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Immunology

Isolation and characterization of cDNAs coding for the β subunit of the high-affinity receptor for immunoglobulin E

(basophilic leukemia cells/Fc receptor/monoclonal/cloning)

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ABSTRACT **Among receptors that bind the Fc region of immunoglobulins ("Fc receptors"), only the one with high affinity for immunoglobulin E (IgE) is known to consist of more than a single polypeptide. In addition to the IgE-binding α chain, the receptor contains a single β chain and two, disulfide-linked, γ chains. From a cDNA library of a rat mucosal mast cell tumor, from which we recently cloned cDNAs coding for the α chain, we have now isolated cDNAs coding for the β subunit. *In vitro* transcription-translation of the cDNA directed the synthesis of a polypeptide reactive with two distinctive anti-β monoclonal antibodies and whose molecular weight was identical to that of authentic β chains. Polyclonal antibodies to β peptides expressed in *Escherichia coli* reacted with intact receptors and isolated β chains. The gene encodes a protein of 243 residues with no leader sequence. A hydropathicity plot suggests that the polypeptide crosses the plasma membrane four times. The epitope recognized by one of the monoclonal antibodies was localized to the NH_2 terminus; that by the other was localized to the COOH terminus. Since those antibodies react with membranes and not with intact cells, we suggest that both ends of the β subunit are cytoplasmic. RNA transfer blots at high stringency failed to reveal mRNA for β chains in a variety of cells (in particular, monocytes) that do not contain the high-affinity receptor for IgE.**

Receptors that bind the Fc region of immunoglobulins ("Fc receptors") mediate their transport across membranes, stimulate a variety of cellular activities induced by antigen–antibody complexes, and may regulate the biosynthesis of antibodies. The cDNAs for several of the Fc receptors have been characterized. Three of the receptors [the receptor for polymeric immunoglobulins (1), the Fc_γ receptors on macrophages and lymphocytes (2), and the high-affinity Fc_ϵ receptor on mast cells and basophils (3–5)] share a common feature: their immunoglobulin-binding portion contains two or more immunoglobulin-like domains.

The high-affinity Fc_ϵ receptor is the only Fc receptor known to consist of multiple subunits. In addition to its immunoglobulin-binding α chain, it contains a β chain and two disulfide-linked γ chains (6). It has not yet been possible to express the cDNA for the α subunit on the surface of transfected cells (3, 4). Possibly, as with other multisubunit receptors, one or more of the other subunits must be cosynthesized to achieve surface expression (7, 8). The role of the β and γ subunits in the mechanism of action of this receptor is also of interest.

In this report we describe the isolation and sequencing of cDNAs that code for the β subunit.§ The polypeptide sequence it predicts suggests a topological model that has been partially tested experimentally.

MATERIALS AND METHODS

Isolation and Sequencing of Peptides. Electroeluted β subunits from polyacrylamide gels were prepared as described (9). Tryptic peptides were separated by high-pressure liquid chromatography and sequenced as before (3).

Cloning and Sequencing of cDNA. RNA extracted from rat basophilic leukemia (RBL) cells by the guanidinium isothiocyanate method (10) was fractionated on an oligo(dT)-cellulose column (11) and used to construct a pUC9 and a λgt11 library (11, 12). Colonies were screened as before (3) using oligonucleotides prepared on a model 380A automated DNA synthesizer (Applied Biosystems, Foster City, CA). cDNA inserts were subcloned into pGEM-4 or pGEM-3Z and the resulting double-stranded DNA was sequenced with the Gemseq/RT sequencing system according to the method recommended by the supplier (Promega Biotec, Madison, WI). Twenty-mer oligonucleotides, corresponding to previously sequenced regions by this method, were used as primers to generate overlapping sequences otherwise difficult to obtain. In some instances, DNA sequencing was performed using Sequenase as recommended by the supplier (United States Biochemical, Cleveland).

***In Vitro* Transcription and Translation.** cDNAs corresponding to the β subunit and various mutated or truncated forms thereof were subcloned into either pGEM-4 or pGEM-3Z transcription vectors (Promega Biotec). Unlabeled RNAs were synthesized using either SP6 or T7 polymerase as recommended by the supplier. Capping reactions were performed as reported (13). After digestion of the template with RNase-free DNase I, the RNAs were purified further by extraction with phenol/chloroform and three precipitations from ethanol. The RNA was then translated with a micrococcal nuclease-treated lysate of rabbit reticulocytes in the presence of [^{35}S]methionine as recommended by the supplier (Promega Biotec). The products of translation were diluted 1:1 with 20 mM detergent {3-[3-(cholamidopropyl)dimethylammonio]-1-propane sulfonate} in borate-buffered saline (pH 8) containing 30 μl of aprotinin per ml, 175 μg of phenylmethylsulfonyl fluoride per ml, 10 μg of leupeptin per ml, and 5 μg of pepstatin per ml and immunoprecipitated with monoclonal antibodies as described (14).

