Further Characterization of the Subunits of the Receptor with High Affinity for Immunoglobulin E

Gisele Alcaraz,[‡,§] Jean-Pierre Kinet,[‡] Teh-Yung Liu,[∥] and Henry Metzger*[,‡]

Section on Chemical Immunology, Arthritis and Rheumatism Branch, National Institute of Arthritis, Musculoskeletal and Skin Diseases, National Institutes of Health, and Division of Biochemistry and Biophysics, Center for Drugs and Biologics, Bethesda, Maryland 20892

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ABSTRACT: The α, β, and γ subunits of the receptor with high affinity for immunoglobulin E were isolated and their compositions assessed by direct amino acid analysis and by incorporation of radioactive precursors. The compositions show no unusual features other than a rather high content of tryptophan in the α chain as assessed from the incorporation studies. The results combined with future sequence data will permit unambiguous determination of the multiplicity of the chains in the receptor. Chymotryptic peptide maps of the extrinsically iodinated subunits show several similar peptides, particularly for α and β. However, these putative homologies were not apparent when tryptic maps of the biosynthetically ([^{3}H]leucine) labeled subunits were analyzed.

Mast cells and related tumor analogues have on their surface a receptor with high affinity for monomeric immunoglobulin E (IgE)[1] (Metzger et al., 1986). Aggregation of this receptor leads to degranulation of the cells. Previous work from this laboratory indicated that the receptor is composed of three types of polypeptide chains: α, β, and γ. The studies we describe here were designed to obtain additional information about the covalent structure of the subunits. In particular, we wished to develop criteria by which to judge the success of ongoing efforts to isolate the genes that code for these polypeptides. We also wished to resolve remaining uncertainties about the multiplicity of polypeptides in a single molecule of receptor. Finally, in connection with continuing efforts to identify molecular changes associated with activation of the cells, we wanted to develop criteria by which small changes in the covalent structure of the receptor polypeptides—such as proteolytic cleavage—could be revealed.

Materials and Methods

Cells and Antibodies. The 2H3-HR+ subline (Barsumian et al., 1981) of rat basophilic leukemia cells (Eccleston et al., 1973; Kulczycki et al., 1974) was used for all experiments. Cells were grown either in spinner cultures or in stationary flasks. When the latter was used, the adherent cells were released by brief exposure to trypsin (Barsumian et al., 1981). The IgE in all of the present studies was derived from the hybridoma Hi-DNP-ϵ-26.82 (Liu et al., 1980) and purified as described (Holowka & Metzger, 1982). For some studies, the IgE was benzenearsonylated (Kanellopoulos et al., 1979). The preparation of purified rabbit anti-mouse IgE was by methods analogous to those used for goat anti-mouse IgE (Taurog et al., 1977). A preparation of the β subunit of transducin was a gift from Drs. P. Giershik and A. Spiegel (NIDDK, NIH).

Purification of Receptors. In all instances, cells were reacted with mouse anti-dinitrophenyl-IgE in order to saturate the receptors, washed, and then dissolved wiih 10 mM CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) at 5×10^7 cells/mL, i.e., at a ρ of ~2.2 (Rivnay & Metzger, 1982). The suspension was centrifuged at 50000G for 1 h at 4 °C and the supernatant applied to Sepharose beads conjugated with trinitrophenyllysine (Holowka & Metzger, 1982). The beads were then placed in a column and washed with 50–100 volumes of 10 mM CHAPS–2 mM tumor phospholipids (Rivnay et al., 1982), then briefly with 10 mM CHAPS at a ratio of 3×10^4 micelles/mol of receptor (Kinet et al., 1985a), and finally with 1–2000 volumes of 2 mM CHAPS (Kinet et al., 1985b) prior to elution with 10 mM (dinitrophenyl)-ϵ-aminocaproate in 2 mM CHAPS. For large-scale isolations, the eluates were concentrated as necessary on a Centricon-10 device (Amicon, Danvers, MA). For one of the large-scale preparations, receptors bound to arsonylated anti-dinitrophenyl-IgE were first purified on an anti-benzenearsonate column (Kanellopoulos et al., 1979) prior to purification on the trinitrophenyllysine column. The results using this double purification were not appreciably different than those obtained with the preparations purified by only a single affinity chromatography.

Purification of Subunits. For large-scale purifications of the subunits, the latter were first partially segregated by phase separation of Triton X-114 (Alcaraz et al., 1984). The eluate containing purified IgE–receptor complexes was concentrated to 200 μL and brought up to 1 mL with 4.76% Triton X-114. The solution was incubated for 2 h at 37 °C to dissociate the IgE–α from β and γ, and the phases were separated by centrifugation. When the pellet was used, it was made 1% in recrystallized $NaDodSO_4$ (Hunkapiller et al., 1983) before addition of 9 volumes of ethanol. After overnight incubation at −20 °C, the sample was centrifuged in a microfuge for 5 min at 4 °C. The supernatant was removed, 100 μL of sample buffer added to the protein pellet, and the tube incubated at 55 °C for 1 h. The sample was then applied to a 12.5% acrylamide gel (0.1 × 16 × 14 cm) with a 1-cm stacking gel

[‡] Section on Chemical Immunology, National Institutes of Health.

