

The crystal structure of IgE Fc reveals an asymmetrically bent conformation

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The distinguishing structural feature of immunoglobulin E (IgE), the antibody responsible for allergic hypersensitivity, is the Cε2 domain pair that replaces the hinge region of IgG. The crystal structure of the IgE Fc (constant fragment) at a 2.6-Å resolution has revealed these domains. They display a distinctive, disulfide-linked Ig domain interface and are folded back asymmetrically onto the Cε3 and Cε4 domains, which causes an acute bend in the IgE molecule. The structure implies that a substantial conformational change involving Cε2 must accompany binding to the mast cell receptor FcεRI. This may be the basis of the exceptionally slow dissociation rate of the IgE-FcεRI complex and, thus, of the ability of IgE to cause persistent allergic sensitization of mast cells.

Immunoglobulin E (IgE) plays a central role in the mechanisms of allergic disease¹, but in one important respect our understanding of the structure of this molecule is incomplete. Although IgE, like IgG, consists of two identical heavy and two identical light chains, the ε heavy chains of IgE contain four constant domains, one more than the γ chains of IgG. These additional (disulfide-linked) Cε2 domains take the place of the hinge region that, in IgG, mediates flexibility between the antigen-binding Fab regions and the Fc. Although many Fab and IgG Fc structures are known, no crystal structure for this (Cε2)₂ domain pair, or that of the homologous (Cμ2)₂ domain pair of IgM, had been determined. The effect of the Cε2 domains upon the structure of IgE has been a matter of speculation for many years, and models of these domains have been proposed^{2–4}. Physical studies of IgE in solution indicate that the molecule is less flexible than IgG^{5,6} and that it adopts a bent structure both when free in solution^{7–9} and when bound to its high-affinity receptor (FcεRI) on mast cells⁸. However, the role played by the Cε2 domains in determining this structure was unknown.

Functionally, IgE is distinguished by its high affinity for FcεRI, which is two to five orders of magnitude greater than that of IgG for its receptors (FcγRI, FcγRII and FcγRIII)¹⁰. IgG binds to FcγRIII, the closest homolog of FcεRI, with an affinity (K_a) of $5 \times 10^5 \text{ M}^{-1}$ (ref. 11), whereas the affinity of the IgE-FcεRI interaction is $1 \times 10^9 \text{ M}^{-1}$ (measured in a comparable surface plasmon resonance experiment¹²). This difference in affinity is determined almost exclusively by the dissociation rate constants: $k_{\text{off}} = 1 \text{ s}^{-1}$ for IgG-FcγRIII¹¹ and $2 \times 10^{-4} \text{ s}^{-1}$ for IgE-FcεRI¹². In cell-binding assays the K_a and k_{off} values for the human IgE-FcεRI interaction are $\sim 10^{10} \text{ M}^{-1}$ and $\sim 10^{-5} \text{ s}^{-1}$ respectively^{13–16}; in tissue, the dissociation rate for IgE is slower still, $k_{\text{off}} \sim 10^{-7} \text{ s}^{-1}$ (calculated from data in¹⁷). The half-life of cell-bound IgE is therefore of the order of weeks, compared with minutes for IgG. It is this exceptionally slow

rate of dissociation of IgE from FcεRI on mast cells and basophils that is responsible for its ability to cause persistent sensitization of these cells to allergenic challenge.

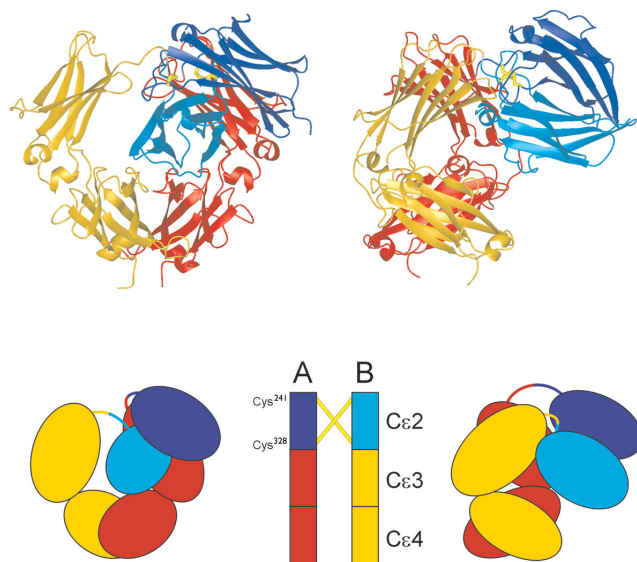
We report here the crystal structure of the complete IgE Fc, which reveals the disulfide-linked Cε2 domain pair and the origin of the bent IgE structure. Taken together with the crystal structures of an IgE Fc fragment that consists of the Cε3 and Cε4 domains¹⁸ (here termed Fcε3-4) and its complex with the soluble extracellular fragment of the FcεRI α chain¹⁹ (sFcεRIα) as well as nuclear magnetic resonance (NMR) evidence for an interaction between the receptor and Cε2¹², the IgE Fc structure implies that a substantial conformational change in the Cε2 and Cε3 domains accompanies receptor binding. This may provide a structural explanation for the unique binding kinetics and consequent biological properties of the IgE molecule.

