

Phase Separation of the Receptor for Immunoglobulin E and Its Subunits in Triton X-114*

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Above its critical micelle concentration, Triton X-114 in solution forms two phases at room temperature: a lower phase containing supramicellar aggregates and an upper phase largely depleted of detergent. This property of the detergent is potentially useful for separating under mild conditions proteins that bind detergent from those that do not (Bordier, C. (1981) *J. Biol. Chem.* 256, 1604-1607). We studied the distribution of the receptor for immunoglobulin E (IgE) and its subunits in the two phases. IgE and IgE complexed either with intact receptors or with the α chains of the receptor alone are principally partitioned into the upper phase, whereas the unliganded receptor as well as the isolated α , and especially the β and γ chains of the receptor, preferentially partition into the lower detergent phase. Chromatography of IgE and of the subunits of the receptor on a hydrophobic support showed that the β and γ chains have a considerably greater hydrophobic surface than the α chains or IgE. These results indicate that the distribution of a protein in the two phases of phase-separated Triton X-114 is not an all-or-none effect based upon whether it binds detergent or not. Rather, it reflects the overall balance between the hydrophobic and hydrophilic properties of the protein's surface.

Mast cells and related cells have a plasma membrane receptor with high affinity for immunoglobulin E which, when aggregated, initiates degranulation of the cells. Although a $M_r = 45,000$ glycoprotein (α) accounts for the IgE¹-binding activity of the receptor, we recently proposed that the receptor must be considered as a tetramer consisting of a single α

chain, a single β chain, and a disulfide-linked dimer of γ chains (1).

The different chains have distinctive characteristics, especially with respect to properties that reflect the relative surface hydrophobicity of the polypeptides (Table I). A unique property of this complex protein is that it dissociates in mild detergents unless lipids are present (14).

In this paper, we describe the properties of the receptor and its subunits in the detergent Tx-114. This detergent contains approximately two less ethoxy groups than the analogous, and more commonly used, Triton X-100. The decrease in ethoxy groups is sufficient to lower the cloud point, the temperature at which supramicellar aggregates form, from 60-100 °C to 25-30 °C (15). Thus, at this or higher temperatures, the detergent solution forms two phases: an upper phase which contains ~95% of the total volume but only a small proportion of the detergent and a lower phase which contains most of the detergent. Bordier (16) proposed that these properties of Tx-114 could be used to characterize and separate membrane proteins. His data indicated that proteins that do not bind detergent preferentially localize in the upper phase, whereas those that bind detergent are concentrated in the lower detergent phase.

Our studies with the receptor for IgE suggest that the manner in which molecules distribute in the two phases may not simply reflect their ability to bind detergent. The distribution resembles rather a partitioning of molecules with a relatively hydrophilic surface to the upper phase and those with a more hydrophobic surface to the lower phase.

MATERIALS AND METHODS

Proteins and Cells—The sources, methods of preparation for the hybridoma mouse IgE (17), antibodies to IgE, and rat basophilic leukemia cells were the same as described previously (4).

Other Materials—Triton X-114 (octylphenoxy polyethoxyethanol) was obtained from Sigma (lot 71F-0158). It was fractionated at the desired temperature as described by Bordier (16). We assumed an average of 7.5 ethoxy groups ($M_r = 537$) and a molar absorption of 1.46×10^3 at 275 nm at pH 8, equivalent to that of the published value for Triton X-100 (18). The concentrations of stock solutions were based on measurements of absorbance at room temperature in 1% sodium dodecyl sulfate. We express concentrations in molar terms; thus, a 1% solution is ~19 mM. The zwitterionic detergent CHAPS was from Pierce Chemical Co. Bromphenol blue (4,4'-(3H-2,1-benzoxathiol-3-ylidene)bis[2,6-dibromophenol] *S,S*-dioxide) was obtained as the sodium salt from J. T. Baker Chemical Co.

Standard Procedures—The procedures we use for analytical electrophoresis in sodium dodecyl sulfate in polyacrylamide gels and for the autoradiography of such gels have been described (2). In this study, all gels were 12.5% in polyacrylamide. The amount of radioactivity in individual components was determined either by measurements of transmission on the autoradiographs using a model 1650 scanning densitometer and a model 1321 recorder (both from Bio-Rad) and measuring peak areas by planimetry, or by direct counting of strips from the dried gel, or both.

