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A Previously Unrecognized Subunit of the Receptor for Immunoglobulin E†

Ruy Perez-Montfort,‡ Jean-Pierre Kinet, and Henry Metzger*

ABSTRACT: Our laboratory previously found that under conditions that stabilized the interaction between the α and β subunits of the receptor for immunoglobulin E, two new components were recovered having apparent molecular weights of 45 000 and 20 000, respectively. In this paper, we characterize the 20-kDa material. We demonstrate that it consists of a disulfide-linked dimer of 10-kDa polypeptides and that these have all the characteristics expected for subunits of the receptor. We propose that they be termed γ chains and that the receptor consists of four chains: one α, one β, and two γ chains. The γ chains share many of the labeling properties of the β chain and, like the latter, are likely to be embedded in the plasma membrane and exposed on the internal but not the external surface of the bilayer.

Mast cells, basophils, and the related rat basophilic leukemia (RBL)[1] cells have a protein on the surface of the plasma membrane that binds monomeric immunoglobulin E (IgE) with high affinity. Aggregation of this receptor induces degranulation of the cells [reviewed in Metzger & Ishizaka (1982)].

The IgE binds to a glycopeptide termed the α chain. Evidence has accumulated that a second polypeptide (β chain) is associated with α in a 1:1 molar ratio, but this chain has been difficult to purify in the absence of chemical cross-linking reagents (Holowka & Metzger, 1982). Recently, we found that by adding phospholipids to the nonionic detergent containing solvents used during purification, the α-β interaction could be stabilized (Rivnay et al., 1982). Surprisingly, under these conditions two new components were observed of molecular weight 45 000 and 20 000, respectively. This paper describes the characterization of the 20-kDa component; the following paper (Kinet et al., 1983), the 45-kDa material.

Materials and Methods

IgE, Derivatives of IgE, and Cells. Monoclonal mouse anti-dinitrophenyl-IgE from hybridoma HI-DNP-e-26.82 (Liu et al., 1980) and human myeloma IgE from patient PS were prepared as described previously (Kulczycki & Metzger, 1974; Holowka & Metzger, 1982). Mouse IgE was iodinated with the chloramine T method (McConahey & Dixon, 1966) or with the iodine monochloride method (Helmkamp et al., 1960). The procedure for conjugation of the IgE with azobenzenearsonate groups has been give in Kanellopoulos et al. (1979). RBL cells were maintained as described previously (Barsumian et al., 1981) and routinely had 3×10^5 receptors/cell. Rat peritoneal mast cells from Sprague-Dawley rats were isolated from peritoneal washings as described by Holgate et al. (1980), omitting the final gradient step. Mast cells were 99% pure as judged by staining with toluidine blue.

Affinity Chromatography Columns. Bio-Gel A5m beads (Bio-Rad, Richmond, CA) were derivatized with (trinitrophenyl)lysyl groups as described previously (Holowka & Metzger, 1982). The coupling of anti-benzenearsonate antibodies to Sepharose 4B (Pharmacia, Piscataway, NJ) has also been described (Kanellopoulos et al., 1979). Generally, the 0.2-mL affinity columns were eluted with 10 mM (dinitrophenyl)caproic acid and 1 mM [(*p*-arsonatophenyl)-azo]tyrosine (Rivnay et al., 1982), respectively.

Solubilization of Receptors. Receptors were solubilized by suspension of cells for 15 min at 4 °C in 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (Hjelmeland, 1980), in borate-buffered saline (0.2 M $NaBO_3$, 0.16 M NaCl, pH 8) containing the protease inhibitors aprotinin (0.2 trypsin inhibitor unit/mL), phenylmethanesulfonyl fluoride (0.5 mM),

† From the Section on Chemical Immunology, Arthritis and Rheumatism Branch, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205. *Received May 20, 1983.* Parts of this work were presented at the 1983 meeting of the Federation of American Societies for Experimental Biology in Chicago, IL (Perez-Montfort et al., 1983).

‡ Present address: Departamento de Inmunologia, Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico, 04510 Mexico D.F.

[1] Abbreviations: RBL, rat basophilic leukemia; IgE, immunoglobulin E; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)-aminomethane; PPO, 2,5-diphenyloxazole.

and pepstatin (10 μg/mL) (all from Sigma) and leupeptin (10 μg/mL) (Boehringer-Mannheim Biochem., Indianapolis, IN). In some experiments this buffer also contained the kinase and phosphatase inhibitors sodium fluoride (50 mM), sodium pyrophosphate (5 mM), and ethylenediaminetetraacetic acid (2 mM) (Chaplin et al., 1980). The cells were solubilized at 5×10^7 cells/mL, which, with 10 mM detergent, gives a value of ρ of approximately 2 in the extract, without the addition of exogenous lipids (Rivnay & Metzger 1982).

The solubilized cells were centrifuged at 50000*g* for 1 h at 4 °C, and the pellet was discarded. In all subsequent procedures, the same concentration of detergent was used, and the solution was supplemented with 2 mM phospholipids derived from solid RBL tumors (Rivnay & Metzger, 1982).

