



Dossier : Allergy

The intrinsic flexibility of IgE and its role in binding FcεRI

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Received 7 November 2006; accepted 8 November 2006

Abstract

The interaction between IgE and its high affinity cellular receptor (FcεRI) is an essential step in the development of allergic responses. Studies have identified the third constant domain of IgE (Cε3) as the receptor binding region. The Cε3 domain has unusual structural features; it was found to be a 'molten globule' structure in an isolated form, only assuming a well structured form upon binding to FcεRI. The conformational flexibility intrinsic to the receptor binding portion of the molecule may be useful to IgE in allowing the large allosteric changes postulated to be required for FcεRI engagement. If allosteric inhibitors can be developed then the dynamic properties of the Cε3 domain may provide opportunities for therapeutic intervention in allergic disorders.

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Keywords: Immunoglobulin E; Molten globule; Fluorescence; CD; NMR; Protein folding; Protein–protein interactions

The increase in the incidence of allergic diseases in most industrial countries over the last 30 years has been described as an 'allergy epidemic' [1]. In the year 2000, it was estimated that approximately one-third of the general population were affected by allergic diseases [2], and the numbers continue to increase [3]. Allergic diseases can be made manifest in virtually any mucosal surface of the body or, in the most serious cases, as a systemic response. As the precise mechanism of the development of allergic diseases remains elusive at present, this creates problems in terms of the therapeutic strategies used for their treatment. Current therapeutic strategies for the management of such diseases tend to be directed towards inhibition of the downstream signalling molecules activated following the initiation of the allergic response by antigen [4]. As the interaction between IgE and its high affinity receptor, FcεRI, is absolutely required for the initiation of the

allergic response [5], interruption of this interaction may provide a universal means of treatment for all allergic diseases. Hence a more detailed investigation of the mechanism of the IgE–FcεRI interaction is of significant pharmaceutical value.

The interaction of IgE with FcεRI is a critical step in the initiation of an allergic response [5]. FcεRI has an extremely high affinity for IgE, of the order of 10^{10} M^{-1} , predominantly due to its very slow dissociation rate. This high affinity is such that most IgE in the body is found fixed to the cells that bear FcεRI, even in the absence of bound antigen [6]. The formation of the IgE–FcεRI complex results in the stabilisation of both proteins, and contributes to mast cell survival [7,8]. This stabilisation, combined with the restricted diffusion that occurs within tissues that contain mast cells, results in an exceptionally long lifetime of the IgE–FcεRI complex *in vivo*. It is the binding of a multivalent antigen that causes cross-linking of the IgE–FcεRI complexes on the surface of the mast cell or basophil. This aggregation of receptors triggers the initiation of the intracellular signalling cascade [9]. The interaction sites for the extracellular domains of FcεRI on IgE have been localised to the third constant domain of IgE (Cε3), both by site-directed mutagenesis [10] and, more recently and