Results

Overall domain topology

The crystal structure of human IgE Fc (the disulfide-linked dimer of Cε2-Cε3-Cε4) was determined from multi-wavelength anomalous dispersion data at a 2.6-Å resolution (**Web Table 1** online and see Methods), and the spatial arrangement of the six domains in the two chains (A and B) is shown (**Fig. 1**). The structure is acutely and asymmetrically bent at the Cε2-Cε3 linker region, with the two Cε2 domains folded back onto the rest of the structure so that one of them—Cε2(B) (shown light blue in **Fig. 1**)—makes extensive contact with Cε3(A) and even touches Cε4(A) (both red). In contrast, Cε2(A) (dark blue) makes very few contacts with Cε3(B) and none with either Cε4 domain. The angle between the local two-fold dyad axis of the (Cε2)₂ domain pair and that of the (Cε4)₂ pair is 62°. The asymmetric nature of these inter-domain contacts is shown (**Fig. 2**) and discussed in more detail below.

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Cε2 dimer structure

The two Cε2 domains are related by an almost exact two-fold axis, and each displays the expected topology of an Ig C-type domain with no conformational differences between them. Although this is the first crystal structure shown to contain these domains the NMR structure of a monomeric Cε2 domain has been determined¹². The only substantial differences between the isolated Cε2 domain and that within IgE Fc are the lengths of the C and D strands (both of which are two residues shorter in the crystal structure) and the consequent location of the CD loop; it is this loop in Cε2(B) that contacts Cε3(A).

The two domains are disulfide-linked between residues Cys²⁴¹ and Cys³²⁸ in a “crossed” arrangement with Cys²⁴¹(A) linked to Cys³²⁸(B) and vice versa; the electron density for this region is shown (Fig. 3). (Sequence numbers are denoted as in²², with residue insertion 253a in Cε2). This pairing was unexpected, as the early observations of differential susceptibility to reduction and alkylation^{23,24} had argued for a “parallel” arrangement, Cys²⁴¹(A)-Cys²⁴¹(B) and Cys³²⁸(A)-Cys³²⁸(B) with the latter more labile, and more recent fragmentation studies with IgE supported this conclusion⁴. In fact, both disulfide bridges are similarly accessible in the bent IgE Fc structure (19 Å² and 20 Å² for the -CH₂-S-S-CH₂- moiety) with the Cys³²⁸ half of each bridge contributing almost all of this (18 Å² and 19 Å², respectively). In the earlier studies, both bridges may in fact have been cleaved upon reduction, but the accessibility of Cys³²⁸ and inaccessibility of Cys²⁴¹ to alkylation may have led to the (erroneous) impression of a differential susceptibility to reduction.

The Cε2 domain pair exhibits a distinctive mode of Ig domain association; not only is this interaction less extensive than between other Ig C domains in Fab (C_H1:C_L) or Fc (Cγ3:Cγ3 or Cε4:Cε4) structures, but it is dominated by polar—rather than the usual nonpolar—buried surface areas (Fig. 4). Thus for Cε2:Cε2, the total buried surface area is 1760 Å², of which only 25% is nonpolar (Fig. 4a,b). In contrast, for Cε4:Cε4, 2326 Å² are buried, of which 63% is nonpolar (Fig. 4c,d). The Cε2:Cε2 interaction involves 11 hydrogen bonds, a further ten bridged by water molecules and a total of nine water molecules buried at the interface (Web Table 2 online). In contrast, the only water molecules trapped at the Cε4:Cε4 interface are at the periphery and none are completely buried.

Figure 1. Domain structure of IgE Fc. Ribbon representations of the polypeptide chain folds within the six domains of IgE Fc, in two orthogonal views, color-coded according to the schematic diagrams below. The two disulfide bridges that link the two heavy chains (designated A and B) between residues Cys²⁴¹ and Cys³²⁸ in Cε2 are shown in yellow: Cys²⁴¹(A)-Cys³²⁸(B) and Cys²⁴¹(B)-Cys³²⁸(A). (Figure generated with MOLMOL²⁰.)