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¹ The abbreviations used are: IgE, immunoglobulin E; Tx-114, Triton X-114; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

TABLE I
Hydrophobic/hydrophilic surface properties of the subunits of the receptor for IgE on rat basophilic leukemia cells

Subunit	No./receptor (2-5) ^a	M _r	Extra membrane exposure	Carbohydrate	Labeling with			Binding by		Hydrophobicity index ^c
					INA ^b (5, 6)	P _i (5, 7)	Palmitate (8)	C ₁₈ support (this paper, Figs. 4 and 5)	Detergents (this paper)	
α	1	45 (10)	Exterior (11)	30% (w/w) (2)	—	—	±	Weak	+ ^d	0.288 (2)
β	1	33 (3)	Cytoplasmic (4, 7, 13)	None (4)	+	+	+	Strong	+	ND ^e
γ	2	10 (5)	Cytoplasmic (5, 13)	None (5)	+	+	+	Strong	+	ND

^a The numbers in parentheses indicate references to the pertinent data.

^b INA, iodonaphthyl azide.

^c See Ref. 9. Peripheral membrane proteins and serum proteins typically yield a value of 0.12 ± 0.16 , integral membrane proteins 0.52 ± 0.11 .

^d Additional evidence for the binding of detergent micelles by the α chains comes from our unpublished studies on purified IgE-α chain complexes by gel filtration in the presence of Triton X-100. The IgE-α complex showed a Stokes radius compared to IgE alone which was much larger than would be expected for the 20% increase in molecular weight. That the α chain of the IgE-α complex can be extrinsically iodinated little or not at all at micellar concentrations of detergent (12), but is substantially iodinated at submicellar concentrations (see "Materials and Methods"), provides additional supportive evidence.

^e ND, no data.

Purification of IgE-Receptor Complexes—Arsonylated mouse anti-dinitrophenyl IgE-receptor complexes in crude detergent extracts of rat basophilic leukemia cells (5) were partially purified on a trinitrophenyl lysine column (4). After absorption of the extract containing ~10 μg of bound IgE on 0.2 ml of the affinity matrix (capacity of 100 μg of free IgE), the latter was washed by gravity flow successively with 200 volumes of 10 mM CHAPS, 2 mM liver phospholipids (14), 20 volumes 10 mM CHAPS and 20 volumes 2 mM CHAPS over a period of ~15 min. The IgE-receptor complexes were eluted from the column three times with 200 μl of 10 mM ε-NH₂ dinitrophenyl caproate in 2 mM CHAPS for 15 min, 15 h, and 4 h, respectively. The IgE-receptor complexes were then extrinsically iodinated by the chloramine-T method and freed of unbound iodine by centrifugation through a 10-ml column of Sephadex G-25 equilibrated with 10 mM CHAPS, 2 mM phospholipids (5). The material was then applied to an antibenzenes arsonate column and washed with the same solutions used for the first column, except that the last two washes were 10 volumes only. The complexes were then eluted from the column by three 1½ h applications (the last two at room temperature) of 2 mM *p*-(arsonic)phenylazotyrosine in 2 mM CHAPS (19). This protocol differs from previous ones and permits all of the receptor subunits to be labeled efficiently while at the same time yielding intact receptor in a relatively simple solvent (2 mM CHAPS).

Reverse-phase Chromatography—Aliquots of extrinsically iodinated purified receptor-IgE complexes, which had been eluted in 2 mM CHAPS, were added to a 1 M pyridine, 0.5 M acetic acid, pH 5.5 buffer (20) with or without 30% propanol. The final volume was 1 ml. They were then loaded on 0.5-ml Sep-Pak C₁₈ cartridges (Waters Associates, Milford, MA) equilibrated with pyridine/acetate alone. The effluent was collected and the columns eluted with varying concentrations of propanol. The recovered solutions were dried in a Speed-Vac concentrator (Savant Instruments, Hicksville, NY), dissolved in sample buffer, and analyzed on gels.

RESULTS

Methodological Aspects—In the absence of adequate concentrations of phospholipids, the subunits of the receptor for IgE dissociate at micellar concentrations of detergents (14). Since we wished, among other things, to determine the distribution of intact receptors in the two phases of Tx 114, we sought conditions under which dissociation would be minimized. Since the dissociation is time-dependent and increases at higher temperatures,² we aimed for conditions that would

promote rapid phase separation at a relatively low temperature.

