# Phospholipids Stabilize the Interaction between the $\alpha$ and $\beta$ Subunits of the Solubilized Receptor for Immunoglobulin E†

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ABSTRACT: The cell-surface component ($\alpha$) which binds monomeric immunoglobulin E with high affinity is associated with a second polypeptide ($\beta$) in the plasma membrane. The latter component tends to dissociate during purification of the $\alpha$ chain from detergent extracts of cells, even at neutral pHs and physiological ionic strengths. We now report that the interaction of $\alpha$ and $\beta$ can be stabilized by maintaining an appropriate phospholipid to detergent ratio. Under such conditions, other discrete components reproducibly copurify with the $\alpha$ and $\beta$ chains. These results suggest that the subunits of this membrane protein—or the interaction of it with other constituents in the cell—may be stabilized in ways not observed with ordinary soluble proteins.

Our laboratory has been investigating the structure and function of a membrane protein on the surface of mast cells and the related rat basophilic leukemia (RBL)[1] cells which binds monomeric immunoglobulin E (IgE) with high affinity (Metzger et al., 1982). Aggregation of this receptor, e.g., by the binding of a multivalent antigen to the surface-bound IgE, induces rapid degranulation of the cells on which the receptor resides (Metzger & Ishizaka, 1982).

When the surface of mast cells or RBL cells is labeled, IgE is added, the cells are solubilized with detergent, the IgE is precipitated with anti-IgE, and the precipitate is dissolved in $NaDodSO_4$ and analyzed on polyacrylamide gels, a single labeled component is observed (Conrad & Froese, 1976; Kulczycki et al., 1976). This "$\alpha$ chain" (Goetze et al., 1981) is a glycopeptide (Kulczycki et al., 1976; Kanellopoulos et al., 1980; Goetze et al., 1981) which for several years was referred to as "the receptor for IgE". We subsequently found that when cells were labeled by photolysis of the lipophilic probe 5-iodonaphthyl 1-azide (Bercovici & Gitler, 1978) a different component was observed in the immune precipitates (Holowka et al., 1981). This polypeptide ("$\beta$ chain") which migrated as a 36K band on gels was difficult to characterize because it tended to disappear when purification procedures other than immunoprecipitation were employed (Holowka et al., 1980; Holowka & Metzger, 1982). However, from experiments in which cross-linking reagents were used, it became clear to us that the $\alpha$ chain on intact cells or in unfractionated extracts of cells was intimately associated with the $\beta$ chain with a stoichiometry of 1:1 (Holowka et al., 1980, 1981; Holowka & Metzger, 1982). Others have also observed traces of a component with a molecular weight of $\simeq$30000 which variably copurified with IgE-$\alpha$ (Kulczycki & Parker, 1979; Helm & Froese, 1981), but the relationship of these components to $\beta$ is uncertain. For example, whether these components, like $\beta$, will cross-link to $\alpha$ or become labeled with iodonaphthyl azide has not been reported.

During the course of studies in which conditions were sought for stabilizing the $\alpha$-$\beta$ interaction, we were also engaged in attempts to reconstitute the receptor into liposomes. Those studies indicated that maintenance of lipid:detergent ratios within rather narrow limits was critical for successful reincorporation (Rivnay & Metzger, 1982). A parameter "$\rho$" was used to characterize this ratio where

$$\rho = \frac{[\text{detergent}] - \text{CMC}_{\text{effective}}}{[\text{phospholipid}]}$$

Here $\text{CMC}_{\text{effective}}$ is that concentration of detergent which when subtracted from the total concentration of detergent leads to equivalent solubilization of cells at a constant value of $\rho$ over at least a limited range of detergent:lipid ratios. When $\rho$ was maintained at around 2.5, complete solubilization of the receptor was achieved, and excellent reincorporation into liposomes was accomplished (Rivnay & Metzger, 1982). We have now found that similar conditions stabilize the interaction between the $\alpha$ and $\beta$ chains.

## Materials and Methods

*Cells and IgE.* Rat basophilic leukemia (RBL) cells were maintained as described (Barsumian et al., 1981) and were routinely found to have $3 \times 10^5$ receptors/cell. Rat IgE from tumor IR162 (Bazin et al., 1974), monoclonal mouse anti-dinitrophenyl-IgE from hybridoma HI-DNP-$\epsilon$-26.82 (Liu et al., 1980), and human myeloma IgE from patient PS were prepared as described elsewhere (Kulczycki & Metzger, 1974; Holowka & Metzger, 1982). The procedures used for iodination, amidination, and modification with benzenearsonate groups of IgE's have all been given (Kulczycki & Metzger, 1974; Holowka et al., 1980; Kanellopoulos et al., 1979).

