

REVIEW

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Impact of oligomerization on the allergenicity of allergens

Amin Moradi Hasan-Abad¹, Mohsen Mohammadi², Hamed Mirzaei³, Mohsen Mehrabi⁴, Hossein Motedayyen^{1*} and Reza Arefnezhad^{5*}

Abstract

Type I hypersensitivity (allergic reaction) is an unsuitable or overreactive immune response to an allergen due to cross-link immunoglobulin E (IgE) antibodies bound to its high-affinity IgE receptors (FcεR1s) on effector cells. It is needless to say that at least two epitopes on allergens are required to the successful and effective cross-linking. There are some reports pointing to small proteins with only one IgE epitope could cross-link FcεR1-bound IgE through homo-oligomerization which provides two same IgE epitopes. Therefore, oligomerization of allergens plays an indisputable role in the allergenic feature and stability of allergens. In this regard, we review the signaling capacity of the B cell receptor (BCR) complex and cross-linking of FcεR1 which results in the synthesis of allergen-specific IgE. This review also discusses the protein-protein interactions involved in the oligomerization of allergens and provide some explanations about the oligomerization of some well-known allergens, such as calcium-binding allergens, Alt a 1, Bet v 1, Der p 1, Per a3, and Fel d 1, along with the effects of their concentrations on dimerization.

Keywords: Oligomerization, Allergenicity, Protein-protein interactions, Cross-linking, High-affinity IgE receptor

Introduction

Hypersensitivity reactions are generally classified into Type I, Type II, Type III, and Type IV. Type I hypersensitivity is an immediate reaction due to immunoglobulin E (IgE) antibodies against a soluble allergen [1]. It reveals itself in a range of troublesome to life-threatening diseases, from atopic eczema, seasonal hay fever, food allergies, drug allergies, asthma, allergic conjunctivitis, angioedema, urticaria (hives), eosinophilia, sweet itch to anaphylaxis [2]. These reactions are also known as atopic allergies. The term atopic is derived from the Greek word *atopos*, which means unusual or inappropriate. Allergic reactions begin with the exposure of the skin and mucosal surfaces to allergens resulting from

IgE production, mast cell and basophil sensitisation and degranulation [2, 3]. The important impacts of the mediators (histamine, leukotrienes, and prostaglandins) are vasodilation and smooth-muscle contraction [4, 5]. Many people suffer from these disorders around the world, in a range of mild discomfort to rapid death. In general, the prevalence of atopic reactions is increasing and occurs in about 20 to 30% of the population [3, 4]. It is somewhat unclear why some people develop inflammatory reactions to these harmless substances. Multiple agents are responsible for susceptibility to allergic reactions, including environmental, hormonal and genetic factors [5].

FcεR1 has a high affinity for the IgE antibody or allergen-IgE complex. It is reported that cross-linking of at least two FcεR1-bound IgEs by an allergen are needed to the degranulation of mast cell and basophils [6]. Previous studies have revealed that two major immunological properties of allergenic proteins play pivotal roles in inducing allergic reactions, including stimulation of B cell receptors (BCRs) to produce allergen-specific IgE

*Correspondence: hmotedayyen@gmail.com; reza.aref1374@gmail.com

¹ Autoimmune Diseases Research Center, Shahid Beheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran

⁵ Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

Full list of author information is available at the end of the article



antibody and capacity of the cross-linking of IgE bound to FcεRI on effector cells [6–8].

The role of oligomerization of monovalent antigens in improving the signaling capacity of BCR

IgE synthesis in allergic reactions is induced following B cell activation through allergens binding to BCR [9]. The stimulation of this receptor results in the activation of Bruton's tyrosine kinase (BTK), a key member of the BCR signaling pathway, leading to internalize BCR bound to allergens. B cells present allergen-derived peptides to CD4+ T helper2 (Th2) cells which play indispensable roles in B cell proliferation and its differentiation into IgE-secreting cells through releasing some cytokines and immune mediators.

Epitopes of a polyvalent antigen are able to cross-link at least two BCRs which in turn enhance B cell activation, proliferation, and differentiation into IgE-secreting cells [10]. However, monomeric soluble antigens may induce B cell unresponsiveness [11]. Recent studies have demonstrated that a monovalent antigen with a length of 17 amino acids is only able to activate B cells, while the oligomer form of short peptides could more effectively activate B cells [11–13]. It is revealed that the oligomer forms of peptides shorter than eight amino acids have the ability to activate B cells [11, 13]. Furthermore, the dimerizations of plant allergenic profilins rHev b 8 (rubber tree) and rZea m 12 (maize) considerably increases the IgE-mediated degranulation in rat basophilic leukemia cells [14]. These observations suggest that oligomerization of monomer forms of allergens more effectively enhances the signaling capacity of BCR through providing at least two identical epitopes and cross-linking of BCR [15].