In working out the appropriate conditions, we found it convenient to use bromphenol blue in the sample. This colored amphiphile was found to distribute itself in the upper and lower phases in parallel with the Tx-114 (Fig. 1). The ability to judge semiquantitatively the relative efficiency of phase separation simply by eye under a variety of solvent conditions in this way is helpful. The physical separation of the phases by pipetting is also accomplished more securely in the presence of the blue marker. We routinely used 40 μM dye which yields an absorbance of ~2 at 590 nm at neutral pH in sodium dodecyl sulfate (see "Materials and Methods"). By fractionation of the stock detergent at 23 °C rather than at 30 °C (16) and by using 0.5 M NaCl in the solvent, efficient separation could be achieved in <15 min at a relatively low temperature (Table II). Since the specimens to be analyzed were sometimes

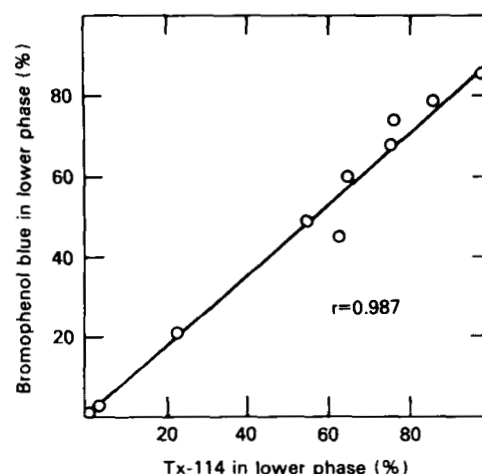


FIG. 1. Distribution of bromphenol blue and Tx-114 in upper and lower phases. This experiment was performed at 25 °C. The extent of Tx-114 depletion in the upper phase was varied by adding different amounts of the zwitterionic detergent CHAPS, or NaCl, or both, to the solution.

² J.-P. Kinet, G. Alcaraz, A. Leonard, S. A. Wank, and H. Metzger, manuscript in preparation.

TABLE II

Effect of prefractionation of detergent, temperature, and ionic strength on the efficacy of phase separation of Tx-114

Samples of 9 mM Tx-114 were incubated for 15 min at the temperature indicated in the second column and then centrifuged at $\sim 300 \times g$ for 2 min. The centrifugations at 37 °C were performed by suspending the small (~ 1 ml) tube containing the sample from the cap of a 50-ml tube freshly filled with water at 37 °C.

Temperature at which Tx-114 was purified	Temperature during phase separation	Concentration of NaCl ^a	Bromphenol blue in upper phase
°C	°C	M	%
30	25	0.15	34.3
	37	0.15	19.9
	25	0.50	16.0
	37	0.50	12.3
23	25	0.15	11.7
	37	0.15	10.3
	25	0.50	6.4
	37	0.50	5.9

^a All solutions also contained 10 mM Tris-HCl, pH 8.0.

TABLE III

Phase separation of IgE-receptor complexes under conditions that minimize chain dissociation

Extrinsically iodinated IgE-receptor complexes were phase separated at 25 °C in 9 mM Tx-114. Aliquots of the phases and the unfractionated material were analyzed on a polyacrylamide gel, and the radioactivity in the components was determined by direct counting of the gel and by tracings of the autoradiograph.

Component	Unfractionated eluate	Phase-separated eluate	
		Upper phase	Lower phase
IgE	"100"	80	20
α	27	18	6.5
β	16	11	6.1
γ	25	17	5.2
α /IgE	0.27	0.22	0.32
$(\beta + \gamma)/\alpha$	1.52	1.56	1.74

in a detergent other than Tx-114, care had to be exercised that the final concentration of this "contaminating" detergent was not sufficient to interfere with the phase separation. We found that the detergent we use most frequently, CHAPS, did not interfere providing it was present at <0.01 mol/mol of Tx-114. The effect of CHAPS therefore corresponds approximately to that of sodium deoxycholate which interferes with the phase separation of Tx-114 at similarly low molar ratios (calculated from Fig. 3 of Ref. 16).

Distribution of IgE and IgE-Receptor Complexes—Virtually 100% of isolated IgE remains in the upper phase when 9 or 19 mM solutions of Tx-114 are phase separated (data not shown).

