

A Conformational Rearrangement upon Binding of IgE to Its High Affinity Receptor*

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One of the critical steps in the allergic reaction is the binding of immunoglobulin E (IgE) to its high affinity receptor (FcεRI). FcεRI is a tetrameric complex composed of an α-chain, a β-chain, and a dimeric γ-chain. The extracellular portion of the α-chain (α-t) is sufficient for the binding of IgE. The Fc portion of IgE contains two copies of the FcεRI binding sites. In contrast, the binding stoichiometry is 1:1. Previously, it was hypothesized that the binding of FcεRI to IgE results in a conformational change in IgE that precludes the binding of a second molecule (Presta, L., Shields, R., O'Connell, L., Lahr, S., Porter, J., Gorman, C., and Jardieu, P. (1994) *J. Biol. Chem.* 269, 26368–26373). Here we characterize the secondary structure of IgE and α-t and analyze their interaction by circular dichroism spectroscopy. Binding experiments show that when IgE interacts with α-t there is a 15–26% decrease of the negative ellipticity at 217 nm. Together, the absence of an α-helix element in α-t and the small contribution of α-t to the spectra of the complex indicate that upon binding, a major conformational rearrangement must occur on IgE. In addition, we analyze the thermal unfolding of α-t, IgE, and their complex. Despite the several domains that constitute IgE and α-t, these molecules unfold cooperatively with two-state kinetics.

The first step in the pathogenesis of allergic disease is the production of an immunoglobulin E (IgE) antibody response to an allergen. The B cells of allergic individuals secrete IgE against various specific antigens (allergens). These IgE molecules then bind to the high affinity IgE receptor (FcεRI)¹ present on mast cells and basophils and, through the interaction with a multivalent antigen, trigger the release of vasoactive mediators.

The FcεRI is a tetrameric complex composed of an α-chain, a β-chain, and a dimer of identical disulfide-linked γ-chains. The β- and γ-chains are required for the insertion of the α-chain into the membrane and for signal transduction. The extracellular portion of the α-chain (α-t) is heavily glycosylated, and it is sufficient for the binding of IgE (1, 2).

IgE is composed of two heavy chains (ε) and two light chains

(κ or λ). The ε-chains are composed of five domains and they are linked through two disulfide bridges between the two Cε2 domains. IgE can be cleaved by papain into Fc and Fab fragments, and the Fc portion is solely responsible for binding to the receptor (3, 4).

The circular dichroism (CD) spectrum of IgE was first reported by Dorrington and Bennich (5). However, the spectrum was not deconvoluted and the secondary structure content was not reported. Although the α-chain of FcεRI has been purified in many laboratories and from several sources, the CD spectrum and the secondary structure composition have not been previously characterized. In this study, we characterize the secondary structure of a chimeric IgE containing human Fc (ch-IgE), mouse IgE (m-IgE), and human α-t.

Ample attention has been dedicated to the characterization of the FcεRI binding site on the Fc (6). In a study performed using protein produced in *Escherichia coli* (7, 8), it has been reported that the binding site on human IgE is located on a sequence of 76 amino acids at the junction of the Cε2 and Cε3 domains. In a later study, using chimeric proteins, the Cε3 was indicated as the principal region in mouse IgE involved in the interaction with FcεRI (9). In contrast, Basu *et al.* (10) have shown that the smallest human Fc fragment capable of binding FcεRI is composed of the whole Cε3–Cε4 domain. Mutagenesis experiments indicate three loops in the Cε3 domain as the binding sites on human IgE (11). Introduction of these loops by protein engineering into the IgG-Fc confers FcεRI binding to IgG (11).

The high homology between IgG and IgE was used by Padlan *et al.* (12) for proposing a molecular model of the IgE-Fc structure. On the basis of sequence similarity, the Cγ2 was chosen as homologous to Cε3 and the Cγ3 as homologous to Cε2 and Cε4.

IgG-Fc has a 2-fold axis of symmetry; therefore, for each element on one heavy chain there is another identical element in the other heavy chain (13). Both protein A and the neonatal Fc receptor bind IgG with a stoichiometry of 2:1 (13, 14). The structural similarity between IgE and IgG and the molecular symmetry suggest that the stoichiometry of the binding of FcεRI to IgE should be 2:1. The location of the three loops on IgE indicated as a binding site for FcεRI (11) also would suggest that a second receptor could bind to the other heavy chain. However, previously published data have clearly shown that the stoichiometry of the binding of IgE to FcεRI both on the surface of the cell membrane and in solution is 1:1 (15–18). Extensive energy transfer studies suggest that IgE is bent at the junction of its Cε2 and Cε3 domains (19–23). Furthermore, the bent structure of IgE in solution appears to be similar in conformation to receptor-bound IgE (19, 23). These data suggest that the binding site for FcεRI could be on the convex surface of IgE and thus the concave surface would not be available for the binding of a second receptor. In contrast to

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¹ The abbreviations used are: FcεRI, high affinity IgE receptor; α-t, extracellular portion of the α-chain of FcεRI; Fc, constant domain of immunoglobulin; ch-IgE, chimeric human IgE containing human Fc; m-IgE, mouse IgE; mAb 15-1, monoclonal antibody anti-FcεRI; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

this hypothesis is the recent observation that an IgE-Fc fragment consisting of only the Cε3 and Cε4 domains still binds FcεRI with a stoichiometry of 1:1 (18).