*Affinity Columns.* The preparations of Bio-Gel A-5m beads (Bio-Rad, Richmond, CA) coupled with trinitrophenyllysyl groups ("TNP column") and Sepharose 4B (Pharmacia, Piscataway, NJ) coupled with anti-benzenearsonate antibodies have been described (Holowka & Metzger, 1982; Kanellopoulos et al., 1979). The former were eluted with 10 mM dinitrophenyl caproate and the latter with 1 mM *p*-arsonatophenylazotyrosine.

*Detergents and Lipids.* The detergent preparations used and their sources have been given as has the preparation of the tumor-derived lipids (Renkonen et al., 1963; Rivnay & Metzger, 1982). Lipids from rabbit liver were prepared in an identical manner. Other lipids used were commercially available: soybean lecithin (type IV S, Sigma, St. Louis, MO), phosphatidylserine (PS) (from bovine brain, no. P6641, Sig-

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[1] Abbreviations: RBL, rat basophilic leukemia; IgE, immunoglobulin E; $NaDodSO_4$, sodium dodecyl sulfate; PS, phosphatidylserine; PE; phosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; DNP, dinitrophenyl; $Cl_3CCOOH$, trichloroacetic acid.

ma), phosphatidylethanolamine (PE) (no. 840021, Avanti Polar-Lipids, Birmingham, AL), dipalmitoylphosphatidylcholine (DPPC) (no. 850355, Avanti), and dioleoylphosphatidylcholine (DOPC) (no. 850375, Avanti). All lipids were quantitated for their phospholipid content by the method of Ames & Dubin (1960). For solvents employing specific lipid:detergent ratios, the parameter $\rho$ was employed (see above) by using the following values of $CMC_{effective}$ for the detergents previously determined (Rivnay & Metzger, 1982): 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (Chaps), 4.7 mM; octyl glucoside, 20.5 mM; sodium cholate, 9.7 mM; Triton X-100, $\leq$0.3 mM.

*Solubilization of Receptors.* Receptors were solubilized by adding detergent to either intact cells or particulate fractions of cells (Rivnay & Metzger, 1982) such that the value of $\rho$ (see above) of the final extract was $\simeq$2 without addition of exogenous lipid. For this purpose, we have found that $5 \times 10^7$ RBL cells (or an equivalent amount of membrane material) yield $\simeq$2 $\mu$mol of phospholipid. In most of the experiments described here, five protease inhibitors (1 mM phenylmethanesulfonyl fluoride, 20 mM iodoacetamide, 1 $\mu$g/mL leupeptin, 3 $\mu$g/mL pepstatin, and $\sim$0.6 trypsin inhibitor unit/mL of aprotinin) were used (Rivnay & Metzger, 1982).

*Extrinsic Labeling of Receptors.* Receptors were labeled either on intact cells by lactoperoxidase-catalyzed iodination (Kanellopoulos et al., 1979; Marchalonis, 1969) or in eluates containing partially purified receptors, by the Chloramine-T method. In the latter instance, the eluate was then filtered through G25 Sephadex by the (excellent!) centrifugation procedure of Tuszynski et al. (1980).

*Intrinsic Labeling of Receptors.* Cells were harvested from stationary flasks on the fourth day and washed 2 times with complete medium and then once in leucine-free medium [Eagle's minimum essential medium 2 with Earl's balanced salt solution, 0.22% $NaHCO_3$ without leucine (NIH media unit no. 921219)] to which we added 0.06% glutamine, 100 units/mL penicillin, 100 $\mu$g/mL streptomycin, 60 $\mu$g/mL tylocine, and undialyzed fetal calf serum to 20%. The cells were then suspended at $3 \times 10^5$ cells/mL in a 250-mL spinner flask in the same medium containing also 0.05 mCi/mL ($8.1 \times 10^{-2}$ $\mu$mol) [$^3$H]leucine (62 Ci/mmol, Amersham Corp., Arlington, IL). After 36 h, the cells (which were $\sim$80% viable by testing with trypan blue) were harvested, washed, incubated with $^{125}$I-labeled mouse IgE, and, after being rewashed, solubilized directly with Chaps at a $\rho \simeq 2.5$ and processed further.

*Cross-Linking.* Cross-linking was performed on solubilized receptors as described by Holowka et al. (1980) and Holowka & Metzger (1982) by using dimethyl dithiobis(propionimidate) (Pierce Chemical Co., Rockford, IL).