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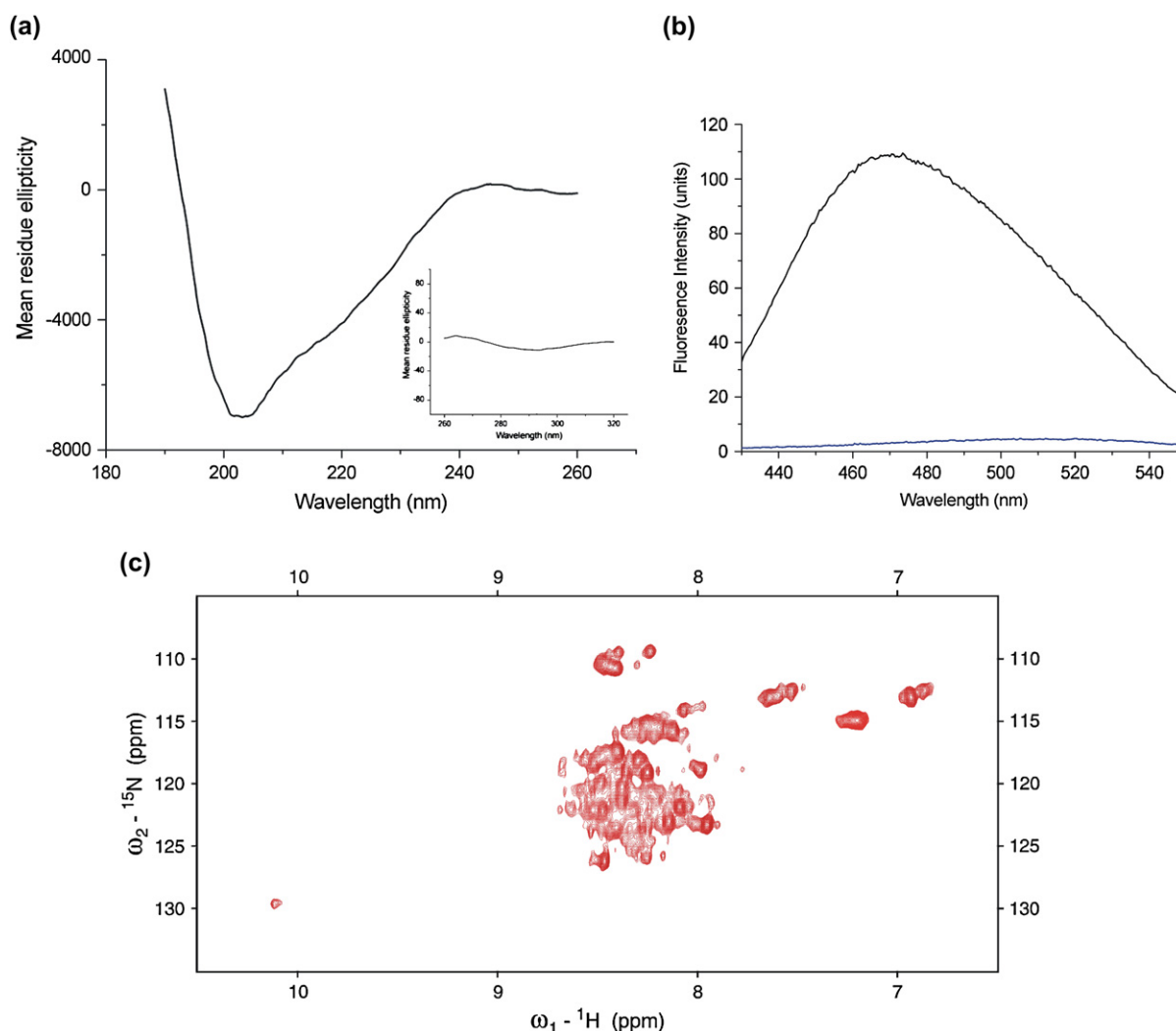


Fig. 1. Spectroscopic properties of Cε3. (a) Far UV CD spectrum (Cε3 at 0.2 mg/ml in PBS at pH 5.0, cuvette pathlength 0.2 mm). The near UV spectrum (Cε3 at 3 mg/ml in PBS at pH 5.0, cuvette pathlength 5 mm) is shown inset. (b) The emission spectra of ANS (17.3 μM) in the presence of Cε3 (0.2 mg/ml) or in PBS pH 5.0 alone are shown in black and blue, respectively. The excitation wavelength was 370 nm. (c) ¹⁵N-HSQC of 0.2 mM ¹⁵N-Cε3 in PBS pH 5.0 (red).

definitively, through the determination of the crystal structure of Cε3–4 dimer in complex with sFcεRIα [11]. The crystal structure of the complex also confirms and explains the stoichiometry of the Cε3–4:sFcεRIα interaction, where the two Cε3 domains contained within one IgE molecule interact with one molecule of sFcεRIα.

Early investigations into the stoichiometry of the IgE–FcεRI interaction led to the suggestion that a major conformational rearrangement occurs within IgE, precluding further binding of an additional FcεRI molecule [12]. This hypothesis was tested using various mixtures of IgE and sFcεRIα [13]. These mixtures were then analysed by CD spectroscopy to monitor any changes that occurred in the secondary structure of the components. It was shown that when IgE interacts with sFcεRIα, a conformational rearrangement does indeed occur, and it was proposed that this rearrangement occurred within the IgE molecule. A later study also confirmed this structural rearrangement, by monitoring the exposure of certain epitopes of IgE in the absence and presence of sFcεRIα

[14]. It was shown that antibodies raised to the Cε3 domain of IgE differentially recognise soluble and receptor-bound IgE. Hence, this study identified the Cε3 domain of IgE as potentially involved in the conformational change that occurs on interaction with sFcεRIα. It is thought that this conformational change provides the additional interactions that would explain the higher affinity of IgE for FcεRI compared with IgG and its receptors [11,15]. It has been suggested that the conformational flexibility that appears to exist within IgE may in fact be important in the regulation of the interactions with its two receptors, FcεRI and CD23 [16].