Extrinsic Labeling of Receptors. Receptors were labeled on intact cells by lactoperoxidase-catalyzed iodination as described previously (Kanellopoulos et al., 1979; Marchalonis, 1969). The preparation of 5-iodonaphthalene-1-azide and the method used for labeling intact cells have been given previously (Bercovici & Gitler, 1978; Holowka et al., 1981).

In some cases, partially purified IgE–receptor complexes attached to trinitrophenylated lysyl-Sepharose or in the eluate of such a column were labeled by the chloramine T method or the iodine monochloride method (Rivnay et al., 1982). The labeled IgE–receptor complexes were then filtered through Sephadex G-25 by the procedure of Tuszynski et al. (1980).

Intrinsic Labeling of Receptors. Cells were harvested from stationary flasks on the fourth or fifth day and washed 2 times with complete medium and then, depending on the experiment, once in medium without leucine or with 10% the normal glucose concentration or once in medium without methionine [Eagle's minimum essential medium 2 with Earle's balanced salt solution, 0.22% $NaHCO_3$, without leucine (NIH Media Unit No. 930700) or with 0.1% glucose (NIH Media Unit No. 923443), or RPMI 1640 without methionine (NIH Media Unit No. 922715)]. The media also contained 0.06% glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, 60 μg/mL tylocine, 80 μg/mL gentamycin, and 20% fetal calf serum.

The cells were suspended at 1×10^6 cells/mL in a 250- or 500-mL spinner flask in the appropriate medium containing either 0.01–0.02 mCi/mL (5 mCi) [^{3}H]leucine (130 Ci/mmol), 1 μCi/mL (250 μCi) [^{14}C]glucosamine (54 mCi/mmol), or 1.8 mCi/mL (5.25 mCi) [^{35}S]methionine (1120 Ci/mmol) (all from Amersham Corp., Arlington IL). Except for the kinetic experiments, the cells were harvested after 20 h of incubation, washed, reacted with ^{125}I-labeled mouse IgE, washed again, and solubilized.

The method for labeling RBL cells with ^{32}P followed exactly that described by Fewtrell et al. (1982). The cells were incubated at 37 °C for 1 h with 10 mCi of carrier-free [^{32}P]-orthophosphoric acid (New England Nuclear, Boston, MA) with shaking, washed, and then solubilized.

Immunoprecipitations. In most cases, a "clearing" precipitation of purified human IgE and purified rabbit anti-human IgE plus 50–100 μL of a 10% suspension of protein A containing *Staphylococcus aureus* (Pansorbin, Calbiochem, LaJolla, CA) (Kessler, 1975) preceded the specific immune precipitation with 10–50 μg of purified rabbit anti-mouse IgE and another addition of 50–100 μL of the 10% suspension of Pansorbin.

NaDodSO$_4$–Polyacrylamide Gel Electrophoresis. Eluates from anti-benzenearsonate affinity columns or immune precipitates adsorbed to Pansorbin were dissolved in 2% sodium dodecyl sulfate ($NaDodSO_4$) in 0.08 M Tris-HCl, pH 6.8, containing 10% glycerol and incubated in a boiling water bath for 2 min. When samples were reduced, 2-mercaptoethanol (10% final) was added, and the samples were reheated. Slab gels were prepared following the method of Ames (1974). High and low molecular weight standards from Bio-Rad and low molecular weight standards from BRL were used to calibrate the gels. These contained myosin heavy chain (M_r 200 000), β-galactosidase (M_r 116 250), phosphorylase B (M_r 92 500), bovine serum albumin (M_r 66 200), ovalbumin (M_r 45 000), carbonic anhydrase (M_r 31 000), α-chymotrypsinogen (M_r 25 700), soybean trypsin inhibitor (M_r 21 500), β-lactoglobulin (M_r 18 400), lysozyme (M_r 14 400), cytochrome *c* (M_r 12 300), bovine trypsin inhibitor (M_r 6200), and insulin (A and B chains, M_r 3000). In general, 12.5% polyacrylamide gels were used. On such gels, a plot of the R_f of the standards vs. log molecular weight is approximately linear in the range 100K–15K but curved upward and downward above and below this range, respectively. Gels were analyzed by slicing and direct counting (for ^{125}I) in a Biogamma counter (Beckman, Palo Alto, CA) and/or after treatment with NCS tissue solubilizer for ^{3}H, ^{14}C, ^{32}P, or ^{35}S. After addition of 18 mL of complete counting cocktail 3a20 (Research Products International Corp., Elk Grove Village, IL), the vials were counted in a tracer analytic Mark III liquid scintillation counter (Elk Grove Village, IL). Autoradiography of dried gels was done at −80 °C with intensifying screens (Dupont, Wilmington, DE) and Kodak X Omatic AR film. For autoradiography of gels containing ^{35}S, ^{14}C, and ^{3}H, gels were treated with either Me_2SO–PPO, as described by Bonner & Laskey (1974), or with sodium salycilate (Chamberlain, 1979) prior to drying. In some cases, preflashed film was used to further increase the sensitivity and linearity (Laskey & Mills, 1975). Radioautographs were scanned on a LKB 2202 ultrascan laser densitometer (LKB Produkter AB, Bromma). A tracing of each of the peaks was cut out and weighed in order to determine the relative intensity.