*Immunoprecipitations.* Immunoprecipitations were performed by using 10 $\mu$g of affinity-purified rabbit antibodies and a maximum of 1 $\mu$g of the appropriate IgE. After 2 h, 20–50 $\mu$L of a 10% suspension of protein A containing *Staphylococcus aureus* (Pansorbin, Calbiochem, La Jolla, CA) (Kessler, 1975) was added.

*Slab Gel Electrophoresis.* The general procedure we use follows that described by Ames (1974). Gels were analyzed by autoradiography (Holowka & Metzger, 1982; Swanstrom & Shank, 1978) or were sliced and counted directly (for iodine) in a Biogamma counter (Beckman, Palo Alto, CA) or after treatment with NCS tissue solubilizer as described previously for $^3$H (Holowka et al., 1980).

## Results

*Use of Cross-Linking Reagent.* In order to examine the conditions required to stabilize the interaction of $\alpha$ with $\beta$, we were faced with several problems. Incorporation of $^3$H-labeled amino acids, in order to label the $\beta$ chain, is relatively inefficient [presumably because the receptor turns over slowly (Isersky et al., 1979)] and is therefore costly and time consuming. The $\beta$ chain can be radiolabeled with the lipophilic probe [$^{125}$I]iodonaphthyl azide (Holowka et al., 1981), but again the yield of radioactivity is very low and several attempts to improve the incorporation were unsuccessful. We therefore decided for the initial experiments to surface label cells (which labels the $\alpha$ chains exclusively), expose the solubilized IgE–receptor complexes to the desired condition, react the material with cross-linking reagent, and isolate the IgE–receptor complexes by immunoprecipitation.

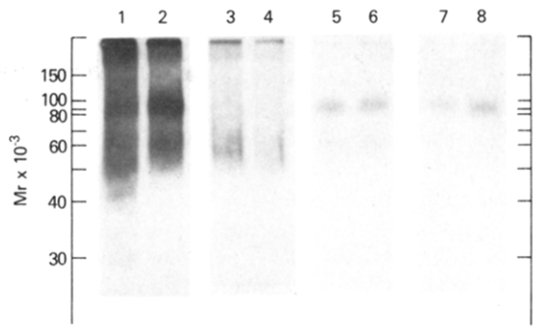


FIGURE 1: Autoradiogram of a 10% polyacrylamide slab gel on which cross-linked IgE–receptor complexes were analyzed. Complexes were solubilized from surface-labeled cells. They were then applied to a hapten affinity column, washed with the desired buffer, eluted with hapten, and only then cross-linked. The material was then immunoprecipitated and analyzed. For each condition used, the entire procedure was performed in duplicate. (Lanes 1 and 2) Material obtained by cross-linking the unpurified detergent extract, i.e., without prior isolation from an affinity column; (lanes 3 and 4) material purified on an affinity column washed with 10 mM Chaps alone; (lanes 5 and 6) material purified on an affinity column washed with a Triton X-100 extract ($\rho \simeq 2.5$) of whole tumor cells; (lanes 7 and 8) material purified on an affinity column washed with tumor lipids/detergent buffer ($\rho \simeq 2.5$).

The presence of $\alpha$–$\beta$ complexes was detected by a shift in the molecular weight of the $\alpha$ chain by using gel electrophoresis (Holowka et al., 1980). Since cross-linking the receptor interferes with the binding of IgE, the latter was added before the bifunctional reagent. Since cross-linking of IgE with the $\alpha$ chain precludes observing the shift in the molecular weight of the $\alpha$ chain, the IgE was amidinated in order to block its lysines (Holowka et al., 1980).

In a typical experiment, mouse anti-dinitrophenyl, amidinated IgE was incubated with surface-labeled cells, the cells were washed and solubilized with detergent, and the supernatant from a centrifugation at 31000*g* for 1 h was applied to a TNP column. The column was washed with the desired solvent, and the bound receptor–IgE complexes were eluted with hapten. The eluate was reacted with the cross-linker dimethyl dithiobis(propionimidate), the reaction was quenched with glycine, and the IgE–receptor complexes were precipitated with anti-IgE and *Staphylococcus* A adsorbant. After the precipitates were washed, they were extracted with $NaDodSO_4$ and were analyzed—without reduction—by gel electrophoresis.