The binding of IgE to both FcεRI and CD23 has been localised to the Cε3 domains [10], suggesting this domain might be an attractive target for structural and functional studies. Indeed, substitution of the equivalent Cγ2 domains in IgG for Cε3 domains confers on the resulting molecule the capacity to bind FcεRI [12], confirming the role of the Cε3 domain in determining the effector functions of IgE. A recombinant human Cε3 fragment was produced in baculovirus Sf9 cells

and was shown to be capable of interaction with FcεRI using a nitrocellulose blot [17]. The recombinant Cε3 domain was also shown to be sufficient for inhibition of the binding of natural IgE to FcεRI, hence implying a potential role for this domain in both diagnosis and therapeutic treatment of allergic diseases. More recently a monomeric recombinant Cε3 domain has been produced in *E. coli* [18] and though it appears to have little tertiary structure or hydrophobic core, this domain was shown to interact with FcεRI using SPR, but with a greatly reduced affinity compared with IgE ($K_A = 5 \times 10^6 \text{ M}^{-1}$ compared to $K_A = 4 \times 10^9 \text{ M}^{-1}$ for IgE).

Spectroscopic characterisation of the recombinant Cε3 domain of IgE has revealed that it possesses a number of unusual properties when compared to other Ig domains. These properties are summarised in Fig. 1. The far UV CD spectrum of refolded Cε3 (Fig. 1a) can be used to give an estimate of the content of elements of secondary structure and indicates that Cε3 contains approximately 35% β-sheet, in good agreement with the approximately 40% β-sheet structure present in the fully folded Cε3 domain within IgE-Fc [15]. However, the near UV spectrum of refolded Cε3 exhibited only very small ellipticities, suggesting an absence of tight packing of side chains in the protein core. As seen by both CD and fluorescence spectroscopies, the unfolding of Cε3 is not a cooperative process; this behaviour would be expected for a protein that lacks stable tertiary structure. ANS is a fluorescent hydrophobic probe which shows a stronger affinity for the ‘molten globule’ state of proteins than for either the fully folded or unfolded states [19]. While free ANS in aqueous solution shows weak fluorescence with a λ_{max} of 515 nm, binding of the dye to the ‘molten globule’ state causes a large increase in the ANS fluorescence intensity, as seen on incubation with the Cε3 domain (Fig. 1b). The ^{15}N -HSQC NMR spectrum of refolded Cε3 is shown in Fig. 1c. The lack of chemical shift dispersion in both dimensions is again characteristic of a protein that lacks native stable tertiary structure and is sampling a series of different conformations during the course of the NMR experiment. Monomeric recombinant Cε3 alone in solution has native-like secondary structure and lacks rigid tertiary structure [20], and is therefore typical of the ‘molten-globule’ state [21]. Other Ig domains, such as the recombinant Cε2 domain, are fully folded alone in solution [22]. It has been suggested that the structural flexibility that Cε3 exhibits, although reduced in the context of the IgE molecule as a whole, can confer a significant advantage to IgE in terms of controlling its interactions [20,23].

Cε3 alone in solution binds to ANS, causing an increase in intensity of fluorescence emission and a blue shift in the λ_{max} compared with free ANS. Any reduction in binding of ANS to Cε3 is an indication of the loss of the ‘molten globule’ character as would be expected if Cε3 adopted a more stable tertiary structure on binding to a ligand (Fig. 2). The fluorescence emission spectrum represents an average of all the conformations of Cε3 present in the sample. The effect that various physiological ligands had on the structure of the Cε3 domain was observed using an ANS fluorescence assay, the use of which was further validated through NMR spectroscopy.