[§] Present address: Centre d'Immunologie, INSERM–CNRS de Marseille-Luminy, 13288 Marseille, Cedex 9, France. Supported largely by the Institut National de la Santé et de la Recherche Medical and by a supplemental grant from DNAX, Palo Alto, Inc.

[∥] Center for Drugs and Biologics.

[1] Abbreviations: IgE, immunoglobulin E; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; $NaDodSO_4$, sodium dodecyl sulfate; kDa, kilodalton(s); HPLC, high-pressure liquid chromatography.

and 1-cm-wide sample wells. Each lane was loaded with no more than ~25 μg of each of the proteins expected to be present. Upon completion of the electrophoresis, the gel was rapidly stained with 0.5% Coomassie blue and destained. The bands of interest were excised and electroluted by using the device and the procedure described by Hunkapiller et al. (1983). The eluates of electrodialysis were vacuum evaporated (Speed-Vac, Savant, Hicksville, NY), and the $NaDodSO_4$ was extracted by the procedure of Konigsberg and Henderson (1983).

When the supernatant of the Triton X-114 phase separation was used, it was also concentrated to 100 μL and applied to a 21.5 × 600 mm column of 3000 SW Spherogel TSK (Beckman Instruments, Inc., Berkeley, CA) in order to separate the IgE from the α chain. The buffer contained 0.15 M NaCl, 0.01 M NaH_2PO_4, pH 6.3, and 0.1% $NaDodSO_4$, and the column was run at room temperature at a flow rate of 0.2 mL/min. The effluent containing largely IgE and α was monitored by absorption at 220 nm. Fractions containing α were pooled, concentrated, and precipitated from 92% ethanol. The pellet was then processed as above. We emphasize the details in these steps since they are necessary in order to obtain adequate yields.

Amino Acid Analyses. The subunits were hydrolyzed and analyzed for their amino acid content as described previously (Kanellopoulos et al., 1980).

Peptide Mapping. Extrinsically iodinated IgE–receptor complexes (Alcaraz et al., 1984) were immunoprecipitated with anti-IgE and Pansorbin (Calbiochem, LaJolla, CA) (Perez-Montfort et al., 1983a). The precipitates were dissolved in sample buffer and electrophoresed on polyacrylamide gels in the presence of $NaDodSO_4$. The gels were dried and analyzed by autoradiography. Segments of the gels containing the individual subunits of the receptor were excised and washed 2 times with 25% 2-propanol and once with 10% methanol, dried, and then incubated with 1 mL of NH_4HCO_3 containing 50 μg of α-chymotrypsin (Sigma Chemical Co., St. Louis, MO; type II 3× crystallized) for 24 h at 37 °C. The supernatant was recovered, vacuum evaporated, and analyzed on thin-layer sheets (Eastman Chromogram sheets, 13255, without fluorescent indicator). For the first dimension, electrophoresis was performed by using a solution of acetic acid/formic acid/water (15:5:80) for 1 h at 1 kV in a thin-layer electrophoresis cell (CAMAG, Wrightsville Beach, NC). The plate was turned 90°, and the peptides were then chromatographed in butanol/pyridine/acetic acid/water (32.5:25:5:20) at room temperature for 2–3 h. The plate was analyzed by radioautography. Alternatively, we employed high-pressure liquid chromatography (HPLC) utilizing a C18 reverse-phase Vydac column and a Beckman Model 344 apparatus. The starting solvent was 0.05% trifluoroacetic acid in water, and a linear gradient was formed with 0.05% trifluoroacetic acid in acetonitrile up to 60% v/v. The eluate was analyzed through an on-line detector (Flo-One Radiomatic Instrument, Thompson Instrument Co., Burke, VA).

Intrinsically labeled subunits (Perez-Montfort et al., 1983a) were analyzed by several different procedures, but for the most detailed experiments described under Results, the following procedure was used. Affinity-purified, immunoprecipitated IgE–receptor complexes in $NaDodSO_4$ sample buffer were loaded on a TSK-3000 column (above) equilibrated with 0.15 M NaCl and 10 mM sodium phosphate (pH 6.3) containing 0.1% $NaDodSO_4$. A flow rate of 250 μL/min was used, one fraction per minute was collected, and aliquots were counted. The peak tubes corresponding to α, β, and γ chains were digested with trypsin (below). The tubes on either side of the peak tubes were pooled, and each such pool was rerun. The three peak tubes were in turn pooled. An aliquot of these pools was taken for analysis on gels and the remainder processed for a separate tryptic cleavage. Prior to tryptic cleavage, 100 μg of bovine serum albumin was added to the sample and the specimen reduced with 10 mM dithiothreitol in $NaDodSO_4$ at pH 8.6–8.8 at 45 °C under N_2 for 2 h. The sample was alkylated with 22 mM iodoacetamide and the detergent extracted. The pellet was taken up in 100 μL of 75 mM NH_4HCO_3 containing 2 mM Ca^{2+} and reacted with 1 μg of HPLC-purified (Titani et al., 1982) trypsin for 4 h at 37 °C and then with an additional 1 μg for 16 h at 37 °C. The digest was vacuum evaporated and taken up in the starting buffer for HPLC.