Figure 1 illustrates such an experiment—the last in a series and the one which yielded the most definitive information. It is an autoradiograph of a 10% polyacrylamide gel on which immunoprecipitated material was analyzed by electrophoresis in $NaDodSO_4$. In lanes 1 and 2 is material from the extract of cells which was not applied to the TNP column but which was directly cross-linked in duplicate and then immunoprecipitated with anti-IgE. In addition to some high molecular

weight material, the following two principal components are seen: the broad band at ~60K, representing un-cross-linked $\alpha$ chains, and the band at ~95K, which we have previously demonstrated to be the cross-linked product of $\alpha$ and $\beta$ chains (Holowka et al., 1980, 1981; Holowka & Metzger, 1982). Lanes 3 and 4 represent material from a similar cell extract which was applied to duplicate TNP columns and washed with 9 mM Chaps before elution, cross-linking, and immunoprecipitation. It can be seen that virtually no cross-linked product was observed, suggesting that the $\beta$ chain had been removed. Similar experiments utilizing Triton X-100 instead of Chaps gave the same result. Lanes 5 and 6 represent material from cells solubilized with Triton X-100, applied to duplicate TNP columns, and washed with a Triton X-100 extract of unlabeled cells prior to elution, cross-linking, and immunoprecipitation. In this instance, the cross-linked product is observed again, demonstrating that the binding to the TNP column, washing, and elution were not responsible for the failure to observe cross-linking in specimens 3 and 4. A similar experiment using for the washing solution a crude extract which had been extensively dialyzed vs. Triton X-100 showed the same result. Lanes 7 and 8 are specimens from cells which had been solubilized with 9 mM Chaps applied to a TNP column and washed with a solvent containing 9 mM Chaps and 2 mM phospholipids ($\rho \simeq 2.1$) extracted from RBL tumors. It can be seen that the addition of lipids alone preserved the ability to generate the cross-linked product as effectively as the unfractionated extract of tumors.

*Labeling of IgE–Receptor Complexes.* Having found conditions under which the interaction of $\alpha$ and $\beta$ could be stabilized during purification, it became possible to extrinsically label the $\beta$ component, thereby greatly simplifying further studies of the $\alpha$–$\beta$ interaction. Benzenearsonylated, amidinated, mouse anti-dinitrophenyl-IgE was reacted with cells. The IgE–receptor complexes in extracts from the cells solubilized with detergent were partially purified by using a TNP column prewashed with a Chaps/lipid buffer. The complexes bound to the beads were labeled with $^{125}I$, the beads were washed again, and the complexes were eluted with hapten in Chaps/lipid buffer. The preparation was then split: half (1) was applied to an anti-benzenearsonate column equilibrated with Chaps only, and the column was washed with 150 column volumes of buffer containing 10 mM Chaps over 3 days. The other half (2) was treated identically except that the column had been equilibrated with a Chaps/lipid buffer and the washing solution also contained 10 mM Chaps and 2 mM tumor lipids ($\rho \simeq 2.6$). The columns were then eluted with benzenearsonate hapten, the released complexes were immunoprecipitated, and the specimens were analyzed on a 10% polyacrylamide gel. An autoradiograph of the latter is shown in Figure 2. Specimen 1 shows an intense band near the origin, representing the unreduced IgE, a weaker band at $M_r$ 116 000 (not yet positively identified), and a faint broad band [seen more distinctly on other films (e.g., Figure 3)] at ~58K, representing the $\alpha$ chain. [It has been previously shown that, even when solubilized, the latter is only poorly labeled extrinsically by oxidative iodination when bound to IgE (Pecoud & Conrad, 1981).] Specimen 2 is dramatically different. It shows prominent bands at 45K and 36K and a weaker band at 21K. On prolonged exposure, an additional weak band at <15K was also observed (see also Figure 3).

Figure 3 is an autoradiogram of a gel used to analyze material prepared by a procedure very similar to that followed for sample 2 (above) with the following differences: One specimen was prepared from a detergent extract of whole cells whereas the other was derived from a detergent extract of a membrane fraction of the cells (Materials and Methods). A second (minor) difference was that the iodination was performed on the eluates from the TNP column and not on the beads themselves. Finally, after the immunoprecipitation (which by accident was only partially effective in precipitating the IgE–receptor complexes), the supernatant was treated with 2.5 volumes of cold acetone. The resulting precipitates were also analyzed.

