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Dissociation of the Receptor for Immunoglobulin E in Mild Detergents†

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ABSTRACT: We previously showed that, in the absence of phospholipids, exposure of the tetrameric receptor for immunoglobulin E to mild detergents dissociates the intact β chain and two γ chains from the α chains. Having developed a practical method for assaying the dissociation, we have now explored a variety of different detergents, detergent concentrations, temperatures, times, salts, pHs, and other factors that influence the detergent-induced dissociation. Our findings should be useful for optimizing the stability of the receptor and for future studies on recombination of the subunits. The data suggest the following: (1) The critical perturbant is micellar detergent. (2) Unlike solubilization of membranes, where a molar ratio of micellar detergent:lipid of 2 is adequate, dissociation of the receptor is incomplete even at molar ratios of micellar detergent:receptor of $>10^5$ and may be limited by a reversible component. (3) Detergents that are best for solubilizing membranes are also best for dissociating the receptors. (4) The latter observation and other data implicate bound lipid as stabilizing the receptor. Our findings may be applicable to the study of interactions between membrane proteins in general.

Mast cells and related cells have plasma membrane receptors that bind immunoglobulin E (IgE)[1] and which when aggregated initiate degranulation of the cells. The IgE interacts directly with a 45-kD glycoprotein (α) exposed on the surface of the cells (Conrad & Froese, 1976; Kulczycki et al., 1976; Kumar & Metzger, 1982), but a variety of data indicate that the unit receptor contains in addition a 33-kDa β chain and two 10-kDa, disulfide-linked, γ chains (Perez-Montfort et al., 1983; Metzger et al., 1984).

An unusual property of the $\alpha\beta\gamma_2$ complex is that intact β and γ_2 dissociate from α in the mild detergents generally used to solubilize cells (Rivnay et al., 1982). In this paper we describe studies on rat basophilic leukemia cells in which we examined the conditions that promote the dissociation of β and of γ_2 from α.

Materials and Methods

Proteins and Cells. Monoclonal mouse anti-dinitrophenyl-IgE from hybridoma H1-DNP-e-26.82 (Liu et al, 1980) and human myeloma IgE from patient PS were prepared as described previously (Holowka & Metzger, 1982; Kulcyzcki & Metzger, 1974). Mouse IgE was iodinated with

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[1] Abbreviations: IgE, immunoglobulin E; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; cmc, critical micelle concentration; HLB, hydrophilic/lipophilic balance; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

the chloramine T method (McConahey & Dixon, 1966). Conjugation of the IgE with azobenzenearsonate groups has been described (Kanellopoulos et al., 1979). In all of the experiments described here we used the 2H3+ line of rat basophilic leukemia cells, and these were maintained as described (Barsumian et al., 1981).

Detergents. The zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was from Calbiochem-Behring Corp. (La Jolla, CA). *n*-Octyl β-D-glucopyranoside (octyl glucoside) was from Aldrich Chemical Co., Inc. (Milwaukee, WI). The sodium salts of 7-deoxycholic acid and of 3α,7α,12α-trihydroxycholanic acid (sodium cholate), the Triton series [octylphenoxypoly(ethoxyethanol)series], Triton X-35, Triton X-45, Triton X-114, Triton X-100, Triton X-165, and Triton X-305, poly(oxyethylene) sorbitan monolaurate (Tween 20), poly(oxyethylene) sorbitan monopalmitate (Tween 40), poly(oxyethylene)sorbitan monostearate (Tween 60), poly(oxyethylene)monoleate (Tween 80), poly(oxyethylene)sorbitan trioleate (Tween 85), poly(oxyethylene) 10 cetyl ether (Brij 56), and poly(oxyethylene) 20 cetyl ether (Brij 58) were all obtained from Sigma Chemical Co. (St. Louis, MO).

Lipids. Preparation of the tumor-derived lipids and of the lipids from rabbit liver has been described (Rivnay et al., 1982).

Other Materials. Bromophenol blue [4,4′-(3*H*-2,1-benzoxathiol-3-ylidene)bis[2,6-dibromophenol] *S*,*S*-dioxide] was obtained as the sodium salt from J. T. Baker Chemical Co. (Philipsburg, NJ); 8-anilino-1-naphthalenesulfonic acid magnesium salt was from Eastman Kodak Co. (Rochester, NY).

Intrinsic and Extrinsic Labeling, Solubilization, and Purification of Receptors. Intrinsic labeling of receptors with [^{3}H]leucine and extrinsic labeling of receptors have been described respectively in Perez-Montfort et al. (1983) and Alcaraz et al. (1984). Solubilization of receptors and purification by two different affinity chromatographies were the same as before (Alcaraz et al., 1984).

Assay for Dissociation of IgE Receptor Complexes. Unless stated otherwise, 2 μL from a preparation containing purified IgE–receptor complexes in 2 mM CHAPS–borate-buffered saline (0.2 M HBO_3, 0.16 M NaCl, pH 8) was incubated at 4 °C for 2 h in 200 μL of a buffer (20 mM Tris-HCl, 0.15 M NaCl, pH 7.6) containing 10 mM CHAPS. The IgE–receptor complexes were immunoprecipitated at 4 °C for 2 h as described previously (Perez-Montfort et al., 1983). When necessary, this immunoprecipitation was performed after quenching the dissociation process by diluting the samples from 10 to 2 mM CHAPS at 4 °C. Immunoprecipitates were analyzed by gel electrophoresis, and radioautography of the dried gels was performed as described previously (Perez-Montfort et al., 1983).

The radioactivity in the individual components was determined by densitometry of the radioautographs (Alcaraz et al., 1984). As will be described under results, the extents of dissociation of β from α and γ_2 from α were equivalent within experimental error. We therefore quantitated the percent of β and γ_2 that had dissociated from the IgE-α by the following relationship: percent dissociation = $[1 - [\alpha_{cont}(\beta + \gamma_2)_{exptl}/\alpha_{exptl}(\beta + \gamma_2)_{cont}]] \times 100$, where exptl and cont refer to the test sample and a sample maintained in 2 mM CHAPS, respectively.