Intrinsic Labeling of Receptors. Biosynthetic incorporation of labeled amino acids and monosaccharides was as described (15). The purification and analysis on gels and by

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Abbreviation: RBL, rat basophilic leukemia.
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§The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03845).

immunoblotting of the IgE–receptor complexes have also been described (14).

RNA Transfer Blotting. Thirty micrograms of total RNA was run on a 1% agarose gel containing 2% formaldehyde and blotted to nitrocellulose filters (11). The filters were hybridized with a restriction fragment of the β cDNA (nucleotides 1–474) as described (11) and washed with 15 mM NaCl/1.5 mM sodium citrate at 65°C.

Antibodies. *Escherichia coli* transformed with an expression vector containing the desired restriction fragments (16, 17) were cultured and induced, and the fraction enriched for the recombinant protein was prepared as described (17). After separation on polyacrylamide gels in sodium dodecyl sulfate ($NaDodSO_4$) the transformant-specific protein was eluted and used to immunize rabbits. Approximately 100 μg of protein was injected in complete Freund's adjuvant; this was followed by a booster injection of 25 μg of protein in incomplete adjuvant. The isolation and characterization of monoclonal anti-β antibodies mAbβ(JRK) and mAbβ(NB) (the latter, a generous gift from David Holowka, Cornell University) have been described (14).

RESULTS

Isolation of Peptides. Since repeated attempts to sequence intact β chains were unsuccessful, we isolated peptides from tryptic digests. A peptide (no. 1) isolated from an initial digest had the sequence Tyr-Glu-Glu-Leu-His-Val-Tyr-Ser-Pro-Ile-Tyr-Ser-Ala-Leu-Glu-Asp-Thr. The same peptide from later digests showed an additional leucine at the NH_2 terminus and an arginine at the COOH terminus. The sequences of three other peptides, each isolated in substantial yields, are indicated in a subsequent figure.

Isolation of cDNA Clones. The initial sequence obtained for peptide 1 was used to construct two 26-mer oligonucleotides of 32-fold degeneracy: 5′-GGIGA$^{A}_{G}$TA$^{G}_{C}$ACATGIA$^{AC}_{GT}$T-C$^{C}_{T}$TCATA-3′ and 5′-GGICT$^{A}_{G}$TA$^{G}_{C}$ACATGIA$^{AC}_{GT}$TC$^{C}_{T}$TC-ATA-3′. A λGT11 library constructed from mRNA of RBL cells was screened with a 1:1 mixture of these oligonucleotides. Six positive clones gave similar restriction patterns. The clone containing the longest insert was sequenced according to the strategy shown in the upper portion of Fig. 1. The sequence predicts possible starting codons at nucleotides 46–48 and 55–57, which would yield a polypeptide of 246 or 243 residues, respectively (Fig. 2*A*). The predicted M_r of about 27,000 is some 20% less than the apparent molecular weight of β subunits when analyzed on polyacrylamide gels (18). In addition, no in-frame stop codon was apparent upstream of the start codon. To rule out the possibility that the true start codon was still further 5′, we rescreened the cDNA library with a restriction fragment (nucleotides 7–474) and with a synthetic oligonucleotide probe (nucleotides 3–32). Twenty-eight additional clones were isolated and their restriction patterns were examined. Twenty were similar to the original clones. Only six additional nucleotides at the 5′ end (nucleotides 1–6, Fig. 2*A*) were identified. Early termination was found in six clones, which otherwise had the same sequence through nucleotide 375 (Fig. 2*B*). One 2.4-kb clone had cytidine 473 substituted with an adenine. This substitution abolishes the *Pst* I site and creates a new *Cla* I site at nucleotide 470. Also thereby, Ala-140 would become Asp-140 (Fig. 2*A*). Finally, one clone extended ≈350 base pairs (bp) in the 5′ direction. The