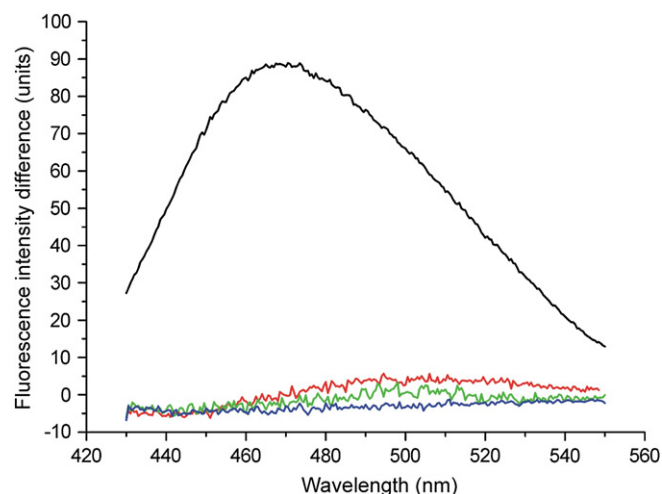


Fig. 2. Fluorescence emission difference spectra for Cε3 and ANS on addition of various ligands. Difference spectra are calculated by subtraction from [Cε3 and ANS] spectrum alone. [Cε3 and ANS] with sFcεRIα added is shown in black; [Cε3 and ANS] with CD23 added is shown in red; [Cε3 and ANS] with Cε2 added is shown in green; [Cε3 and ANS] with Cε4 is shown in blue. Each ligand is added in a 1:1 molar ratio. Each line represents an average of three separate experiments, and each of those an average of three fluorescence emission scans. A positive difference shows that ANS is dissociating from Cε3.

The capacity of the Cε3 domain to bind ANS was not affected by incubation with the Cε2 and Cε4 domains of IgE, or with derCD23. This would indicate that the Cε3 domain interacts with these ligands through local interaction surfaces, not dependent on a fully folded Cε3 domain. However, incubation with sFcεRIα caused a global rearrangement in the Cε3 domain inducing it to adopt a folded conformation with defined tertiary structure, as indicated by the release of ANS.

The interaction of the Cε3 domain with sFcεRIα was also investigated using NMR spectroscopy. Observation of the interaction by NMR gives an insight into the folding of the Cε3 domain with the potential for atomic level resolution, permitting the definition of groups of residues that appear to fold with similar kinetic characteristics. Addition of sFcεRIα to a ^{15}N -Cε3 sample clearly causes the Cε3 to adopt a folded structure, as seen by an improvement in the chemical shift dispersion in both dimensions. Following incubation with sFcεRIα, the observed ^{15}N -HSQC for Cε3 contains approximately 100 peaks, corresponding to the expected number of observable backbone amides. A well-dispersed ^{15}N -HSQC can be used as the foundation for sequential assignment, allowing correlation of each peak to a specific backbone residue of Cε3. Following addition of 1% (10 μM) sFcεRIα to a 1 mM ^{15}N -Cε3 sample, a series of seven sequential ^{15}N -HSQCs were recorded to observe Cε3 folding. An overlay of the ^{15}N -HSQCs collected is shown in Fig. 3a. It can be seen that after 6 h (Fig. 3b; shown in magenta) the conformation of Cε3 appears to be approaching the ^{15}N -HSQC for the fully folded Cε3 observed previously [20].

Generally, each of the peaks of the ^{15}N -HSQC spectrum represents a backbone amide of Cε3, and appears at a characteristic position according to the chemical environment

