Thermally Induced Structural Changes in Immunoglobulin E*

(Received for publication, July 27, 1973)

K. J. Dorrington and H. Bennich‡

From the Department of Biochemistry and Institute of Immunology, University of Toronto, Toronto M5S1A8, Canada

SUMMARY

The ability of immunoglobulin E (IgE) to bind to the cell membranes of basophiles and mast cells is progressively lost when the molecule is heated at 56°C. In the present study, we have investigated the structural changes induced by heating under conditions known to destroy this biological function, although direct measurements of cytotoxic activity were not made.

After 30 min at 56°C, circular dichroism spectrum of intact IgE showed changes in both the aromatic side chain and peptide bond spectral regions. These effects were only partially reversible upon cooling to 25°C. Ultraviolet absorption difference spectra suggested that tyrosine and tryptophan residues were exposed following the heat treatment. The structural effects observed upon heating could be reversed by complete unfolding in guanidine HCl and subsequent refolding upon removal of the guanidine.

Parallel studies with proteolytic fragments of IgE (F(ab')2, Fc', and Fc) indicated that the irreversible thermal effects were restricted to the COOH-terminal regions of the heavy (a) chain of IgE. F(ab')2, and Fc' were unaffected by exposure to 56°C as judged by circular dichroism and difference spectra. The irreversible transition seen in IgE was localized in the Fc region, which is known to carry the cytophilic site.

Antigenic analysis showed that determinants localized in the Fc' region were unaffected by exposure to 56°C for up to 150 min. However, determinants restricted to the two COOH-terminal domains of the ε chain were progressively lost upon heating IgE over the same time period.

The data indicate that the thermal sensitivity of IgE is restricted to that region which interacts with cell membranes and is not due to a general unfolding of the molecule.

Immunoglobulin E represents a minor but distinct class of proteins in the serum of man and higher primates and also in the serum of other species (1). Antibodies of this class (reagins) participate in immediate-type hypersensitivity reactions (such as hay fever and allergic asthma) and play a role in resistance to certain parasitic infestations (2).

The molecular basis for the mediator role of IgE antibodies involves their firm attachment to membrane receptors on basophilic granulocytes and mast cells (3, 4). The combination of cell-bound antibody with specific antigen (allergen) triggers the release of pharmacologically active substances (e.g. histamine). The pharmacological effects of these substances characterize the allergic response. The discovery of IgE myeloma proteins (5, 6), which resemble reaginic antibodies with respect to antigenic characteristics (7) and cytotoxic properties (8), has provided a means of studying IgE at the molecular and cellular levels. Studies on the cytotoxic properties of various proteolytic fragments of the IgE myeloma protein have provided strong evidence that they are located in the Fc fragment (9, 10).

One of the characteristic properties of reaginic antibodies, observed long before IgE was recognized as a distinct class of immunoglobulin, is their thermal lability (11). When reaginic sera were heated at 56°C for one-half to several hours, a progressive and irreversible loss of cytotoxic activity was apparent. In the present study, we have attempted to delineate the structural changes accompanying this thermally induced loss of activity. Further, the availability of well defined fragments of the IgE molecule has allowed us to localize these thermal transitions to that region of the molecule known to carry the cytotoxic site.

MATERIALS AND METHODS

Isolation of IgE—IgE myeloma proteins were purified from the serum of two patients (ND (5) and FS (6)) by ion exchange chromatography and gel filtration, as previously described (5, 12). Analysis of the purified proteins by immunoelectrophoresis showed a single precipitin line against antisera to normal human serum, λ chain, and ε chain. Quantitative immunological analysis by the Mancini method (13) indicated that the preparations contained less than 1% IgG contamination.

Fragments of IgE—Three types of fragment have been used in this study, and the relationship between them is shown diagrammatically in Fig. 1. The F(ab')2+ε fragment was produced by pepsin digestion of IgE(ND) for 8 hours in 0.1 M sodium acetate buffer, pH 4.5, at 37°C. The enzyme to substrate ratio was 1:100 (w/w). The F(ab')2+ε was purified by gel filtration on...
The Sephadex G-150 and chromatography on DEAE-Sephadex A-50, and 2Fe-ε fragment was prepared by papain digestion of IgE(ND) for 3 hours at 30°C using an enzyme to substrate ratio of 1:120 in 0.1 M Tris-HCl, pH 7.0, and NaCl-0.1 M. The Fc-ε fragment was isolated by gradient chromatography on DEAE-Sephadex A-50 using 0.1 M Tris-HCl, pH 8.0, containing 10 mM cysteine and 2 mM EDTA. The digestion was terminated by the addition of iodoacetamide to a final concentration of 11 mM. The Fe-ε was isolated from a peptic digest of F(ab')2ε (1).