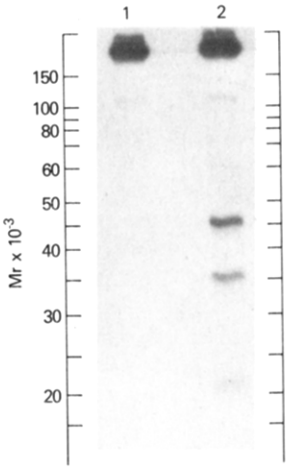


FIGURE 2: Autoradiogram of a 10% polyacrylamide gel on which immunoprecipitated IgE–receptor complexes prepared from mouse benzenearsonylated anti-dinitrophenyl—IgE were analyzed. The complexes were first bound to a TNP column and washed with a buffer containing Chaps and tumor lipids. They were then iodinated in situ, washed, and eluted with dinitrophenyl caproate. Half of the material was applied to one anti-benzenearsonate column and washed with buffer containing 10 mM Chaps before being eluted with hapten (sample 1). The other half was treated similarly except that the wash buffer contained 2 mM tumor lipids in addition (sample 2).

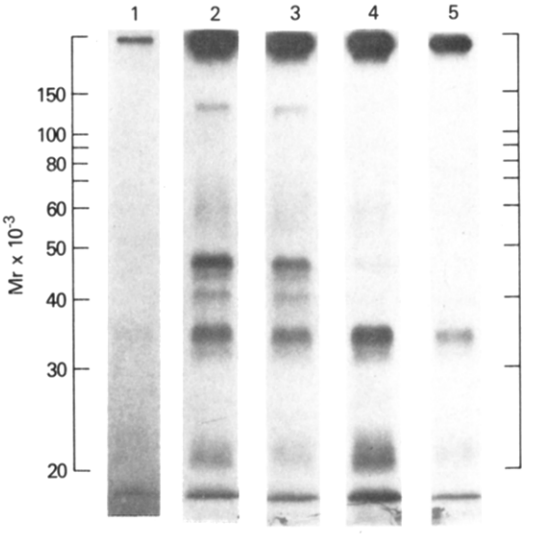


FIGURE 3: Autoradiogram of a 10% polyacrylamide gel on which IgE–receptor complexes purified and extrinsically labeled in a manner very similar to that used for sample 2 in Figure 2 (i.e., using buffers which at all times contained 2 mM lipids and 10 mM Chaps) were analyzed. (Lane 1) The $NaDodSO_4$ extract of the human IgE–anti-human IgE (control) precipitate prepared from mouse IgE–receptor complexes purified from a detergent extract of whole cells. A similar precipitate from the particulate material gave exactly the same result (not shown). (Lane 2) Complexes from the same material used in lane 1 except that anti-mouse IgE was used for the immunoprecipitation. (Lane 3) Complexes prepared from a particulate fraction of cells and specifically precipitated with anti-mouse IgE. (Lane 4) Material precipitated by acetone from the supernatant of the immune precipitate shown in lane 2. (Lane 5) Materials precipitated by acetone from the supernatant of the immune precipitate shown in lane 3.

Table I: Quantitative Analysis of Gel Patterns of IgE–Receptor Complexes Precipitated with Anti-IgE[a]

| component | | complexes prepared from | |
|---|---|---|---|
| $M_r \times 10^{-3}$ | identity | whole cells[c] | membrane fraction[c] |
| 56 | α | 4.6 | 4.0 |
| 45 | ? | 13.7 | 13.4 |
| 36 | β | 8.8 | 9.7 |
| 20 | ? (β1)[b] | 6.3 | 5.8 |
| 15 | ? (β2)[b] | 6.9 | 6.4 |

[a] The analysis was performed on lanes 2 and 3 of the gel the autoradiograph of which is reproduced in Figure 3. [b] β1 and β2 refer to the domains of the β chain which we postulated were generated by proteolytic cleavage of the chain (Holowka & Metzger, 1982; Metzger et al., 1982). [c] Values are the percentage of counts in the IgE bands.

As can be seen in Figure 3, lane 1, the control precipitate, prepared by adding human IgE and anti-human IgE to the eluate from the anti-benzearsonate columns, yielded a weakly radioactive band at the position of IgE only. The specific immune precipitates showed the same principal bands previously noted. The broad α-chain band is somewhat more prominently seen as is the band at <15K. The material derived from a particulate fraction of the cells (lane 3) yielded the same pattern observed with the material derived from whole cells (lane 2). A quantitative analysis of the bands is given in Table I. When normalized for the number of counts in the band representing IgE, each of the other major bands can be seen to be equivalently represented in the two preparations. Thus, all of the components appear to be associated with the cell membrane fraction.

In lanes 4 and 5, which contain the material precipitated with acetone, the 45K band is virtually absent. We have observed this on several occasions but have not yet determined its cause. It is notable that these precipitates contain only components observed in the immune precipitate, so that all the peptides seen appear to be associated with the receptor.