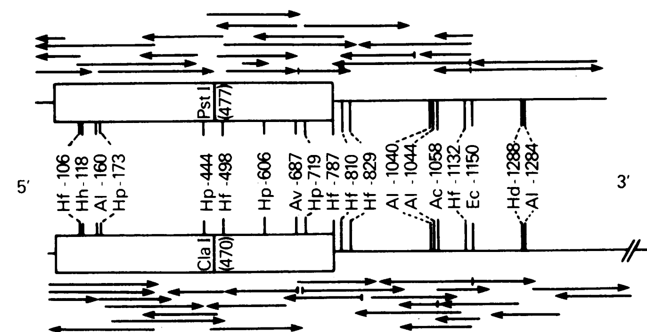

FIG. 1. Restriction maps for β cDNAs and strategy by which they were sequenced. The open rectangle indicates the sequence predicted to code for the β subunit; the lines indicate the 5′ and 3′ untranslated regions. The upper scheme shows the 1.5-kilobase (kb) clone containing a *Pst* I cleavage site. The lower scheme shows a 2.4-kb clone containing a *Cla* I cleavage site. The 3′ region of the latter has been truncated as indicated by the slashes. Its untranslated portion was sequenced as completely as the rest of the clone. Restriction sites are indicated by vertical bars: Hf, *Hin*fI; Hh, *Hha* I; Al *Alu* I; Hp, *Hph* I, Av, *Ava* II; Ac, *Acc* I; Ec, *Eco*RI; Hd, *Hin*dIII. The horizontal arrows show the direction and extent of sequencing by the dideoxynucleotide chain-termination method.

