 0.050$, $b = 0.60$, $c = 0.035$, $\phi_1 = 3$ μs, and $\phi_2 = 63$ μs) yield the smooth line through the lower curve.

a ϕ value of 63 μs, identical to the value of ϕ_2 for the two-exponential fit. This indicates that the faster component revealed by the two-exponential analysis is easily separable from the slower component. Furthermore, fitting the data from 15 to 200 μs instead of to 350 μs does not change either the magnitude of ϕ or the value of the residual anisotropy (data not shown). Because of the lower density of some mutant receptors on the transfected cells (Mao et al., 1993), the signal-to-noise ratio for these cases limits our comparative analysis to single-exponential fits of the data between 15 and 200 μs.

In an earlier study, we found that binding of the monoclonal antibody B1E3 caused the loss of most of the phosphorescence anisotropy decay between ~10 and 150 μs (Myers et al., 1992). B1E3 is a rat IgG that binds to a single site in the Cε4 domains of receptor-bound mouse IgE (Keegan et al., 1991; Grassberger, 1989), and it fails to trigger a Ca^{2+} response or degranulation of RBL-2H3 cells by itself but synergizes with another bivalent ligand to trigger these cellular responses (Posner, 1991). As shown in Figure 1, upper curve, addition of B1E3 to the transfected wild-type IgE−receptor complexes (IgE−R) on P815 cells with B1E3 also causes a similar immobilization effect. The fast component that dominates the decay of the uncross-linked IgE−R anisotropy within the first 15 μs is apparently not altered by B1E3 binding. The anisotropy after the first 15 μs for B1E3-cross-linked IgE−R slowly decays to a residual anisotropy of 0.075, a value significantly greater than that for monomeric IgE−R. In the present study we did not attempt to fit the shallow decay curves of the B1E3-cross-linked IgE−R. However, the rotational dynamics of the transfected wild-type receptors appear to be very similar to those of the endogenous receptors on RBL-2H3 cells.

We next carried out TPA experiments on the transfected mutant IgE receptors on P815 cells in the presence and absence of B1E3. The structural features of these mutants are summarized in Table 1. Representative TPA experiments are shown in Figure 2 B−E, with the best fits for the monomeric IgE−R between 15 and 180−200 μs indicated for each case. Table 2 summarizes the best fit parameters

Table 1. Structural Aspects of FcεRI Examined in These Studies

transfected receptor	number of residues in cytoplasmic domains[a]				
	α	β_N	β_C	γ	total
wild-type	19	59	43	2 × 42	205
α_{Ct}	4	59	43	2 × 42	190
β_{Nt}	19	2	43	2 × 42	148
β_{Ct}	19	59	5	2 × 42	167
γ_{Ct}	19	59	43	2 × 42[b]	205 (125)[b]
α_H−GPI	0				0

[a] The size of the cytoplasmic domains is estimated on the basis of hydropathicity plots of the amino acid sequences as defined from the cDNA sequences for the subunits of the rat FcεRI (Blank et al., 1989). In that paper evidence was presented that the cytoplasmic domains of the γ subunits might be truncated, but more recent data rule this out (Letourneur et al., 1991). [b] As noted in the text, although the P815 mouse mastocytoma cells were transfected with constructs coding for truncated γ chains which would be predicted to have a cytoplasmic domain only four residues long (so that the total number of cytoplasmic residues would be expected to be 125), analysis of the receptor in these transfectants revealed that the endogenous mouse γ chains had become assembled with the transfected α and β subunits (Alber et al., 1991), so that the number of cytoplasmic residues in the expressed receptor would be the same as for the wild-type.

for three or more experiments with the wild-type and each of the mutant receptors. The mutant α_{Ct}, with a truncated COOH-terminal cytoplasmic segment, shows a decay pattern (Figure 2B) similar to that of the wild-type receptor (Figure 2A) both in the uncross-linked and the B1E-cross-linked state. As with the wild-type receptor, the data between 15 and 200 μs are well-fit with a single-exponential expression. For three separate experiments with α_{Ct}, we obtained an average value of $\phi = 60 \pm 3$ μs, and this, as well as the other fitting parameters, is indistinguishable from those of the wild-type receptor (Table 2, lines 1 and 2). β_{Nt}, the mutant with a truncated N-terminal cytoplasmic segment of the β subunit, also shows similar decay patterns in the absence and presence of B1E3 (Figure 2C), and the average value of ϕ for three such experiments with uncross-linked IgE−R is 72 ± 6 μs. This value is slightly larger than that for wild-type receptor, but this difference is not statistically significant.