*Intrinsic Labeling.* If all of the components observed by extrinsic iodination were present in substantial amounts, it can be anticipated that they should also be observed in receptors isolated from cells labeled biosynthetically. Figure 4 shows the result of such an experiment using an extract from cells grown in [$^3$H]leucine. It can be seen (bottom panel) that the receptors isolated in the presence of tumor lipids show the same components on gel electrophoresis as those observed with the iodinated material. There is substantial incorporation into the α chain, the 45 000 molecular weight component, the β chain, and the 20 000 molecular weight component. Only components observed by extrinsic iodination are apparent, demonstrating that the latter procedure labels all the principal components associated with the receptor when isolated in this manner. All except one of the principal components observed after iodination show considerable incorporation of [$^3$H]leucine. This suggests that these components are not simply trace contaminants which had been disproportionately heavily iodinated. Only the ~14 000 molecular weight component (cf. Figure 3) was not observed after incorporation of [$^3$H]leucine. However, since such a component has been previously observed in material isolated from cells which had incorporated a mixture of $^3$H-labeled amino acids (Holowka et al., 1980) or $^{32}$P (Fewtrell et al., 1982), we are inclined to view this component as significant; it may simply not contain very much leucine.

The top panel in Figure 4 shows that by omitting added lipid in the washing buffer, all of the 45 000 molecular weight

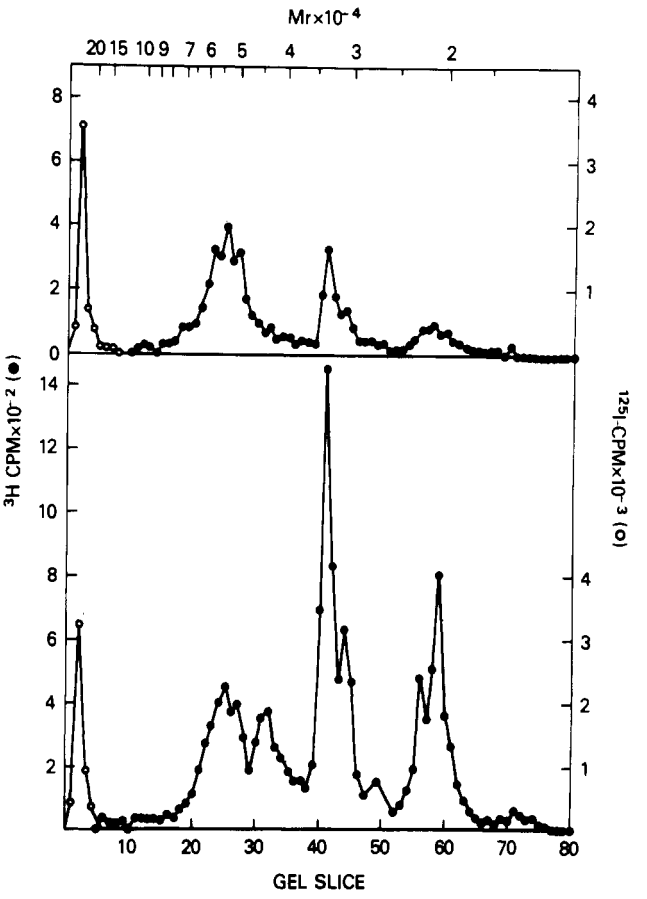


FIGURE 4: Analysis of IgE–receptor complexes isolated from cells which had been grown in the presence of [$^3$H]leucine. The cells were saturated with $^{125}$I-labeled IgE and solubilized with Chaps at $\rho = 2.5$, and the IgE–receptor complexes were isolated by affinity chromatography on successive columns conjugated with TNP and anti-benzenearsonate antibodies. Elution was with the appropriate hapten in each instance. The eluates were then reacted with anti-IgE, and the precipitates were extracted with $NaDodSO_4$. After electrophoresis on a 12.5% polyacrylamide gel, the latter was dried, cut into 2-mm slices, and counted for $^{125}$I and $^3$H. (Top panel) Material isolated from the eluate of the anti-benzene column which had been washed over 48 h with ≃200 column volumes of 10 mM Chaps. (Bottom panel) Exactly like the top panel except that the washing buffer also contained 2 mM phospholipids.

component and most of the β chain and the 20 000 molecular weight material are lost. This confirms that the components other than the α chain are differentially lost. When analyzed on a separate gel, a control precipitate performed by adding human IgE and anti-human IgE to the eluate yielded no counts above background anywhere on the gel (not shown).