It is apparent that the currently available data are not sufficient for explaining why there is a 1:1 stoichiometry. A possible explanation is that binding of one receptor causes a major conformational rearrangement on IgE, precluding the binding of a second receptor (11).

Energy transfer experiments led Baird and Holowka (21) to suggest that a conformational change in IgE accompanies its binding to FcεRI. Kinetic studies (24) have also predicted that in the binding of IgE to its receptor, a conformational transition might occur. However, no direct evidence has been presented to confirm a conformational rearrangement upon binding. Furthermore, later energy transfer studies suggest that the bent conformation of IgE does not change significantly upon binding (19, 23).

CD spectroscopy is one of the most widely used techniques for studying the protein conformation in solution. The far UV region (170–250 nm) of the spectrum is dominated by contribution of the peptide bond electronic transitions and can be used to predict the content of secondary structure elements (25, 26). The near UV region (250–300 nm) contains bands mostly due to aromatic amino acids and disulfide bonds (25, 26). These bands depend on the immediate structural and electronic environment and thus this region is a fine criterion for the native structure of a protein (25, 26). The far UV spectra can be employed to monitor secondary structure changes in the interaction between a cellular receptor and a ligand. For example, conformational rearrangement using CD spectroscopy were observed for the insulin receptor (27) and the epidermal growth factor receptor (28).

Here, we use CD spectroscopy to study the interaction between IgE and FcεRIα with the aim of characterizing a possible conformational rearrangement that could occur upon binding. Our data indicate that a change in the secondary structure accompanies the binding of α-t to IgE, supporting a model where the binding of one receptor induces a conformational change in IgE precluding the binding of a second receptor molecule.

EXPERIMENTAL PROCEDURES

Materials—Mouse IgE antidinotrophenyl was purchased from Sigma and rabbit muscle aldolase was obtained from Pharmacia Biotech Inc.

Production and Purification of mAb 15-1—The hybridoma cell line expressing the mouse monoclonal antibody anti-FcεRI (mAb 15-1) was described previously (29). The cell supernatant was diluted 1:1 with binding buffer (Pierce) and then loaded on a column containing protein A immobilized to agarose beads (Pierce) equilibrated with binding buffer. The column was washed with 10 volumes of binding buffer, and then elution of the antibody was carried out using the mild elution buffer (Pierce).

Production and Purification of Chimeric Human IgE—The hybridoma cell line secreting the chimeric human IgE was generously donated by Z. Eshar (9). This IgE is a hybrid containing a mouse light-chain, a mouse anti-(4-hydroxy-3-nitrophenyl)-acetic acid V_H region and a human Fc (9). In this manuscript we refer to this chimeric IgE as ch-IgE. The purification was carried out essentially as described previously (30). Briefly, the cell supernatant was loaded on a column containing Sepharose 4B (Pharmacia) to which bovine serum albumin coupled to (4-hydroxy-3-iodo-5-nitrophenyl)-acetic acid was covalently linked. The IgE was then eluted with 5 M MgCl₂.

Chimeric IgE was preferred to human IgE because no hybridoma secreting human IgE with known antigenic specificity was available and the antigenic specificity was essential for a simple purification of sufficient quantities of fully active protein.

Expression and Purification of α-t—A clone of Chinese hamster ovary cells with glycosylation deficiency (ldld.Lec1) was used for generating stable transfectant secreting α-t. Transfection and selection of the clone secreting α-t was essentially done as described previously (2). A detailed

purification procedure will be described elsewhere.²

Determination of the Concentration and Activity—The protein concentrations were estimated spectrophotometrically by measuring the absorbance of the samples at 280 nm. The extinction coefficient of α-t at 280 nm was determined from the content of aromatic amino acids (31) and was found to be 2.6 cm²/mg. For the estimate of the concentration of m-IgE, ch-IgE, and mAb 15-1 an extinction coefficient of 1.4 cm²/mg was used.

The activity of m-IgE was calculated by labeling the proteins with ¹²⁵I and determining their capacity of binding to rat basophilic leukemia cells. ch-IgE was more sensitive to the iodination; therefore, the activity was estimated by labeling the protein with biotin and determining its capacity for binding to rat basophilic leukemia cells, which express the human receptor. The activity of α-t was calculated by its capacity of inhibiting the binding of iodinated m-IgE to rat basophilic leukemia cells. All preparations of proteins used in the structural studies were fully active.

Circular Dichroism—Spectra were recorded on a Jasco J-500/DP-501N circular dichroism spectropolarimeter, calibrated with D-10-camphorsulfonic acid (32). The full spectrum was scanned at room temperature from 260 to 190 nm using a 0.1-mm path Helma QS sandwich cell. All samples were dialyzed against a buffer consisting of 10 mM Na₂HPO₄, pH 7.2. The conformational content of the protein was calculated using Provencher's spectral deconvolution program (33). For the deconvolution of the IgE spectra, we used the previously reported values of 184,500 for the molecular weight and 1498 for the number of amino acids (34). The number of residues for α-t is 173, and the calculated molecular weight from the amino acid sequence is 20,063 (1).

For the binding experiments, the two proteins were placed into two separate 4.37-mm path length cells of a tandem cuvette (Helma). Spectra were recorded and the buffer baseline was subtracted (before binding). The protein solutions were then mixed by inversion and incubated for 10 min at room temperature, and CD spectra were recorded as above (after binding). The differential spectra (after binding – before binding) were recorded using the DP-501N data system of the CD spectrometer. These samples were dialyzed against 50 mM NaH₂PO₄, 50 mM Na₂HPO₄, and 10 mM NaCl before analysis.