Dissociation of α from IgE was assessed similarly by comparing the α:IgE ratio from the experimental sample with that from the control specimen. The β:γ ratio allowed us to look at a possible preferential dissociation of one of the chains from the α chains.

Determination of the Critical Micelle Concentration of Detergents. Two methods were used to determine the cmc value of detergents. The first method using the fluorescent probe 8-anilino-1-naphthalenesulfonate has been reported (De Vendittis et al., 1981). We also used bromophenol blue, the spectrum of which is modified by its interaction with micelles. The spectral shift of this dye was measured in a recording spectrophotometer (Beckman Instruments, Columbia, MD) at the temperature used in the experiment of dissociation. The concentration of dye was 10 μM. Maximal absorbance (λ_{max}) shifted from 590 to 606 nm by increasing the concentration of micelles. The actual solutions used to determine the cmc of the detergents and containing fluorescent probe or bromophenol blue were used in the experiments on dissociation.

RESULTS

Methodological Aspects. We previously described a method by which the receptors can be purified without exposing them to conventional denaturants (Kanellopoulos, et al., 1979). In this procedure, haptenated IgE is reacted with the receptor, and the IgE–receptor complexes are absorbed on an anti-hapten affinity column. After washing of the latter, the complexes are eluted with hapten. Such purified complexes were employed in all the studies described here.

In order to examine the dissociation of β and of γ_2 from IgE–α, it was necessary to have each of the chains labeled so that the α:β:γ ratio could be analyzed. Such labeling can be accomplished biosynthetically, e.g. with [^{3}H]leucine, but the small amount of incorporation makes analyses of large numbers of samples prohibitively expensive and time consuming, especially relative to what could be done if the purified receptor were extrinsically labeled by oxidative iodination.

The problem with the latter technique is that under the conditions employed in the past the IgE-bound α chains do not become labeled[2] and the β and γ chains have a relatively low specific activity. These considerations were a major impasse in the initial stages of this study. Ultimately the problem was overcome by two findings. First, we observed that in a solvent containing 2 mM of the detergent CHAPS the receptor–IgE complex was as stable as it is in the mixture of 10 mM CHAPS–2mM phospholipids previously shown (Rivnay et al., 1982) to maintain the integrity of the receptor. This is documented by the data shown in Table I.

Second, we noted that if the IgE–receptor complexes bound to the affinity beads were quickly (≤1 min) washed with 20–30 volumes of 10 mM CHAPS and then maintained in 2 mM CHAPS for all subsequent steps, they on the one hand retained their normal α:β:γ chain ratio (see below) and on the other hand could be efficiently labeled in all the chains (Figure 1).

For three such preparations purified "identically" and for which complete data were obtained, labeling with 1 mCi of ^{125}I yielded a specific activity of 54 (±25) Ci/mmol for the IgE–receptor complex. These values are within the range we routinely obtain with IgE itself. The average distribution of counts ±1 SD in IgE, α, β, and γ_2 was 0.71 (±0.032), 0.1 (±0.005), 0.07 (±0.004), and 0.12 (±0.008), respectively. These proportions are not very different than those expected if the labeling had been proportional to the peptide molecular

[2] The α chain of IgE–receptor complexes can be iodinated by using the Bolton–Hunter reagent (Kanellopoulos et al., 1979). However, the latter is also expensive and in our experience less efficient than oxidative iodination. We do not know whether the β and γ chains are labeled efficiently with this reagent.

Table I: Quantitative Analysis of Gel Patterns of IgE–Receptor Complexes Eluted from Immunoadsorbants That Had Been Incubated in Different Solutions[a]

incubation solution	% of total counts in the receptor		
	α	β	γ_2
10 mM CHAPS–2 mM tumor lipids	30	47	23
10 mM CHAPS–2 mM liver lipids	30	42	28
10 mM CHAPS[b]	69	19	12
2 mM CHAPS[b]	27	47	26

[a] Cells grown in [^{3}H]leucine were reacted with [^{125}I]-labeled arsonylated IgE and solubilized with CHAPS such that the final concentration of CHAPS was 10 mM and of cells was 5×10^7/mL (which yields 2 mM phospholipids). The high-speed supernatant was adsorbed on anti-benzenearsonate beads and the latter washed with 250 volumes of 10 mM CHAPS–2 mM tumor phospholipids over 48 h at 4 °C. By use of the ^{125}I counts, equivalent amounts of Sepharose-bound receptor–IgE complexes were aliquoted and the beads shaken for 48 h with 50 volumes of the solution shown in the first column of the table. The complexes were eluted with hapten in the appropriate solvent and immunoprecipitated with anti-IgE, and the precipitates were run on a polyacrylamide gel. A radioautograph of the dried gel was analyzed by densitometry. [b] The cmc measured in this buffer is 4.5 mM.

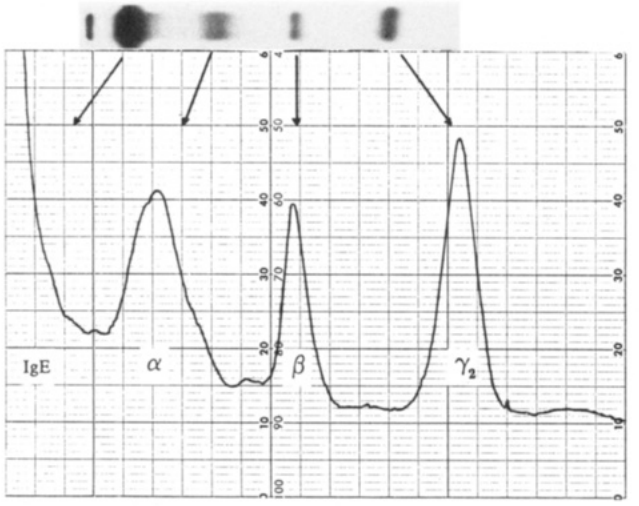

FIGURE 1: Distribution of radioactivity in components of radioiodinated IgE–receptor complexes. The upper part of the figure shows a radioautogram of a gel on which was analyzed an immunoprecipitate of labeled complexes prepared by the standard procedure used in this paper. The lower part is a densitometric tracing of the radioautogram on the basis of which α:β:γ_2 was determined.

weights (0.66, 0.13, 0.13, 0.08). Thus, not only were all three chains labeled, but hundreds of samples could be analyzed from a single preparation of receptors derived from (2–3) × 10^8 cells.