Functional studies have shown that the α_{Ct} and β_{Nt} mutants are indistinguishable from the wild-type receptor in mediating early signaling events including stimulation of tyrosine phosphorylation, phosphatidylinositol hydrolysis, and Ca^{2+} mobilization on the P815 cells (Alber et al., 1991, 1992). In contrast, cells transfected with constructs in which the COOH-terminal cytoplasmic tail of β or γ is truncated (β_{Ct} and γ_{Ct}, respectively) mediated these signaling events poorly (β_{ct}) or not at all (γ_{ct}) (Alber et al., 1991). Figure 2D shows the TPA decay for the β_{Ct} mutant in the presence and absence of B1E3. The general features of these decay curves are similar to those of the wild-type receptor (Figure 2A), and the single-exponential fit between 15 and 200 μs for the unaggregated receptor is also good. As shown in Table 2, line 4, the average value of ϕ for three experiments with β_{Ct} (44 ± 6 μs) is about two-thirds the value for the wild-type receptor, and this difference is statistically significant by Student's *t* test. The results for γ_{Ct} from a single representative experiment are shown in Figure 2E, and these show that TPA for the monomeric form of this mutant decays even faster than that for β_{Ct}, with an average value for ϕ of 32 ± 3 μs (Table 2, line 5). The similarity of the values of a ($a = r_\infty$) and b ($a + b = r_0$) for all of these variants (eq 4; Table 2) makes it reasonable to interpret the values of ϕ as