Cε3-Cε4 domains and the interface with Cε2

The structure of the Fcε3-4 region of the IgE Fc is asymmetric with respect to the Cε3 domains, which make no contact with each other, whereas a local two-fold axis relates the closely associated Cε4 domains. It is instructive to compare the Fc structure in this region with that of the uncomplexed Fcε3-4 crystal structure¹⁸ and its complex with sFcεRIα¹⁹. The Cε3 domains in the free Fcε3-4 are related by an exact (crystallographic) two-fold axis, but are closer together than in the complexed structure, in which this symmetry is perturbed. The three Fcε3-4 structures are compared (Fig. 5), superimposed on their Cε4 domain pairs. All Cε4 domains superimpose well, but the Cε3 domains of IgE Fc represent a hybrid of the other two structures: Cε3(A) adopts a position close to that of the “closed” structure of the uncomplexed Fcε3-4 (Fig. 5a, right), whereas Cε3(B) adopts the more “open” structure of the complexed Fcε3-4 (Fig. 5b, left). This is only an approximate description, for neither Cε3 superposes exactly, and thus the precise details of the Cε3-Cε4 interfaces are slightly different in each case. As noted by others¹⁸, these are more extensive than the homologous Cγ2-Cγ3 interaction, and the angle between the Cε3 and Cε4 domains of IgE Fc is smaller than between Cγ2 and Cγ3 in IgG Fc, which leads to a more compact structure. This difference in quaternary structure explains why attempts to solve the structure by molecular replacement with IgG Fc were unsuccessful.

It is the Cε3 domain in the closed conformation, Cε3(A), with which the Cε2 domain pair makes contact. In fact, Cε2(B) makes extensive contact with both Cε3(A) and Cε4(A), burying surface areas of 938 Å² and 582 Å², respectively. The former involves five hydrogen bonds (two bridged by water) and one salt bridge, Lys³²⁷(B)-Asp³³⁰(A); and the latter involves one water-bridged hydrogen bond and two salt bridges, Glu²⁷⁰(B)-Lys⁴⁹⁷(A) and Arg³⁰⁸(B)-Glu⁴⁷²(A). Both are predominantly polar interactions. In contrast, Cε2(A) makes relatively little contact (306 Å²) with Cε3(B) and none with Cε4(B) (Fig. 2).

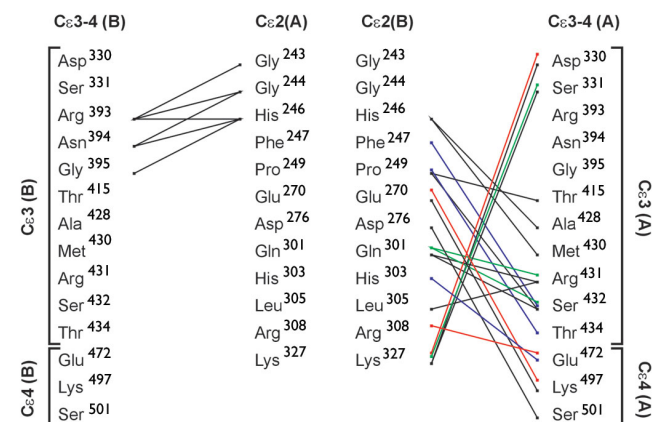
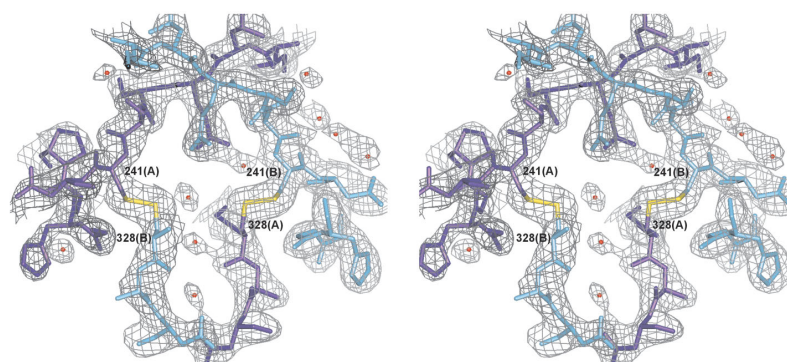


Figure 2. Asymmetry in the contacts between each of the Cε2 domains and the Cε3 and Cε4 domains. Lines connect contacting residues and are color-coded as follows: salt bridge (red), hydrogen bond (green), water-bridged hydrogen bond (blue), van der Waals contact (black). (Contacts calculated with CONTACTSYM²¹.)