Thermal Unfolding—Spectra for temperature denaturation studies were recorded as above using a 1-mm path water-jacketed cell. The ellipticities were recorded from 210 to 250 nm, raising the temperature 5 °C at a time and equilibrating the sample for 10 min between data points. For the unfolding of the complex, a 4.5 μM preparation containing equimolar amounts of ch-IgE and α-t was incubated for 10 min at room temperature before recording the first spectrum. The concentrations used for the unfolding of ch-IgE and α-t in separate solutions were 1.15 and 10 μM, respectively.

Model of the Binding between IgE and α-t—With this model we would like to visualize a possible mechanism that explains the experimental data that are available to date. In drawing the model we kept in consideration the following points: 1) the 1:1 stoichiometry of the binding (14–17); 2) the bent configuration of the molecule (19–23); 3) the presence of three loops in the Cε3 domain essential for the binding to FcεRIα (11); 4) that the extracellular portion of FcεRIα consists of two domains (1), and the binding region resides mainly within the membrane-proximal domain (16); 5) that a major conformational rearrangement occurs upon binding on IgE.

RESULTS

Protein Purification—The purification of α-t expressed in ldld.Lec1 cells was achieved in two simple chromatographic steps from the cell culture supernatant (data not shown). The purified protein is shown in Fig. 1, lane 4. The glycosylation deficiency of the ldld.Lec1 cells limits the protein glycosylation to the high mannose. To show that the heterogeneity seen after purification is due to glycosylation, we treated α-t with Endo-H (Fig. 1, lane 5). As expected from the calculated value, based on the amino acid sequence, the deglycosylated protein shows an apparent molecular weight of 20,000. In Fig. 1 we also show the purified preparation of m-IgE, ch-IgE, and mAb 15-1. The homogeneity of the proteins is further confirmed with the activities data.

Conformational Characterization of α-t, m-IgE, and ch-IgE—Although IgE was previously isolated from several species and

² S. Sechi, manuscript in preparation.

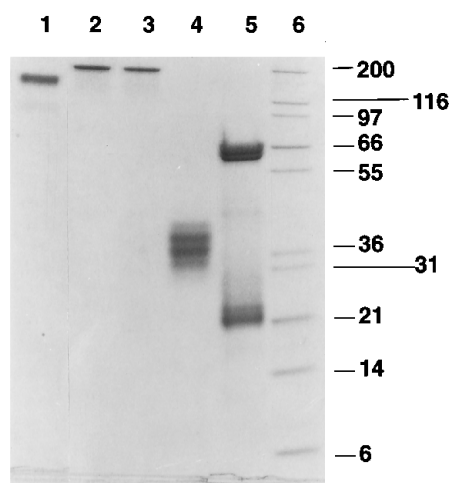


FIG. 1. SDS-polyacrylamide gel electrophoresis analysis of mAb 15-1, m-IgE, ch-IgE, and α -t. Lane 1, 1.5 μ g of purified mAb 15-1; lane 2, 1.5 μ g of purified m-IgE; lane 3, 1.5 μ g of purified ch-IgE; lane 4, 3.5 μ g of purified α -t; lane 5, bottom band, 3.5 μ g of α -t after 1 h incubation at 37 °C with 5.4 μ g of Endo H_f (New England Biolabs, Inc., Beverly, MA); lane 5, top band, Endo H_f used for the digestion of α -t. The purification procedures are described under "Experimental Procedures." Lane 6, molecular weight standards. The apparent values are denoted on the right margin. Samples were run under nonreducing conditions on a 10% Tricine SDS-polyacrylamide gel electrophoresis (38) and stained with Coomassie Blue.

with different antigen specificities, the tertiary structure and the secondary structure content have not been reported. Here we report the secondary structure components of a mouse IgE and a chimeric IgE containing human Fc. In Fig. 2 we show the far UV CD spectra of m-IgE and ch-IgE from 190 to 260 nm.

The secondary structure composition for m-IgE, calculated according to Provencher and Glockner (33) is $1 \pm 2\%$ α -helix, $66 \pm 3\%$ β -sheet, and $33 \pm 4\%$ remainder. Similarly, the estimated secondary structure composition for ch-IgE is $5 \pm 2\%$ α -helix, $74 \pm 3\%$ β -sheet, and $20 \pm 3\%$ remainder. The CD spectra for these two IgEs are very similar, with an ellipticity maximum at 199 nm, a crossing point a 206–207 nm, and a minimum at 217 nm (Fig. 2). The conformational content is typical of proteins with high β -sheet content as expected for immunoglobulins.

In Fig. 2 we also show UV CD spectrum of the extracellular portion of FcεRI α . The spectrum shows a maximum at 205 nm, a minimum at 214 nm, and another maximum at 228 nm. The conformational content estimated as above is $0 \pm 0\%$ α -helix, $76 \pm 2\%$ β -sheet, and $24 \pm 2\%$ remainder. The high β -sheet content typical of an immunoglobulin-like protein is in agreement with what was previously suggested based on the sequence homology with other proteins of the immunoglobulin superfamily. Noteworthy on the CD spectrum of α -t is the uncommonly large positive ellipticity peak with a maximum at 228 nm. It has been reported that positive CD bands in the region 220–230 nm are almost certainly due to aromatic side chains (35). In most proteins this band is canceled by the high intensity negative CD band with a maximum in the 210–220 nm range. In this range, the ellipticity is mainly due to the α -helical conformational components. For these reasons, we conclude that since α -t does not contain α -helix, the CD band at 228 nm remains a major component in the CD spectrum. Furthermore, such a large positive ellipticity peak at 228 nm also indicates that a significant number of aromatic residues are in a highly ordered conformation.