A

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ACGTTTCTGTGTAACAATATCTTTTATTCCTGGATAGTCCAATTAATGAAAAAA  ATG GAC ACA GAA   66
                                               (M   K   K)   M   D   T   E     4
AAT AAG AGC AGA GCA GAT CTT GCT CTC CCA AAC CCA CAA GAA TCC CCC AGC GCA  120
 N   K   S   R   A   D  <L   A   L   P   N   P   Q   E   S   P   S   A    22
CCT GAC ATT GAA CTC TTG GAA GCG TCC CCT CCT GCA AAA GCT CTA CCA GAG AAG  174
 P   D   I   E   L   L   E>  A   S   P   P   A   K   A  <L   P   E   K    40
CCA GCC TCA CCC CCA CCA CAG CAG ACA TGG CAG TCA TTT TTG AAG AAA GAG TTG  228
 P   A   S>  P   P   P   Q   Q   T   W   Q   S   F   L   K   K   E   L    58
GAG TTC CTG GGC GTA ACC CAA GTT CTG GTT GGT TTG ATA TGC CTT TGT TTT GGA  282
 E   F   L   G   V   T   Q   V   L   V   G   L   I   C   L   C   F   G    76
ACA GTT GTC TGC TCC ACA CTC CAG ACT TCA GAC TTT GAC GAC GAA GTG CTT TTA  336
 T   V   V   C   S   T   L   Q   T   S   D   F   D   D   E   V   L   L    94
TTA TAT AGA GCA GGC TAC CCA TTC TGG GGT GCA GTG CTG▼TTT GTT TTG TCT GGA  390
 L   Y   R   A   G   Y   P   F   W   G   A   V   L   F   V   L   S   G   112
TTT TTG TCA ATT ATG TCC GAA AGG AAA AAC ACA CTG TAT CTG GTG AGA GGC AGC  444
 F   L   S   I   M   S   E   R   K   N   T   L   Y   L   V   R   G   S   130
CTG GGA GCA AAC ATT GTC AGC AGC ATC GCT GCA GGC TTG GGG ATC GCC ATA TTG  498
 L   G   A   N   I   V   S   S   I   A   A   G   L   G   I   A   I   L   148
ATT CTC AAT CTG AGC AAC AAC TCC GCT TAT ATG AAC TAC TGC AAG GAT ATA ACC  552
 I   L   N   L   S   N   N   S   A   Y   M   N   Y   C   K   D   I   T   166
GAA GAC GAT GGT TGC TTC GTG ACT TCT TTC ATC ACA GAA CTG GTG TTG ATG TTG  606
 E   D   D   G   C   F   V   T   S   F   I   T   E   L   V   L   M   L   184
CTG TTT CTC ACC ATC CTG GCC TTT TGC AGT GCC GTG CTG CTC ATT ATC TAT AGG  660
 L   F   L   T   I   L   A   F   C   S   A   V   L   L   I   I   Y   R   202
ATT GGA CAA GAA TTT GAG CGT AGT AAG GTC CCC GAT GAC CGT CTC TAT GAA GAA  714
 I   G   Q   E   F   E   R   S   K   V   P   D   D   R  <L   Y   E   E   220
TTA CAT GTG TAT TCA CCA ATT TAC AGT GCG TTG GAA GAC ACA AGG GAA GCG TCC  768
 L   H   V   Y   S   P   I   Y   S   A   L   E   D   T   R> <E   A   S   238
GCA CCA GTG GTT TCA TAA GAATCAAGGGGCCAGGACAATCTGATTCCAGTATAGTCTTGAGAGTC  833
 A   P   V   V   S>                                                        243
GATCTTTTTGCAACATTATGGCAACATTTCTGTTTCCTCCGCACTCTATCAACTTTTCAATTGGATTGTTC  904
TGTAGATACCCCTGTTTCAGTTATGATGCCTCTGGTCTTTAATTATCTCCCTTTTTGTGGATATCGTTCAA  975
TCCAGTTTTCTTGTTTTGTGTCACAGTCTCACATACAACCTTTCTGGAAAGTCATCAAAAACAAGCTAGCT 1046
TTTATTGCATGTCTACTTTCATGAACAAAAGGAAGGAGGAGTTATTTTGAGAGTTTAACTAAACTTAGATA 1117
ATCAGGTAATATTTGACTCTTAGTTCATTTTAGAATTCTCAACAATACTTGTGCATGATATATGCCCACCA 1188
TATCAAGCCTTCTATATATATTTAATATGGTATTTACTTTTCTATGTAGATAGATTTTCCACCCTCAATAA 1259
TAATGGGTTTTTCAGAGACATAAAGCTTTATGAAAAGACACATATTATCTAATTCATGGTATATTCACTA 1330
ATACAGTTGTTGCTCAGTGGTGTTTACTACTTGGTGGGTAGTAGGTAATAGAGAACATTATTAAATCATTC 1401
AGTGTAGTGAGATGCATAGGTAAAATCAGGGACACTGTGAGTGTGTATATCTTTTGGTAAGACATGTGTGA 1472
AAATGAAGAATAAACTGATGAAGACTTGAGCTGGAAAGTAGTCAATGGGAATGACAAGAAATGATTGTGTA 1543
TAACACTTGTAGATAAATAACTACCAACAATTGGTAGAGATTGCCATGTATGCCTAAAATCTCCCAGCCCA 1614
AGGCCAGCCTCTGTTACACAGTGAGTTAGAGGCCAGTCTGGGCTACACAAGATCATACATCAAAGGACGAA 1685
AGAAGATGTTGGTTCAAACTGTTAACACAGTAAGGGATATTTAAACAAACAGAAGTTTGACTGATATATTG 1756
AGTGCTTGAGTTTTTAATAAAACTGAATGAATAACATTGCGGGGGAGGGGAGCAGTGATGCAGAAGTCTGG 1827
ATGATGGAGGAGTAGCAGAATCAGATGAAACATTGAAACGTATTTCCAGACTTTTGTTCTGAGATGGTTAT 1898
AAGAGCAATCACCATTAAATGAAGAAGGTCAAGACACCAAAAGAATTATTTTGAGATAGAATTAAGACAGT 1969
CAAAATCCACATGCCTATACTTAGAAGGTGAAGTAAGGATCAAAAGTAGAAAGCCTAACGATTAGTTGGAA 2040
AAGCATATTACGTTAGGCAGCAGATGTCTATAGTGGAGAAAAGTTAAACAAGGAGAAATAATGAACCACCA 2111
GAGACTCTACATGTTGGTTTGGGAAATAAGAGAAAATAGCAATTCTAAACGAATGCAAACTCTGAAGAAGC 2182
ATTTCCCAAAGGGTGTGGGCAGAGGACCAGAACATTTGCAAATGTACCTAGAGAGCAAACCTGAATAGGAG 2253
GTAAAATGGGGGAAAAGCAGCTAAGAAAATGATTTTGTTGCTGTTATTTAGATTTTAAAAGAAACAAAAAG 2324
AGTCATTAAAAATCTGTTTGCTGGGATCAGTTATTGTGTTCTCTGTGTATGTCCAAAGTACAGGTAACTTT 2395
TCTAAATCTTCCTGTAAGGCTCACCTCATATGTCTCTTCACATAGCCACACCCTTGATTCACAGTTACTCT 2466
ACCACAGTAGTAAACTGTGCTTGTGGTCTCCTTATGTATCTTCCACTAGTGTTTATAAAATAAATCAGAAT 2537
TATTTAAA                                                                2545
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B

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GTG AGA ACA TAT CTG TAA TTGTTTCTGAAATGATGCTAACCAGAGATTTTATTTTAATCAAAGAC  440
 V   R   T   Y   L                                                       112
AACTAATTTTCTTTTAATCAAGTGCTTATCTCTAGCCTTTCAATAATATCTACAGTTCTTCATTTATATGC  511
ACATAGCCATCTATAAATGTAGTTTCCAAAGCACTCTCTACATATACTCATTAACAAGAGCAAATACACTC  582
ACCACAGTTAACTATGGTTTAACCCATTACTATACTTTTATTGACTGAAAACCTTGAGACTGTAC(A)14   661
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FIG. 2. (*A*) Nucleotide and deduced amino acid sequences of the cDNA coding for the β subunit. Beginning at the arrowhead (▼), an alternative sequence (*B*) was observed in six clones. The putative transmembrane domains are underlined. The tryptic peptides of the β subunit, from which the amino acid sequences were determined directly, are bracketed (⟨ ⟩). A putative poly(A) signal near the end is underlined. (*B*) Continuation of the nucleotide sequence of the deleted form of β cDNA, 3′ to the junction indicated in *A* (▼).