Figure 3. Electron density map showing the two inter-C ϵ 2 domain disulfide bridges. Stereo image of the $2F_o - F_c$ map contoured at 1.2σ showing the “crossed” arrangement of the two inter-C ϵ 2 domain disulfide bonds, Cys²⁴¹(A)-Cys³²⁸(B) and Cys²⁴¹(B)-Cys³²⁸(A). Chain A (dark blue), chain B (light blue), sulfur atoms (yellow), water molecules (red). (Figure generated with QUANTA.)



Carbohydrate conformation

IgE is glycosylated in the Fc region, and at residue Asn³⁹⁴ in each C ϵ 3 domain (structurally homologous to Asn²⁹⁷ in C γ 2 of IgG Fc) well ordered electron density reveals two branched carbohydrate chains that occupy a central location in the structure. The density indicates a high mannose-type structure, with two *N*-acetyl-glucosamine and five mannose units visible in each chain. This is consistent with carbohydrate composition analysis of the recombinant IgE Fc from which the crystals were grown [(GlcNAc)₂-(Man)₅₋₈]²⁵; almost identical compositions were reported in the early studies of IgE myeloma proteins PS [(GlcNAc)₂-(Man)₆]²⁶ and ND [(GlcNAc)₂-(Man)₆]²³. The weaker electron density for the terminal residues of the chains are consistent with this reported heterogeneity in composition²⁵, but could also reflect flexibility and/or static disorder. Both oligosaccharide chains interact principally with the C ϵ 3 domain to which they are attached (each covering a surface area of 450 Å², 50% of which is hydrophobic), but terminal mannose units just reach within hydrogen-bonding distance of the C ϵ 4 domains at Gln⁴⁹⁴. The two chains make no contact with each other.

The interaction of IgE Fc with Fc ϵ RI α

The disposition of the C ϵ 3 domains in IgE Fc is more asymmetric than in either of the two previously determined Fc ϵ 3-4 structures^{18,19}. C ϵ 3(A) of IgE Fc adopts the “closed” conformation seen in the uncomplexed Fc ϵ 3-4¹⁸, whereas C ϵ 3(B) is “open”, as in the Fc ϵ 3-4 complex with sFc ϵ RI α ¹⁹ (Fig. 5a,b). Consequently, if sFc ϵ RI α (taken from the structure of the complex) is docked onto IgE Fc, it can engage with C ϵ 3(B) but not C ϵ 3(A) (Fig. 6a). In the Fc ϵ 3-4-sFc ϵ RI α

complex¹⁹, the receptor interacts with the two C ϵ 3 domains at sites termed 1 and 2. With the same terminology, site 2 on C ϵ 3(B) is accessible in IgE Fc, whereas site 1 on C ϵ 3(A) is not.

IgE Fc differs, therefore, from the uncomplexed Fc ϵ 3-4 fragment¹⁸ in which both C ϵ 3 domains are closed, and both sites are inaccessible to the receptor. Fc ϵ 3-4 differs in another respect, however: in this recombinant fragment the two chains are linked by a disulfide bridge between cysteine residues at position 329. Not only are these cysteines displaced by one residue from their natural location at position 328, but we now know that in IgE Fc they form “crossed” pairings with Cys²⁴¹ (Fig. 3). The effect of this unnatural constraint on the affinity and kinetics of Fc ϵ RI binding is unpredictable, and it applies to all studies with Fc ϵ 3-4 fragments that contain the Cys³²⁸-Cys³²⁸ linkage^{16,27-29}. However, the proximity of the two chains in this region of IgE Fc (Fig. 3)—and the fact that this segment of chain in the Fc ϵ 3-4 crystal structure is disordered¹⁸—suggests that this disulfide bridge may not impose very different constraints on the C ϵ 3 domains in the Fc ϵ 3-4 fragment compared with IgE Fc.

If IgE Fc interacts with the receptor in the same manner seen in the Fc ϵ 3-4-sFc ϵ RI α complex, then it is clear that a conformational change involving the C ϵ 3(A) domain must occur. However, closer inspection of the structural superposition (Fig. 6a) reveals that the sFc ϵ RI α docked onto C ϵ 3(B) of IgE Fc clashes with C ϵ 3(A) at the CC' loop in the α (2) domain of the receptor. This might suggest that the receptor cannot access site 2 after all, but if the structure of the free receptor^{30,31}—rather than that taken from the Fc ϵ 3-4 complex—is docked in this way, there is no conflict. The CC' loop adopts a different conformation in the free structure (shown purple in Fig. 6a),