When a fresh preparation of extrinsically iodinated IgE-receptor complexes were phase separated in Tx-114 at 25 °C over <10 min, 80% of the IgE was recovered in the upper phase (Table III). There was a slight depletion of α relative to the IgE, a phenomenon regularly observed (see below), but the ratio of $\alpha:\beta:\gamma$ in the upper phase corresponds closely to that observed in the starting material.

Phase Separation of Unliganded Receptors—An unfractionated cell extract, containing 5×10^7 cell equivalents/ml in 9 mM Tx-114, 0.5 M NaCl, was phase separated in the presence of bromphenol blue as a marker, in 10 min at 25 °C. The upper and lower phases were adjusted to 3.7 mM Tx-114 and 0.15 M NaCl, 0.02 M Tris-HCl, pH 8, the cell equivalents then being $\sim 1 \times 10^7$ cells/ml. The separated phases, as well as an

unfractionated control sample, were then incubated for 45 min at 0 °C with 7 μ g/ml ^{125} I-IgE with or without prior addition of a 15-fold excess of unlabeled IgE. Bound IgE was determined using an assay for solubilized receptors which employs $(\text{NH}_4)_2\text{SO}_4$ (21). Table IV shows the results from two of several such experiments. Approximately 60% of the recovered binding activity was found in the detergent pellet and 40% in the upper phase. The overall recovery was excellent. The results were similar whether the extracts were phase separated with 9 mM (experiment 1) or 19 mM Tx-114 (experiment 2).

The values for the partitioning of bromphenol blue (65% in experiment 1, 83% in experiment 2) should be considered approximate since they were determined in separate tubes and since we do not know the precise correlation of the distribution of the dye with that of the detergent in the presence of lipids. Nevertheless, they show that there was considerable phase separation and that the unliganded receptor is distributed in the lower phase to a substantial extent. If the average value of 75% for the distribution of the bromphenol blue is taken as an indicator of the completeness of phase separation, it can be calculated that had the phase separation been complete, 80% of the unliganded receptor would have been distributed in the pellet of detergent (i.e. $0.59:0.75 = 0.79$). Rephrased, this indicates a preferential distribution of the $\alpha\beta\gamma_2$ complex in the detergent phase of 4:1. This result is in marked contrast to the behavior of the liganded receptor which was preferentially distributed into the aqueous phase to a similar extent (Table III).

Distribution of Dissociated β and γ Chains and IgE- α —Extrinsically labeled IgE-receptor complexes in 2 mM CHAPS were reacted for 30 min at 37 °C with either 9, 19, 37, or 75 mM Tx-114. Each solution was then diluted 10-fold and adjusted such that the final concentration was 9 mM in Tx-114. The phases were separated and the upper phases saved. The lower phases were repurified by resuspending them at 0 °C with buffer and detergent to bring the Tx-114 concentration to 19 mM and, after reincubation at 37 °C for 15 min, separating the phases again. This time, only the lower phases were saved. Fig. 2 is an autoradiograph of the gel on which the four upper and lower phases were analyzed along with a sample of the unfractionated preparation. Fig. 3 illustrates tracings taken from this autoradiograph. It is clear that the β and γ chains have dissociated from the α chains almost completely under these conditions, and that the β and γ subunits preferentially partition into the lower phase, whereas

TABLE IV

Assay of unliganded receptor in crude extract of cells before and after phase separation with Tx-114

Each experiment was performed in duplicate, and each specimen was assayed in triplicate. In experiment 1, the phase separation was conducted in 9 mM Tx-114 in the presence of 2 mM phospholipid. The corresponding values in experiment 2 were 18 and 3 mM.

	Experiment 1	Experiment 2
IgE binding capacity		
	molecules/cell equivalent $\times 10^{-6}$	
Unfractionated extract	4.4	4.9
Upper phase of Tx-114	2.1 ± 0.26	1.7 ± 0.05
Lower phase of Tx-114	3.0 ± 0.26	2.4 ± 0.13
Recovery in lower phase		
	%	
Activity	59	59
Bromphenol blue ^a	65	83

^a The partitioning of bromphenol blue was performed in separate tubes.