Peptide Maps. One-dimensional peptide maps were performed exactly as described by Gard et al. (1979), with addition of 1 μg of *S. aureus* protease V8 (Miles, Elkhart, IN) or incubation of the gel slices with 100 μg of trypsin (Worthington Biochemical Corp., Freehold, NJ) overnight at 37 °C.

Results

Initial Observations. The experiment illustrated in Figure 1 shows the 20-kDa component, the analysis of which forms the basis of this paper. IgE–receptor complexes bearing arsonylated mouse anti-dinitrophenyl-IgE antibodies were partially purified by adsorption on a (trinitrophenyl)lysine column and eluted with (dinitrophenyl)caproate. The preparation was labeled with ^{125}I by the chloramine T method and then further purified by adsorption on an anti-benzenearsonate column and elution with [(*p*-arsonatophenyl)azo]tyrosine (Kanellopoulos et al., 1979). Throughout the purifications, the solvent contained 10 mM detergent and 2 mM phospholipids (Rivnay et al., 1982). Figure 1 is a radioautograph of a 10–30% gradient polyacrylamide gel on which the eluate was analyzed. The left lane shows the unreduced specimen. Near the origin is a heavily labeled band formed by the unreduced IgE. Below it—but not visible in this radioautography—is the α chain of the receptor with an apparent mass of 55 kDa. It has been noted previously that when complexed to IgE, this component of the receptor is iodinated inefficiently (Conrad & Froese, 1976; Pecoud & Conrad, 1981). The band at 33 kDa is the β subunit previously described (Holowka et al., 1981), and that at ~20 kDa is the component that was only recently delineated

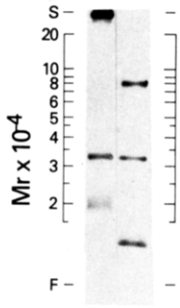

FIGURE 1: Radioautograph of a 10–30% polyacrylamide gradient gel on which IgE–receptor complexes extrinsically labeled with radioiodine were analyzed. The IgE–receptor complexes, prepared with benzenearsonylated IgE, were first bound to a (trinitrophenyl)lysine column and washed with a buffer containing detergent and tumor lipids. They were eluted with dinitrophenylcaproic acid, and the eluate was iodinated by the chloramine T method. After separation of the free iodine, the material was applied to an anti-benzenearsonate column and washed with buffer containing detergent and lipids. Aliquots of the eluate of this second column were analyzed in the absence (left lane) and presence (right lane) of reducing agent. The molecular weight scales are based on the standards listed under Materials and Methods. S marks the end of the stacking gel; F is the dye front. A quantitative analysis of a similar preparation is given in Figure 2 (filled circles).

in our laboratory (Rivnay et al., 1982).

The right lane in Figure 1 shows the result of reducing the preparation with 10% 2-mercaptoethanol. The IgE is reduced, but because the (28-kDa) light chain of this IgE iodinates poorly (Fewtrell et al., 1982), only the 80-kDa ϵ chain is seen. As has been noted previously, the β chain of the receptor is unchanged in its apparent molecular weight after reduction (Holowka et al., 1981; Holowka & Metzger, 1982). However, the 20-kDa material was quantitatively converted into a component of about half the molecular mass. Several experiments with appropriate standards yielded an apparent mass of 8.5–10 kDa for this component.

Labeling Characteristics of 20-kDa Component. (1) Extrinsic Labeling. (a) Oxidative Iodination of Solubilized Receptors. Regardless of whether the chloramine T or iodine monochloride method was used and whether the receptors were iodinated while adsorbed on the haptenated beads or after elution with hapten, the pattern seen on gels was essentially the same as that shown in Figure 1, when the eluates were examined directly. Variably, the band representing the β chain was more prominently a doublet.

(b) Surface Iodination of Intact Cells. RBL cells were labeled with ^{125}I by the lactoperoxidase method for iodinating surface proteins. The cells were then incubated with ^{125}I-labeled arsonylated IgE (of low specific activity) and solubilized and the receptor–IgE complexes partially purified on a (trinitrophenyl)lysine column. Half of the preparation was iodinated with ^{125}I by the chloramine T method whereas the other half was sham iodinated with the same reagents by omitting the radioisotope. The receptors were then separately purified on anti-benzenearsonate columns, immunoprecipitated with anti-IgE, and then analyzed on separate lanes of the same polyacrylamide gel.