The digestion was performed in 0.01 M HCl, for 14 hours at 37°C using an enzyme to substrate ratio of 1:50. The Fe-ε was purified on Sephadex G-150 in 0.2 M NaCl-0.1 M Tris-HCl, pH 7.7. The Fe-ε fragment was prepared by papain digestion of IgE(ND) for 3 hours at 30°C using an enzyme to substrate ratio of 1:120 in 0.1 M sodium phosphate buffer, pH 8.0, containing 10 mM cysteine and 2 mM EDTA. The digestion was terminated by the addition of iodoacetamide to a final concentration of 11 mM. The Fe-ε was isolated by gradient chromatography on DEAE-Sephadex A-50 using 0.1 M Tris-HCl, pH 8.0, as starting buffer and 1.0 M NaCl-0.1 M Tris-HCl, pH 8.0, as the final buffer. Further purification was achieved by gel filtration on Sephadex G-150 in 0.2 M NaCl-0.1 M Tris-HCl, pH 7.7.

The purity of the several fragments was assessed by immunoelectrophoresis, and their antigenic relationships were determined by immunodiffusion using a specific rabbit antiserum to IgE (5).

**Circular Dichroism**—CD spectra were recorded with an ORD/CD-15 spectropolarimeter (Durrum/Japan Spectroscopic Co.), equipped with the SS-20 CD modification, at 24°C. The performance characteristics of this instrument have been described previously (15). Between 250 and 320 nm, protein concentrations of 1.0 to 1.25 mg per ml were used in cells of 1.00-cm path length. Spectra were accumulated using a time constant of 4 s and scanning speeds of 1.1 to 3.6 nm per min. The results are presented as mean residue ellipticity (deg cm² dmole⁻¹) calculated in the usual way (15). The mean residue weight was taken as 110 for IgE and the fragments. No Lorentz corrections for the dispersion of the solvent refractive index have been made. The solvent used was 0.1 M NaCl-0.05 M Tris, pH 7.7. All solutions were filtered through cellulose ester membranes (Millipore, average pore size, 0.45 μm) prior to spectral analysis.

Protein concentrations were determined spectrophotometrically using a value of E₁%₀ = 15.3 at 280 nm for IgE(ND) and the fragments, and 12.5 for IgE(PS) (16).

**Ultraviolet Difference Spectra**—Difference spectral measurements, between 250 and 320 nm, were performed on a Shimadzu MP-40 double-beam recording spectrophotometer using matched quartz cells of 1.00-cm path length. The reference solution was maintained at room temperature (25 ± 1.0°C) for the duration of the experiment, while the temperature of the second cell was adjusted by circulating water through the jacketed cell holder from an external thermostated water bath. Baselines were determined with both cells at room temperature. Difference spectra were obtained 30 min after the temperature of the test protein solution had reached 56°C and after the solution had been cooled to room temperature. No corrections for light-scattering effects have been made. In each case, the protein concentration was approximately 1.0 mg per ml in 0.1 M NaCl-0.05 M Tris, pH 7.7.

**Thermal Unfolding of IgE and Fragments**—Solutions of IgE or the various fragments were heated in closed vessels at 56°C for 30 min in a thermostatic water bath and subsequently cooled to room temperature. In all cases, the protein concentration was near 1.0 mg per ml in 0.1 M NaCl-0.05 M Tris, pH 7.7. In some experiments, solutions were heated at 56°C for up to 4 hours, and serial samples were taken at predetermined intervals (see “Results”).