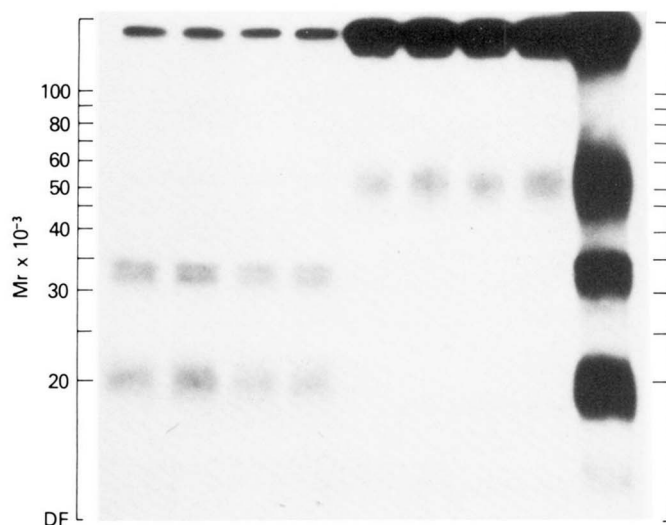


FIG. 2. **Effect of concentration of Tx-114 on dissociation of the subunits of the receptor.** The figure shows the autoradiograph of a polyacrylamide gel on which samples of the lower and upper phases of a Tx-114 phase separation were analyzed. The Tx-114 concentration during the phase separation itself was made 19 mM in each case by diluting the specimens with appropriate amounts of buffer and detergent. The lower phases were recondensed once. Lanes 1-4, lower phases of specimens incubated for 30 min at 37 °C with 9, 19, 37, or 75 mM Tx-114 prior to a phase separation. Lanes 5-8, upper phases of same specimens. Lane 9, starting material ($\sim 3\times$ as much as in the phase-separated material). DF, dye front.

the opposite is true of the IgE- α complex.

We tested to see if the β and γ chains were associated with each other, by reacting such lower phases containing β and γ_2 subunits with the cross-linking reagent dimethyl 3,3'-dithio-bispropionimidate (22) (Pierce Chemical Co.) (3, 4). Although an internal control (aprotinin dimer) showed that the cross-linking reagent was effective in the sample assayed, no cross-linked $\beta:\gamma_2$ product was observed. This result suggests, but doesn't prove, that the β chain and dimer of γ chains (γ_2) are not associated with each other in the Tx-114 pellet.

Phase Separation of α Chains—In order to assess the distribution of isolated α chains, extrinsically labeled IgE-receptor complexes were first incubated in Tx-114 in order to dissociate most of the β and γ chains (see above). Thereafter, the sample was centrifuged, and the supernatant was recovered. Tx-114 was added to the upper phase to restore the detergent concentration to ~ 19 mM, and after a further brief incubation at 37 °C, the solution was recentrifuged. In order to dissociate the IgE from the α chains in the upper phase, a 0.2 M glycine buffer was added to bring the final pH to 2.9. After 30 min at room temperature, the pH was returned to neutrality with a concentrated Tris buffer, and the specimen was divided into three aliquots. One portion of the specimen was examined directly on the same gel on which the pellets and supernatants of the other aliquots were analyzed. A second portion was immunoprecipitated with anti-IgE. This permitted us to assess the extent of dissociation of α from the IgE. A third aliquot had added to it sufficient Tx-114 and NaCl to bring the concentrations to ~ 19 mM and 0.5 M, respectively, and subjected to phase separation by centrifugation after an additional brief incubation at 37 °C.

The specimen examined directly (aliquot 1) showed that $\sim 80\%$ of the β and γ chains had been dissociated from the α chains during the initial incubation at 37 °C in Tx-114. The data from the immunoprecipitation (aliquot 2) showed that 65% of the α chains had dissociated from the IgE after the acidification step (Table V). The phase-separation experi-