Effect of the Type and Concentration of Detergent. IgE–receptor complexes in 2 mM CHAPS were diluted 100-fold with solutions containing increasing concentrations of CHAPS and after a suitable time reacted with anti-IgE. The α:β:γ ratio was then assessed by analyzing the immune precipitates on gels. Figure 2A shows the result of such an experiment. The filled circles show that there was a progressive dissociation of both β and γ_2 from the IgE–α. The extent of dissociation closely paralleled the concentration of micelles as assessed by the change in λ_{max} of trace amounts of bromophenol blue (triangles). The analysis further showed that regardless of the extent of dissociation equivalent amounts of β and γ_2 were released (open circles). Indeed, in all the experiments described in this paper the extent of dissociation of β and γ_2 from α was equivalent within experimental error. In our discussion of the data we shall, therefore, treat the $\beta\gamma_2$ triad as a unit, even though we have no persuasive evidence that permits us

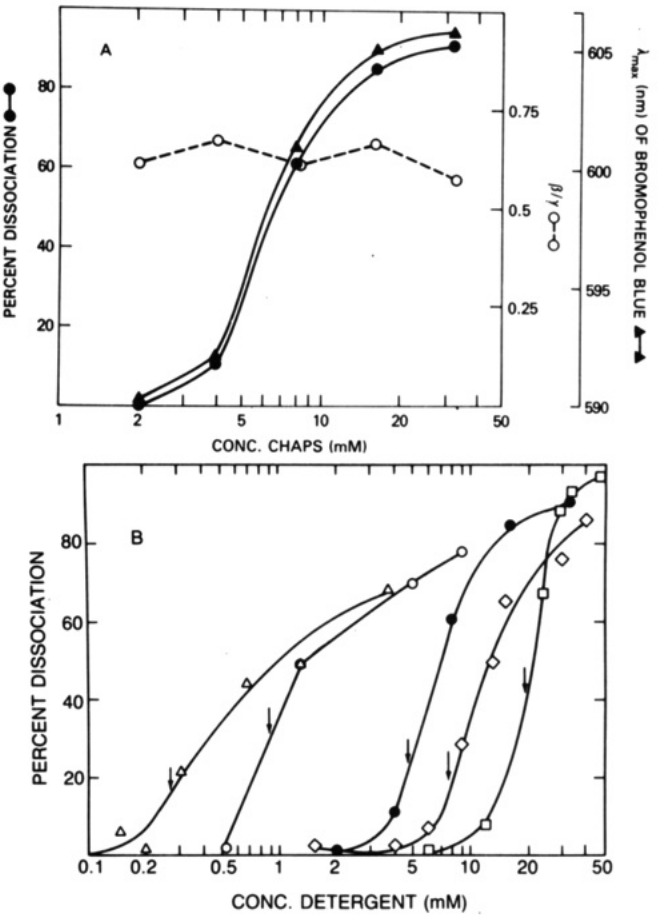

FIGURE 2: Effect of detergents on the dissociation of the receptor for IgE. Two microliters of a solution of purified IgE–receptor complexes in 2 mM CHAPS was incubated at 4 °C with 200 μL of solvent containing varying concentrations of different mild detergents. The complexes were immunoprecipitated with anti-IgE prior to analysis on gels. The total time that the complexes were exposed to the mild detergents was 4 h. The percent dissociation was calculated as described under Materials and Methods. (A) Effect of varying concentrations of CHAPS: (●) dissociation of β and γ_2 from α; (○) ratio of β:γ_2; (▲) λ_{max} of bromophenol blue in the samples. (B) comparative effects of different detergents: (△) Triton X-114; (○) sodium deoxycholate; (●) CHAPS; (◇) sodium cholate; (□) octylglucoside. The solvent contained 0.15 M NaCl and 0.02 M Tris-HCl at a pH of 7.6 (Triton X-114, CHAPS, and octyl glucoside) or 8.5 (sodium cholate and sodium deoxycholate). The arrows indicate the cmc shown in Table II.

to know whether β and γ_2 interact with each other even on the intact receptor. However, the dissociation data themselves make such an interaction likely.

Figure 2B shows comparative data from experiments in which we examined the dissociation induced by five different detergents whose cmc's encompass a 75-fold range (Table II). In each case substantial dissociation was evident only when appreciable concentrations of detergent micelles were present. Notably, the slopes of the dissociation curves are substantially different; those detergents with higher cmc's induced progressively increasing dissociation per *proportional* change in the concentration of detergent.

These findings suggested that a critical factor inducing dissociation was the absolute concentration of micellar detergent. In Figure 3A we have taken data from Figure 2B and plotted the percent of dissociation of a fixed concentration of receptors against the estimated absolute concentration of *micellar* detergent for the five detergents. It is apparent that regardless of the detergent the absolute concentration of micellar detergent correlates with the degree of dissociation.