Figure 2 shows the results. The top panel shows the distribution of counts in the two preparations before reduction. The material that had been radioiodinated with chloramine T (solid circles) shows a pattern very similar to that seen in Figure 1 (left lane) except that in addition to the peaks representing IgE, α chain, β chain, and the 20-kDa component, an additional component is seen with an apparent mass of 45 kDa. The nature and origin of this latter material is discussed in the following paper (Kinet et al., 1983). After reduction

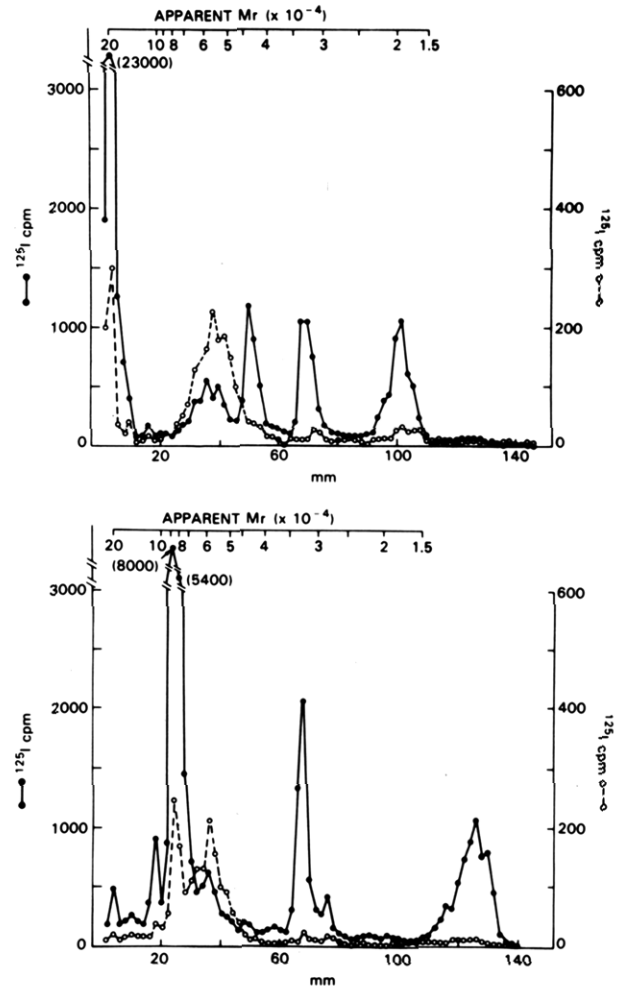

FIGURE 2: Analysis of IgE–receptor complexes isolated from cells that had been surface labeled with radioactive iodine by the lactoperoxidase method. Cells were first saturated with benzenearsonylated IgE and solubilized with detergent. The IgE–receptor complexes were partially purified on a (trinitrophenyl)lysine column. Half of the material was reiodinated by the chloramine T method, and the other half was sham iodinated by omitting only the radioactive isotope from the procedure. After a further purification on separate anti-benzenearsonate columns, the eluates were reacted with anti-IgE, and the precipitates were extracted with $NaDodSO_4$. After electrophoresis on 12.5% polyacrylamide gels in the absence (upper panel) and presence (lower panel) of reducing agent, the gels were dried, cut into 2 mm slices, and counted for ^{125}I. (●) Profile of radioactivity from the doubly labeled material; (○) material radiolabeled by surface iodination only.

(lower panel), the pattern is exactly like that shown in the right lane of Figure 1. The preparation that was only sham iodinated with chloramine T (open circles) shows the counts on the labeled IgE and on those components of the receptor that were modified by the surface labeling. It is evident that only the α chain was modified and that neither the β chain or the 20-kDa component (upper panel) nor the reduction product of the latter (lower panel) were derivatized by this technique.

(c) Labeling with 5-Iodonaphthalene-1-azide. Cells were labeled with the intramembrane probe, 5-[^{125}I]iodonaphthalene-1-azide as described previously. The receptors bearing arsonylated ^{125}I-labeled IgE were isolated on anti-benzenearsonate columns, immunoprecipitated, and analyzed on polyacrylamide gels before and after reduction. This experiment was performed at a time when we were experiencing a deterioration of one or more of the protease inhibitors, but this accident led to results that were more informative than they might otherwise have been.

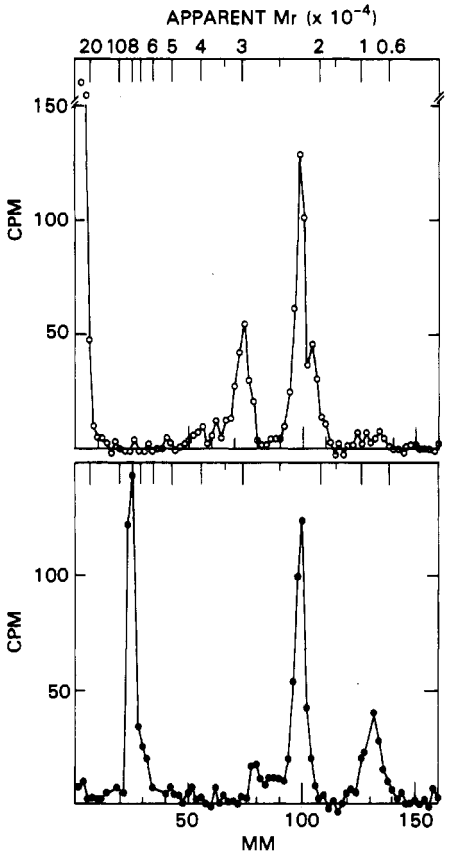

FIGURE 3: Analysis of IgE–receptor complexes isolated from cells labeled with 5-[^{125}I]iodonaphthalene-1-azide. The cells had been previously reacted with ^{125}I-labeled benzenearsonylated IgE and were solubilized after labeling with 5-iodonaphthalene-1-azide. The IgE–receptor complexes were isolated by affinity chromatography on an anti-benzenearsonate column. The eluate was reacted with anti-IgE and the precipitate extracted with $NaDodSO_4$. Samples were electrophoresed in the absence or presence of reducing agent on a 13.5% polyacrylamide gel. The gel was dried, cut into 2 mm slices, and counted for ^{125}I. The upper panel shows the distribution of counts from the sample that was not reduced and the lower panel that of the reduced sample.