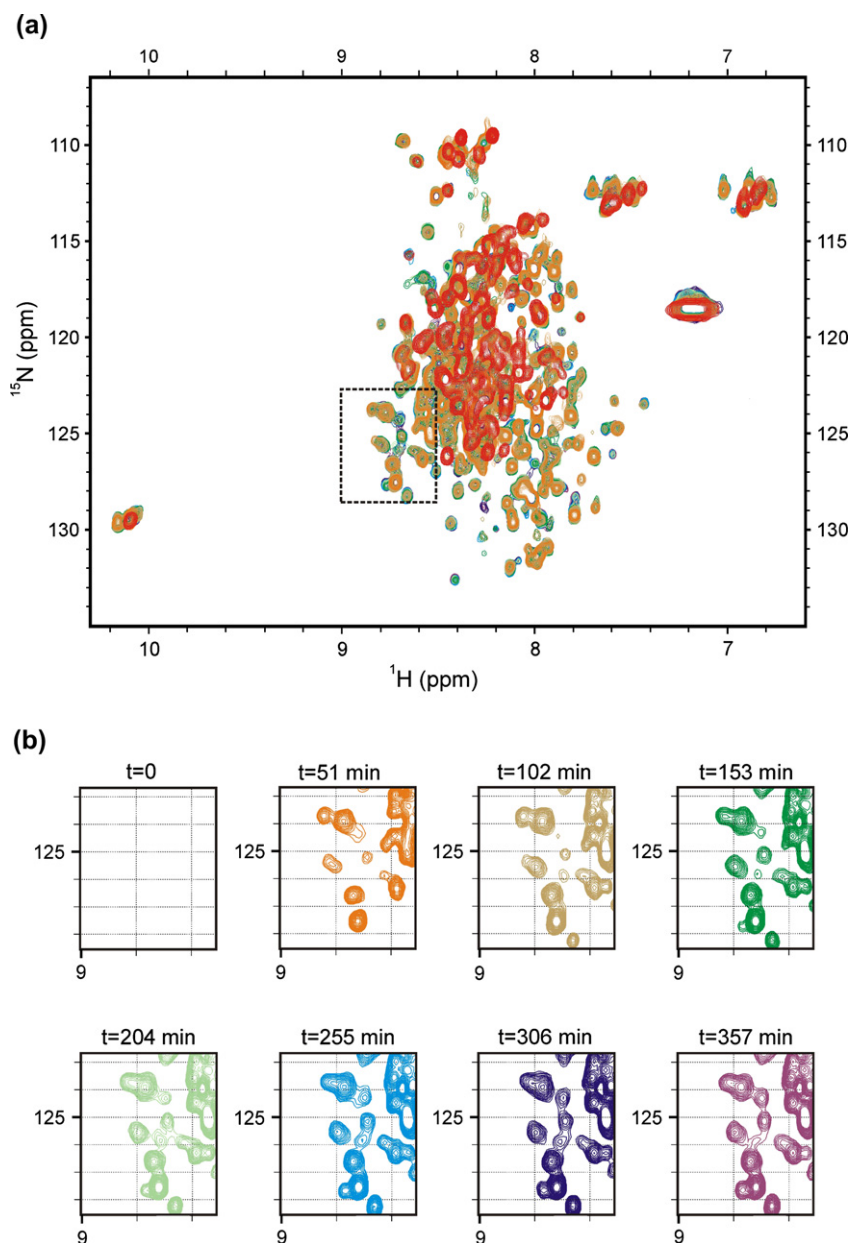


Fig. 3. ^{15}N -HSQC time course overlay to observe ^{15}N -C ϵ 3 folding. (a) Initial ^{15}N -HSQC of 1 mM ^{15}N -C ϵ 3 in PBS shown in red. Following addition of 10 μM sFc ϵ RI α (1%), seven consecutive ^{15}N -HSQC were recorded at 51 min intervals and are shown overlaid. (b) Expanded view of the boxed region from (a) shown. Each of the ^{15}N -HSQC collected is shown independently. Through the course of the folding reaction the spectra are shown (earliest first) in red, orange, gold, dark green, light green, blue, purple, magenta. (Reprinted by permission of Federation of the European Biochemical Societies from reference [24].)

sampled. In this case each of the peaks present has been given an arbitrary label in order to follow its intensity changes during the course of the C ϵ 3 folding reaction. From this analysis it is possible to divide the peaks broadly into five groups based on their rates of appearance and/or disappearance [24]. The division of residues into such groups would suggest that some areas of C ϵ 3 fold more rapidly, forming a folding nucleus around which the rest of the molecule can fold more slowly. Assignment of the peaks in the ^{15}N -HSQC of folded C ϵ 3 would allow this kinetic information to be localised to specific regions at atomic resolution and indicate which parts of C ϵ 3 fold at which rates. It was possible to trace a number of assigned backbone residues through the course of the

folding reaction and locate them on the structure of the folded C ϵ 3 domain based on the structure of IgE-Fc [15]. The residues of the C ϵ 3 domain that appeared to adopt their final conformation most rapidly were those that are located at the site of interaction with sFc ϵ RI α [11]. The observation of groups of residues that appear to fold at different rates indicates that the C ϵ 3 folding reaction being observed does not occur by a simple two-state folding mechanism of the type reported for a number of other protein folding systems [25,26]. Instead, it appears that the C ϵ 3 domain folds via an atypical mechanism on interaction with sFc ϵ RI α , in which the exposed loops are held in a specific conformation by sFc ϵ RI α , leading to stabilisation of the hydrophobic core of the domain and