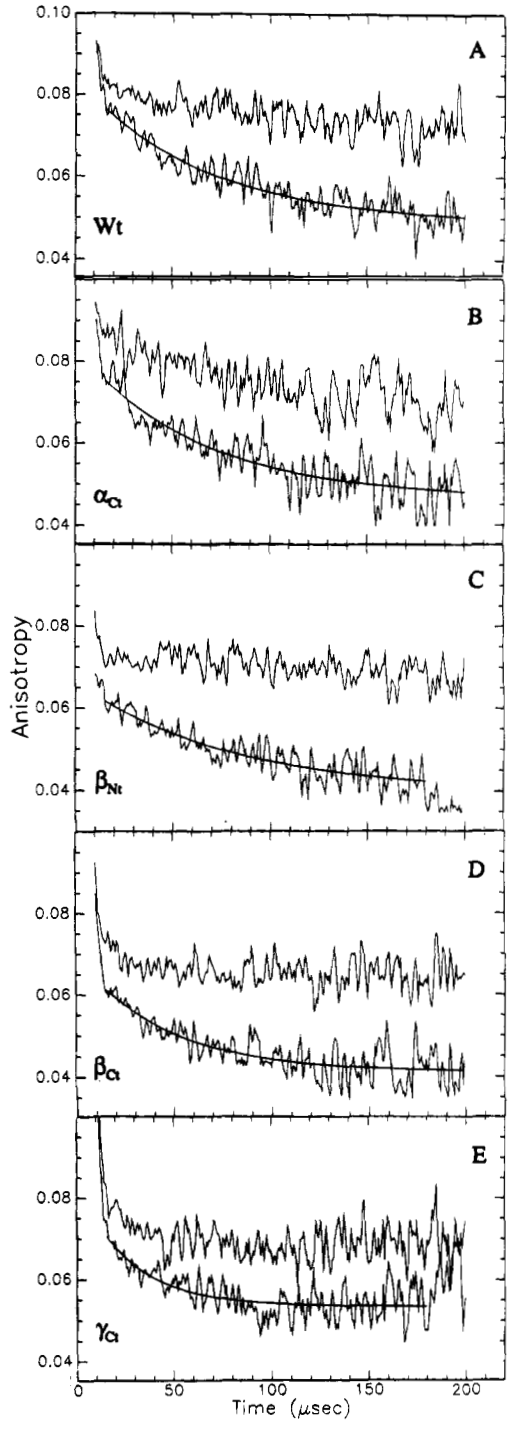

FIGURE 2: Phosphorescence anisotropy decay of transfected IgE receptors on P815 cells. Representative anisotropy decay curves for each receptor construct before and after cross-linking with 30 nM B1E3 are shown in panels A−E, and the smooth curve represents the single-exponential fit of the data between 15 and 200 μs for A, B, and D and between 15 and 180 μs for C and E. The best fit parameters for each transfectant are (A) $a = 0.049$, $b = 0.035$, $\phi = 63$ μs for wild-type (Wt); (B) $a = 0.047$, $b = 0.037$, $\phi = 63$ μs for α_{Ct}; (C) $a = 0.040$, $b = 0.027$, $\phi = 78$ μs for β_{Nt}; (D) $a = 0.041$, $b = 0.028$, $\phi = 46$ μs for β_{Ct}; and (E) $a = 0.053$, $b = 0.029$, $\phi = 29$ μs for γ_{Ct}.

being representative of the rate of rotation of a fixed subpopulation of these different receptors in the plasma membrane of the P815 cells. This interpretation is a simplification of two-dimensional rotational diffusion predicted by theory, since even a simple uniaxially rotating ellipsoid should exhibit two values of ϕ that are different in magnitude by a factor of 4 (Kawato & Kinosita, 1981). The

Table 2: Anisotropy Decay Parameters for Transfected Er−IgE−R on P815 cells[a]

receptor	a	b	ϕ (μs)
Wt ($n = 3$)	0.050 ± 0.010	0.033 ± 0.003	65 ± 9
α_{Ct} ($n = 3$)	0.042 ± 0.007	0.038 ± 0.002	60 ± 3
β_{Nt} ($n = 3$)	0.039 ± 0.004	0.034 ± 0.008	72 ± 6
β_{Ct} ($n = 3$)	0.051 ± 0.009	0.031 ± 0.006	44 ± 6
γ_{Ct} ($n = 4$)	0.050 ± 0.003	0.030 ± 0.007	32 ± 3

[a] All experiments were performed at 25 °C. Average values for a, b, and ϕ were calculated from the best single-exponential fits $r(t) = a + b \exp(-t/\phi)$ (eq 4) for each individual experiment. Uncertainties represent the standard deviations of the best fit parameters from the data used to calculate the averages, and n = number of experiments, each carried out on a separate day.

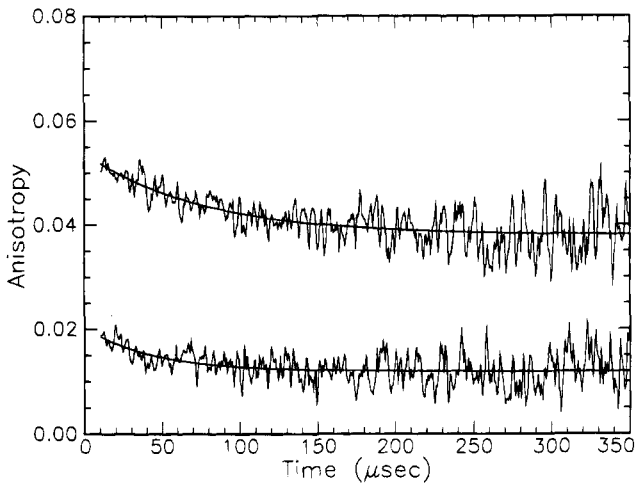

FIGURE 3: Representative phosphorescence anisotropy decay of Er−IgE bound to α_H−GPI on transfected CHO cells. Lower and upper curves show anisotropy decay before and after cross-linking with 100 nM B1E3, respectively. The smooth line through lower curve represents the one-exponential fit of the monomer curve. The fitting parameters in this case are: $a = 0.012$, $b = 0.0084$, and $\phi = 43$ μs.

low signal-to-noise in the data due to the poor phosphoresence quantum yield of Er at 25 °C and the relatively low density of transfected receptors does not justify a more complex analysis. The relatively high (nonzero) values of r_∞ for all of these constructs raise the possibility that some receptors may be rotating at a much slower rate than is detectable by the Er probe, but the fraction of receptors rotating with values of ϕ between ~30 and 75 μs appears to be constant for all of these. Taken together, the results suggest a correlation between the functional competence of the mutant and wild-type receptors and the values of ϕ observed for the monomeric IgE−R.