Figure 3 shows an analysis of the gel. The top panel shows the pattern of the unreduced specimen. There are principal peaks at 200 (IgE), 30, and 23 kDa with a "shoulder" at 20 kDa. As noted previously, there is no labeling of the 55-kDa chain (Holowka et al., 1981). Upon reduction (lower panel), peaks are seen at 80 kDa (ϵ chain of IgE), a very small peak is seen at 28 kDa (light chain of IgE), and peaks are seen at 23 and 8 kDa. The 30- and 20-kDa components are absent. As a check on our assignments, the 30- and the 20–23-kDa bands from the unreduced pattern were individually isolated, reduced, and examined on a new polyacrylamide gel; the IgE was also electrophoresed (data not shown). The labeled component at 23 kDa represents the breakdown fragment of the β chain that we have called β_1 and that was previously shown to be the portion of the β chain that becomes labeled by 5-iodonaphthalene-1-azide (Holowka et al., 1981; Holowka & Metzger, 1982). It is unchanged by reduction. The labeled material at 20 kDa is the component that is the focus of this paper. It is weakly labeled by the probe and upon reduction is quantitatively converted to the 8-kDa component. The 30-kDa component is not the β chain; it disappears upon reduction whereas the β chain, as already noted, is unchanged by this procedure. When isolated and reduced, the 30-kDa band generated two components whose relative intensity (on the radioautogram) and molecular weights were identical with those of the 23- and 8-kDa components seen in the pattern

Table I: Incorporation of [^{3}H]Leucine into Components of Receptor for IgE

component	ratio (SD)[a]
α	0.26 (0.041)
β (unreduced)[b]	0.46 (0.046)
β (reduced)[c]	0.46 (0.012)
20 kDa (unreduced)[b]	0.26 (0.013)
10 kDa (reduced)[c]	0.25 (0.031)

[a] The tritium counts associated with the individual component were divided by the total counts in the gel. In all the experiments, incorporation was for approximately 20 h. [b] In four experiments, the specimens were examined without prior reduction. These preparations contained variable amounts of the 45-kDa component. Since this material is a complex of the β and 20-kDa component (Kinet et al., 1983), the counts in it were assigned to the β and 20-kDa components in proportion to the ratio of counts in the uncomplexed forms of these peptides. [c] Data from three experiments.

for the reduced material shown in Figure 3. We believe that the 30-kDa material is analogous to the 45-kDa component. The latter is a disulfide-linked complex of the β chain and 20-kDa component (Kinet et al., 1983). The 30-kDa component appears to be a similar complex of the β_1 fragment and the 20-kDa component [see also Kinet et al. (1983)].

(*2*) *Intrinsic Labeling.* (*a*) [^{3}*H*]*Leucine.* Labeling cells with [^{3}H]leucine yielded patterns for the affinity-purified receptor that were exactly like those for the iodinated receptor (Figures 1 and 2) both before and after reduction [see also below and Figure 4 in Rivnay et al. (1982)]. Table I presents a quantitative analysis of several such experiments. It is apparent that each of the three components shows a substantial and highly reproducible incorporation of the [^{3}H]leucine.

The data in Table I were derived from experiments in which the cells had been grown in [^{3}H]leucine for a relatively fixed time: ~20 h. It was of interest to see whether, if followed kinetically, the incorporation into the α, β, and 20-kDa components was proportional. Cells were grown in [^{3}H]leucine and samples removed at 3, 6, 12 and 24 h. The cells were reacted with arsonylated IgE and extracted with detergent, and the receptors from each specimen were isolated individually. Figure 4 shows the autoradiogram of a gel on which the receptors were analyzed. The principal components seen are the iodinated IgE, the broad band of α chains, the 45-kDa component, the β chain, and the 20-kDa component. A quantitative analysis was performed by scanning the radioautogram and integrating the areas under each peak by weighing. The relative amount of radioactivity in each component was multiplied by the total counts in the sample. These results and additional data are shown in Figure 5. The total counts in the eluates of the affinity columns (expressed as [^{3}H]leucine/μg of IgE) increased linearly (squares) and were equal to about 0.1% of the counts found in the detergent extract prior to purification.

The counts associated with the α chain (open circles), β chain (half-filled circles), and the 20-kDa component (filled circles) increased proportionately. The insert gives the ratios of the β to α and β to 20-kDa components at 6, 12, and 24 h. (The incorporation at 3 h was insufficient to permit an accurate assessment—see Figure 4.) The value for these ratios taken from the data in Table I is also included (diamonds). Although there could be a slight drift in these ratios, this is principally due to the data from the 6-h specimen, which had the least counts. We think it likely that this drift reflects experimental error.