In an attempt to determine the sequence fragments containing α -helical or β -sheet elements we applied the Chou-Fasman and Robson-Garnier prediction programs (36) to the human Fc

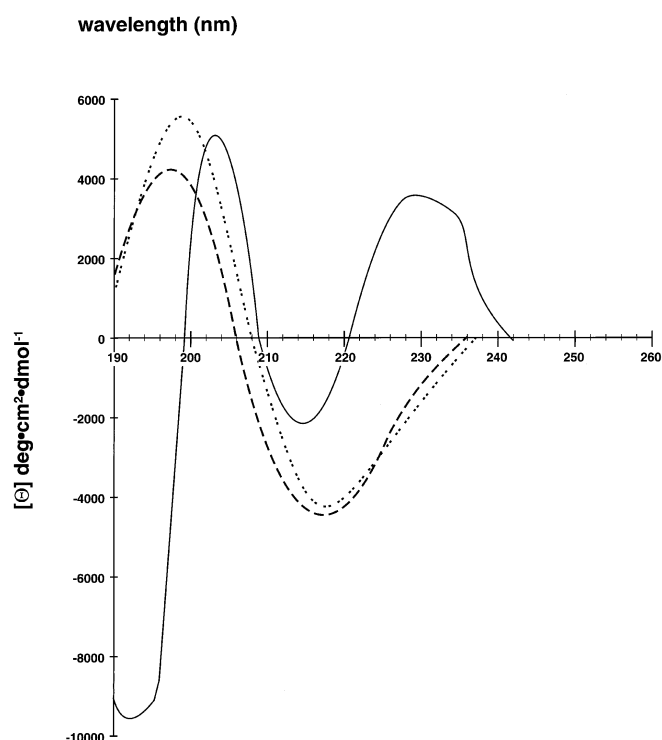


FIG. 2. Far UV CD spectra of m-IgE, ch-IgE, and α -t. All of the samples were dialyzed against a buffer consisting of 10 mM Na₂HPO₄ (pH 7.2) and placed in a 0.1-mm path sandwich cell. The protein concentrations for m-IgE, ch-IgE, and α -t were 3.3, 3.3, and 10.4, respectively. Measurements were made at room temperature. The molar residue ellipticities (Θ) were calculated using the number of residues and the molecular weights reported under "Experimental Procedures." Dashed line, ch-IgE; solid line, α -t; dotted line, m-IgE.

and α -t sequences (data not shown). However, the accuracy of these programs is approximately 60% (37), and the values obtained with either method failed to yield a coherent result with the circular dichroism experimental data.

Conformational Characterization of the Interaction between IgE and α -t—In this study, we analyze the interaction between IgE and FcεRI α by circular dichroism spectroscopy. The goal is to determine if a conformational change occurs upon binding. For this purpose, we use a tandem cell that has two separate compartments where two different proteins can be placed simultaneously in the path of light and the spectrum recorded. The resulting spectrum is the equivalent of the sum of the two individual spectra. The proteins can then be mixed by simple inversion, since the upper part of the cuvette does not have the two compartments separated, and after a 10-minute equilibration another spectrum is recorded. Subtracting the spectrum before mixing from this later spectrum (after binding – before binding), we obtain a differential spectrum that represents the changes in ellipticity after mixing the two individual components.

In Fig. 3A, we show the far UV (left side) and near UV (right side) CD spectra of ch-IgE and α -t using the same tandem cuvette and the same concentrations that are used in the binding experiments (Fig. 3B). Due to the reduced signal-to-noise ratio below 200 nm, it was not possible to extend the measurements far into the ultraviolet region. The relative contribution of α -t in the far UV (Fig. 3A) to the spectrum of the complex (Fig. 3B) is much less than that of ch-IgE (Fig. 3A). This is expected, since in this region the ellipticity is mainly due to the peptide bond electronic transitions and per each molecule there are approximately 10 times more amino acids in ch-IgE than α -t. In the near UV, the contribution of α -t is comparable to