Table II: Characteristics of Some Mild Detergents[a]

	Triton X-114	deoxycholate	CHAPS	cholate	octyl glucoside
chemical name	octylphenoxy-poly(ethoxy-ethanol)	7-deoxycholic acid sodium salt	3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate	cholic acid sodium salt	*n*-octyl β-D-glucopyranoside
charge	nonionic	negative	zwitterionic	negative	nonionic
cmc (mM); aggreg no.[b]					
this paper[c]					
$\Gamma/2 = 0.17$, $T = 4$ °C	0.25; ND[d]	0.95; ND	4.4; ND	7.5; ND	19; ND
literature					
$\Gamma/2 > 0.15$, $T \sim 22$ °C	ND	0.89;[e] 8.7[e]–15[f]	ND	2.8,[e] 3.2;[g] 2.5,[e] 5[f]	17;[h] ND[i]
$\Gamma/2 = 0$, $T \sim 22$ °C	0.21;[j] 140[k]	5;[l] ND	8;[m] 10[m]	12;[g] ND	25;[h] ND

[a] The values from the literature are taken from the original sources. We found that several compilations contained numerous errors of transcription. [b] Aggregation number, i.e., monomers/micelle. [c] All cmc values were determined with the fluorescent probe 8-anilino-1-naphthalenesulfonate in 0.15 M NaCl–0.02 M Tris at pH 7.6 (Triton X-114, CHAPS, octyl glucoside) or pH 8.5 (deoxycholate, cholate) at 4 °C. [d] ND = no data. [e] Vochten & Joos, 1970. [f] Small, 1968. [g] Small, 1971. [h] Shinoda et al., 1961. [i] A value of near 100 has been reported for the aggregation number of octyl glucoside [D. McCaslin, unpublished data; cited in Mimms et al. (1980)]. However, it appears that with this detergent there is a dispersion of the sizes of the micelles and that the aggregation number is sensitive to other components in the medium (D. McCaslin, personal communication). [j] Crook et al., 1963. [k] Value for the related detergent Tx-100 from Kushner & Hubbard (1954). [l] Norman, 1960. [m] Hjelmeland et al., 1983.

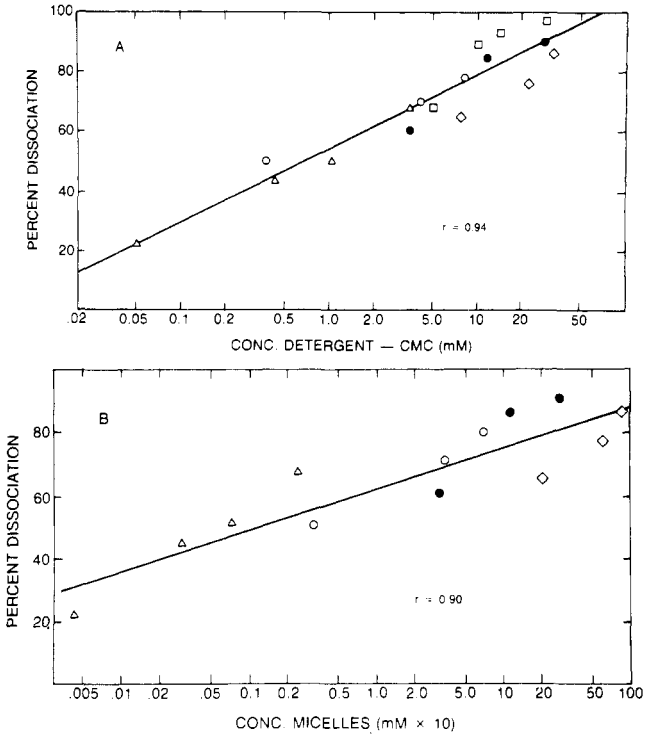

FIGURE 3: Dissociation of the receptor for IgE by detergent micelles. (A) The data from Figure 2 have been used to relate the extent of dissociation to the concentration of micellar detergent. The latter was calculated by substracting the experimentally determined cmc from the total concentration of detergent. (B) The extent of dissociation of the receptor as a function of the concentration of micelles. The concentration of micelles was calculated by dividing the values shown on the abscissa of (A) by the aggregation number (monomers/micelle) for each detergent shown in Table II. Since this number is not known for octyl glucoside, the data with this detergent have been omitted.

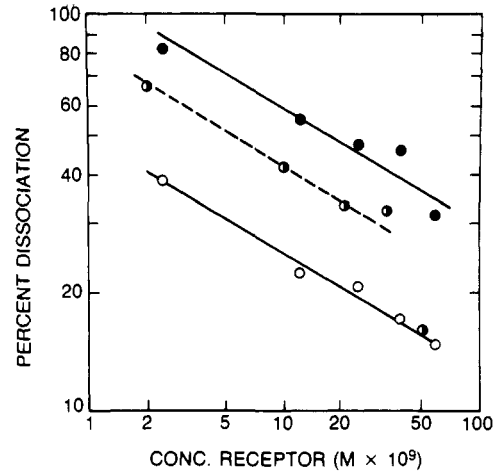

FIGURE 4: Dissociation of receptors as a function of their concentration. Two microliters of two different preparations containing $\sim 1.2 \times 10^{-7}$ M purified IgE–receptor complexes was incubated with increasing volumes of 10 mM CHAPS at 25 °C for 1 h. The solutions were then placed at 4 °C and diluted such that the concentration of CHAPS was decreased to 2 mM and all the volumes were equivalent. The complexes were then immunoprecipitated, and the extent of dissociation was assessed. The open circles are the data for freshly prepared receptors; the filled circles are the data for a preparation that had been stored for 10 days in 2 mM CHAPS. The half-filled circles show the results from the latter preparation replotted after correction for the dissociation (~15%) that had taken place prior to the start of the experiment. In this case the values on the abscissa represent the concentration of undissociated receptor in the aged preparation.

For the four detergents for which data are available, the cmc bears an approximately inverse relationship to their aggregate number (Table II). It is not surprising, therefore, that a similarly good correlation is observed if dissociation is plotted vs. the absolute concentration of micelles (Figure 3B). It should be noted that even the lowest concentration of micelles shown, $\sim 5 \times 10^{-7}$ M, is 1000 times greater than the concentration of the receptors ($\sim 5 \times 10^{-10}$ M).

All of the experiments described above were performed with an equivalent concentration of receptors incubated at 4 °C for a total of 4 h at the indicated concentration of detergent. The dissociation was calculated in relation to an equivalent concentration of receptor incubated for an equivalent length of time in 2 mM CHAPS. Several factors influenced the absolute amount of dissociation at a fixed concentration of detergent. One was the age of the preparation. Even in 2 mM CHAPS a moderate amount of β and γ_2 dissociated from IgE–α over prolonged times. For example, up to 30% after 2 weeks. This did not appear to result from slow proteolysis since analysis of the older preparations on polyacrylamide gels showed no evidence of proteolytic degradation of any of the chains.