(*b*) [^{35}*S*]*Methionine.* RBL cells were grown in the presence of [^{35}S]methionine and the receptors isolated as before and

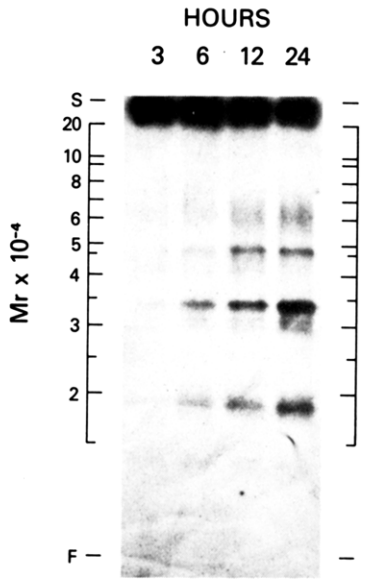

FIGURE 4: Kinetic study of the incorporation of [^{3}H]leucine into the receptor. Cells were grown in the presence of [^{3}H]leucine and samples removed at the times indicated above the figure. The cells were then incubated exactly 30 min with ^{125}I-labeled benzenearsonylated IgE, washed, and subsequently solubilized with detergent. The IgE–receptor complexes for each time point were purified separately and precipitated with anti-IgE. The $NaDodSO_4$ extracts of the immune precipitates were analyzed before and after reduction on 12.5% polyacrylamide gels. A radioautograph of the gel on which the unreduced samples were examined is shown.

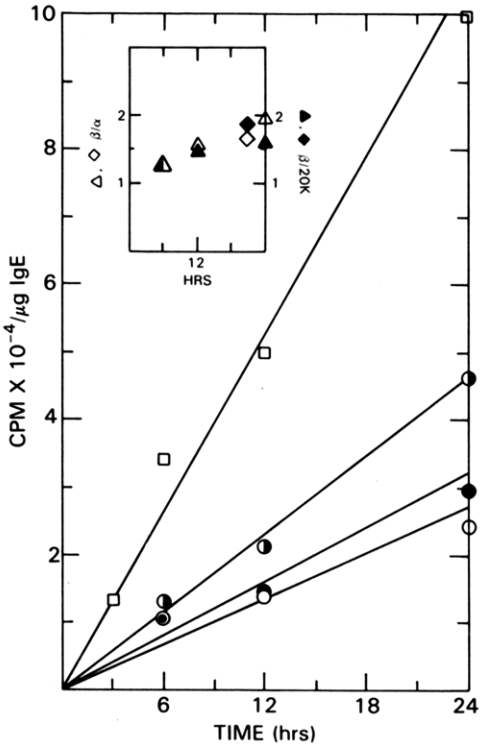

FIGURE 5: Kinetic study of the incorporation of [^{3}H]leucine into components of the receptor. The samples analyzed were the same as those shown in Figure 4. The fraction of the total tritium counts contributed by each component was multiplied by the counts in the total eluate after the latter had been normalized to ^{3}H counts/μg of IgE (□). Corrections for the counts in the 45-kDa component were made as in Table I. The insert shows the ratios of incorporated leucine in the principal components to each other. The values for 20 h (diamonds) are taken from the data in Table I. Counts associated with the α chain (○), β chain (◑), and with the 20-kDa component (●).

analyzed on polyacrylamide gels. Labeling of the α chain and β chain but not of the 20-kDa component (or its reduction product) was observed. More detailed data with this label are given in the following paper (Kinet et al., 1983).

(*c*) [^{14}*C*]*Glucosamine.* RBL cells were grown in the presence of [^{3}H]leucine or [^{14}C]glucosamine in leucine-deficient or glucosamine-deficient media, respectively. The cells were mixed after being washed and reacted with arsonylated IgE. The receptors were purified on anti-benzenearsonate columns and analyzed on a polyacrylamide gel.

Figure 6 shows the results of such an experiment. The pattern of tritium counts (open circles) shows the 55-kDa α chain, the 33-kDa β chain, and the 20-kDa component. The pattern of ^{14}C counts (filled circles) shows labeling of the α chain but not of the two other components.

(*d*) ^{32}*P.* Figure 7 shows a radioautograph of a polyacrylamide gel on which purified receptors from cells that had been incubated with [^{32}P]orthophosphate were analyzed. The right lane shows the unreduced specimen. The α chain was not labeled, but the β chain and a component at 23 kDa are observed. The left lane shows the reduced specimen. The 23-kDa material has disappeared, and a component at ~14 kDa has taken its place. These molecular weights are slightly greater than those typical for the 20-kDa component and its reduction product, but as discussed elsewhere (Perez-Montfort et al., 1974), the data are consistent with phosphorylation of the 20-kDa components.

Peptide Analysis. The 20-kDa component shares many of the labeling properties of the previously described proteolytic cleavage product of the β chain (see Discussion). To clarify further the possible relationship between these components, receptors were iodinated with chloramine T and purified, and the bands corresponding to the β chain and 20-kDa component were isolated from a preparative polyacrylamide gel. They were digested with protease V8, and the peptides were analyzed. Figure 8 shows a radioautograph of such an analysis.