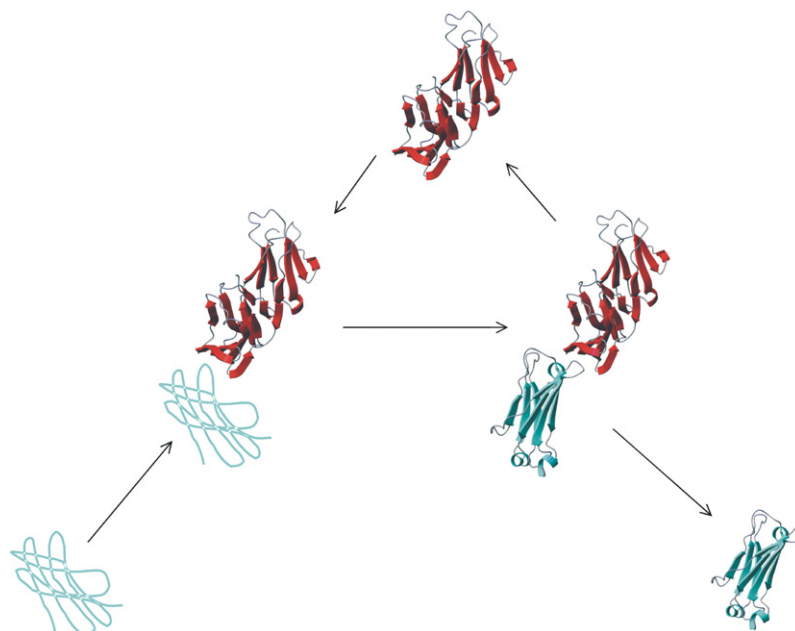


Fig. 4. A schematic representation of the interaction between C ϵ 3 and sFc ϵ RI α . C ϵ 3 present as a 'molten globule' alone in solution, binds to sFc ϵ RI α causing it to adopt a stable tertiary structure. Following the interaction, folded C ϵ 3 dissociates from sFc ϵ RI α allowing it to be recycled to bind to another 'molten globule' C ϵ 3. C ϵ 3 is shown in cyan, and sFc ϵ RI α is shown in red.

subsequent adoption of tertiary structure. Such a scheme is different from folding pathways that have been described in detail for model protein domains [27]. In these cases the folding of proteins, such as barnase and CI2, initially depends on formation of the hydrophobic core, rather than stabilisation of surface loops. However, after the initiation by sFc ϵ RI α via surface loops, the folding of the C ϵ 3 domain could be accommodated within a nucleation-condensation scheme of protein folding.

Over the last two decades the native state of proteins has been increasingly viewed as consisting of a dynamic ensemble sampling a variety of conformational states. It has been shown that there is an uneven distribution of structural stability throughout proteins with sites involved in mediating protein–protein interactions often characterised by the presence of regions with low structural stability, thus providing additional means to regulate these interactions [28]. It was observed previously that IgE may contain such a region of disorder,