Additional confidence in the interpretation of the cytoplasmic tail-deletion mutants described above was given by an investigation of the TPA decay for a GPI-linked ectodomain of the α subunit. This construct is permanently expressed on CHO cells at a moderately high level (Mao et al., 1993). As seen in Figure 3, the value of r_0 for the monomeric Er−IgE−α_H-GPI complex is very low and is followed by only a small decay to a value of r_∞ that is significantly lower than that observed with the tetrameric form of the receptor (including mutants). These observations support the likelihood that the rotation of lipid-anchored complexes in the membrane is too fast to be detected by our methodology. Freely rotating lipid-anchored molecules would be expected to have a rotational correlation time of <5 μs at 25 °C.

Addition of B1E3 to the GPI-linked construct causes a substantial increase in r_0, followed by a small amount of decay to a value for r_∞ much larger than that for the monomeric species. Similar results were obtained in five separate experiments with this construct, and these indicate that the loss of rotational motion observed after dimer formation by B1E3 is not dependent on specific interactions of transmembrane or cytoplasmic segments of the receptor subunits with other membrane components.

DISCUSSION

The present work extends previous studies (Mao et al., 1991, 1992, 1993; Alber et al., 1991, 1992) on the functional roles of the cytoplasmic segments of multisubunit FcεRI as well as on the effect of cross-linking by the monoclonal anti-IgE antibody B1E3 on the rotational dynamics of the IgE−FcεRI (Myers et al., 1992). Our studies show that on P815 cells the transfected wild-type receptor labeled with Er−IgE has TPA characteristics qualitatively similar to those exhibited by the endogenous receptor on RBL cells, both before and after cross-linking by B1E3. The value for ϕ of 65 μs for monomeric IgE−FcεRI on the P815 cells (Table 1) is slightly larger than the value of 40−50 μs previously reported for this complex on RBL cells at 25 °C (Myers et al., 1992), but the significance of this difference is unclear. Experimental differences such as signal-to-noise can influence the absolute values of ϕ determined when such differences are substantial, since different amounts of contributions from faster or slower modes of motion may be more or less resolvable. Differences in the relative contributions from slower or faster modes of motion from one cell type to another can also affect the values of ϕ obtained. Both of these factors could affect the apparent difference in ϕ observed for wild-type receptors in RBL cells and P815 cells. For these reasons, it is important to compare the phosphorescence anisotropy decay of the mutant receptors to that of the wild-type receptors on the same cell type with similar signal-to-noise and identical methods of analysis.

One feature of the TPA for IgE−FcεRI on the P815 cells that was not detected on the RBL cells in any previous studies (Myers et al., 1992; Rahman et al., 1992; Pecht et al., 1991; Zidovetzki et al., 1986) is a fast component that is observed during the first 15 μs of the measurable decay (Figures 1 and 2). This component of decay appears to reflect a segmental mode of motion in the Er−IgE−R complex that has a ϕ value of several microseconds. This component is too fast to represent rotation of the $\alpha\beta\gamma_2$ receptor complex in the plane of the membrane: such a complex is expected to have an average ϕ value of ~15 μs in a fluid bilayer at 25 °C on the basis of experimental (Cone, 1972; Cherry & Godfrey, 1981) and theoretical results (Saffman & Delbrück, 1975) for a membrane protein with seven transmembrane segments, as predicted for FcεRI (Blank et al., 1989). It is possible that on P815 cells there is a mode of segmental motion of IgE−FcεRI that is slow enough to be at least partially observable on the time scale of our measurements. The functional significance of this fast component of TPA remains unclear, but it appears to some extent in all of the transfected receptors (Figure 2). As on RBL cells, faster modes of segmental motion and/or dye rotation also contribute to an r_0 that is well below that expected for a rigid rotating membrane protein [see Myers et al. (1992)].