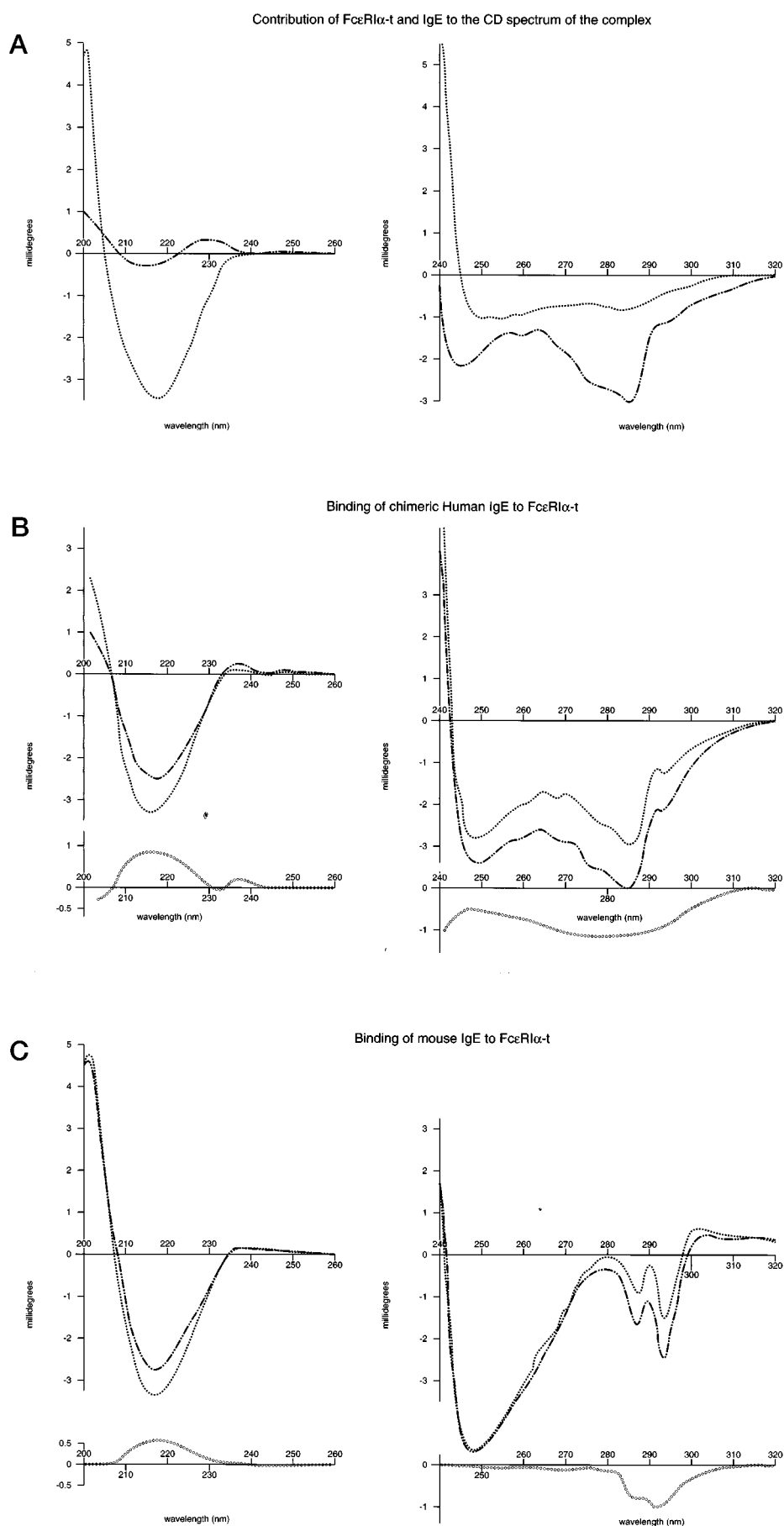


FIG. 3. CD spectra of α -t, IgE, and their complexes. *Panel A* shows the CD spectra of α -t (---) and ch-IgE (···). The samples are placed one at a time in one single 4.7-mm path length compartment of a tandem cuvette while the other compartment is filled with buffer. *Panel B*, before-binding CD spectra of ch-IgE and α -t placed separately into the two 4.37-mm compartments of a tandem cuvette (···), and after-binding spectra of the same samples, mixed and incubated at room temperature for 10 min (---). *Panel C*, CD spectra of m-IgE and α -t placed separately into the tandem cuvette (before binding) (···); m-IgE and α -t after mixing and 10 min incubation (after binding) (---). At the bottom of panel B and C are shown the differential spectra (·····) obtained by subtracting the spectra before and after binding. The far (200–260 nm) and near (240–320 nm) UV CD spectra are shown on the *left* and *right*, respectively. The sample concentrations for the spectra in the far and near UV were 100 μ M and 9 μ M, respectively. The ellipticities are expressed in millidegrees. Details are reported under “Experimental Procedures.”