*Isolation of Dissociated Components.* The results described in the previous sections indicated that by changing the solvent to which the IgE–receptor complexes are exposed it should be possible to isolate the components of interest as well as to develop an efficient assay which would permit more detailed analysis of the interaction of these components with the α chain. Preparations, such as those described in Figures 2 (sample 2) and 3, were rebound to a TNP column, employing a solvent containing 10 mM Chaps and 2 mM phospholipids throughout. The column was then incubated in a buffer containing 10 mM Chaps without lipid and eluted *without addition of hapten* at 24 and 48 h. Figure 5 shows an autoradiogram of a gel on which the $Cl_3CCOOH$ precipitates of the eluted material were analyzed, and Table II is the quantitative analysis of the gel. It is apparent that the eluted material is substantially enriched in the β chain and that it is virtually devoid of IgE and the α chain which remain bound

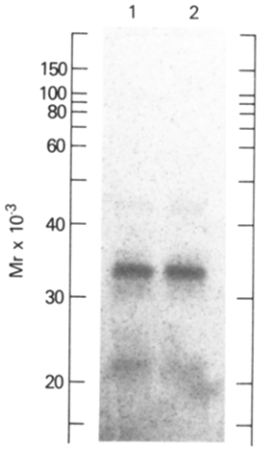


FIGURE 5: Autoradiogram of a 12.5% polyacrylamide gel on which acetone-washed $Cl_3CCOOH$ precipitates of material which dissociated from labeled IgE–receptor complexes in the presence of 10 mM Chaps without lipids were analyzed. (Lane 1) Material which dissociated during the first 24 h of incubation. (Lane 2) Material which dissociated during a subsequent 24 h of incubation.

Table II: Quantitative Analysis of Gel Patterns of Detergent Eluate from Bound IgE-Receptor Complexes

| | component | |
|---|---|---|
| | $\alpha$[c] | $\beta$[c] |
| unfractionated material[a] | 4.5 ± 1.9 | 8.4 ± 3.9 |
| detergent eluate[b] | (12) | 720 |

[a] Mean ± SD of three separate analyses. One specimen had been immunoprecipitated; the other two were $Cl_3CCOOH$ precipitates. [b] $Cl_3CCOOH$ precipitates. The autoradiograph of the gel which was analyzed is shown in Figure 5. Slices from lanes 1 and 2 were pooled for this analysis. The band at the position for IgE contained a net of 110 cpm above background (85 cpm) representing ≃ 1% of the total counts attributable to IgE which was bound to the TNP column. All calculations are based on 10-min counts. The value in the parentheses is likely to be highly inaccurate because the counts on which it is based were only ≃2% above the background. [c] Values are the percentage of counts in the IgE band.

to the column under these conditions. It can also be calculated that ~80% of the $\beta$ chain was recovered. The $Cl_3CCOOH$ precipitates had been washed with acetone prior to analysis. Possibly because of this, the 45K component was lost (above). The latter results suggested that simply by monitoring the radioactivity in the washes the dissociation of $\beta$ and the other non-$\alpha$ components could be estimated. Table III presents an example of how this protocol can be employed. Some dissociation of radioactivity occurred in all of the samples, but the protective effect of pure phospholipids, as well as by the more complex mixtures of lipids, is apparent. A more complete analysis will now be possible in order to define more precisely the conditions under which the interaction of the $\alpha$ chain with the other components can be selectively stabilized or disrupted.

## Discussion

The principal aim of this study was to discover conditions under which the association of the $\alpha$ and $\beta$ chains of the receptor for IgE could be preserved during purification of the solubilized receptor. We have now found that by maintaining throughout the purification procedures the phospholipid:detergent ratio used during solubilization, this goal was achieved. Furthermore, we have demonstrated that by appropriate manipulation of the solvent, conditions can be found by which the $\beta$ chain can be recovered in the washing solution, thereby

Table III: Release of Label from Receptors Bound to the TNP Column

| incubation buffer | | | | | |
|---|---|---|---|---|---|
| detergent | | | | % of total counts released[a] | |
| type | concn (mM)[b] | lipid (2 mM) | $\rho$ | 2 h | 9 h |
| Chaps | 10 | | ∞ | 4.5 ± 0.43 | 5.1 ± 0.15 |
| octyl glucoside | 26 | | ∞ | 5.5 | 5.9 |
| sodium cholate | 15 | | ∞ | 5.4 | 5.7 |
| Triton X-100 | 6 | | ∞ | 6.5 | 7.9 |
| Chaps | 10 | tumor | 2.6[c] | 1.6 | 2.9 |
| Chaps | 10 | liver | 2.6 | 1.5 | 2.5 |
| Chaps | 10 | soybean | 2.6 | 2.4 | 2.4 |
| Chaps | 10 | PS | 2.6 | 3.0 | 3.6 |
| Chaps | 10 | PE | 2.6 | 3.1 | 4.3 |
| Chaps | 10 | DPPC | 2.6 | 3.4 | 4.8 |
| Chaps | 10 | DOPC | 2.6 | 2.5 | 3.0 |