Another factor that influences the amount of dissociation is the concentration of the receptor. Data relating to this aspect are shown in Figure 4 for two different preparations studied at a fixed concentration of detergent over a 25-fold range in concentration of the receptor. Both preparations show substantially greater dissociation at lower concentrations. In this type of graph a simple reversibly dissociating system of the type $AB \rightleftharpoons A + B$ yields a virtually linear plot. It can be seen that within experimental error the dissociation of both

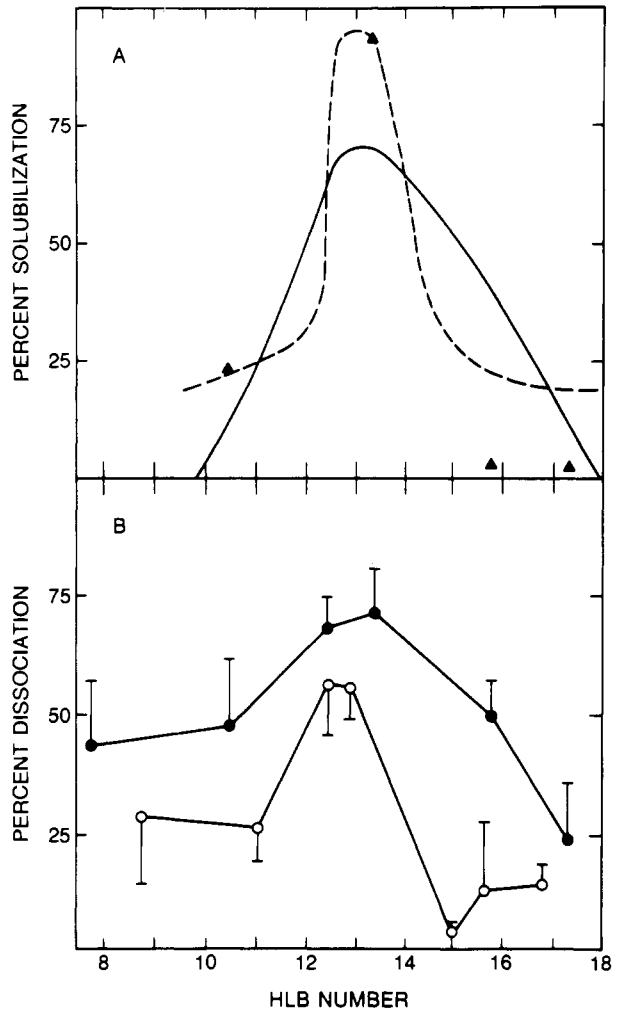

FIGURE 5: Effect of hydrophilic/lipophilic balance (HLB number) on the solubilization and dissociation of the receptor for IgE. (A) Cells were reacted with iodinated IgE, washed, and then incubated with 1% solutions of the following detergents: [(▲) from left to right] Triton X-45, Triton X-100, Triton X-165, and Triton X-305. The percentage of receptors solubilized was calculated from counts in the supernatant after a high-speed centrifugation compared to the total cell-bound counts. The dashed and solid lines are reproductions of the curves published by Umbreit & Strominger (1973) and Egan et al. (1976), which relate solubilization to HLB numbers for the systems they employed. (B) Two microliters of purified IgE–receptor complexes was incubated at 4 °C for 48 h with 200 μL of 1% solutions of the following detergents: [(●) left to right] Triton X-35, Triton X-45, Triton X-114, Triton X-100, Triton X-165, and Triton X-305; [(○) left to right] Span 20, Tween 85, Brij 95, Brij 55, Tween 60, Tween 40, Tween 20. At least two determinations of the extent of dissociation were performed for each detergent, and the values shown are the means ±1 SD.

preparations shows a concentration dependence consistent with such a mechanism. The preparation shown by the filled symbols was older than that shown by the open symbols and showed ~15% dissociation compared to when it had been freshly prepared. Even if the data are corrected for this "spontaneous" dissociation, the plot of the dissociation of the remaining intact receptor as a function of its concentration is displaced upward (half-filled symbols) compared to the other preparation, although the slopes of all the plots are very similar. This is what one would expect if the affinity of the subunits for each other was decreased. We have insufficient data to discriminate whether this is due to slight differences in the original preparation or to changes with time. In either case it underscores some of the variabilities that make it difficult to deal with this system.

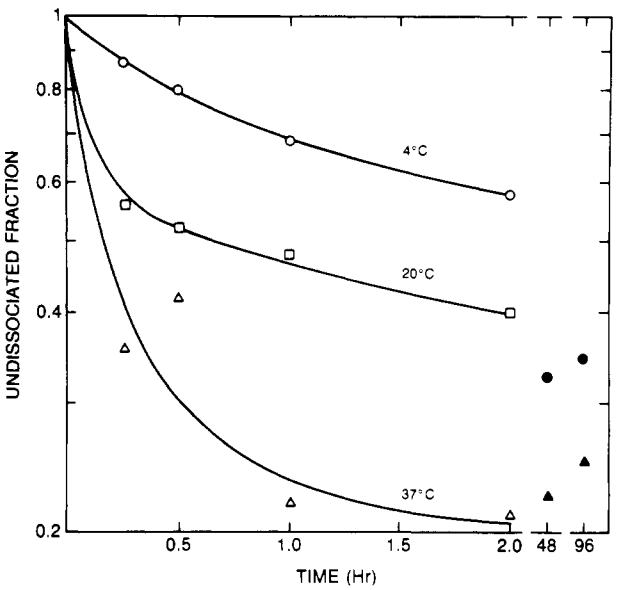

FIGURE 6: Effect of temperature on the dissociation of the receptor. Two microliters of purified receptor–IgE complex was added to 200 μL of 10 mM CHAPS at 4 (○, ●), 20 (□), and 37 °C (△, ▲). At various times the aliquots were quenched as described under Materials and Methods. The data shown by the open and filled symbols are from different experiments performed on the same preparation.