We found that we could make the clearest comparisons between the slower component of TPA decay for all of these IgE−R by analyzing the decay data after 15 μs with a single-exponential function (see Materials and Methods). This analysis showed that transfectants expressing the wild-type receptors and receptors containing α_{Ct} and β_{Nt} all exhibit values of ϕ in the range from 60 to 72 μs. In contrast, P815 cells transfected with receptors containing γ_{Ct} and β_{Ct} exhibit TPA decay curves with values of ϕ (32 and 44 μs, respectively) that are significantly lower than those for α_{Ct} and β_{Nt}, whereas the values for a, b, and r_∞ are not significantly different for all of these transfected receptors on the basis of the standard deviations of the averaged measured parameters (eq 4; Table 2). Although the transfected P815 cells express different number of receptors per cell, with γ_{Ct} containing the fewest receptors (Mao et al., 1993), the reduced values for ϕ associated with β_{Ct} and γ_{Ct} are not likely to be attributable to variations in receptor number, since the β_{Ct} mutant expresses more receptors than any other variant except the wild-type receptor. As indicated in Figure 2, the signal-to-noise for the different constructs is not substantially different. Our results are also unlikely to have resulted simply from a reduced bulk of the cytoplasmic portion of the receptor. As can be seen by comparing Tables 1 and 2, the number of residues remaining in the cytoplasmic domains does not correlate with the rotational mobility. Although this is seen most strikingly by comparing the wild-type and γ_{Ct}, the lack of correlation is also seen by comparing β_{Nt} with β_{Ct}. Instead, our results suggest that truncations of the COOH-terminal segments of either the β or γ subunits of FcϵRI reduce the association of the receptors in the uncross-linked state with unidentified cellular component(s).

Er−IgE bound to α_H−GPI exhibited very little decay following a very low r_0 as would be expected for fast rotational motion around the lipid tail in the membrane. To our knowledge this is the first reported measurement of rotational diffusion for a GPI-linked protein, and it indicates that this lipid anchored protein is located in a relatively fluid environment prior to aggregation. In contrast, all Er−IgE receptor complexes, including Er−IgE−α_H-GPI, exhibited a substantial loss of TPA decay after cross-linking receptors with B1E3. These results suggest that the effect of B1E3 on FcϵRI that was previously reported by Myers et al. (1992) is not dependent on specific interactions of the receptor's transmembrane or cytoplasmic segments. It is possible that IgE itself or the ectodomain of α is capable of mediating specific interactions with other membrane components. Recent studies indicate that aggregation of this α_H−GPI complex can mediate the activation of certain lipid metabolizing pathways, but not all of the signalings necessary for cellular degranulation (Lung et al., 1994). The association of aggregated GPI-linked proteins with caveolae-like membrane domains at the cell surface (Mayor et al., 1995) may be relevant to the decrease in rotational motion and possibly to the signaling events that are stimulated by cross-linking. It is also possible that these domains play a role in the loss of rotational mobility observed with the other FcϵRI constructs cross-linked by B1E3.

In view of the functional studies of Alber et al. (1991, 1992) which showed that the P815 transfectants β_{Ct} and γ_{Ct} are relatively inactive in receptor-mediated protein tyrosine phosphorylation, phosphoinositide hydrolysis, and Ca^{2+} mobilization, the data presented here suggest that unidentified cellular components normally associated with wild-type receptor in the uncross-linked state are important in FcϵRI-mediated signaling. A surprising finding in the study by Alber et al. (1991) was the observation that endogenous wild-type γ chains of the P815 cells co-immunoprecipitated with the $\alpha\beta$ complex, whereas the truncated mutant γ did not. This implies that the functional defect in this transfected cell line is caused by an indirect effect of the transfected mutant γ chain. Since the rotational correlation time for Er−IgE−FcϵRI in the γ_{Ct} transfected cells is less than that of the wild-type receptor, our result suggests that the mutant γ chain somehow prevents the association of additional protein(s) with the receptor complex, possibly by associating with such protein(s) at some stage in the biosynthetic pathway.