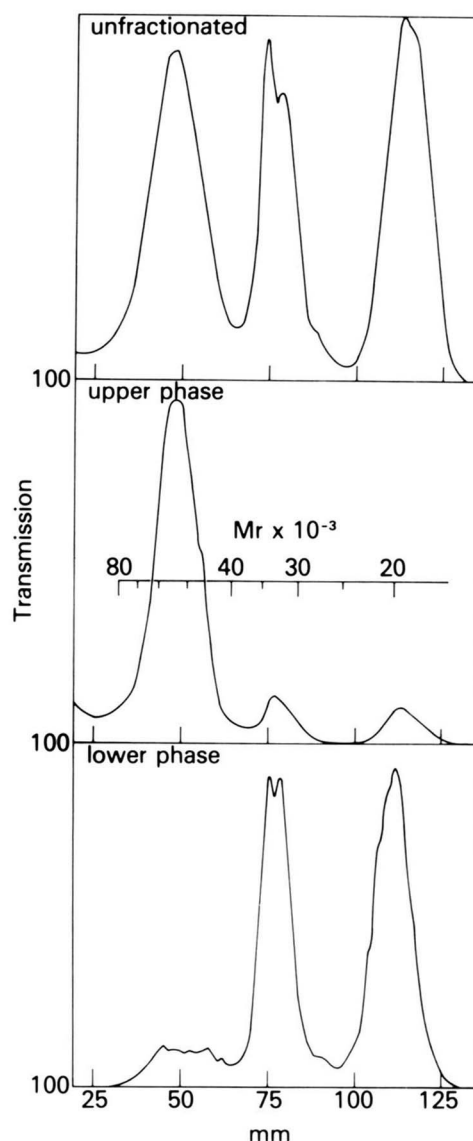


FIG. 3. **Densitometric scans of the samples phase-separated from 19 mM Tx-114 shown in lanes 2, 6, and 9 of Fig. 2, respectively.**

ment (aliquot 3) indicated that 80% of these dissociated α chains were partitioned in the pellet of detergent (Table V).

Studies with Hydrophobic Chromatography—The results obtained by phase separation in Tx-114 are interesting to compare with those obtained by hydrophobic chromatography on a C_{18} support. Extrinsically labeled IgE-receptor complexes in pyridine/acetate, pH 5.5, were applied to the column in the presence or absence of 30% propanol, and after rinsing with the same solvent, the column was washed with increasing concentrations of propanol. The eluted material was dried, separated on gels in sodium dodecyl sulfate, and analyzed by autoradiography.

Fig. 4A, lanes 1-3, shows a sample that had been added in buffer alone. The effluent showed no counts (lane 1). When the column was washed with 30% propanol, only IgE and α chains were eluted (lane 2). When the propanol concentration was increased to 60%, the β and γ chain dimer were released (lane 3). If the sample had been equilibrated with 30% propanol, IgE and α chains failed to bind to the column (lane 4) and could be cleanly separated from the β and γ chains; the latter, as before, were released with 60% propanol (lane 5).

TABLE V
Partitioning of dissociated IgE and α subunits

Extrinsically labeled IgE-receptor complexes were incubated in 18 mM Tx-114 in order to promote dissociation of the β and γ chains from the α chains. The preparation was then phase separated. Relative to the α chains, the upper phase contained 20% of the β and γ seen in the starting material. The solution was adjusted to pH 2.9 at 25 °C and, after 30 min, neutralized. The specimen was either reacted with anti-IgE or phase separated.

	Recovery		Ratio of counts, α /IgE
	IgE	α	
	%		
Acidified first Tx-114			
Upper phase	"100"	"100"	0.269 ± 0.000^a
Immunopre- cipitation with anti-IgE			
Supernatant	11.4 ± 2.2	68.5 ± 1.1 (65) ^b	1.59 ± 0.30
Pellet	88.5 ± 2.2	31.4 ± 1.1	0.09 ± 0.003
Phase separation			
Upper phase	90 ± 1	48 ± 1 (20) ^c	0.142 ± 0.003
Lower phase	9.2 ± 1	52 ± 1 (80)	1.50 ± 0.17

^a Average of duplicate determinations and range.

^b Estimated per cent dissociation calculated by correcting for incomplete immunoprecipitation of IgE.

^c The values in the parentheses were calculated by adjusting for the degree of dissociation of α from IgE estimated from the results of immunoprecipitation (see Footnote b).

That the α chains were not merely eluting from the column because they were bound to the IgE is shown by the experiment illustrated in Fig. 4B. Here, the sample and column were equilibrated with buffer alone. Lane 1 shows the effluent and lanes 2–7 the material eluted after rinsing the column with 20, 30, 40, 50, 60, and 100% propanol, successively. It is clear from the relative intensities of the band of IgE and α in lanes 2–5 that the α chains are dissociated from IgE and that they bind less tightly to the support than does IgE.

We interpret these results as follows. IgE, although incapable of binding substantial amounts of detergent (23), binds to the support even in 20% propanol. The α chain although clearly able to interact with detergent (Table V and footnote d, Table I) also binds to the support but less firmly than IgE. It seems likely that this is related to the fact that the α chain has a peptide molecular weight only one-fifth as great as that of IgE (32,000 (10) versus 160,000 (17)). On the other hand, although the peptide molecular weights of β and the γ chain dimer are, respectively, equal to and two-thirds as great as that of the α chain (Table I), they bind much more firmly to the support because of their greater hydrophobicity.