**Immunological Studies**—The fate of different antigenic determinants on IgE during heat treatment was followed by the radioimmunosorbent test. This test has been described in detail elsewhere (1, 12). In the present study, two separate tests were performed on each sample, with particle-bound antibodies specific for the D,1 antigens and D,2 antigens, respectively. The molecular localization of these determinants is shown in Fig. 1. Samples of IgE(ND) and the F(ab')2ε and Fe-ε fragments, heated at 56°C for various times as described above, were centrifuged at 100,000 X g for 60 min at 4°C, and the supernatants were Millipore-filtered (0.45 μm). The antigenicity of the heated samples was expressed as a percentage of the activity present in the unheated control samples, normalized to equal protein concentrations.

**RESULTS**

**Effect of Heating on the Conformation of Intact IgE**—Fig. 2 shows the CD spectra of the two IgE myeloma proteins between 210 and 320 nm. The spectral region above 250 nm reflects the optical activity of aromatic side chains and cysteine held in disymmetric environments. The marked differences between the spectra of the two proteins in this region probably reflect unique folding of the variable regions of the ε and λ chains. This conclusion is based on previous studies with IgG myeloma proteins (15). Both IgE proteins show some qualitative similarities in the spectral region above 280 nm where the transitions may be attributed to tryptophan (i.e. bands at 295, 291, 288, and 283 nm) (17).

Below 250 nm, both IgE(ND) and IgE(PS) show a principal CD band near 217 nm and a second transition near 230 nm, which is particularly well resolved in IgE(PS) (Fig. 2). The 217 nm band is a common feature of all immunoglobulins and has been attributed to peptide bonds in the β conformation (18). Again, there are considerable differences in the level of optical activity between the two proteins below 250 nm.

Heating the IgE proteins at 56°C for 30 min followed by cooling to 24°C caused a marked change in the CD spectrum, indicating that the proteins had undergone an irreversible conformational transition (Fig. 2). The gross changes were similar in both proteins; a decrease in optical activity above 250 nm and an over-all increase and broadening of the 217 nm band.

When the CD spectra of IgE(ND) was measured at 56°C (Fig. 3a), changes were greater than seen upon subsequent cooling to room temperature, indicating that the conformational changes were partially reversible. Similarly, the thermal effects were time-dependent. Samples of IgE(ND) taken after 1 hour and 2 hours at 56°C showed greater spectral changes than after 30 min (Fig. 3b).

Since the thermal effects were only partially reversible, we
performed some experiments with 6.0 M guanidine HCl to test the reversibility of more general unfolding of the IgE molecule. CD studies (not presented) indicated that IgE(ND) was converted to a random coil in guanidine HCl except for the restrictions imposed by the intact disulfide bonds. Slow removal of the guanidine HCl by dialysis against 0.1 M NaCl-0.05 M Tris, pH 7.7, resulted in a complete recovery of the native conformation as judged by CD (Fig. 4). IgE(ND), which had been previously heated to 56°C for 30 min, was also subjected to the same treatment. After refolding, the CD data suggested that the native IgE conformation had been substantially recovered; the spectrum of the heated and guanidine HCl-treated IgE(ND) was more similar to that of the native protein than the sample which had only been heated (Fig. 4). The structural changes characterized by the CD studies were confirmed by ultraviolet difference spectra (Fig. 5). At 56°C a “blue-shifted” spectrum was obtained, the fine structure of which suggested that tryptophan and probably also tyrosine residues, which were buried in the
native protein, had been exposed. The difference peaks near 292 and 268 nm may be attributed to tryptophan; the peak near 280 to tyrosine and that at 286 probably represents mixed contributions from both types of residue (19). The spectral changes were not reversed upon cooling the IgE to 24°C (Fig. 5). This is consistent with the irreversibility of the conformational changes apparent in the CD data. The difference peaks associated with tryptophan are somewhat different in the cooled sample: a peak at 292 is not apparent, the principal difference being associated with the 298 nm band. Ananthanarayanan and Bigelow (20) have presented data suggesting that the long wave length difference peaks associated with tryptophan arise as a result of electrostatic perturbation of the indole moiety. The present data would suggest that the partial reversibility of the thermal transitions seen in CD is also reflected by the environment of certain tryptophan residues.

Effect of Heating on Fragments from IgE(ND)—Three types of fragments (Fig. 1) were studied in an attempt to localize the structural changes seen upon heating intact IgE. The CD spectra of the native fragments and the spectra obtained after the fragments had been heated at 56°C for 30 min are shown in Fig. 6.