Analysis on Polyacrylamide Gels. Electrophoresis on 12.5% polyacrylamide gels was performed as described (Perez-Montfort et al., 1983a) using the following standards (molecular weights in parentheses) from Bio-Rad (Richmond, CA): lysozyme (14 400), soybean trypsin inhibitor (21 500), carbonic anhydrase (31 000), ovalbumin (45 000), bovine serum albumin (66 000), and phosphorylase *b* (92 500). Electrophoresis in the presence of 8 M urea (Swank & Munkres, 1971) was performed exactly as recommended by the distributor of the standards used (Sigma Tech Bulletin MWS-877P). In those gels run with the urea-containing buffers, the Sigma SDS molecular weight markers (MW-SDS-17 kit) were used: myoglobin (16 950), myoglobin fragments I and II (14 400), fragment I (8160), fragment II (6210), and fragment III (2510).

Results

Amino Acid Composition. The isolated subunits were subjected to amino acid analysis, and the results are shown in Table I. The table also compares the composition previously reported by our laboratory for the α chain (Kanellopoulos et al., 1980). In addition, we analyzed the β chain of transducin using the same procedures we employed for isolating and analyzing the subunits of the receptor.

As can be seen from the data in columns 6 and 7, our findings for the β chain of transducin, which was isolated from the same gel used for one of the preparative isolations of the receptor subunits, corresponded reasonably well with the cDNA-derived sequence of Sugimoto et al. (1985), suggesting that the methods we employed are basically reliable. [Actually, our data show even better correspondence to the compositional data published by Manning and Gilman (1983).] With respect to the α chain of the receptor, there are some substantial variations in the results from the separate analyses, but overall there is reasonable agreement with our previous study which involved quite different methods of isolation (Kanellopoulos et al., 1980).

In Figure 1, we have grouped the homologous amino acids and compared the compositions of the chains. It is apparent that the overall compositions of the α, β, and γ chains are similar to each other and not particularly distinctive when compared to the average composition of a large collection of proteins in general.

Incorporation Studies. Specific aspects of the amino acid composition were also analyzed by using biosynthetic incorporation of four different radiolabeled amino acids (Table II). The data are compared to the data from the direct compositional analyses (Table I). It can be seen that results of these different methods are in reasonable agreement. For tryptophan, the ratio of incorporated counts in α, β, and γ_2 was (7.9 ± 0.2):1:0 (Figure 2). A single previous analysis had yielded

Table I: Amino Acid Composition Analyses on the Receptor for IgE and the β Subunit of Transducin[a]

	receptor					
	α				β-transducin	
residue	Kannelopoulos et al. (1980) (2)[b]	this paper (6)[c]	β (4)	γ (3)	(1)	Sugimoto et al. (1985)
Asx	13.03 ± 0.09	10.71 ± 0.97	9.34 ± 1.35	9.04 ± 0.93	16.40	14.78
Thr	5.63 ± 0.15	4.77 ± 0.94	5.39 ± 0.74	6.78 ± 0.12	7.66	8.81
Ser	10.51 ± 0.61	9.28 ± 1.48	9.18 ± 0.55	5.77 ± 0.17	7.66	8.81
Glx	7.64 ± 0.13	9.48 ± 1.26	11.20 ± 0.41	12.59 ± 0.92	8.29	6.60
Pro	2.93 ± 0.13	3.29 ± 1.25	5.57 ± 1.06	4.29 ± 0.82	1.60	1.57
Gly	6.29 ± 0.06	8.72 ± 1.77	7.74 ± 1.98	7.19 ± 0.71	10.52	8.49
Ala	4.76 ± 0.27	5.24 ± 1.14	7.33 ± 0.12	6.38 ± 0.17	9.09	9.43
Val	7.90 ± 0.30	6.0 ± 0.81	5.79 ± 0.73	4.63 ± 0.09	5.26	5.35
Met	0.91 ● 0.08	1.16 ± 0.48	1.66 ± 0.48	0.66 ± 0.63	1.96	2.52
Ile	7.12 ± 0.10	6.82 ● 0.84	5.37 ± 0.60	5.45 ± 0.39	4.55	5.66
Leu	7.76 ● 0.14	9.54 ● 2.07	12.84 ± 0.84	13.39 ± 0.25	8.29	9.43
Tyr	4.39 ● 0.07	4.74 ± 1.59	3.06 ± 0.57	4.44 ± 0.78	1.69	2.52
Phe	4.19 ± 0	4.65 ± 1.47	4.30 ± 0.22	2.53 ± 0.40	3.30	3.46
His	1.96 ± 0.05	2.08 ± 0.79	0.80 ± 0.09	1.44 ± 0.19	2.58	2.52
Lys	9.92 ± 1.01	7.69 ± 2.04	4.20 ± 0.10	6.18 ± 0.47	4.55	3.14
Arg	2.72 ± 0.11	3.74 ± 0.34	4.25 ± 0.34	5.24 ± 0.19	6.60	6.92
Cys	2.36 ± 0.02	1.94 ± 0.57	2.24 ± 0.54	3.24 ± 0.05	ND[e]	
Zp[d]	0.288	0.314	0.360	0.279		0.187