A further factor that influences the amount of dissociation observed, is the length of time that the preparation is exposed to the perturbant. The percent dissociation in experiments such as those shown in Figures 2 and 3 would have been different had we examined the receptors at alternative times. Examples of the kinetics of dissociation are shown in a different context below.

Effect of Hydrophilic/Lipophilic Balance (HLB Number) of Detergent. Of the many properties by which detergents can be characterized, the empirically determined HLB number (Griffin, 1949) appears to correlate best with the detergents' capacity to solubilize cellular membranes (Umbreit & Strominger, 1973). It was of interest to see if the capacity of detergents to dissociate the β and γ chains from the α chain correlated with their effectiveness in solubilizing plasma membranes. The data shown in Figure 5 indicate that this is the case. In the upper panel (Figure 5A) we have reproduced the solubilization curves published by Umbreit & Strominger (1973) (dashed line) and Egan et al. (1976) (solid line) for the systems they examined. Detergents having HLB numbers in the 12–14 range were optimal. The triangles give our results on the solubilization of the receptor for IgE from rat basophilic leukemia cells with four different Triton X detergents. It is clear that solubilization of the receptor for IgE is also maximal when detergents having HLB numbers in the 12–14 range are used. The lower panel (Figure 5B) presents our analysis of the relationship between the HLB number of various detergents and their effectiveness in dissociating the receptor for IgE. The upper curves shows an experiment using six different detergents of the Triton-X series. The lower one shows the results with Span, Tween, and Brij detergents that encompass HLB numbers in the range 9–17. Detergents with HLB numbers that are optimal for disrupting biomembranes also appear to cause maximum dissociation of the receptor.

Effect of Time and Temperature. Figure 6 shows the effects of time and temperature on the dissociation of the receptors at a fixed concentration of detergent. If the system was relatively simple, i.e., involving only the interaction between the receptor and micellar detergent, then because the receptors

Table III: Effects of pH and Salts on the Dissociation of the IgE–Receptor Complex Induced by Detergent[a]

dissociation examined	pH	ions		% dissociation[a]
		type	concn (M)	
α from IgE	3	NaCl	0.15	80
α from IgE	4	NaCl	0.15	60
α from IgE	4.5	NaCl	0.15	10
α from IgE	5–9	NaCl	0.15	1
α from IgE	10	NaCl	0.15	1
$\beta\gamma_2$ from α	3	NaCl	0.15	100
$\beta\gamma_2$ from α	4	NaCl	0.15	55
$\beta\gamma_2$ from α	4.5	NaCl	0.15	40
$\beta\gamma_2$ from α	5–9	NaCl	0.15	35
$\beta\gamma_2$ from α	10	NaCl	0.15	53
$\beta\gamma_2$ from α	7.5	NaCl	0.05	40
$\beta\gamma_2$ from α	7.5	NaCl	0.10	45 ± 0.7
$\beta\gamma_2$ from α	7.5	NaCl	0.30	59 ± 3
$\beta\gamma_2$ from α	7.5	NaCl	0.50	70
$\beta\gamma_2$ from α	7.5	KCl	0.10	43
$\beta\gamma_2$ from α	7.5	KCl	0.30	45 ± 9
$\beta\gamma_2$ from α	7.5	KI	0.3	50 ± 4
$\beta\gamma_2$ from α	7.5	KSCN	0.3	71 ± 1

[a] For the experiments in which the pH was varied, 2 μL of a preparation of purified–IgE receptor complexes was incubated at room temperature with 200 μL of a solution containing 10 mM CHAPS and whose pH varied by 0.5 pH units over a pH range of 3–10. After *15 min* the samples were placed at 4 °C and adjusted to pH 7.5, and the concentration of CHAPS was lowered to 2 mM by dilution with an appropriate buffer. The complexes were immunoprecipitated and analyzed for dissociation of β and γ_2 from α as before and for α from IgE by comparing the ratio of α:IgE with a suitable control. For the experiments on the effects of salts, the purified IgE–receptor complexes were incubated for a total of *4 h* at 4 °C with 10 mM CHAPS and variable concentrations of various salts prior to being assayed for dissociation as before. For those experiments performed 3 times, the mean ± the standard deviation is shown.

are at a very low concentration compared to the detergent, it is reasonable to assume that the dissociation will follow pseudo-first-order kinetics. Were the system in addition homogeneous, then the log of the concentration of the undissociated receptors should fall off linearly with time. This is clearly not the case. At each temperature the rate of dissociation decreases more rapidly than predicted for a homogeneous simple system following pseudo-first-order kinetics. Indeed, even after prolonged incubation *in a fixed volume* there remains a substantial amount of undissociated receptor (filled symbols in Figure 6). This accords with our previous experience, which showed that, in order to get complete dissociation of β and γ_2 with moderate concentrations of detergent at neutral pH and 4 °C, prolonged exposure to large volumes of detergent (preferably with replacement of the solvent, e.g., by washing of receptor bound to beads) is required [cf. Figure 4 in Rivnay et al. (1982)].

Effect of H^+ and Other Ions. The effects of pH are shown in Table III. No unusual pH effects were observed. We also examined the condition used in a procedure by which receptors are purified by binding them to an IgE affinity column and rapidly eluting them with 0.5 M acetic acid at 4 °C and then neutralizing rapidly (Kulczycki & Parker, 1979). We observed that even after only a 5-s exposure to these conditions 85% of β and γ_2 were lost relative to α; if the ~30% loss of α is taken into consideration, the loss of $\beta\gamma_2$ relative to the IgE was almost 90%. This explains the failure to observe the β and γ_2 subunits in those studies.