The spectrum of F(ab′)_2-E was similar to that of intact IgE. This observation is consistent with the idea that the variable region conformations make an important contribution to the CD characteristics since F(ab′)_2-E also contains these regions. Any conformational change induced by heating at 56°C is essentially reversible, since the CD spectrum of the heated fragment is closely similar to the native species (Fig. 6). Slight differences in the environment of certain side chain chromophores are apparent above 250 nm, but the over-all secondary folding of the polypeptide chains appear to have been preserved below 250 nm. Parallel experiments on the difference spectra generated at 56°C and after cooling to 24°C confirmed the CD observations (Fig. 7). At 56°C a blue-shifted spectrum was obtained, the characteristics of which suggested the exposure of both tyrosine and tryptophan side chains normally buried in the native fragment. However, unlike the comparable experiments performed on intact IgE(ND), the difference spectral changes were completely reversed when the F(ab′)_2-E solution was cooled to 24°C.

![Intact IgE(ND) CD Spectrum](image)

**Fig. 5.** Ultraviolet absorption difference spectra observed when intact IgE(ND) was heated to 56°C for 30 min (---) and subsequently cooled to 24°C (----). The reference cell contained an equal concentration (about 1 mg per ml) of IgE(ND) maintained at 24°C. The difference in absorbance, ΔA, is plotted as a function of wavelength. No corrections for light-scattering effects have been made. The solvent was 0.1 M NaCl-0.05 M Tris-HCl, pH 7.8.

![CD Spectra of Fragments](image)

**Fig. 6.** Effects of heating at 56°C for 30 min on the CD spectra of the F(ab′)_2, Fc′, and Fc fragments derived from IgE(ND). The spectra of the native fragments are represented by solid curves, and the heated fragments are represented by dashed curves. Other details as in Fig. 2.
This shows that F(ab')\textsubscript{2}-\epsilon undergoes an essentially reversible conformational transition at 56°.

The CD characteristics of the Fc'-\epsilon fragment are interesting (Fig. 6). The partially resolved transition near 230 nm seen in the spectra of intact IgE, Fc-\epsilon and F(ab')\textsubscript{2}-\epsilon is clearly contributed by the Fc'-\epsilon region. An unambiguous assignment of this transition is not possible, although it is almost certainly contributed by an aromatic residue. Tyrosine is the most likely candidate from consideration of other work on model compounds and synthetic oligopeptides (21). The aromatic optical activity above 250 nm is also quite distinct from the intact IgE and the other fragments studied. The spectrum of Fc'-\epsilon heated for up to 4 hours (30 min shown in Fig. 6) and subsequently cooled was identical to the unheated fragment. Any conformational change occurring at elevated temperatures is therefore completely reversible. Since Fc'-\epsilon also forms a part of the F(ab')\textsubscript{2}-\epsilon fragment (Fig. 1), the data obtained with the two fragments are entirely compatible. The thermal stability of Fc'-\epsilon was confirmed by analysis of the spectral data obtained upon heating Fc'-\epsilon support the con-

irreversible changes seen with intact IgE result from structural changes in that portion of chain COOH terminal to Fc'-\epsilon. This region also contains D,2 (Fig. 1) which are thermolabile as shown in Fig. 8. With increasing time of exposure to 56°, there is a progressive loss of antigenicity compared to unheated Fc. This loss also occurs when intact IgE is heated at 56° (Fig. 8).

**DISCUSSION**

The objectives of the present study were 2-fold: (a) to define the structural changes accompanying the "classical" thermal inactivation of reaginic (IgE) antibodies; (b) to determine how far any observed changes were localized in those regions of the molecule responsible for the cytotropic activity. This was considered essential, since the thermal effects may have reflected a general unfolding of the molecule and not a special sensitivity of the cytropic sites.

Direct structural studies on specific IgE antibodies were not feasible because of the low levels of such antibodies even in the serum of chronically sensitized individuals (2). The use of IgE myeloma proteins was considered entirely justified in view of the considerable evidence, indicating that such molecules have the characteristic effector function of antibodies of this class (8–10).