[a] The data are expressed as mole percent excluding any tryptophan that may be present. Labeling studies indicate that γ chains contain no tryptophan but that α and β contain tryptophan in the α:β ratio of 8:1 (see text and Figure 2). In three of the analyses on α (performed by K. Williams, New Haven, CT), cysteine was not determined. For these, we assumed an average of 2.15 mol % (the average of the percentage found in the prior and present analyses). The value of 1.94 shown in column 3 is the mean of the three analyses performed for this study in which a determination was made. [b] Number of analyses. [c] Three of these amino acid analyses were performed by using α isolated from the supernatant of the Triton X-114 phase separation and the other three on residual α in the pellet (see Materials and Methods). There were no consistent differences between these analyses. [d] Discriminant function of Barantes (1975). [e] ND, not determined.

Table II: Ratios of Leucine, Cysteine, Methionine, and Tryptophan in the Subunits of the Receptor[a]

	incorporation studies[b]			amino acid analyses[c]		
amino acid	α	β	γ_2	α	β	γ_2
leucine (7)[d]	0.52 ± 0.19	1.0	0.40 ± 0.10	0.62	1.0	0.42
cysteine (7)	1.1 ± 0.07	1.0	0.64 ± 0.08	0.83	1.0	0.60
methionine (7)	0.78 ± 0.07	1.0	0	0.60	1.0	0.16
tryptophan (2)	7.9 ± 0.20	1.0	0			

[a] All results are presented relative to the composition of the β subunit. [b] The mean and standard deviations are indicated. The data for methionine are from Kinet et al. (1983, 1985). [c] The ratios are calculated from the data in Table I with the following assumptions: The molecular weight of β is assumed to be 34 000 (Holowka & Metzger, 1982) so that it contains about 40 leucines, 7 cysteines, and 5 methionines. The molecular weight of the peptide portion of the α subunit is assumed to be 30 000 (Kumar & Metzger, 1982) and that for the γ chain 6800. Two γ chains per one α and one β chain were assumed (see text). [d] Number of analyses.

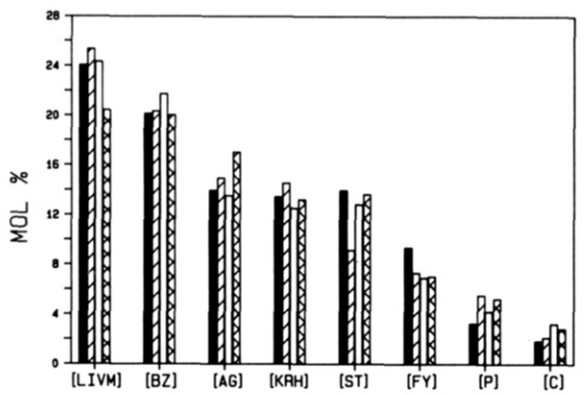

FIGURE 1: Comparison of the amino acid compositions of the subunits of the receptor for IgE with each other and with the average composition of a large collection of random proteins (Dayhoff et al., 1978). Each bar shows the sum of the mole percent of a homologous set of amino acids. (■) α; (▨) β; (□) γ; (▩) random proteins. The one-letter designation for amino acids is used.

2.5 residues of tryptophan per α chain (Kanellopoulos et al., 1980), but this value might be falsely low because of destruction during hydrolysis. Even if the β chain contains only one tryptophan, our incorporation data suggest an unusually high tryptophan content for the α chain.