junction with the sequence shown in Fig. 2*A* was AATAAAACAAAAAAAAAAAAATG, the last two nucleotides of the newly generated ATG corresponding to nucleotides 8 and 9 of the previous sequence. It is likely that this clone simply resulted from the ligation of two independent cDNAs. Screening of the puc-9 library revealed three clones. However, the sequence of none of these extended 5′ beyond nucleotide 84.

RNA Transfer Blotting. RNA transfer blotting was performed under high stringency using a *Pst* I fragment probe (nucleotides 1–474). RBL cells yielded two major bands at ≈2.7 kb and 1.75 kb, with the upper band having about twice the intensity of the lower one. A minor band at 1.2 kb was also noted. Negative results were obtained with a variety of cells that do not express high-affinity IgE receptors: the rat pituitary line GH3 (American Type Culture Collection no. CCL82.1), the rat glial cell line C6 (no. CCL107), the mouse Leydig cell line I-10 (no. CCL83), and, notably, the mouse monocytic line J774 (no. T1B67) and the rat lymphoma "NTD" (14).

***In Vitro* Expression.** The β clone containing the *Pst* I site was transcribed *in vitro* with T7 RNA polymerase, and the resulting mRNA was translated with a lysate of rabbit reticulocytes in the presence of [^{35}S]methionine. The unfractionated translated material showed a major component at M_r ≈32,000 compared to the control from which the RNA had been omitted or an alternative RNA (brome mosaic virus) had been substituted (data not shown). The monoclonal anti-β antibodies mAbβ(JRK) and mAbβ(NB) (14) (Fig. 3*A*, lanes 2 and 3)—but not an irrelevant antibody (lane 5)—precipitated radioactive material, which on polyacrylamide gels in NaDodSO$_4$ showed a major band at M_r 32,000. This band had the identical mobility as the upper band of the doublet precipitated by mAbβ(JRK) from an extract of labeled RBL cells (lane 1). Although not seen well in the reproduction, the autoradiogram showed that the material synthesized *in vitro* also contained the lower molecular weight component seen in the *in vivo* synthesized β chains. The mobility of the *in vitro* synthesized protein was unaltered by reduction as has been previously observed with the β subunit. The clone containing the *Cla* I site (which incidently lacks the first ATG codon) led to the synthesis of a protein whose mobility on gels was indistinguishable from that for the clone containing the *Pst* I site. On the other hand, an aberrant clone containing the newly generated ATG (above) induced the synthesis of a somewhat larger protein with an apparent M_r of 33,500 (data not shown). *In vitro* translation of a transcript coding for the NH$_2$-terminal 21 amino acids of the β subunit led to a product precipitable by mAbβ(JRK) (Fig. 3*B*).

***E. coli* Expression.** Two *Hin*fI fragments (A, nucleotides 106–498; B, nucleotides 499–787) were individually subcloned into an *E. coli* expression vector, and extracts were prepared from the induced cultures. The results of one immunoblotting experiment are shown in Fig. 3*C*. The material extracted from the bacteria transformed with a vector containing the *Hin*fI fragment B exhibited a M_r 14,000 component reactive with mAbβ(NB) but not with mAbβ-(JRK) (Fig. 3*C*). The extract from the transformants containing the more NH$_2$-terminal *Hin*fI fragment A (residues 17–148) reacted with neither antibody (compare with above). Rabbit antibodies generated by fragment A reacted on immunoblots with purified receptors exactly at the position