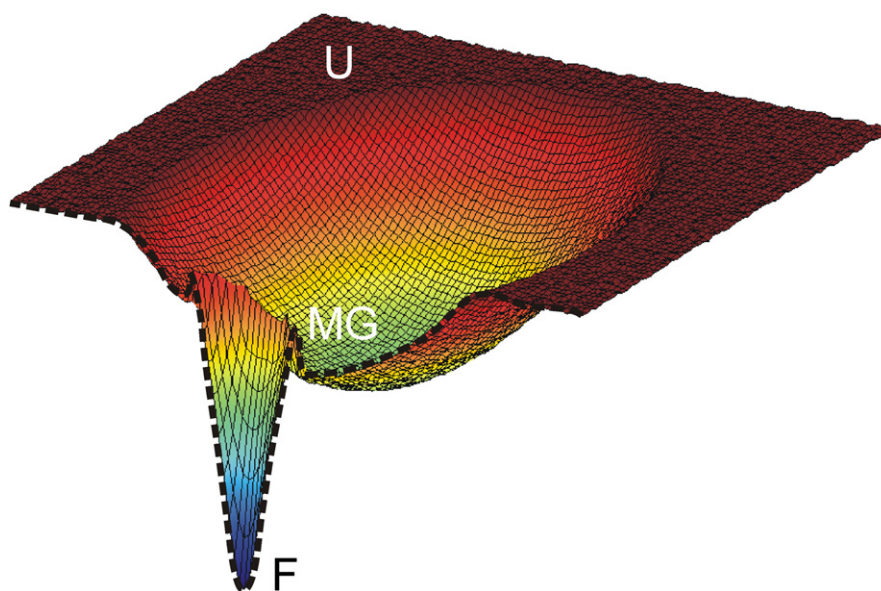


Fig. 5. A three-dimensional representation of the proposed energy landscape for the isolated C ϵ 3 folding pathway. The topology is similar to the 'champagne glass' landscape [37], with the addition of a significant energy barrier protecting the low-energy state. sFc ϵ RI α binding acts as a catalyst, allowing C ϵ 3 to overcome this energy barrier and reach its folded state. The topological representation is sliced (indicated by dashed line) to allow a view of the funnel of the lowest energy folded state. U, unfolded; MG, 'molten globule'; F, folded state.

as evidenced by its lower melting temperature when compared with IgG [29]. Hence, even when the Cε3 domain is present within the context of whole IgE, it may retain some conformational flexibility, thus permitting fine-tuning of the specificity and affinity of IgE interactions. Such an arrangement may facilitate ligand-induced conformational changes and can offer the advantage of allowing fine-tuning of specificity and affinity between ligands and proteins [30]. It has been estimated that 36–51% of cellular proteins or protein domains exist in a partially disordered state [31]. When such a protein, existing as an ensemble of conformers, binds to a ligand one protein conformer may be selectively stabilised, perturbing the equilibrium and causing a population shift in favour of this conformer [32,33].

From these observations we have proposed a scheme of the interaction between the Cε3 domain and sFcεRIα (Fig. 4). In this scheme we assume that Cε3 in its ‘molten globule’ state is competent for binding to sFcεRIα. Binding to sFcεRIα induces Cε3 folding, and subsequent dissociation of folded Cε3 allows ‘recycling’ of sFcεRIα so it is ready to bind and fold another Cε3. Previous characterisation of Cε3 binding to sFcεRIα by SPR indicates that the off-rate of the interaction is rapid, an observation that is in agreement with this hypothesis [18]. The rate of Cε3 folding is slow compared with typical rates of protein folding, which for many simple single domain proteins is completed on a timescale of milliseconds *in vitro* [34]. Presumably in this case the rate of Cε3 folding is limited by its interaction with sFcεRIα, which must occur with the right energy and orientation to proceed along the folding pathway.

The energy landscape concept has been utilised for several years to describe protein folding pathways [34,35], and currently it seems likely that such a concept could be useful in the understanding of protein–protein interactions. We have used this concept to propose a three-dimensional energy landscape to describe the folding reaction of Cε3, shown in Fig. 5. Cε3 present as a ‘molten globule’ alone in solution can obtain the energy necessary for overcoming the folding energy barrier through its interaction with sFcεRIα.

The conformational flexibility of the Cε3 would be expected to be enhanced by the isolation of this domain from the context of the IgE molecule. However, it seems reasonable to suggest that, even within the IgE molecule itself, the Cε3 domain could retain a large degree of conformational flexibility. This suggestion is consistent with earlier observations of the differences in melting temperature between IgE and IgG [29,36]. The positioning of a less structured domain at sites where protein–protein interactions are known to occur would offer IgE a degree of flexibility in its ligand interactions, allowing fine tuning of specificity and affinity according to the particular conditions present at that time. The observation of conformational flexibility of the Cε3 domain of IgE offers insights into potential therapeutic strategies for the management of allergic diseases. Evidently the Cε3 domain allows modulation of the ligand binding properties of IgE in terms of specificity and affinity according to the particular physiological conditions. It was also seen that the alteration of the

conformational flexibility of the Cε3 domain would affect its interactions with ligands. From these results, it can be suggested that modulation of the binding properties of IgE *in vivo* by introduction of a suitable external agent could cause the interaction with FcεRI to become unfavourable, and thereby provide a route to a universal anti-allergy therapeutic.

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