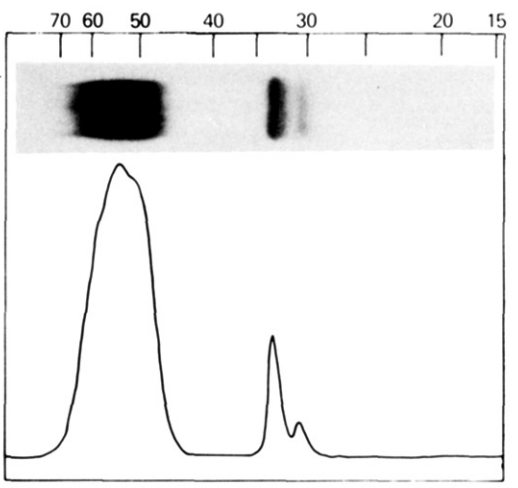

FIGURE 2: Incorporation of [^{3}H]tryptophan in the subunits of the receptor for IgE. The photograph is of a fluorograph from a gel on which an immunoprecipitate of the affinity-purified intrinsically labeled receptor was electrophoresed under nonreducing conditions. The molecular weight scale indicated on the upper abscissa is based on two sets of standards run in the same gel in adjoining lanes. The lower part of the figure shows a densitometric scan of the fluorograph, from which the ratio of counts in α (at 55 kDa) to those in β (at 34 kDa) was determined. The oligomer of γ chains is at ~20 kDa but shows no evidence of incorporation.

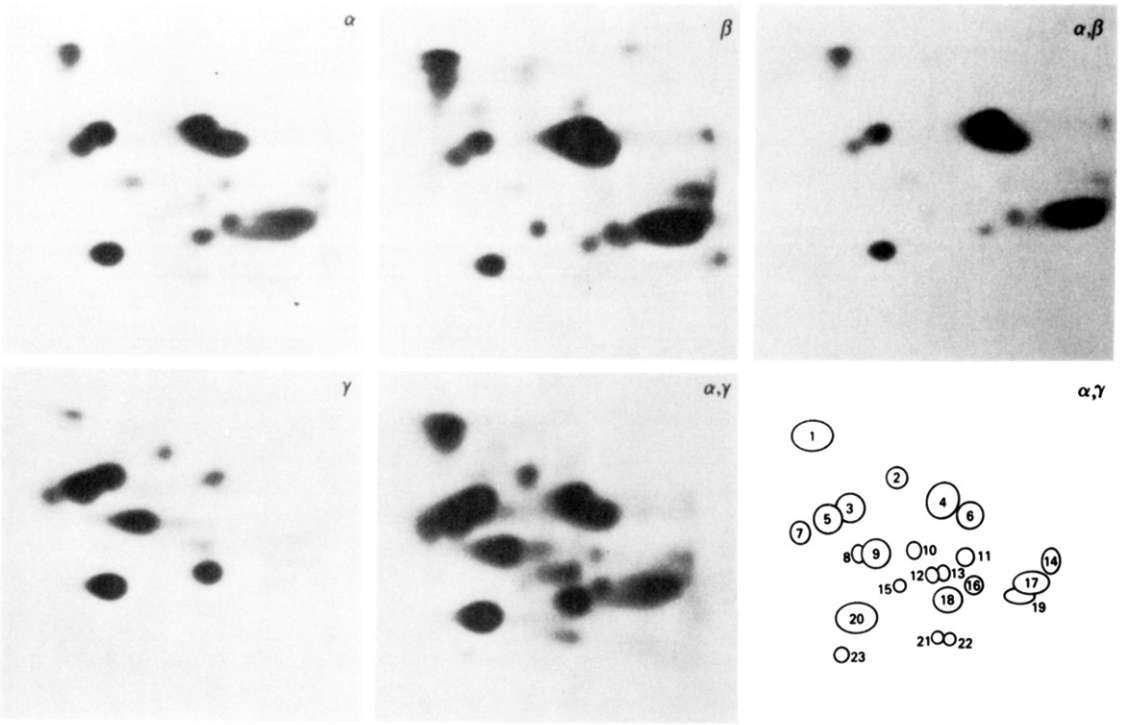

FIGURE 3: Thin-layer peptide maps of chymotryptic digests of oxidatively iodinated subunits of the receptor for IgE. The figure shows the autoradiographs of the maps. Electrophoresis was from bottom (+) to top (−), and chromatography was from left to right. The panel at the bottom right is a tracing of the autoradiograph to its left. The circles representing intense spots have the number inside; those indicating weaker spots have the number to one side.

Molecular Weight of γ Chains. In the absence of reducing agent, the purified receptor as analyzed on polyacrylamide gels in the presence of the strong detergent sodium dodecyl sulfate shows the α chain, the β chain, and a "γ" component which has an apparent molecular weight of about 20000 (Perez-Montfort et al., 1983a). Upon reduction, the latter material is quantitatively converted to a component having an apparent molecular weight of about 10000 when analyzed under the same conditions. On the basis of these results and other findings, we proposed that the band at M_r 20000 represented two disulfide-linked γ chains. When such gels are run using a somewhat modified protocol which includes urea (Swank & Munkres, 1971), the apparent molecular weight of the unreduced γ component was found to be 18300 whereas the reduced γ yielded an apparent molecular weight of 6600–6900 (Table III). Because the accuracy of molecular weight estimates on unreduced specimens is particularly suspect, it is clear that we cannot distinguish on the basis of these results alone whether the receptor contains two or three γ chains. The data on the amino composition (Table I) and those derived from the incorporation studies (Table II) are insufficient to yield an unambiguous answer. However, once the sequence of the γ chains is known, then the incorporation data will define the multiplicity of the γ chains in the receptor.