Our data provide strong presumptive evidence that the classical lability of the cytotropic activity in reaginic antibodies, upon heating, results from an irreversible conformational change in the IgE molecule, although the loss of activity was not measured in the present study. These transitions have been followed by alterations in the optical activity of IgE, the exposure of previously buried, aromatic chromophores, and changes in the antigenic determinants associated with \epsilon chain. The irreversibility of thermally induced conformational changes is well known (22) and contrasts with the complete reversibility of more profound changes produced by other unfolding agents (e.g. guanidine HCl). In some cases, the irreversibility has been attributed to secondary disulfide interchange reactions (22). With IgE this is unlikely to be the explanation, since the presence of alkylating agent during the heating period did not affect the irreversibility. In addition, the heat-denatured IgE could be unfolded in 6.0 M guanidine HCl and subsequently refolded into a conformation closely similar to the native protein. The difference spectrum experiments on F(ab')\textsubscript{2}-\epsilon and Fc-\epsilon clearly showed that, although...
both regions of IgE undergo structural transitions at 56°, only those in the F(ab')2 fragments were fully reversible. This marked asymmetry in the responses of different regions of the IgE molecule to heating form the basis for our conclusions regarding the correlation between structural changes and the loss of cytotoxic activity. The heat stability of F(ab')2 and Fe', as judged by antigenic analysis, has also been observed by Ito et al. (23).

The "compact domain" concept of the gross folding of immunoglobulin subunits formulated by Edelman et al. (24) has proved useful in discussions of structure to function relationships within IgG. Each domain consists of some 110 residues folded into a globular unit stabilized by a single intrachain disulfide bond. A comparison of the molecular size of the polypeptide portion of the chain (59,000) and the chain (49,000) suggests that the former is larger by an amount corresponding approximately to one domain (25). This conclusion has been contested (16) based on comparisons between the molecular sizes of the μ chain and the chain. Since μ chain is also likely to be a five domain subunit (26), we are unconvinced by these arguments. Fe-c, the only fragment which has been shown to compete with IgE for sites on the membranes of basophils and mast cells (9, 10), probably consists of three domains. The first domain (C2), would correspond to the Fe" fragment. This fragment does not participate in cytotoxic reactions and has been shown to be unaffected by heat treatment. It seems likely, therefore, that the cytotoxic activity is a property of either the C3 or C4 domains. Our present data also indicate that the thermolabile structures are present in this region. Fragments corresponding to the C-terminal domain (C4) have been isolated which do not possess cytotoxic activity. However, recent CD studies on these fragments indicate that they are partially unfolded even when isolated under the mildest possible conditions and that heat treatment does not result in significant further unfolding of these fragments (unpublished observations). Recent sequence data (27) on these fragments showed a 33% homology between this region and the analogous (C3) domain of the chain. With regard to this homology, it is interesting to note that there is evidence that C3 participates in the cytotoxic activity of IgG (28, 29). Clearly, these homologies must be treated cautiously until the complete sequence of the Fe region of chain has been determined, the existence of three domains confirmed, and the various levels of homology with C2 and C3 calculated.

The cytotoxic properties of IgE may not be an intrinsic property of any one domain but rather formed as a result of cooperation between domains. Alternatively, the site(s) may be located within one domain, but its structural integrity may be dependent upon interactions with neighboring domains. The existence of noncovalent interactions between domains within Fe is supported by the observation that the products of limited peptic cleavage (F(ab')2, C3 and C4 fragments) do not completely dissociate at neutral pH but only upon exposure to acid conditions; and the same is true for the C-terminal fragment produced by cyanogen bromide cleavage (unpublished observations). These putative interactions may be lost or modified upon heating as a result of structural changes within either C3 or C4 or both. These various possibilities are represented diagrammatically in Fig. 9.

The consistent failure to isolate fragments corresponding to C3 in good yield severely limits any further analysis of the location of the cytotoxic site. Efforts are currently being made to devise methods for the isolation of biologically active domains from the COOH-terminal region of the molecule.

REFERENCES


FIG. 9. A schematic representation of the Fe region of IgE showing three possible but not mutually exclusive thermally unfolded states of the C3 and C4 domains.

![Diagram of the Fe region of IgE](https://example.com/diagram.png)
Thermally Induced Structural Changes in Immunoglobulin E
K. J. Dorrington and H. Bennich


Access the most updated version of this article at http://www.jbc.org/content/248/24/8378

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/248/24/8378.full.html#ref-list-1