The effects of the type and concentrations of some simple cations and anions are given in the lower portion of Table III. High concentrations of salts appear to favor dissociation, and the stabilizing effect of K^+ vs. Na^+ and Cl^- vs. I^- vs. SCN^- follows the patterns observed for the denaturation of many other proteins (von Hippel & Schleich, 1969). A variety of other cations were tested. At 2 mM concentrations, Ca^{2+}, Mg^{2+}, Hg^{2+}, and Cu^{2+} did not affect the dissociation induced by CHAPS. The total exclusion of salts had no substantial effect either.

Discussion

In this study we exposed IgE–receptor complexes to a variety of physical and chemical conditions and analyzed the degree to which the integrity of the receptor was affected. We had several purposes in mind. One obvious and practical consideration was to find optimal conditions for purifying and maintaining intact receptors. Our data are clearly helpful in this respect.

A second consideration relates to our plans to perform chain-recombination studies as an approach to investigating the contributions of each of the chains to the function of the receptor. Again, the data described here give potentially useful clues about relatively gentle reversible conditions that could be examined for this purpose.

When other perturbants were added in addition to the detergent, variable results were obtained. Raising the temperature, which should enhance hydrophobic interactions and diminish electrostatic interactions, markedly promoted the disruptive effect of the detergents. High ionic strength should behave similarly and had the same effect. On the other hand, the progressively increasing disruption induced by substituting I^- and SCN^- for Cl^- implicates hydrophobic interactions. There are multiple hydrophobic interactions that these anions might affect: interactions between lipids that may be important components of the receptor (below), interactions between detergent molecules, and interactions between the latter and the receptor. Many more concentrations of detergents, ions, and temperatures would have to be assessed and analyzed for their effect on the detergent alone, as well as the receptor, before a more complete understanding of the effect of each of these perturbants could be achieved. Because of the complexity of the experimental analyses, we limited our exploration to a general survey of conditions designed to reveal any unusual phenomenon. When more is learned about the mechanism of dissociation, a more focussed exploration may well be justified.

A third consideration of our studies was to see if we could learn something about the mechanism of the unusual dissociation of the receptor in "mild" detergents. Our success in this respect is more difficult to determine.

There is good evidence that only micellar detergent destabilizes the receptor (Figures 2 and 3). That in some instances dissociation was observed at concentrations of detergent below the so-called critical micelle concentration (cmc) (Figure 2B) only appears contradictory if one presumes that below this value no micelles are present. The slippery nature of the cmc as an absolute value and as a concept has been well described by Mukerjee & Mysels (1971) and by Tanford (1980). This is not to gainsay the convenience of using the cmc as a rough measure of the concentration of detergent above which any added detergent largely adds to the concentration of micelles. The molar ratio of micellar detergent to receptor that is required to dissociate the receptor even partially was $\sim 10^5$ (Figures 3A and 4). This ratio is much higher than that necessary to solubilize cells, or to disperse lipids fully, where molar ratios of 2–10 are sufficient (Rivnay et al., 1982; Mimms et al., 1981).

Two mechanisms can be envisioned for the action of the detergent micelles. In one model, the micelles compete for the presumed hydrophobic interface between α and $\beta\gamma_2$:[3]

$$\alpha\beta\gamma_2\cdot\text{mic} + \text{mic} \rightleftharpoons \alpha\cdot\text{mic} + \beta\gamma_2\cdot\text{mic} \quad (1)$$

where mic refers to the micellar detergent.

Alternatively, more complex models can be postulated in which the stability of the receptor is dependent upon tightly bound lipid. For example, the action of the detergent could be to displace lipid:

$$\begin{array}{ccccc} \text{mic} + \alpha(\text{lip})_n\beta\gamma_2\cdot\text{mic} & \rightleftharpoons & \text{lip}\cdot\text{mic} + \alpha(\text{lip})_{n-x}\beta\gamma_2\cdot\text{mic} & & \\ \uparrow\downarrow & & \downarrow & & (2) \\ \text{lip} + \text{mic} + \alpha\cdot\text{mic} + \beta\gamma_2\cdot\text{mic} & & & & \end{array}$$

where lip refers to lipid.

Such a model postulates that receptor-bound lipid is in equilibrium with mixed micelles of lipid and detergent and that the receptor irreversibly dissociates when a critical amount of lipid is stripped from it. The effects observed at increasing concentrations of detergents (Figures 2 and 3) or decreasing concentrations of receptor (Figure 4) are consistent with either mechanism. Both equations posit a reversible component to the reactions. In principle, they must be, but we have not so far observed this experimentally. Certainly over the times employed in these studies, simply diluting out the detergent to submicellar concentrations does not lead to reassociation of β and γ_2 with α (data not shown).

If the displacement of lipid such as is postulated in eq 2 were reversible, this could explain the nonlinear kinetic data illustrated in Figure 6. As the reaction proceeded to the right, the increasing concentrations of the released lipid would progressively compete with the detergent micelles for binding sites on α and $\beta\gamma_2$.

As shown in Figure 5, the optimum HLB number of a detergent for solubilization of biomembranes corresponds closely to the optimum for dissociation of the receptor. The former process chiefly entails disrupting lipid–lipid and lipid–protein interactions. It can be argued that the detergent is likely, therefore, to play a similar role in dissociating the receptor. However, this argument is rather indirect and is weakened by the paucity of data showing that the disruption of hydrophobic interactions between proteins requires qualitatively different properties for the detergents. [A suggestion that this might be the case comes from the work of Helenius & Simons (1975) and Robinson & Capaldi (1977) discussed below.]

We previously showed that when added to detergent solutions of receptors, phospholipids protect against dissociation (Rivnay et al., 1982; Kinet et al., 1984; Table I). The explanation of this protective effect is uncertain. If the detergent acts by eq 1, one could postulate that by interacting with detergent micelles the added lipid reduces the capacity of the micelles to compete for the interacting groups on α and $\beta\gamma_2$. It is difficult to understand, however, why the capacity of different lipids to protect against dissociation should then be so different (Rivnay et al., 1982; Kinet et al., 1984).