[a] Based on data such as those in Table I, it can be calculated that ≃30% of the bound counts should eventually have been released if the IgE and the $\alpha$ chain attached to it had remained bound to the column. [b] The concentrations of detergent were such that when the $CMC_{effective}$ was subtracted the difference was ~5 mM. [c] The parameter $\rho$ was calculated on the basis of a value for $CMC_{effective}$ determined in the presence of excess tumor-derived lipids (Rivnay & Metzger, 1982).

greatly simplifying future characterization of this component of the receptor.

When IgE–receptor complexes are isolated under conditions where the detergent to lipid ratio is such that $\rho \simeq 2$–3, subsequent analysis shows several components in addition to the $\alpha$ and $\beta$ chains (Figures 2–5). The 20K and ~14K components have been observed previously (Holowka et al., 1980; Holowka & Metzger, 1982; Fewtrell et al., 1982). Several results were consistent with these being proteolytic breakdown products $\beta$ ("$\beta$1" and "$\beta$2", respectively) (Holowka & Metzger, 1982; Fewtrell et al., 1982). It is apparent that these components dissociate or remain associated with the $\alpha$ chains under approximately those conditions under which the 36K $\beta$ chain dissociates from or remains associated with the $\alpha$ chain. Now that these components can be reproducibly isolated and labeled, it should be possible to test their relationship to the $\beta$ chain directly, e.g., by peptide mapping.

Focusing on the two largest components associated with the $\alpha$ chain, the $\beta$ chain and the 45 000 molecular weight component, it is clear that they are both membrane associated (Figure 3, Table I) and are cosolubilized and copurified with the $\alpha$ chain. Cross-linking studies which demonstrated that $\alpha$ chains and $\beta$ chains are associated in a 1:1 complex on intact cells and in detergent extracts led us to consider the $\beta$ chain a "subunit" of the receptor (Holowka & Metzger, 1982). Further experiments will be required to define the stoichiometric relationship of the 45K component to $\alpha$ and $\beta$. We have occasionally observed components of this molecular weight previously and raised the possibility that they represented breakdown products of the $\alpha$ chain (Holowka et al., 1980). However, our new observations that it can be extrinsically labeled in the presence of bound IgE, that it dissociates at high values of $\rho$, and that it has somewhat unusual solubility properties (i.e., with respect to acetone) appear to differentiate it from the $\alpha$ chain. On gels, its mobility is like that of rabbit actin (unpublished experiments). Again, since we can now isolate it, its further characterization should be feasible. It will also be of special interest to determine whether the $\beta$ chain and other components found to be associated with the $\alpha$ chain in addition independently interact with other membrane receptors. Antibodies specifically directed toward the $\beta$ chain

etc. should provide an efficient tool to answer this question.

There are abundant examples in which specific detergents or lipids have been found to protect the native conformation or function, or both, of solubilized membrane proteins (Baron & Thompson, 1975; Rizzolo & Tanford, 1978; Rizzolo, 1981; Parsegian, 1982). However, our data appear to be the first to demonstrate that membrane lipids (mainly phospholipids) preserve an interaction between two or more separate polypeptides. It is becoming clear that the stability of some membrane proteins depends on the hydrophobic environment of the lipid bilayer or even on interactions with specific lipid components. Such proteins will dissociate or suffer significant conformational changes when the bilayer is disrupted even by "mild" detergents. Maintenance of an environment sufficient for solubilization, but as close to the native one as feasible, enhances the likelihood of preserving the original relationships.

Further analysis is required to determine whether the less prominent "bands" (Figure 3) or "peaks" (Figure 4) which copurify with the principal components represent additional significant elements. Our experience with the $\beta$ chain illustrates that it is unwise to discount these prematurely as simple contaminants.

We wish to emphasize that there is no sharp dividing line between subunits of a membrane receptor and other cellular elements with which it may be associated. The critical question with regard to such polypeptides is not one of terminology, i.e., whether they are to be considered subunits, but of their functional relevance. As noted elsewhere, it is possible that the receptor for IgE undergoes no functionally significant alterations itself but rather that its aggregation causes changes in other cellular elements with which it interacts, by an "extrinsic mechanism" (Metzger, 1983). The procedures employed in the present study may be useful for studying this question.

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