Peptide Mapping Studies. It was of interest to compare the products of proteolytic digestion of each of the subunits (Discussion). The receptors were labeled extrinsically by oxidative iodination or intrinsically by biosynthetic incorporation of [^{3}H]leucine. The receptors were purified, and the subunits were then separated on polyacrylamide gels, the appropriate slices reacted with protease directly, and the released peptides analyzed by thin-layer techniques or HPLC. Alternatively, the subunits were separated by gel filtration, digested in solution, and analyzed.

Figure 3 presents a matched set of autoradiographs of thin-layer plates on which chymotryptic digests of iodinated subunits were analyzed. The top row shows from left to right an analysis of the peptides from α, the peptides from β, and a mixture of the peptides from α and β. The three patterns are very similar with respect to both the more prominent and the less intense spots. It is particularly striking that the number of spots generated by the mixture of α and β (Figure 3) is not significantly larger than the number in the patterns derived from the individual chains. The lower row shows the pattern given by the digest from γ alone (left), and the next two panels show the mixture of γ + α and a tracing of the latter, respectively. The pattern from the γ chain shows some of the same prominent spots seen in the patterns derived from the α and β chains (e.g., peptides 3, 5, and 20), but others are missing (e.g., peptides 6 and 17). In addition, peptide 9 is strongly positive in γ whereas it is only very weakly present in α and β. As anticipated, the mixture of β and γ (not shown) appeared identical with the mixture of α and γ, and both mixtures were equivalently more complex than for γ alone. A detailed scoring of the presence and intensity of the 23 peptides shown in Figure 3 for each of the patterns gave consistent results; i.e., the mixtures showed the pattern expected from the patterns of individual subunits.

Table III: Analysis of Molecular Weight of β and γ Chains on Polyacrylamide Gels

analysis	% cross-linking	urea	reduction	mol wt β	mol wt γ
1	10	−	−	34.2/33.3	19.7
2	12	−	−	34.4	22.3
2	12	−	+	32.8	ND[a]
3	14	−	−	33	21.4
3	14	−	+	33.2	ND
4	12.5	+	−	ND	18.3
4	12.5	+	+	ND	6.6
5	12.5	+	+	ND	6.9 ± 1

[a] ND, not determined.

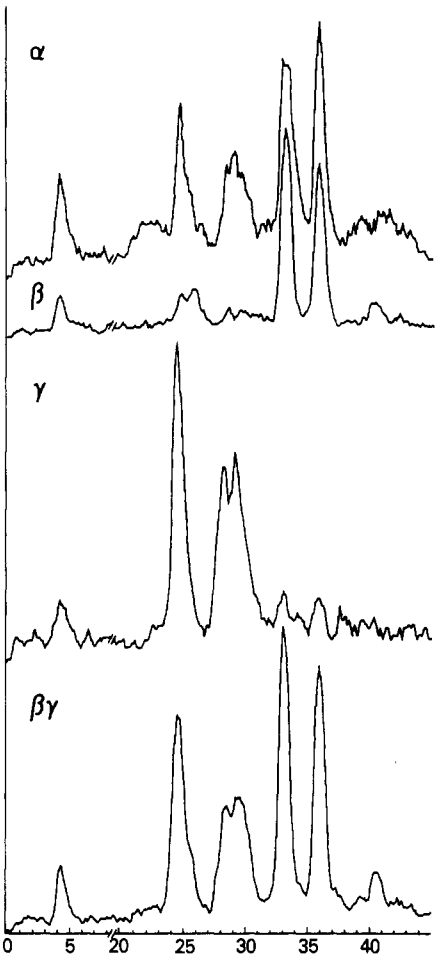

FIGURE 4: Reverse-phase HPLC of chymotryptic digests of oxidatively iodinated subunits of the receptor. The column was eluted with a linear gradient of 0–60% acetonitrile in H_2O/0.05% trifluoroacetic acid. The flow rate was 1 mL/min. Radioactivity was monitored by continuous flow counting. For the peptides from α, full scale was 100 cpm, and the highest peak registered 71 cpm. The corresponding values for β were 250 and 145 cpm, for γ 100 and 88 cpm, and for the mixture of βγ 250 and 208 cpm.