Oligomerization effects of monovalent antigens on enhancing cross-linking capacity of FcεRI on the effector cells

For the successful cross-linking of the FcεRI-bound IgE antibodies on the effector cells, allergens must have at least two IgE-binding sites (IgE epitopes) [16]. Furthermore, two different allergen-specific IgE antibodies with complementary paratopes are required for the degranulation of effector cells. These antibodies must bind to their receptors with an appropriate distance [17, 18].

Most of multivalent antigens are large molecules, but some allergenic proteins have low molecular weight. Monomeric allergens rarely have two identical epitopes due to their low molecular weight. Since the oligomer structure of an allergen can present a repetitive array of the identical IgE epitope, oligomerization of allergens displaying only one epitope is sufficient to cross-link FcεRI-bound IgE antibodies on effector cells and initiates

an allergic reaction via the release of immune agents [7, 19, 20]. Moreover, the oligomer structure of allergens can cross-link BCRs and subsequently induce allergen-specific IgE synthesis more potent than their monomeric forms [7, 19, 21].

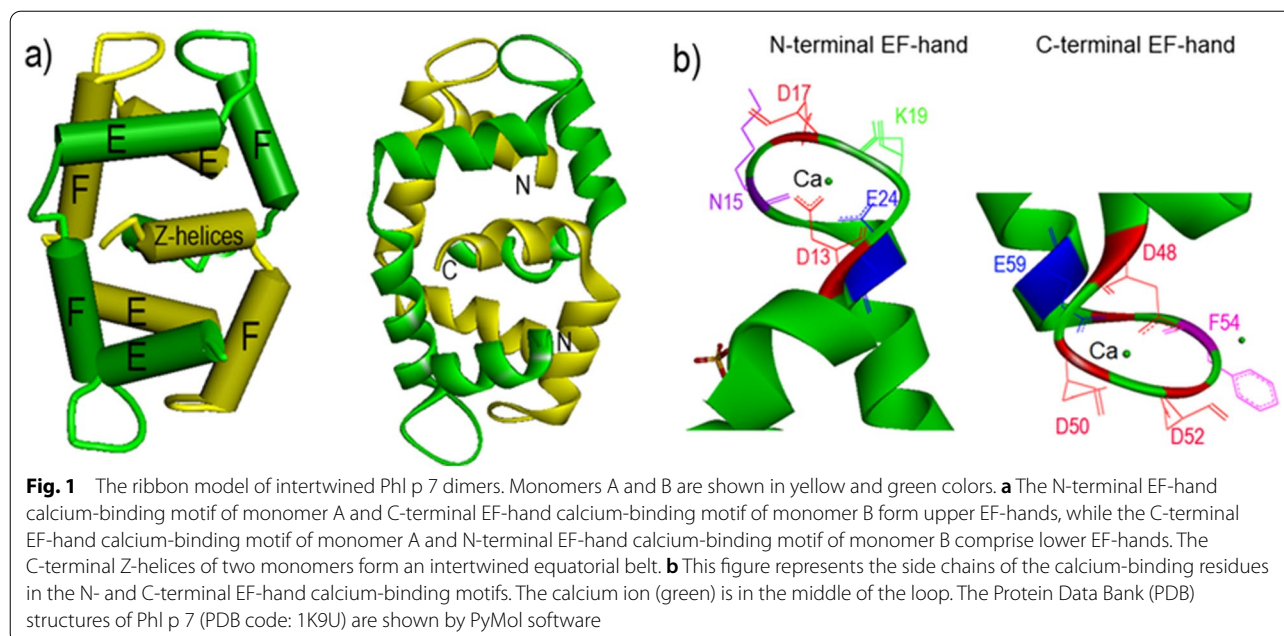
Although there are some reports pointing to the importance of the oligomerization phenomenon in allergenic properties of soluble antigens [7], the possible mechanism(s) involved in the dimerization of allergens has not been well identified yet. Therefore, we review protein-protein interactions, such as amino acid-base interactions and peptide bonds, resulting in the formation of the homo-oligomer structures in allergens. As mentioned above, these homo-oligomerization could increase the cross-linking of BCRs on IgE-B cells and FcεRI-bound IgE on other effector cells by providing at least two epitopes.