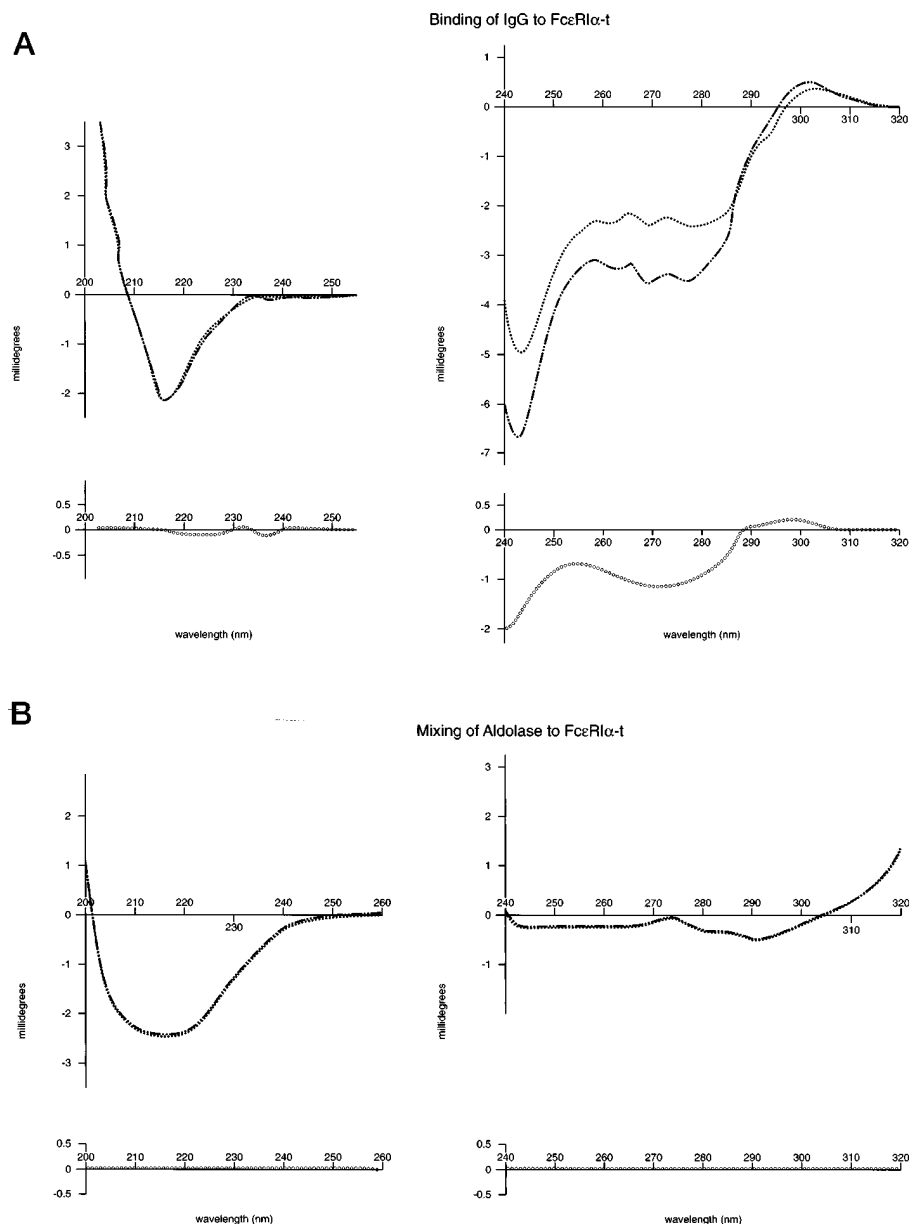


FIG. 4. CD spectra of mAb 15-1 and aldolase before and after mixing with α -t. Solutions containing 100 nM of mAb 15-1 and α -t (panel A, left) and 9- μ M solutions (panel A, right) were placed separately into the two compartments of a tandem cuvette and the CD spectra were recorded (before binding) (---). Similarly to Fig. 3, the samples were then mixed and incubated for 10 min and the spectra were recorded (after binding) (—). In panel B, a similar experiment was performed (same conditions and concentrations as in panel A) using aldolase and α -t. At the bottom of the panels the differential spectra are shown (-----). The far and near UV CD spectra are shown on the left and right, respectively. Details are reported under "Experimental Procedures."

that of ch-IgE. This may be explained by the relatively high number of aromatic residues present in α -t.

The near UV CD spectrum of proteins is often too complicated to interpret, due to the many overlapping and canceling effects of different chromophores in this region. It is, however, a very fine criterion for the native structure of the protein. In Figs. 3 and 4 (right panels) we report the near UV CD spectra and the CD spectral changes that occur upon binding of m-IgE, ch-IgE, and mAb 15-1 to α -t. However, the changes seen in the near UV CD spectrum might not reflect a conformational change, since the direct effect of the ligand (bound asymmetrically) could be a responsible factor. For these reasons, we will analyze the far UV CD spectrum in much greater detail.

For an easier visual evaluation of the conformational changes at the bottom of each panel of Figs. 3 and 4, we report the differential spectra (after binding – before binding).

The changes in the far UV CD spectrum that occur upon combining IgE with α -t indicate that major conformational rearrangements accompany binding (Fig. 3, B and C). The conformational changes are reflected in a 17% decrease of the negative residue ellipticity at 217 nm when using m-IgE and a

26% decrease when using ch-IgE (Fig. 3, B and C, left). Also in the near UV region, strong changes of the ellipticity were observed upon binding. At 286 nm the negative ellipticity maximum increases; when using m-IgE or ch-IgE these changes are 21 and 75%, respectively. The increase of the negative ellipticity at 293 nm is 61% when using m-IgE and 78% when using ch-IgE (Fig. 3, B and C). This suggests a major involvement of aromatic chromophores in the binding.

The decrease in ellipticity observed when ch-IgE binds to α -t is larger than when using mouse IgE (compare differential spectra of Fig. 3, B and C). Previous kinetic studies (38, 39) have shown that the affinity of murine IgE to the human FcεRI is approximately 7–10-fold lower than that of human IgE. Since the affinity constants of murine IgE and human IgE for the human FcεRI are on the order of 10^9 M⁻¹ and 10^{10} M⁻¹, respectively (39), we should expect that in our experimental conditions (100 nM of IgE and α -t) approximately 90 and 97% of m-IgE and ch-IgE, respectively, are complexed with α -t. Thus the larger decrease in ellipticity observed when using chimeric human *versus* murine IgE cannot be explained solely by the difference in affinity. Another possible explanation is that the

TABLE I
 T_m values (°C) obtained from the thermal denaturation of
 α -t, ch-IgE, and their complex

The conformational stability of the proteins was studied by CD spectroscopy. The spectra were recorded as described under "Experimental Procedures" from 210 to 250 nm, raising the temperature 5 °C at a time. The T_m values (in °C) at 236, 230, 225, and 215 nm are reported.