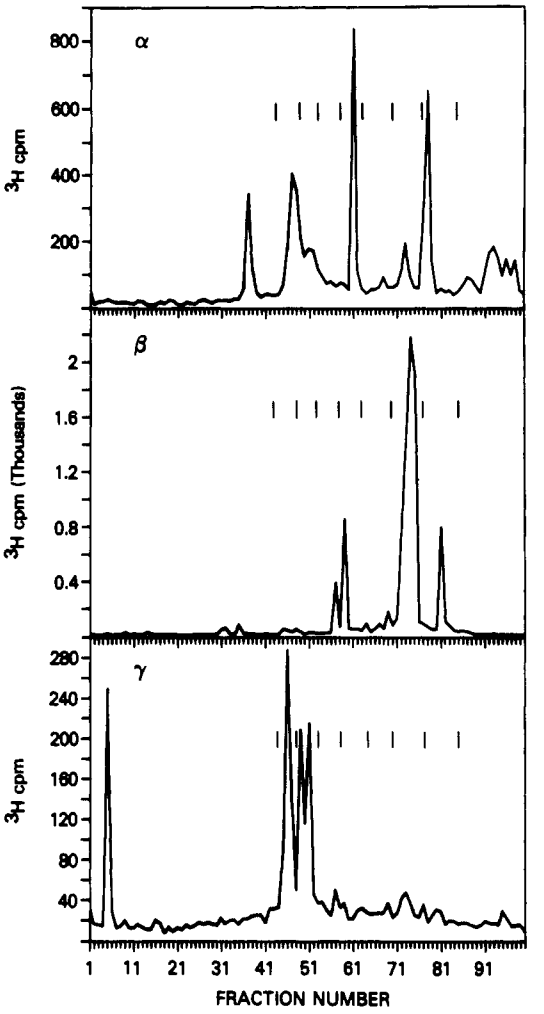

FIGURE 5: Reverse-phase HPLC of tryptic digests of the subunits labeled intrinsically with [^{3}H]leucine and unlabeled bovine serum albumin. Peptides of the latter were monitored at 220 nm. The vertical bars show the position of 8 peptides selected from over 70 because of their usefulness in standardizing the precise position of the labeled peaks. The gradient was the same as that described in Figure 4. The flow rate was 0.5 mL/min, and one fraction per minute was collected for measurement of radioactivity.

The iodinated peptides were also analyzed by HPLC (Figure 4). It can be seen that the digest of α chains yielded four principal peaks (α1–4), the second being complex and likely representing at least two discrete entities. The digest of the β chain showed two principal peaks whose retention times were essentially identical with those of α3 and α4. The digest of the γ chains showed two peaks with retention times virtually the same as α1 and α2. Mixtures of βγ (Figure 4), and of αβ and αγ (data not shown), showed the expected pattern: in each case, the same four peaks were seen in roughly the expected proportion.

Intrinsically Labeled Chains. Although several different types of experiments were performed, the most complete studies were performed on isolated chains digested in solution with trypsin in the presence of excess unlabeled carrier protein (bovine serum albumin). Receptors from cells that had incorporated [^{3}H]leucine were isolated on affinity columns in the usual way and then subjected to fractionation on a TSK 3000 gel filtration column in $NaDodSO_4$. Upon rerunning the principal peaks of ^{3}H-containing material, it was possible to separate cleanly each of the subunits, so that on polyacrylamide gels only a single band was observed for each (data not shown). Each subunit was digested separately, and the digest was analyzed by HPLC. The absorbance at 220 nm was used to detect the peptides derived from albumin. The latter patterns showed about 70 prominent peaks and many smaller ones and were highly reproducible both within the experiment (i.e., with the samples of the three subunits individually digested) and also between the two completely separate studies performed. Figure 5 presents the data on the peptides labeled with leucine that were derived from the α, β, and γ chains, respectively. The vertical hatch marks indicate the positions of eight of the peptides from albumin that were particularly useful for comparing the patterns from the labeled chains. It is clear that each of the chains yielded a distinctive pattern. Although small differences were observed in another separate experiment, the basic patterns were very similar to those shown in Figure 5.

Discussion

The principal purpose of these studies was to characterize further the covalent peptide structure of the receptor for IgE. The absolute composition for each of the chains must be derived from sequence data. However, the information we have newly obtained provides important criteria by which to judge whether the cDNAs we are currently attempting to isolate in order to obtain these sequences are the correct ones. Potentially, other criteria can be applied to arrive at such a decision, but several commonly used ones present special problems for this receptor. There are still a paucity of suitable antibody

reagents. There are as yet no reports of polyclonal reagents that react specifically with any of the chains using Western blot procedures and only a limited number of monoclonal antibodies for α and β (none yet for γ). The reactivity of these with the peptide portion of the chains uniquely, and not with carbohydrate or with other cellular proteins, remains to be proven. Furthermore, immunological identifications must be supplemented with other evidence since they could reflect cross-reactivity between homologous and, particularly because of "exon shuffling", even nonhomologous proteins (Gilbert, 1985; Südhof et al., 1985). Expression of the individual gene products may be difficult, since our previous reincorporation data suggest that at least the α chain would not (re)incorporate in the absence of β and γ (Rivnay & Metzger, 1982). Furthermore, there are still no specific biochemical functions, other than the binding of IgE to the α chain, by which the receptor can be assessed (Metzger et al., 1986). For all of these reasons, the compositional data will provide useful, if not essential, bench marks. A case in point relates to the cDNA that codes for an IgE binding moiety recently isolated by Liu and his colleagues (Liu & Orida, 1984; Liu et al., 1985; Albrandt et al., 1986). They utilized a fraction of mRNA derived from RBL cells that led to expression of a 31-kDa IgE binding protein when injected into oocytes. A cDNA isolated by use of this messenger fraction in turn led to expression of a similar protein that reacted with an antiserum directed to IgE binding proteins from RBL cells exclusively. The cDNA they have isolated codes for a polypeptide that contains 15.4 mol % proline, 14.1 mol % glycine, and 11.6 mol % alanine (Albrandt et al., 1986). These values are all substantially higher than those we previously reported (Kanellopoulos et al., 1980), and have confirmed in this study, for the α chain (Table I). The data of Albrandt et al. (1986) are also inconsistent with our analyses of the β and γ chains (Table I).