If eq 2 is correct, then presumably the effect of added phospholipids would be to shift the equilibrium to the left. In a solvent containing radioactive lipids, one should, therefore, observe progressive incorporation of counts into the receptor providing the lipids were of the protective kind. This is a testable prediction.

Although we are not aware of other multisubunit proteins that show precisely the same structural instability as the receptor for IgE, some related observations have been published. Helenius & Simons (1975) found that solubilization of Semliki Forest virus by deoxycholate induced concomitant dissociation of the spike protein complex. However, Triton X-100, although equally capable of solubilizing the virus, did not induce such disruption. Similarly, Robinson & Capaldi (1977) reported that although several mild detergents were roughly equivalent in their capacity to delipidate the cytochrome C oxidase protein complex, deoxycholate uniquely also caused dissociation of the native dimer into monomers. The monomeric structure—itself composed of several different polypeptides—appears not to have been disrupted. There are of course many examples of other membrane proteins whose function is inhibited by so-called mild detergents; doubtless, these induce more subtle conformational perturbations.

An example of a non-membrane protein complex that is affected by several mild detergents is the complex of aminoacyl-tRNA synthetases. Dissociation of this complex has recently been analyzed by Sihag & Deutscher (1983). Interestingly, they found an additive effect of high salt, chaotropic ions, elevated temperature, and detergent, just as we have for the receptor for IgE. They also observed that addition of lipids counteracted the disruptive effect of the detergents.

It is evident that the general phenomena we have explored with the receptor for IgE are not unique to it. Moreover, we think it likely *that there are interactions between discrete membrane proteins that have a similar lability when removed from the milieu of the lipid bilayer.*[4] We anticipate that the approaches we have taken in exploring the receptor for IgE and our findings will, therefore, have a wider applicability in the study of how membrane proteins interact with each other.

Registry No. CHAPS, 75621-03-3; Triton X-35, 9002-93-1; Triton X-114, 9036-19-5; Tween 20, 9005-64-5; Tween 40, 9005-66-7; Tween 60, 9005-67-8; Tween 80, 9005-65-6; Tween 85, 9005-70-3; Span 20, 1338-39-2; Brij 95, 9004-98-2; Brij 55, 9004-95-9; H^+, 12408-02-5; K, 7440-09-7; Na, 7440-23-5; Cl, 16887-00-6; I, 20461-54-5; SCN, 302-04-5; Ca, 7440-70-2; Mg, 7439-95-4; Hg, 7439-97-6; Cu, 7440-50-8; NaCl, 7647-14-5; KCl, 7447-40-7; KI, 7681-11-0; KSCN, 333-20-0; octyl glucoside, 29836-26-8; 7-deoxycholic acid sodium salt, 302-95-4; sodium cholate, 361-09-1.

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[3] We have presented evidence elsewhere that both the intact receptor and the dissociated chains interact with detergent micelles (Alcaraz et al., 1984).

[4] After submission of the manuscript, two papers appeared that may be examples of such phenomena (Shimomura et al., 1984; Arad et al., 1984). The first shows that lipid helps to integrate the iron–sulfur protein into a complex with heart ubiquinol-cytochrome *c* oxidoreductase of the mitochrondral respiratory chain; the second shows that phospholipids can prevent the detergent-induced dissociation of adenylcyclase from the GTP binding stimulatory protein.

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Binding and Activation Properties of Human Factor XII, Prekallikrein, and Derived Peptides with Acidic Lipid Vesicles†

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ABSTRACT: The binding of human factor XII and prekallikrein to vesicles of various compositions and the relationship to activation of factor XII were studied. Factor XII, factor XII_a, and the 40-kilodalton binding fragment of factor XII bound tightly to all of the negatively charged lipids investigated, including sulfatide, phosphatidylserine, and phosphatidylethanolamine, but not to the neutral lipid phosphatidylcholine. Binding could be reversed by high salt, and the dissociation constant for binding to sulfatide vesicles was in the nanomolar range at an ionic strength of 0.15 M. Prekallikrein did not bind significantly to either sulfatide or phosphatidylethanolamine vesicles under the conditions used. Stopped-flow studies showed that the association rate for the factor XII–sulfatide interaction was biphasic and very rapid; the faster rate corresponded to about 30% collisional efficiency. The kinetics of activation of factor XII was investigated and was in agreement with previous studies; sulfatide promoted activation but phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine did not. Autoactivation rates correlated closely with the packing density of factor XII and factor XII_a on the vesicle surface. In contrast, kallikrein activation of factor XII correlated with the amount of sulfatide-bound factor XII and was relatively insensitive to the density of factor XII on the vesicle surface. When the concentration of factor XII was reduced to only several molecules per vesicle, the autoactivation rate dropped very low whereas kallikrein activation held relatively constant. These results indicated that the autoactivation and the kallikrein activation of factor XII were dependent on different properties of the surface component.

The human plasma proteins factor XII, factor XI, prekallikrein, and high molecular weight kininogen compose the contact activation system of blood coagulation [for a review, see Griffin & Cochrane (1979)]. The contact system is initiated when plasma in plastic tubes comes into contact with certain negatively charged surfaces such as kaolin, glass (Margolis, 1963), ellagic acid–metal complex (Bock et al., 1981), dextran sulfate (Kluft, 1978), and sulfatide vesicles (Fujikawa et al., 1980). Factor XII_a[1] appears to play the central activator role through limited proteolysis of prekal-

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[1] Abbreviations: factor XII, single-chain factor XII; factor XII_a, two-chain factor XII which is enzymatically active; factor XII_f, 28-kDa fragment of factor XII which is enzymatically active; factor XII_{bf}, 40-kDa N-terminal nonenzymatic binding fragment of factor XII; PC, egg phosphatidylcholine; PE, egg phosphatidylethanolamine; PS, bovine brain phosphatidylserine; DMPS, synthetic dimyristoyl-PS; dansyl, 8-(dimethylamino)-1-naphthalenesulfonyl; dansyl-PE, *N*-dansyl-PE; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; kDa, kilodalton(s); Tris, tris(hydroxymethyl)aminomethane.

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