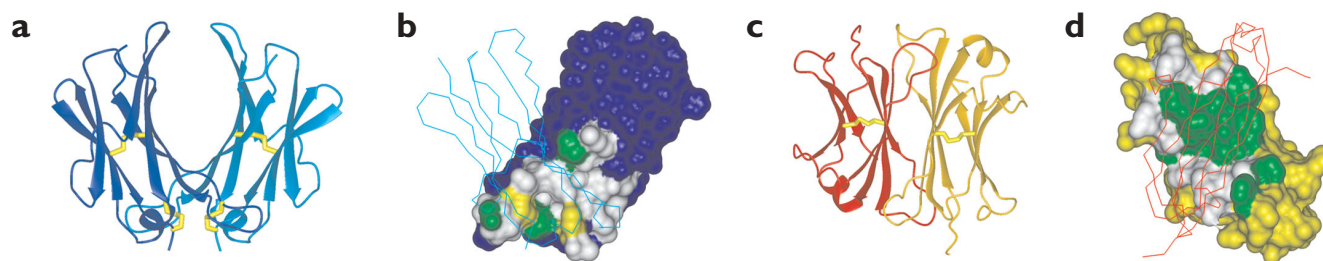


Figure 4. Comparison of the C ϵ 2:C ϵ 2 and the C ϵ 4:C ϵ 4 interfaces. (a) Ribbon representation of the C ϵ 2:C ϵ 2 domain pair showing the two intradomain and two interdomain disulfide bridges (yellow). Domains colored as in Fig. 1. (b) Surface of C ϵ 2(A) showing the contact region with C ϵ 2(B), the latter represented as a C α trace. Color-coding: white, hydrophilic contact residues; green, hydrophobic contact residues; yellow, interdomain cysteine residues; blue, noncontact residues. (c) Ribbon representation of the C ϵ 4:C ϵ 4 domain pair showing the two intradomain disulfide bridges (yellow). Domains colored as in Fig. 1. (d) Surface of C ϵ 4(B) showing the contact region with C ϵ 2(A), the latter is represented as a C α trace. Color-coding: white, hydrophilic contact residues; green, hydrophobic contact residues; yellow, noncontact residues. (Figure generated with INSIGHT II (Accelrys) and MOLMOL²⁰.)

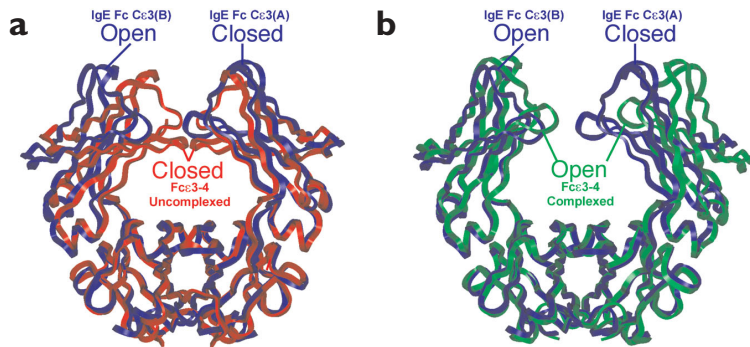


Figure 5. Variation in quaternary structure of the C ϵ 3 and C ϵ 4 domains of IgE Fc. (a) The C ϵ 3 and C ϵ 4 domains of IgE Fc (blue) and the uncomplexed Fc ϵ 3-4 fragment¹⁸ (red), superimposed on their C ϵ 4 domains. (b) The C ϵ 3 and C ϵ 4 domains of IgE Fc (blue) and the complexed Fc ϵ 3-4 fragment¹⁹ (green; receptor not shown) superimposed upon their C ϵ 4 domains. The C ϵ 3 domain of IgE Fc chain A (blue, on the right) adopts the "closed" conformation of the uncomplexed Fc ϵ 3-4 structure in a. In contrast, the C ϵ 3 domain of IgE Fc chain B (blue, on the left) adopts the "open" conformation of Fc ϵ 3-4 seen in the complex with sFc ϵ RI α in b. (Figure generated with INSIGHT II.)

and comparison of several uncomplexed sFc ϵ RI α structures shows substantial conformational variability in this region³¹. Thus the free receptor can indeed engage fully with the bent IgE Fc at site 2 in C ϵ 3(B), whereas conformational changes in IgE Fc (to open up site 1 in C ϵ 3(A)) and the receptor (in the CC' loop) are required for binding at site 1.