The decrease in the value of ϕ for the β_{Ct} mutant also implies that the IgE−R complex normally associates with other membrane components and that these interactions are due at least in part to the cytoplasmic segment of the β subunit. The difference in ϕ values for β_{Ct} and the wild-type receptor is ~20 μs, and, in a fluid membrane, this difference could represent associated proteins with a transmembrane volume that is slightly larger than that of bacteriorhodopsin, which has a reported value of $\phi = 15$ μs in reconstituted membranes (Cherry & Godfrey, 1981). Likewise, the difference in ϕ values for γ_{Ct} and wild-type is ~30 μs, so this could represent the association of a protein with a transmembrane volume equal to bacteriorhodopsin with each of the two γ chains in the wild-type cells. These estimates are subject to a large amount of uncertainty but serve to provide some idea of the size of associated proteins that could account for the differences observed. Although such a complex would be much larger than the $\alpha\beta\gamma_2$ complex of FcϵRI that is isolated upon detergent solubilization under conditions that stabilize these subunit interactions (Kinet et al., 1985), it is possible that other components normally associated with FcϵRI $\alpha\beta\gamma_2$ are dissociated under most conditions of solubilization. Chemical cross-linking of RBL cells with a membrane impermeable reagent results in an almost 2-fold increase in the Stokes radius of CHAPS-solubilized IgE−receptor complexes (D. Holowka, K. Field, and B. Baird, unpublished results), consistent with the association of $\alpha\beta\gamma_2$ with additional polypeptides in the plasma membrane. In related chemical cross-linking studies, some associated proteins have been characterized (Mao et al., 1995; Yamashita et al., 1995).

It is also possible that the reduction in the value of ϕ for IgE−FcϵRI observed with β_{Ct} and γ_{Ct} reflect more dynamic or nonspecific effects, such as the localization of these receptors in a more fluid membrane environment. The relatively large value of ϕ for the wild-type receptor (60−70 μs, Table 2) compared with an average value of ~15 μs expected for a seven transmembrane protein in a fluid membrane (Saffman & Delbrück, 1975; Cone, 1972; Cherry & Godfrey, 1981) could be due to a more viscous lipid environment in the vicinity of this receptor. In this model, β_{Ct} and γ_{Ct} mutant receptors would reside in a more fluid membrane domain in which their ϕ values approached those expected in a completely fluid bilayer and in which their signaling capabilities would be impaired. In a related model, the density of other membrane proteins in the vicinity of the receptor might be higher for the wild-type receptor resulting in a collision-dependent retardation of the rate of rotational diffusion of IgE−receptor complexes. However, these models do not explain how the truncated γ chain could

control the local environment of an apparently normal $\alpha\beta\gamma_2$ complex. Possibly, the mechanisms by which β_{Ct} and γ_{Ct} give similar functional and rotational phenotypes are different. Previous studies have shown that, compared to wild-type, β_{Ct} (but not γ_{Ct}) has a reduced immobilization (Mao et al., 1991) and detergent insolubility (Mao et al., 1992) that accompanies receptor aggregation. Our present results suggest that this defect in aggregated receptors could result from the failure of the β_{Ct} variant to interact with another membrane component even prior to aggregation.

Human α can associate with γ in the absence of β and the ability of this complex to trigger early signaling events appears unimpaired (Alber et al., 1991). However, recent evidence suggests β may play an important role in recruiting the tyrosine kinase lyn to the receptor complex (Eiseman & Bolen, 1992b; Jouvin et al., 1994), and it is possible that this interaction is relevant to the change in rotational motion that we observed. Although it is unlikely that the association of a myristoyl-anchored membrane protein such as lyn could itself alter the rotational motion of the receptor complex, such an interaction may mediate the association of additional proteins (Cantley et al., 1991).

In summary, deletion of the cytoplasmic segments of either the β or γ subunits of FcϵRI results in greater rotational freedom of the IgE–receptor complex in the plasma membrane of transfected P815 mastocytoma cells. These changes correlate with a loss in signaling capability of these two variants and suggest that both phenomena result from a loss of interactions between the receptors and other membrane components that normally occur prior to receptor aggregation by antigen.

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