These analyses did not reveal any unusual aspects with respect to any of the polypeptides—although the incorporation data suggest a 2-fold higher than average tryptophan content for α (Dayhoff et al., 1978). The compositional data also indicate that each of the chains has a moderately elevated discriminant function, Zp, a function that reflects the overall hydrophobicity of a protein (Barrantes, 1975). Thus, for ordinary soluble proteins, this function has a value of 0.12 ± 0.16 whereas for the integral membrane proteins in Barrantes' original survey the value was 0.52 ± 0.11 (cf. Table I). Our previous work (Alcaraz et al., 1984) indicated that β and γ had rather hydrophobic properties, but the compositional data do not reflect this. Instead, the substantial number of charged residues in both β and γ is consistent with other information that suggests exposure of these chains on the cytoplasmic side of the plasma membrane (Holowka & Metzger, 1982; Perez-Montfort et al., 1983a; Holowka & Baird, 1984).

We previously demonstrated that a molecule of receptor contained a single copy of the α chain and of the β chain (Kanelopoulos et al., 1980; Holowka et al., 1980). On the basis of our initial estimates of the molecular weight of the γ chains and of the disulfide-linked oligomer of these chains, it appeared that there were two γ chains (Perez-Montfort et al., 1983a). The new molecular weight data (Table III) raise the possibility that there may be two or three ~7-kDa rather than two ~9-kDa polypeptides. Neither the compositional analysis—which defines the *relative* number of residues per unit weight—nor the incorporation studies—which define the ratios of particular residues in the subunits per unit receptor—alone or together are sufficiently precise to resolve this question. However, once the *absolute* compositions of the chains are defined by their sequences, then our incorporation data for cysteine and leucine should be sufficient to define unambiguously the multiplicity of γ chains per unit receptor.

The peptide mapping data provide additional criteria by which the individual chains can be characterized and will be useful for future topological studies. Such studies using combined labeling and immunological techniques must complement and validate the predictions made from sequence data since topological models based on the latter alone may be incorrect (Ratnam et al., 1986).

There is an apparent discrepancy between the results of the peptide maps derived from extrinsically labeled and intrinsically labeled material. It seems reasonable to await sequence data to see if the discrepancy is more apparent than real. Despite the virtual identity of the α and β chains as assessed by mapping the digests of the iodinated chains, it is clear from the compositional data that α cannot simply represent a glycosylated β chain. Thus, β has palpably more methionine and leucine than α, and α significantly more lysine and histidine. In addition, we have so far failed to observe any immunological cross-reactivity by using two monoclonal anti-α, a polyclonal anti-α, and two monoclonal anti-β antibodies in Western blotting assays (unpublished studies).

The tryptic peptide map (Figure 5) for each of the leucine-labeled chains is distinctive, but this finding must also be interpreted cautiously. Thus, the amino acid analyses suggest that if α has a peptide molecular mass of 30 kDa there could be as many as 30 sites for tryptic cleavage. One can easily think of many factors and combinations of such factors that could account for the relatively simple pattern observed. The same is true for the patterns derived from the β and γ chains where respectively ~25 and 6–10 peptides could theoretically have been generated but of course not necessarily observed using labeled leucine to detect them. Despite the obvious limitations of these data, it is clear that they provide useful ways of characterizing the chains.

In particular, it may permit us to detect small covalent changes in the peptide chains associated with activation of the receptor. Our prior analysis failed to detect any evidence for removal of substantial segments from the ends of the chains or for internal cleavages, but small changes could have been missed (Perez-Montfort et al., 1983b). The peptide mapping can provide a more discriminating tool to explore this question.

Added in Proof

Since submission of the manuscript, a cDNA that codes for the α subunit has been isolated (J. P. Kinet, H. Metzger, J. Hakimi, and J. Kochan, submitted for publication). It predicts an amino acid sequence containing eight tryptophans (see text) and whose overall composition is in good agreement with the data for α shown in Table I. The principal discrepancy is the lower content of glycine. Likely, the value derived from the compositional analysis was falsely high because of incomplete elimination of the glycine buffer used during the preparative gel electrophoresis. The sequence also predicts a maximum of eight leucine-containing tryptic peptides (cf. Figure 5).

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