Calcium-binding allergens

Some members of the calcium-binding protein family show important characteristics which can participate in allergic reactions in polysensitized individuals [21, 22]. Calcium-binding allergens, such as polcalcin, consist of two EF-hand calcium-binding motifs (helix-loop-helix domains) that connect to a hydrophobic helix (Z-helices) in C-terminal through a short linker (Fig. 1). To date, polcalcins from *timothy grass* (Phl p 7), *birch* (Bet v 4), and *common lambs quarters* (Che a 3) have been characterized and their structures in three dimension have been determined [21, 23, 24].

It is demonstrated that calcium-binding polcalcin possesses very high allergenic properties, while the calcium-depleted form of polcalcin (apo-polcalcin) fails to bind to IgE [22]. As shown in Fig. 1, monomeric polcalcin produces a dimer form according to a head-to-tail arrangement through the interactions between the helix-helix of EF-hand calcium-binding motifs, making a barrel shape with a hydrophobic cavity. This barrel is formed by calcium-binding domains in both the top and bottom of the barrel, and the E- and F-helices, which are located in upper and lower part of side, respectively [21].

Several studies have been performed on calcium-binding effects on the oligomerization of polcalcin [25, 26]. It is revealed that reconstruction of the dimer structure of polcalcin and its correct folding after thermal denaturation are largely related to the presence of calcium [21, 26]. The dimer form is the dominant structure of the calcium-binding polcalcin. Hypoallergenic polcalcin correlated with a mutation in a gene coded for calcium-binding sites is unable to make the dimer form. In addition to polcalcin, parvalbumin, as the main allergen of fish, is another



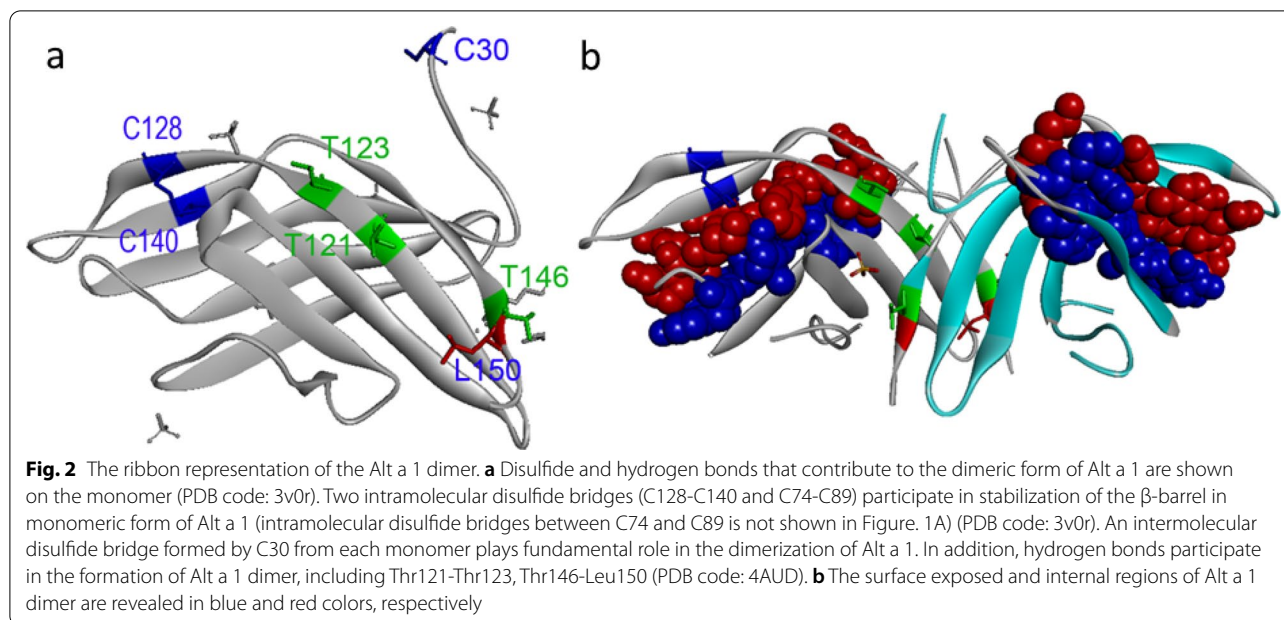
allergen which could provide dimer forms through two EF-hand calcium-binding motifs [27–30].

Alt a 1 allergen

Alt a 1 is a protein in the cell wall of *Alternaria* spores with unknown functions [14, 31]. It is known as the main allergen of *Alternaria alternata* fungus that induces an allergic reaction in approximately 90% of individuals suffering from *Alternaria* allergic [32–34]. In a study conducted by Chruszcz et al. on crystal structure of recombinant Alt a 1, it was indicated that the monomeric structure of Alt a 1 consists of a unique β -barrel form which can assemble to the dimer structure, a highly symmetric butterfly-like homodimer [14].