DISCUSSION

In 1981, Bordier (16) showed that membrane proteins would differentially distribute in the two phases formed by the nonionic detergent Tx-114 at moderate temperatures. He envisioned that those proteins capable of binding detergent would segregate to the lower hydrophobic phase, whereas those that did not bind detergent would remain in the upper, detergent-depleted, phase.

We have explored the behavior of the receptor for IgE in Tx-114 both to see what could be learned about the receptor as well as to test the usefulness of this detergent for preparative purposes.

The preparative implications of our results are clear. As shown in Figs. 2 and 3, the β and γ chains once dissociated from α are readily separated from latter, providing the α chains remain attached to IgE. The simplicity of the separa-

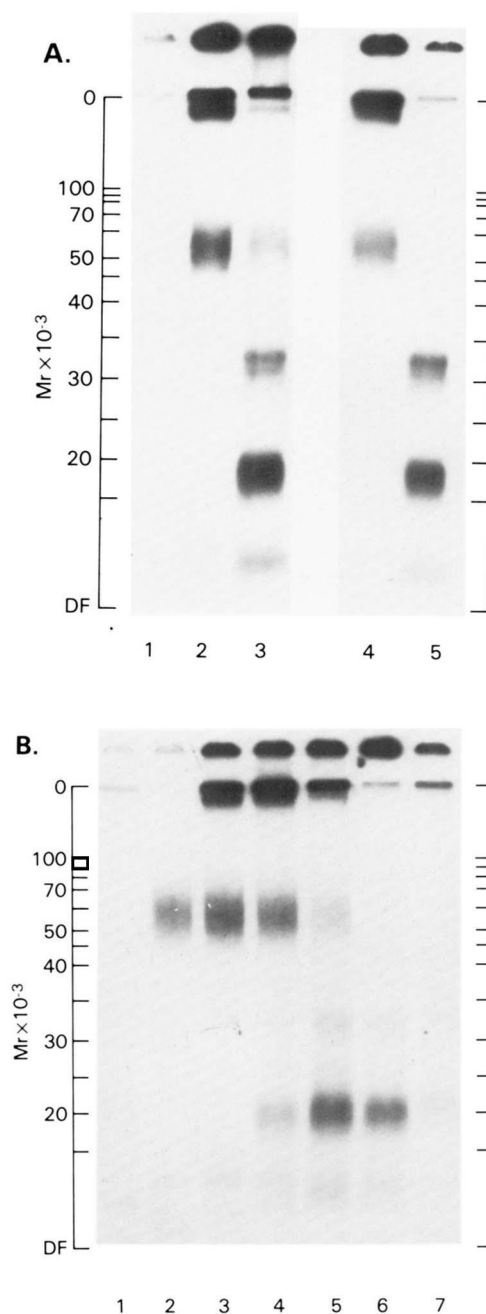


FIG. 4. Chromatography of extrinsically labeled IgE receptor complexes on a C_{18} support. Autoradiographs of gels on which the eluted material was separated are shown. A, two-step elution. Lanes 1–3, sample and column both in buffer without added propanol. Lane 1, effluent; lane 2, 30% propanol eluate; lane 3, 60% propanol eluate. Lanes 4 and 5, sample in buffer plus 30% propanol and column in buffer only. Lane 4, effluent; lane 5, 60% propanol eluate. B, multistep elution. The sample and column were equilibrated with buffer alone. Lane 1, effluent; lanes 2–7, eluates from column with 20, 30, 40, 50, 60, and 100% propanol, respectively. The small amount of material of $M_r < 10,000$ is likely to represent free γ chains (5).

tion and the mildness of the conditions needed to effect it may be especially useful for future studies in which one wishes to test the function of receptors reformed from isolated subunits. We are already employing Tx-114 for preparative isolations of the chains intended for structural studies.

All three subunits of the receptor for IgE interact with detergent, although the labeling studies (Table I) and the studies with hydrophobic chromatography strongly suggest

that the β and γ chains have a more hydrophobic surface than the α chains. Individually, the chains preferentially segregate into the detergent phase (Table V, Figs. 2, 3), and this is also true of the $\alpha\beta\gamma_2$ complex (Table IV). Nevertheless, when bound to the hydrophilic IgE, the α chain (Figs. 2 and 3) and indeed the entire complex (Table III) can be "pulled" into the aqueous phase.