The C ϵ 3(A) domain in IgE Fc is locked in the closed conformation by contact with the (C ϵ 2)₂ domain pair. If C ϵ 3(A) is to open up, the C ϵ 2 domains must also move. Indirect evidence for such a conformational change comes from an NMR study of monomeric C ϵ 2 that demonstrated a direct interaction with sFc ϵ RI α ¹². The contact residues that were identified are shown (Fig. 6a, space-filling representation in green), and their location is clearly such that a movement of the C ϵ 2 domains away from C ϵ 3 and towards the receptor would bring them into contact. The disposition of this modeled complex on the cell surface and the proposed conformational change are shown (Fig. 6b). Studies both in solution and on cells, which used a variety of techniques, have reported evidence for conformational change in IgE upon receptor binding^{32–34}.

Discussion

In the crystal structure of IgE Fc, the angle of 62° between the dimer axis of the C ϵ 2 domains and the approximate two-fold axis of the Fc ϵ 3-4 region is the most acute angle possible, as C ϵ 2(B) packs against both C ϵ 3(A) and C ϵ 4(A). Does this conformation represent a rigidly bent structure or merely one extreme of a range of conformations accessible to the Fc and, by implication, IgE in solution? X-ray and neutron scattering analysis of the same recombinant IgE Fc was consistent only with an extremely bent and compact structure⁹, and the crystal structure now shows marked agreement with the solution scattering data. The nature and extent of the interfaces of C ϵ 2(B) with C ϵ 3(A) and C ϵ 4(A)—a total buried surface of 1520 Å² with six hydrogen bonds and three salt bridges—implies a substantial binding interaction, which has been detected in solution. A *K*_D of 4.5 μM was measured for the interaction between monomeric C ϵ 2 and an Fc ϵ 3-4 fragment, with a strong dependence upon ionic strength¹², consistent with the three salt bridges. Thus, although intermolecular packing contacts may assist formation of this compact structure in the crystal, the solution scattering data⁹ indicate that this extreme conformation also predominates in solution.

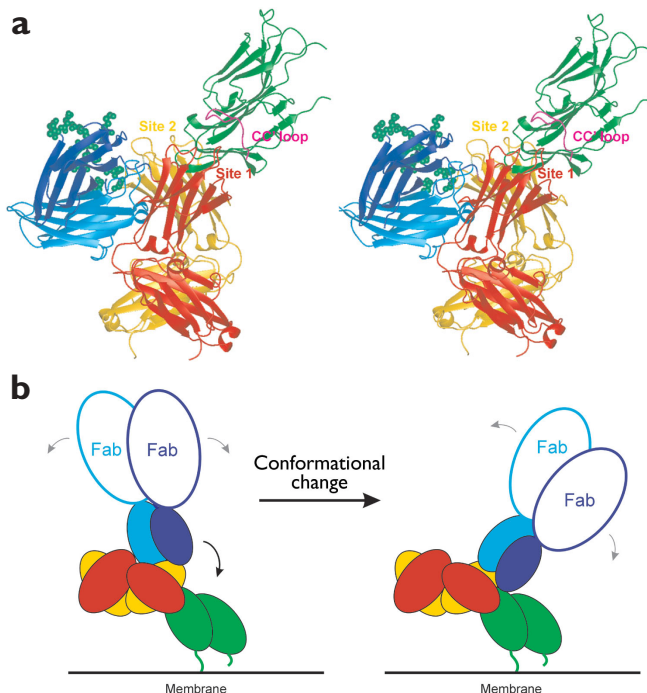


Figure 6. Modeling of the initial complex formed between IgE Fc and sFc ϵ RI α implies a conformational change upon receptor binding. (a) The two extracellular domains of Fc ϵ RI α (green) taken from the crystal structure of the complex with Fc ϵ 3-4¹⁹ are docked onto C ϵ 3(B) in IgE Fc (yellow) by superposition of C ϵ 3(B) in the complex structure (data not shown) on C ϵ 3(B) of IgE Fc. Part of the sFc ϵ RI α (2) domain (the CC' loop, in green) clashes with C ϵ 3(A) (red) in IgE Fc, but if the structure of an uncomplexed sFc ϵ RI α chain is taken³¹, in which the CC' loop adopts a different conformation (shown purple), no steric conflicts occur. Thus free receptor and free IgE Fc may interact in this way at subsite 2 on C ϵ 3(B). Residues on C ϵ 2 identified by NMR as contact residues in sFc ϵ RI α binding¹² are shown in space-filling representation, colored green. Their location implies a substantial movement of C ϵ 2 relative to the C ϵ 3-4 region that may also "open up" the C ϵ 3 domain in chain A, allowing interaction with sFc ϵ RI α at subsite 1 also, as seen in the crystal structure of the complex¹⁹. A conformational change in the CC' loop (from the purple to the green conformation) of sFc ϵ RI α (2) would also be required. (Stereo image generated with MOLMOL²⁰.) (b) Schematic representations of the modeled complex formed initially between IgE and Fc ϵ RI α on the cell surface (on the left) and following the proposed conformational change of the C ϵ 2 domains relative to the C ϵ 3 and C ϵ 4 domains (on the right). The degree of flexibility of the Fab regions relative to the C ϵ 2 domains is unknown; this is indicated by the gray arrows. Color-coding as in a.