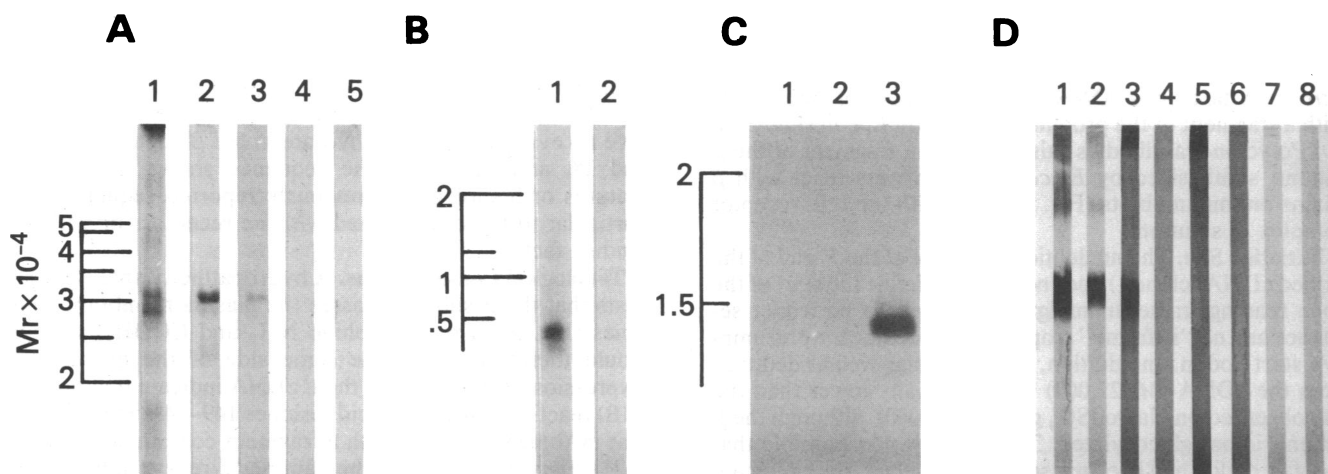

FIG. 3. Expression of cDNA coding for the β subunit. (*A*) Comparison of *in vivo* and *in vitro* translation products. RBL cells were grown in [^{35}S]cysteine-containing medium. The detergent extract of the cells was precipitated with mAbβ(JRK) and, after vigorous washing, extracted with sample buffer and electrophoresed (lane 1). This experiment employed concentrations of detergent high enough to dissociate the receptor completely. A transcript from the β cDNA was translated *in vitro* in [^{35}S]methionine-containing medium (lanes 2, 3, and 5). A control incubation contained no cDNA (lane 4). The mixtures were allowed to react with monoclonal antibodies to the β subunit after a clearing immunoprecipitation. The specific washed precipitates were dissolved in sample buffer and electrophoresed: lanes 2 and 4, mAbβ(JRK); lane 3, mAbβ(NB); lane 5, irrelevant monoclonal antibody [mAb(LB)]. An autoradiograph of the 12.5% polyacrylamide gel on which the specimens were analyzed under reducing conditions is shown. (*B*) Localization of one epitope to the NH$_2$-terminal peptide of the β subunit. A β cDNA-containing vector was digested with *Hha* I before transcription using T7 polymerase. The resulting mRNA was translated to generate an NH$_2$-terminal peptide of the β subunit (amino acid 1–21) labeled with [^{35}S]methionine. The mixture was allowed to react with mAbβ(JRK) (lane 1) and the irrelevant mAb(LB) (lane 2). The precipitates were analyzed on a 17% gel under nonreducing conditions. (*C*) Expression by *E. coli* of a COOH-terminal fragment of the β subunit. A *Hin*fI fragment, containing nucleotides 499–787, was subcloned into an *E. coli* expression vector (16) and extracts were prepared. The proteins were electrophoresed as in *A* and transferred to nitrocellulose paper. The latter was allowed to react sequentially with monoclonal antibody mAbβ(NB), developed with alkaline phosphatase-conjugated goat anti-mouse IgG (Fc), and developed in the usual way (14). An enlargement of the lower half of the immunoblot is shown. Lane 1, extract from transformant without insert; lane 2, extract from transformant with insert in wrong direction; lane 3, extract from transformant with insert correctly oriented. (*D*) Reactivity of β subunits with polyclonal antibodies induced by *E. coli*-expressed *Hin*fI fragments. Purified IgE–receptor complexes were electrophoresed, transferred to nitrocellulose paper, and allowed to react with antibodies and subsequently with an appropriate alkaline phosphatase-conjugated anti-immunoglobulin antibody. Lane 1, mAbβ(JRK); lane 2, mAbβ(NB); lane 3, immune serum to fragment A; lane 5, immune serum to fragment B; lanes 4 and 6, preimmune sera corresponding to the immune sera in lanes 3 and 5, respectively; lanes 7 and 8, second antibody only. This gel was run without molecular weight standards.

where the two monoclonal anti-β antibodies reacted (Fig. 3*D*, lanes 1–3) and quantitatively precipitated intact ^{125}I-labeled IgE–receptor complexes from unfractionated detergent extracts of RBL cells (data not shown).