Proteins evaluated	Wavelength			
	236 nm	230 nm	225 nm	215 nm
	°C			
α -t	50.7	50.0	50.7	51.0
ch-IgE	65.0	66.0	65.0	65.0
α -t/ch-IgE	62.0	62.2	64.1	64.1

conformational rearrangement that occurs upon binding to α -t is larger when using ch-IgE than when using mIgE.

Here we would like to focus attention on the negative peak at 217 nm, since at this wavelength the CD spectrum is particularly sensitive to changes in the secondary structure. The conformational transition that we observed upon binding of ch-IgE to α -t is reflected in a 26% decrease of the ellipticity at 217 nm, and the contribution of α -t to the spectrum of the complex at this wavelength is only about 10%. Clearly the major conformational rearrangement must happen in the IgE molecule, although a conformational change also in α -t may not be excluded.

In a control experiment to demonstrate that the changes in the CD spectrum that we observed upon binding of IgE to FcεRIα are specific for this interaction, we analyzed the binding of an antibody to FcεRIα.

Previously, we had shown that the monoclonal antibody mAb 15-1 specifically binds to properly folded α -t (2). In Fig. 4A, we analyze the binding of mAb 15-1 to α -t by circular dichroism. In the far UV, no significant changes were observed upon binding, although minor differences can be noticed in the 220–240 nm range. Considering the previous studies on the contribution of tryptophan side chains on the CD spectra (35), we interpret these spectral changes as a result of tertiary structure or aromatic residues rearrangements.

We wanted to confirm with an additional control experiment that there is no change when we mix α -t with a protein that does not bind to α -t. For this purpose we chose a protein that has a molecular weight similar to an antibody and also is commercially available. In Fig. 4B, we show the CD spectral analysis of the mixing of aldolase with α -t. As expected, no changes in the near or far UV CD spectra were seen, and the spectra before and after mixing are virtually identical.

Thermal Unfolding—To further investigate the interaction between IgE and FcεRIα, we examined the thermal unfolding of ch-IgE, α -t, and the complex ch-IgE/ α -t. Since the CD spectrum of α -t is complicated by a positive ellipticity maximum in the range 220–230 nm, we decided to monitor the thermal unfolding at several wavelengths. The denaturation isotherm curves of ch-IgE, α -t, and the complex ch-IgE/ α -t are similar (data not shown). These isotherms are all symmetrical, and they are typical of a single stage transition. In Table I, we report the T_m values at 215, 225, 230, and 236 nm. The T_m values for α -t at all wavelengths are between 50 and 51 °C. This confirms that the denaturation is cooperative, indicating that elements of the tertiary structure unfold cooperatively with the secondary structure. Similarly, the T_m values for ch-IgE do not change significantly with the wavelength. Although the isotherms of the complex are typical of monophasic unfolding curves (data not shown), the T_m values change slightly with the wavelength, indicating that some elements of the tertiary structure unfold with different kinetics from the secondary structure.

DISCUSSION

The secondary structure content and the tertiary structure of FcεRIα and IgE have not been previously reported. Here we report the CD spectrum of α -t. We also determine the conformational composition of IgE and α -t. IgE contains mostly β -sheet components and 1–5% α -helix. Our data are consistent with the expected structure, based on sequence homology with IgG. The deconvolution of the CD spectrum of α -t indicates that the secondary structure content is 0% α -helix and 76% β -sheet. The far UV CD spectrum of α -t is characterized by an ellipticity minimum at 214 nm and a maximum at 228 nm. Recently it was reported by Woody (35) that positive CD bands in the 220–230 nm region could be attributed to the interactions between tryptophan side chains and the peptide amide bonds. Possibly the unusually large positive ellipticity with a maximum at 228 nm (Fig. 2) is explained by the coincidence of several factors, such as the high β -sheet content, the absence of α -helix elements, and highly ordered orientation of the aromatic amino acid side chains.

One of the fundamental steps in allergic reaction is the binding of IgE to FcεRI. Sequence homology between IgE and IgG1 has led Padlan and Davies (12) to propose a molecular model for the IgE-Fc. Mutagenesis studies based on this model have identified several amino acids that are localized in three loops as potential binding sites. In this model, the Fc portion contains two binding sites for FcεRI. Furthermore, IgG binds protein A and the neonatal Fc receptor with a stoichiometry of 1:2. In contrast, the stoichiometry of the binding of IgE to FcεRI is 1:1. This apparent contradiction could be explained by a conformational change in IgE that results from the binding to FcεRI, thereby modifying or covering the second binding site (11).

Another possibility for explaining the 1:1 stoichiometry is that occupancy by one receptor obstructs the binding of a second receptor, through spatially covering the binding site on the other ϵ -chain. However, no evidence has been presented in support of this hypothesis. Furthermore, a rough estimation of the size of the putative binding domains doesn't favor this hypothesis.

In this study, we demonstrate that the binding of IgE to α -t results in a 17% decrease of the ellipticity at 217 nm when using m-IgE and 26% when using ch-IgE (Fig. 3, A and B). We propose that this results from a major conformational rearrangement in IgE that occurs upon its binding to FcεRIα. It is possible that portions of the loops that are in the receptor binding sites of m-IgE do not fit the human receptor as well as they fit the ch-IgE. A consequence could be that more bonds or hydrophobic interactions are involved in the binding of ch-IgE with α -t versus m-IgE with α -t. This could explain the lower affinity of m-IgE for the human receptor.