The hypothesis that IgE Fc may engage the receptor through only one Cε3 domain initially, and that a (slow) conformational change is required for engagement of the second Cε3, implies a biphasic binding interaction. Biphasic kinetics have indeed been observed^{12,29,35} both in association and dissociation, with the slower phase dominating the dissociation kinetics^{28,29}. The correspondence between experimentally determined biphasic dissociation kinetics and the existence of the two sites has been reported by others¹⁹, and a two-step mechanism has also been detected in a kinetic study of IgE binding to cells³².

The Cε2 domains, through conformational change and the additional interactions that they may provide for receptor binding, might thus contribute to the characteristically slow off-rate for IgE. Comparison of the dissociation rate constants for IgE ($1.8 \times 10^{-4} \text{ s}^{-1}$), IgE Fc ($1.9 \times 10^{-4} \text{ s}^{-1}$) and Fcε3-4 ($3.4 \times 10^{-3} \text{ s}^{-1}$) shows that dissociation is faster when the Cε2 domains are absent (figures quoted are weighted average k_{off} values for the two phases¹²). Despite these different off-rates, the overall affinities recorded for IgE, IgE Fc and Fcε3-4 are all similar^{12,15,16,27,28}, due to compensatory differences in the on-rates. Similarly, the explanation for the higher affinity of IgE for FcεRI compared to IgG for its receptors does not lie with Cε2, but rather the additional hydrophobic, hydrogen bonding and electrostatic interactions seen in the Fcε3-4–sFcεRIα complex compared to the IgG Fc–sFcγRIII complexes^{19,36–38}. If Cε2 does contribute additional interactions to receptor binding, then presumably the free energy gain is compensated by the energy cost of the conformational change. However, the effect of the Cε2 domains is manifest in the slower dissociation rate of the IgE–FcεRI complex. It is precisely this property that is responsible for the longer residence time of IgE on mast cells and basophils and the resulting long-term sensitization of these cells.

Before the crystal structure of the Fcε3-4–sFcεRIα complex was determined, attempts to map the receptor-binding site had given conflicting results. For example, mutations in the NH₂-terminal sequence of Cε3 that affected receptor binding²⁸ could not easily be reconciled with the similar effects of others in the AB loop at the interface with Cε4 and elsewhere in the domain^{39,40}. In addition, antibodies raised to a peptide in the Cε4 domain were capable of interfering with FcεRI-mediated activities⁴¹, although the consensus was that Cε3 contained the site. However, the acutely bent structure of the Fc, with unanticipated interactions between Cε2, Cε3 and Cε4, resolves many of these paradoxes. Several residues that have been implicated⁴⁰ are now known to be involved in Cε2–Cε3 interactions (including Thr⁴¹⁵ and Met⁴³⁰), and others may serve to destabilize the Cε3 domain (such as Arg³⁷⁶ and Glu⁴¹⁴). The candidate vaccine peptide in Cε4 (residues 497–503)⁴¹ includes precisely those residues with which the Cε2 domain makes contact in the bent structure: Lys⁴⁹⁷ and Ser⁵⁰¹ in Cε4(A) form a salt bridge and several van der Waals interactions, respectively to Glu²⁷⁰ and Asp²⁷⁶, in Cε2(B). It is now clear that antibody binding to this region of Cε4 could alter the quaternary structure of the Fc and its ability to undergo conformational change, thus interfering with receptor binding.

The bent structure of IgE Fc, together with the crystal structure of the Fcε3-4–sFcεRIα complex¹⁹ and evidence for involvement of the Cε2 domains¹², implies that receptor binding is accompanied by a conformational change not only in Cε3¹⁸ but also in Cε2. This identifies a new target for therapeutic intervention: if the IgE molecule could be locked into the acutely bent structure seen in the Fc crystal structure, only one part of the site for receptor binding would be accessible and any interaction with Cε2 would be impossible. The IgE molecule might be left with a low affinity and more significantly a faster off-rate that would render it incapable of persistent mast cell sensitization.

Finally, IgM—with its Cμ2 domain homologous to Cε2—undergoes substantial conformational change. Upon binding to an antigenic surface, the subunits of the pentamer can bend as much as 90° between Cμ2 and Cμ3, exposing sites for complement binding^{42,43}. Thus the Cε2 and Cμ2 domains of IgE and IgM may both serve to facilitate structural transitions in these antibody molecules, but for different functional purposes.