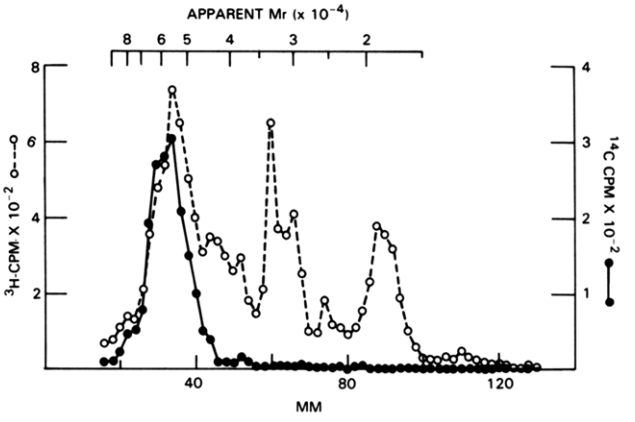

FIGURE 6: Analysis of IgE–receptor complexes isolated from cells grown in the presence of [^{14}C]glucosamine or [^{3}H]leucine. The $NaDodSO_4$ extract of the IgE–receptor complexes that had been immunoprecipitated was analyzed on a 12.5% polyacrylamide gel.

Lanes A and C show the results with the β chain. The unreduced specimen (lane C) shows complete digestion of the 32-kDa chain and conversion into components with apparent masses of 21, 11, and 6 kDa, respectively. The size of these peptides was not altered by reduction (lane A). With the same treatment with protease, the 20-kDa component is not cleaved (lane D) and after reduction (lane B) shows the expected conversion to a 10-kDa component.

A similar experiment in which trypsin was substituted for the protease V8 showed a single 6-kDa product from the β chain, which was unchanged by reduction. The 20-kDa com-

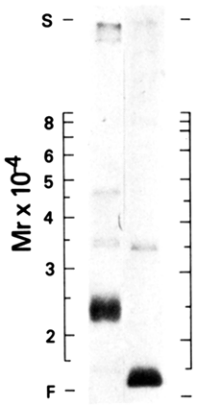

FIGURE 7: Radioautograph of a 12.5% polyacrylamide gel on which IgE–receptor complexes isolated from cells labeled with [^{32}P]orthophosphate were analyzed in the absence (left lane) and presence (right lane) of reducing agent.

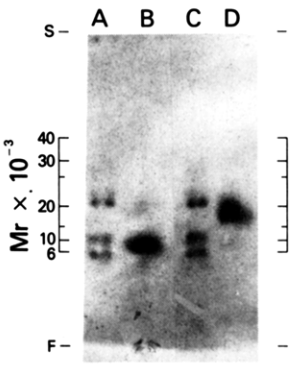

FIGURE 8: Radioautograph of a 10–30% polyacrylamide gradient gel on which peptides generated by treatment of radioiodinated components of the receptor for IgE with protease V8 were analyzed. Bands on a 12.5% polyacrylamide gel corresponding to the β chain (lanes A and C) and the ~20-kDa component (lanes B and D) were digested in the presence (lanes A and B) and absence (lanes C and D) of reducing agent in the second gel.

ponent yielded 10- and 8-kDa components, and these also did not change after reduction (data not shown).

Studies with Rat Mast Cells. Purified peritoneal mast cells were reacted with arsonylated mouse IgE and solubilized, and the receptors were partially purified on a (trinitrophenyl)lysine column, iodinated, and further purified on an antibenzenearsonate column. The analysis showed patterns before and after reduction similar to the results with the RBL cells. The 20-kDa component, which was reduced to a ~10-kDa component, was clearly evident.

Discussion

In this paper we have examined the 20-kDa component that copurifies with the α and β subunits of the receptor for IgE, under conditions where the interaction between α and β are stabilized (Rivnay et al., 1982). We wished to explore the following questions: Is the component simply a copurifying contaminant? If not, is it a breakdown product of either α or β? If not a breakdown product, should it be considered a subunit of the receptor or a "receptor-associated protein"? Finally, what is its topological disposition in relationship to α and β?

That the 20-kDa is not a contaminant is most convincingly demonstrated by its fixed (reproducible) stoichiometry with respect to the α and β chains in numerous and rigorous purifications. That the molar ratio of the 20-kDa component to the α and β chains appears to be a small but integral number (below) would also be a curious coincidence for a simple contaminant. Furthermore, that its rate of intrinsic labeling (Figures 4 and 5) was at a constant ratio with respect to the α and β chains would be highly fortuitous. Finally, that it becomes cross-linked to the α (and/or β) chain during chemical cross-linking (Rivnay et al., 1984) and to the β chains during immunoprecipitation (Kinet et al., 1983) shows that it is closely associated with the previously identified chains of the receptor.