Thus, pairs which can be expected to bind similar amounts of detergent,³ IgE· α : α and IgE· $\alpha\beta\gamma_2$: $\alpha\beta\gamma_2$, distribute very differently. These results indicate that the distribution of a polypeptide or complex of polypeptides in the phases formed by Tx-114 is not simply related to the protein's ability to bind detergent or not. It appears, rather, that the distribution of a component reflects the overall hydrophilic-hydrophobic properties of its surface; if so, then, on the one hand, phase separation with Tx-114 may not allow for as simple a preparative procedure as might be true otherwise. On the other hand, it may prove to be even more useful for characterizing differences between integral membrane proteins than originally envisioned (16).

REFERENCES

- Metzger, H., Kinet, J.-P., Perez-Montfort, R., Rivnay, B., and Wank, S. A. (1983) in *Progress in Immunology*, V (Yamamura, Y., and Tada, T., eds) pp. 493-501, Academic Press, Orlando, FL
- Kanellopoulos, J. M., Liu, T.-Y., Poy, G., and Metzger, H. (1980) *J. Biol. Chem.* **255**, 9060-9066
- Holowka, D., Hartmann, H., Kanellopoulos, J., and Metzger, H. (1980) *J. Receptor Res.* **1**, 41-68
- Holowka, D., and Metzger, H. (1982) *Mol. Immunol.* **19**, 219-227
- Perez-Montfort, R., Kinet, J.-P., and Metzger, H. (1983) *Biochemistry* **22**, 5722-5728
- Holowka, D., Gitler, C., Bercovici, T., and Metzger, H. (1981) *Nature (Lond.)* **289**, 806-808
- Fewtrell, C., Goetze, A., and Metzger, H. (1982) *Biochemistry* **21**, 2004-2010
- Perez-Montfort, R., Alcaraz, G., and Metzger, H. (1984) *Fed. Proc.* **43**, 1664 (Abst. 1446)
- Barrantes, F. J. (1975) *Biochem. Biophys. Res. Commun.* **62**, 407-414
- Kumar, N., and Metzger, H. (1982) *Mol. Immunol.* **19**, 1561-1567
- Kulczycki, A., Jr., McNearney, T. A., and Parker, C. W. (1976) *J. Immunol.* **117**, 661-665
- Pecoud, A., and Conrad, D. (1981) *J. Immunol.* **127**, 2208-2214
- Holowka, and Baird, B. (1984) *J. Biol. Chem.* **259**, 3720-3728
- Rivnay, B., Wank, S. A., Poy, G., and Metzger, H. (1982) *Biochemistry* **21**, 6922-6927
- Goldfarb, J., and Sepulveda, L. (1969) *J. Colloid Interface Sci.* **31**, 454-459
- Bordier, C. (1981) *J. Biol. Chem.* **256**, 1604-1607
- Liu, F.-T., Bohn, J. W., Ferry, E. L., Yamamoto, H., Molinaro, C. A., Sherman, L. A., Klinman, N. R., and Katz, D. H. (1980) *J. Immunol.* **124**, 2728-2736
- Robinson, N. C., and Tanford, C. (1975) *Biochemistry* **14**, 369-378
- Kinet, J.-P., Alcaraz, G., Wank, S. A., and Metzger, H. (1984) *Fed. Proc.* **43**, 1625 (Abst. 1223)
- Rubinstein, M., Stein, S., Gerber, L. D., and Udenfriend, A. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 3052-3055
- Rossi, G., Newman, S. A., and Metzger, H. (1977) *J. Biol. Chem.* **252**, 704-711
- Wang, K., and Richards, F. M. (1974) *Isr. J. Chem.* **12**, 375-389
- Newman, S. A., Rossi, G., and Metzger, H. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74**, 869-872

³ Given the amount of receptor available, it is impractical to assess directly the amount of detergent bound. Nevertheless, even if a difference in the amount of detergent bound was the case, e.g. between α and IgE· α , the argument would change only slightly, i.e. that distribution in the phases of the detergent is not simply a reflection of whether detergent is bound but reflects the amount bound. The concept that the balance between hydrophobic and hydrophilic surface structures is the determining factor covers both cases.