Biosynthetic Incorporation. By using biosynthetic incorporation of two different amino acids labeled distinguishably, we determined their ratio in the subunits of the receptor (Table 1, right part). The ratios of four distinctive amino acids to each other was in satisfactory agreement with the ratios predicted from the β cDNA clone (Table 1, right part, columns 1–3). Because the cDNA for the β subunit predicts three potential glycosylation sites, we also performed a double-labeling experiment using [^{3}H]mannose and [^{35}S]cysteine. Based on the relative carbohydrate data reported for the α subunit (19) and correcting them on the basis of the peptide molecular weight for this chain predicted from the cDNA, we calculated that the α subunit contains ≈20 mol of mannose per mol. We were therefore able to determine the mannose/cysteine ratio in the β subunit from the double-labeling experiment. The results showed only 0.05 mol/mol of cysteine or 0.3 mol/mol of the β subunit (Table 1, right part, column 4).

DISCUSSION

cDNA Codes for the β Subunit. There is ample evidence that the cDNAs we isolated code for the β subunit. (*i*) *In vitro* transcription of the cDNA and translation of the derived mRNA produce a protein whose apparent molecular weight on gel electrophoresis is indistinguishable from that of authentic β chains (Fig. 3*A*). (*ii*) The cDNA accurately predicts the sequence of four peptides isolated from a tryptic digest of β chains (Fig. 2*A*) and a composition that agrees well with direct analyses and biosynthetic incorporations (Table 1). (*iii*) Two monoclonal antibodies reactive with discrete epitopes on the β subunit (14) precipitate the protein synthesized *in vitro* from the cloned cDNA (Fig. 3*A*), and one of them reacts with a fragment of the protein expressed in *E. coli* (Fig. 3*C*). (*iv*) Polyclonal antibodies raised against a fragment of the β subunit synthesized by *E. coli* transformants react with β chains on immunoblots (Fig. 3*D*) and with the IgE–receptor complex in solution.

Initiation Site. The nucleotide sequence at the 5′ end of the cloned cDNA (clone 1) does not in itself define the start of the open reading frame unambiguously. There is no leader sequence and no "in frame" stop codon preceding the presumptive start codon. In addition, the molecular weight deduced from the cDNA (M_r 27,000) is substantially lower than the one observed on $NaDodSO_4$ gels (M_r 32,000), although the β subunit is not glycosylated. Therefore, it was possible that the start codon had been missed. Nevertheless, the aggregate data provide strong evidence that the full coding sequence for the β subunit has been recovered. (*i*) Extensive attempts failed to reveal cDNAs in either of two separate libraries with a more extended 5′ sequence. (*ii*) The major species generated by 5′ extension studies terminated precisely at the point at which most of our clones started. (*iii*) The second ATG codon at the 5′ end meets the consensus characteristics of known initiation sites (20). That it is preceded by a nearby 5′ ATG codon is uncommon, but not rare (20), and has been observed for the human α subunit (4, 5). (*iv*) As already noted, *in vitro* translation of an mRNA transcribed from the cDNA containing only the second ATG codon gives a polypeptide indistinguishable in length from the authentic β chains. An aberrant clone containing a start codon 48 nucleotides 5′ to the presumed start codon directed the *in vitro* synthesis of a polypeptide with an apparent molecular weight appropriately greater than that of the β subunit (*Results*). Therefore, the correspondence in apparent molecular weight between authentic β chains and the protein synthesized *in vitro* from clone 1 is meaningful. The RNA transfer blotting data show an mRNA of ≈2.7 kb, precisely what would be anticipated from the cDNA we have sequenced (Fig. 2), given a poly(A) tail of ≈200 nucleotides. In the discussion that follows we will assume that the β chain begins with the methionine residue coded for by the second ATG and is, therefore, 243 residues long.

Alternative Forms of the β Subunit. Only a single clone containing the *Cla* I restriction site was observed among the 37 clones analyzed. This clone likely resulted from a single base mutation during the cloning and is unlikely to represent a normally occurring mRNA. Conversely, six clones showing the deleted sequence (Fig. 2*B*) were observed and likely reflect an authentic species of mRNA. If translated, it would code for a M_r 14,000 protein with only a single transmembrane segment.

Sequence Characteristics. The sequence of the β subunit contains potential sites for N-linked glycosylation at residues 5, 151, and 154. However, past and new incorporation data give no evidence for carbohydrate in the β subunit (refs. 15 and 18, and Table 1). The sequence shows no unusual features or homology to previously reported sequences, in particular to those associated with Fc receptors or with Fc binding factors.