Only if major conformational rearrangements occur in IgE can such a large decrease of the ellipticity in the far UV CD spectrum upon binding to α -t be explained. This statement is justified by the following points: 1) α -t accounts for only 10% of the spectral intensity at 217 nm in the spectra of the complex (compare Fig. 3, A and B). Therefore, a 17–26% decrease of the ellipticity cannot possibly be due solely to α -t and must be principally due to a conformational rearrangement on IgE. 2) If we simplify the equation that represents the CD spectrum by using only the components α -helix, β -sheet, and random coil, we can write $S = a(\alpha) + b(\beta) + c(r)$, where S is the amplitude of the ellipticity at 217 nm; α , β , and r are the molar residue ellipticity values for 100% α -helix, β -sheet, and random coil conformational components, respectively; and a , b , and c are the percentages for each component. In this formula, α would be approximately 7 times larger than β . Therefore, a 17–26%

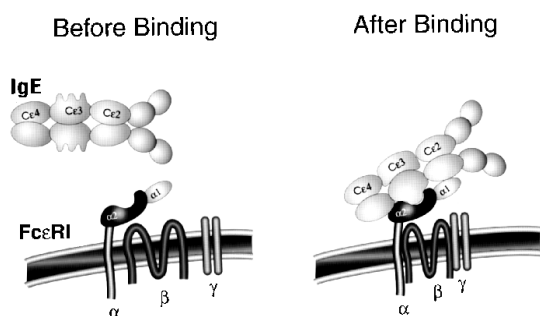


FIG. 5. **Schematic representation of the binding of IgE to its high affinity receptor.** The immunoglobulin domains in the Fc portion of IgE and in FcεRIα are labeled. In accordance with previously published data, IgE has a bent conformation. The drawing is in perspective. The Fc portion is projected on the plane of the paper, while the Fab portion is projected under the plane. The three loops on the Cε3 domain, demonstrated to be essential in the binding, are shown schematically with crests and valleys. This model represents the hypothesis that the conformational rearrangement observed upon binding of IgE to α-t has an effect on the other ε-chain, precluding the binding of a second receptor. This is consistent with the CD spectroscopy data and with the 1:1 stoichiometry. More details on the rationale of this model are described under "Experimental Procedures."

decrease in ellipticity should in part reflect a loss or a change in the α-helix segments, and α-t does not contain α-helix, whereas IgE has 1–5% α-helix (see deconvolution of the spectra under "Results").

Although it is possible that conformational changes occur also in α-t, clearly the major rearrangement must happen in the IgE molecule. This could be due to a major loss of β-sheet component or, to a lesser extent, loss of α-helix. As mentioned above, it is easier to explain such a substantial change as a loss of α-helix content. Interestingly, Glu-452 is one of the amino acids that have a primary role in the binding (11), and based on the proposed model, it is part of an α-helix segment in the Cε3 domain. Unfolding of this segment could partially justify the conformational change that we observed.

Although there is clear evidence that three loops on the Fc are involved in the binding, this model would not completely explain the data that we are presenting. Therefore, we suggest that other parts of the Fc molecule, most likely containing α-helix elements, must be involved in the binding. This would be consistent with the previously published data, in which insertion of these loops in IgG could confer FcεRI binding capacity but with lower affinity (11).

In Fig. 5 we propose a possible mechanism for explaining the 1:1 stoichiometry between IgE and FcεRI. In this model, when FcεRI binds to IgE a conformational rearrangement occurs on the Cε3 domain, this conformational rearrangement affects the conformation of the other Cε3 domain, modifying the second binding site for FcεRI and therefore precluding the binding of a second molecule. This hypothesis is just one of the possible explanations of the presented data, and more work is necessary for further testing our model. Nevertheless, our model is a good working hypothesis for future experiments.

To obtain more information on the conformation of the complex IgE/α-t, we thought to analyze the thermal unfolding of the complex and of the single components. Both ch-IgE and α-t are multidomain proteins, and often multidomain proteins unfold in a multiphase manner. It was previously shown for IgG1 that the unfolding is cooperative and it is a two-state transition, indicating that there are not apparent intermediates in the process of the unfolding (40). Our data show that IgE, α-t, and the complex of these two molecules have a two-state unfolding profile, similar to what was previously shown for IgG. This indicates a strong interaction between the domains and is

consistent with the idea that immunoglobulin-like proteins such as α-t have the same folding topology as immunoglobulin (41). The complex IgE/α-t also has a two-state unfolding kinetic; this is indicated from the thermal unfolding curve (data not shown) and from the similarity of the T_m values at different wavelengths (Table I). These data suggest that the interaction between the two molecules is strong to the point that the complex unfolds in a manner like a single domain protein. This is consistent with the substantial conformational rearrangement that we observed upon binding.

The interaction between the Fc regions of antibodies and the Fc receptors is one of the key steps in the immunoresponse and therefore is an area of intensive study. Here we have used CD spectroscopy to monitor a conformational change upon binding of an antibody to its Fc receptor. Our approach for studying the interaction between IgE and its receptor can be extended to other immunoglobulin/receptor systems. For example, the binding stoichiometry of IgG to its high affinity receptor is also 1:1 (42). Considering the high homology between IgE and IgG, it is reasonable to expect that the structural explanation for the 1:1 binding stoichiometry of IgE to FcεRI is analogous to the explanation for the 1:1 stoichiometry between the high affinity IgG receptor and IgG.

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