The 20-kDa component is also not a fragment of either the α chain or the β chain. The evidence for this is as follows: If it were a fragment of the α chain, it would have to contain the binding site for IgE. Since previous results indicated that there is only one α chain per bound IgE (Kanellopoulos et al., 1980), it would not have purified under the conditions we used were the binding site missing. Thus, it might have been expected to be labeled by surface labeling [which appears to preferentially label the combining site (Conrad & Froese, 1976)] and possibly to contain carbohydrates as does the α chain (Kulczycki et al., 1976; Kanellopoulos et al., 1980); yet, neither was true. Since the interaction between IgE and the α chain is relatively insensitive to nonionic detergents and is unaffected by the presence or absence of lipids, it would also be difficult to understand why, if the 20-kDa component were a fragment of α chains, its recoverability during purification of the receptor should be so sensitive to the detergent to lipid ratios (Rivnay et al., 1982). That after exposure to reducing agents the 20-kDa component is cleaved, whereas α has an unaltered molecular weight, is also a marked difference, although this evidence is confirmatory rather than definitive. That the 20-kDa component in the presence of bound IgE is readily iodinated after solubilization, whereas the α chain is not (Pecoud & Conrad, 1981; Rivnay et al., 1982; Figure 1), is further evidence. Finally, that the 20-kDa components become labeled with ^{32}P whereas the α chains on RBL cells do not (Fewtrell et al., 1982; Perez-Montfort et al., 1984; Figure 7) is additional persuasive evidence.

The 20-kDa component is also not a fragment of the β chain. The peptides produced by exposure of the 20-kDa component and the β chain, to protease V8 and trypsin, respectively, are distinctive (Figure 8). Although the β chain does yield a cleavage product, β_1, of similar (slightly larger) molecular weight (Holowka & Metzger, 1982; Figure 3; Kinet et al., 1983), this fragment is not reducible and contains methionine (Kinet et al., 1983) whereas the 20-kDa component is reducible and fails to incorporate methionine.

Thus the 20-kDa component is a discrete entity, unrelated to α and β. Should it be considered a subunit of the receptor or a "receptor-associated" protein? We previously noted that this is a somewhat semantic question (Rivnay et al., 1982). Nevertheless, some points are worth considering. A "true" subunit should be present at a (usually small) integral molar ratio with respect to the multimer of which it forms a part. This appears to be so for the 20-kDa component. On gels, the receptor would be expected to have an apparent mass of 100 kDa if it consisted of 1 mol each of the α, β, and 20-kDa components. This is close to the value observed when cross-linked receptors known to contain α, β, and the 20-kDa component are analyzed (Rivnay et al., 1984). The data on leucine incorporation are also consistent with such a model. Our best estimates of the mass of the peptide portion of the α chain is ~32 kDa (Kumar & Metzger, 1982). If we assume that the α chain, β chain, and 20-kDa component have similar fractional leucine contents, then the latter components should incorporate $\sim{}^{20}/_{85}$ or 24% of the total leucine incorporated into the receptor providing the molar ratio of the components

is 1:1:1. Although we do not give undue weight to the excellent agreement between this estimate and our experimental findings (Table I), the numbers are at least in an appropriate range. Further evidence for there being an approximately 1:1:1 ratio between the α, β, and 20-kDa component is the observation that under conditions where the 45-kDa component (a 1:1 complex of the 20-kDa and β components; Kinet et al., 1983) is generated, there is a parallel decrease in the residual β and 20-kDa components. Were the ratio of β and the 20-kDa components very different than 1:1, this would not be expected. Compositional analyses should define the fractional leucine content of the β chain and the 20-kDa component and with the results of the incorporation studies allow for a more definitive assessment of the stoichiometry.

A final criterion that is expected for a true subunit but not necessarily for a receptor-associated component is that the putative subunit is coordinately synthesized along with the other subunits. The data in Figures 4 and 5 show that this is so for the 20-kDa component.

On the basis of all of these considerations, it is reasonable to conceptualize the 20-kDa component as being an integral part of the receptor for IgE. This component is always completely reducible. The products of reduction have an indistiguishable molecular weight that is exactly half that of the unreduced material, and in preliminary experiments (G. Alcaraz and J.-P. Kinet, unpublished observations), the two portions appear to have an identical isoelectric point. For these reasons, it is more appropriate to consider the 10-kDa material as the fundamental entity. We therefore propose the term γ chain for it and further that the receptor for IgE must now be considered as a protein consisting of four subunits: an α, a β, and two (disulfide-linked) γ chains.

That the γ chains are also subunits of the receptor for IgE on normal cells was substantiated by the experiment with peritoneal mast cells. An interesting question is whether they are exclusively associated with the receptors with high affinity for IgE or might be subunits of other receptors also. Elsewhere, we have shown that a β-like component is associated with a demonstrably different receptor for IgE on macrophages (Finbloom & Metzger, 1983). It will be interesting to explore this question with regard to the γ chains.

What is the disposition of the γ subunits? At this stage we can only provide circumstantial evidence. That the γ chains are not modified by surface labeling and show no evidence for carbohydrate are consistent with a lack of exposure on the surface. That they label with 5-iodonaphthalene-1-azide, are dissociated with nonionic detergents, and interact with detergent micelles (S. A. Wank et al., unpublished results) suggest that they have an intramembranous portion. That they can be intrinsically phosphorylated suggests that they may be exposed on the cytoplasmic aspect of the membrane. There is at least preliminary evidence that they interact directly with the β_1 region of the β subunit (Kinet et al., 1983; Figure 3), but further data are required to clarify this point.

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