Methods

Preparation and crystallization. The human IgE Fc construct that starts at Val²²⁴—preceded by two leader sequence residues, aspartic acid–isoleucine—and containing the two mutations Asn²⁶⁵→Gln²⁶⁵ and Asn³⁷¹→Gln³⁷¹ (to remove potential heterogeneity in N-linked glycosylation) and Cys²²⁵→Ala²²⁵ (which, in IgE, bridges to Cε1) was expressed in mammalian NS0 cells and purified as described¹⁵. Crystals were grown at room temperature in hanging or sitting drops from 2 mg/ml of protein solution (in 50 mM Tris HCl at pH 8.5, 10 mM NaCl and 0.05% sodium azide) and diluted 1:1 with reservoir solution (100 mM Tris HCl at pH 8.5, 40–45% ammonium sulfate and 0.1% sodium azide). Crystals appeared after 1–2 weeks and were stable for several months.

Data collection. Four multi-wavelength anomalous dispersion (MAD) data sets—at the absorption peak of the Pt L_{III} edge (E-λ2), the inflection point (E-λ1) and at two remote wavelengths (E-λ3 and E-λ4)—were collected at the EMBL outstation (DESY, Hamburg, Germany) beamline BW7A, to a 2.5-Å resolution (Web Table 1 online), from one single frozen IgE Fc crystal soaked in 2.5 mM dichloro(2,2':6',2''-terpyridine)Pt(II) for 24 h. A second set of MAD data at three wavelengths—the absorption peak (B-λ2), the inflection point (B-λ1) and a remote wavelength (B-λ3)—were collected at Brookhaven National Laboratory, NSLS beamline X12-C, to a 2.8-Å resolution (Web Table 1 online), from a single smaller crystal with the same soaking conditions. A native data set was also collected to a 2.6-Å resolution (Web Table 1 online). All data were collected on cryo-cooled crystals at 100 K with 30% glycerol as cryoprotectant. Data were indexed and integrated with the program DENZO⁴⁴ from the HKL package and were scaled and merged with SCALA⁴⁵ and TRUNCATE⁴⁶. The space group is P2₁2₁2 with unit cell dimensions $a = 129.31 \text{ Å}$, $b = 74.83 \text{ Å}$ and $c = 78.65 \text{ Å}$ and one IgE Fc molecule in the asymmetric unit.

Structure determination and refinement. Two Pt atoms were located with SOLVE⁴⁶, and refinement and initial phase calculation was done with MLPHARE⁴⁵, yielding a poor figure of merit of 0.38 for 15–2.5 Å with the EMBL–BW7A data. The electron density map was uninterpretable and density modification (DM⁴⁵) only gave indications of domain boundaries. A molecular fragment that consisted of the core seven β-strands of an Ig domain was used as a search model in the program FFFEAR⁴⁷ on the original map, and solutions were manually examined with QUANTA (Accelrys, Cambridge, UK) against the original and DM maps. Five solutions were selected. Rigid-body refinement (3 Å) was followed by simulated annealing with CNS⁴⁸ and model building with QUANTA, with $2F_o - F_c$ and $F_o - F_c$ electron density maps and a composite omit map generated by CNS. The refinement did not progress beyond $R_{\text{cryst}} = 0.45$ ($R_{\text{free}} = 0.53$).

Further MAD (2.8 Å) and native (2.6 Å) data from BNL–X12C were then collected, from which the same two Pt sites could—with prior knowledge—be detected. Initial phases from MLPHARE gave a poorer phasing power (Web Table 1 online) and figure of merit (0.30 for 15–2.8 Å) than before. Again the map was uninterpretable, but a DM map was similar to that generated from the EMBL BW7A data, and the same fragment search with FFFEAR yielded four common solutions. The model was refined by iterative rounds of simulated annealing and group B factor refinement with a bulk solvent correction applied, and manual model building, with data between 54 and 2.6 Å. As refinement progressed, the missing domains were located, all inter-domain connections were built and main chain density was finally traced from residues 228 to 545 in chain A and residues 228 to 546 in chain B, although density was poor for three loops (BC, DE and FG) of Cε2(B). Water molecules were then added automatically by CNS and manually inspected; carbohydrate and sulfate ions were added manually. The final $R_{\text{cryst}} = 0.217$ ($R_{\text{free}} = 0.297$).

Coordinates. Atomic coordinates and structure factors have been deposited in the Protein DataBank⁴⁹ with accession code 1LS0.

Note: Supplementary information is available on the Nature Immunology website.

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Competing interests statement

The authors declare that they have no competing financial interests.

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