Topological Considerations. A hydropathicity analysis suggests that the β subunit crosses the plasma membrane four times (Fig. 4). The hydrophilic NH_2 and COOH terminus would therefore be on the same side of the membrane. Expression of fragments of the β cDNA indicate that mAbβ-(NB) reacts within amino acid residues 149–243 (Fig. 3*C*) and that mAbβ(JRK) reacts with a fragment containing residues 1–21 (Fig. 3*B*). Since neither antibody reacts appreciably with intact cells but both react strongly with cell sonicates, the combined results are consistent with the NH_2 and COOH

Table 1. Amino acid composition of β subunits

	cDNA versus compositional analysis for the β subunit																		cDNA versus incorporation data			
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Cys	Trp	Met/His	Cys/His	Cys/Trp	Man/Cys
Deduced from β cDNA	20	12	23	24	15	12	19	17	4	15	36	9	12	1	8	8	6	2	4	6	3	—
Direct analysis*	22	13	22	27	13	19	18	14	4	13	31	7	10	2	10	10	5	ND				
Double-labeling studies†																			4.2	5.1	2.5	0.05

*The mol % of each amino acid as reported by Alcaraz *et al.* (9) was multiplied by 241—the number of residues, excluding tryptophan—predicted from the cDNA. ND, not determined.

†IgE–receptor complexes were purified from RBL cells incubated with a mixture of two precursors labeled with differentiable radioisotopes. The subunits were separated on a polyacrylamide gel. The gel was sectioned into 2-mm slices, extracted, and assayed for radioactivity by scintillation spectroscopy. The ratio of cpm of ^{35}S/^{3}H was individually calculated for α, β, and γ subunits. The ratio in the α subunit is proportional to the known molar ratio of the ^{35}S-labeled and ^{3}H-labeled residues in the α subunit. Hence, the corresponding ratio in the β subunit (and the γ subunit) predicts the ratio of the same residues in the latter subunits.

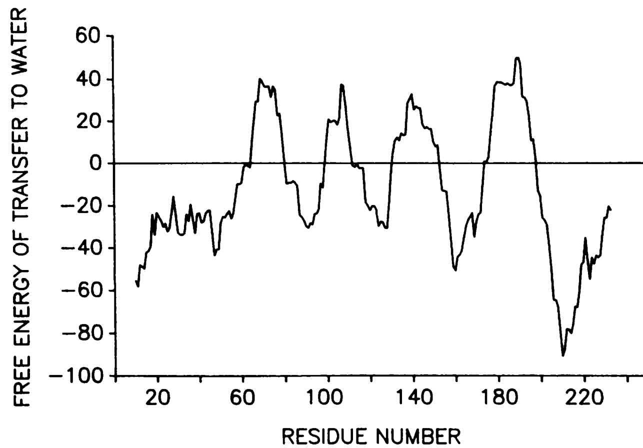

FIG. 4. Hydropathicity plot of predicted sequence for the β subunit. The procedure and hydropathicity scale recommended by Engelman *et al.* (21) was used. The net hydropathicity value for the 20 amino acids for each successive "window" is plotted at the position corresponding to the 10th residue. A net free energy of >20 kcal (1 cal = 4.18 J) for transfer to water suggests a transmembrane segment (21).

terminus being on the cytoplasmic side of the plasma membrane.

Earlier studies had suggested that the β chain contained a M_r 20,000 "β_1" domain resistant to proteolysis while membrane bound (18). This portion also contained those residues that were modified by an intrabilayer labeling reagent (18, 22) and became linked to the α and/or γ subunit when chemical crosslinking reagents were used (18) and to the γ subunit when spontaneous disulfide linkage between the β and γ_2 subunits occurred (23). The remainder, "β_2," appeared to contain the serine residues that became phosphorylated *in situ* (24, 25) but has never been positively identified as a discrete fragment. The sequence predicted by the cDNA for the β subunit suggests that part or all of either the NH_2-terminal 59 residues or the COOH-terminal 44 residues, or of both, is cleaved off to generate the β1 fragment.

Cotransfection Experiments. The full-length coding sequences of the α and the β subunits were cotransfected in COS 7 cells by using a vector for transient expression. So far, no IgE-binding sites were expressed at the surface of transfected cells. Possibly all of the subunits will be necessary to achieve surface expression of the receptor.

β Subunits in Other Cells? Studies of the receptor with low affinity for IgE on macrophages revealed a component that could be chemically crosslinked to the IgE-binding portion and that had an apparent molecular weight similar to the β subunit of the high-affinity receptor (26). The peptides generated from this component by protease digestion appeared to differ from those released from β subunits, but it raised the possibility that other Fc receptors also contained β-like subunits that had heretofore escaped detection (see also ref. 14). So far, we have no evidence for this from RNA transfer blot experiments conducted at high stringency. In particular, J774 cells are known to contain Fc_γ receptors whose immunoglobulin-binding chain shows considerable homology to the α chain of the high-affinity receptor for IgE (3). However, we could not detect mRNA for β chains by the methods we employed. Similarly, NTD lymphoma cells gave negative results even though they have Fc_γ receptors and show a low molecular weight component that reacts with mAbβ(JRK) on immunoblots (14). We of course cannot exclude that Fc_γ receptors have β-like subunits.

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