The natural form of Alt a 1 is a dimer protein with a molecular weight of 30 kDa, showing two bands of 16.4 and 15.3 kDa, under reducing conditions on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [35]. Five disulfide bridges are presented in the Alt a 1 dimer, four of them are intramolecular and stabilize the β -barrel in each monomer. The last disulfide bridge contributes to the formation of the Alt a 1 dimer, and N-terminal cysteine (C30) covalently links to the equivalent residue in each monomer. This disulfide bridge holds two dimers in a “butterfly-like” configuration. Disulfide bonds and the mixture of polar and hydrophobic interactions donate high-temperature stability to the Alt a 1 dimer (Fig. 2). The main IgE epitope of the Alt a 1 dimer locates on the surface with the spatial condition which can cross-link Fc ϵ RI-bound IgE [14, 36].

In addition to the disulfide bond, hydrophobic and polar interactions, hydrogen bonds (Thr121, Thr123, Thr146, and Leu150), and N- and C-terminal regions contribute to the stabilization of the dimer structure of Alt a 1 (Fig. 2) [14]. Previous studies have shown that Alt a 1 forms a tetrameric structure, which can be attached to the flavonoid quercetin [37]. The stability of tetrameric Alt a 1-quercetin complex is pH-dependent, as observed in the other quercetin-bearing proteins. Tetrameric Alt a 1-quercetin complex is secreted from *Alternaria* spore. It is constant even in the low acidic environment such as bronchial epithelium with a pH value of 6, whereas this complex is broken down at pH less than 5.5 [36, 38, 39]. In a study conducted in 2012, the three-dimensional structure of this allergen was determined using X-ray crystallography and reported that there are four IgE antibody binding regions on each monomer of this protein, all of them are located on the surface of the protein. Among these, two peptides (K41-P50 and Y54-K63) are located on the β 1 and β 2 strands of each protein monomer which strongly bind to serum IgE antibodies from *Alternaria alternata* allergies patients. Although some evidence pointing to the oligomerization of Alt a 1, as a classic and dimeric structure, is an important prerequisite for its allergenicity [14, 19], others have revealed that dimerization of this allergen may participate in its allergenicity, but is not essential. As reported mentioned in previous studies [19, 40], many important allergens, such as cockroach allergen Bla g 2, are protein monomers.



Bet v 1 protein

Bet v 1, a member of pathogenesis-related (PR) proteins, is a major allergen of *birch pollen* [41, 42]. This allergen is highly conserved in pollen and plant foods. It is one of the most common allergens responsible for cross-reactivity between aeroallergens and food allergens [43, 44]. Various studies have reported that the cross-linking capacity and allergenic properties of Bet v 1 are largely dependent on the dimerization of this allergen [7, 45]. Schöll et al. reported that the Bet v 1 dimer can induce skin reaction in the skin prick test and activate specific B cells for IgE synthesis more effective than the monomeric form of this allergen [7]. Thus, the dimerization of Bet v 1 provides two IgE-binding sites that are required for the cross-linking of IgE on effector cells and BCRs on B lymphocytes.

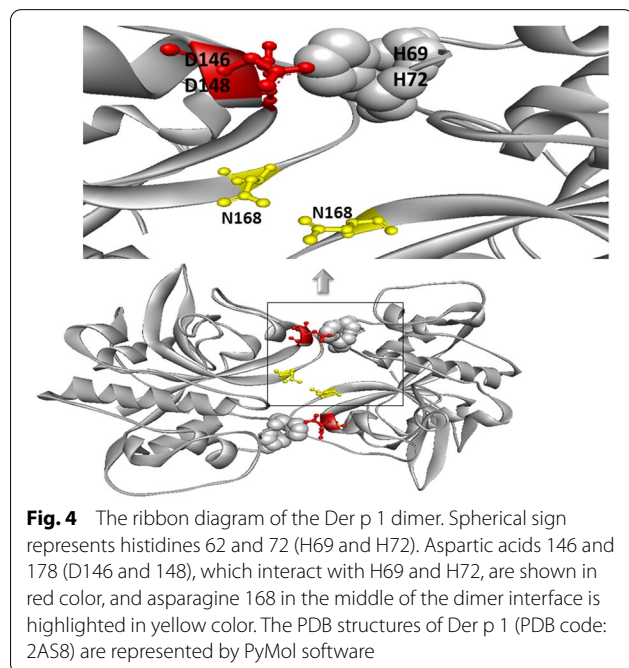
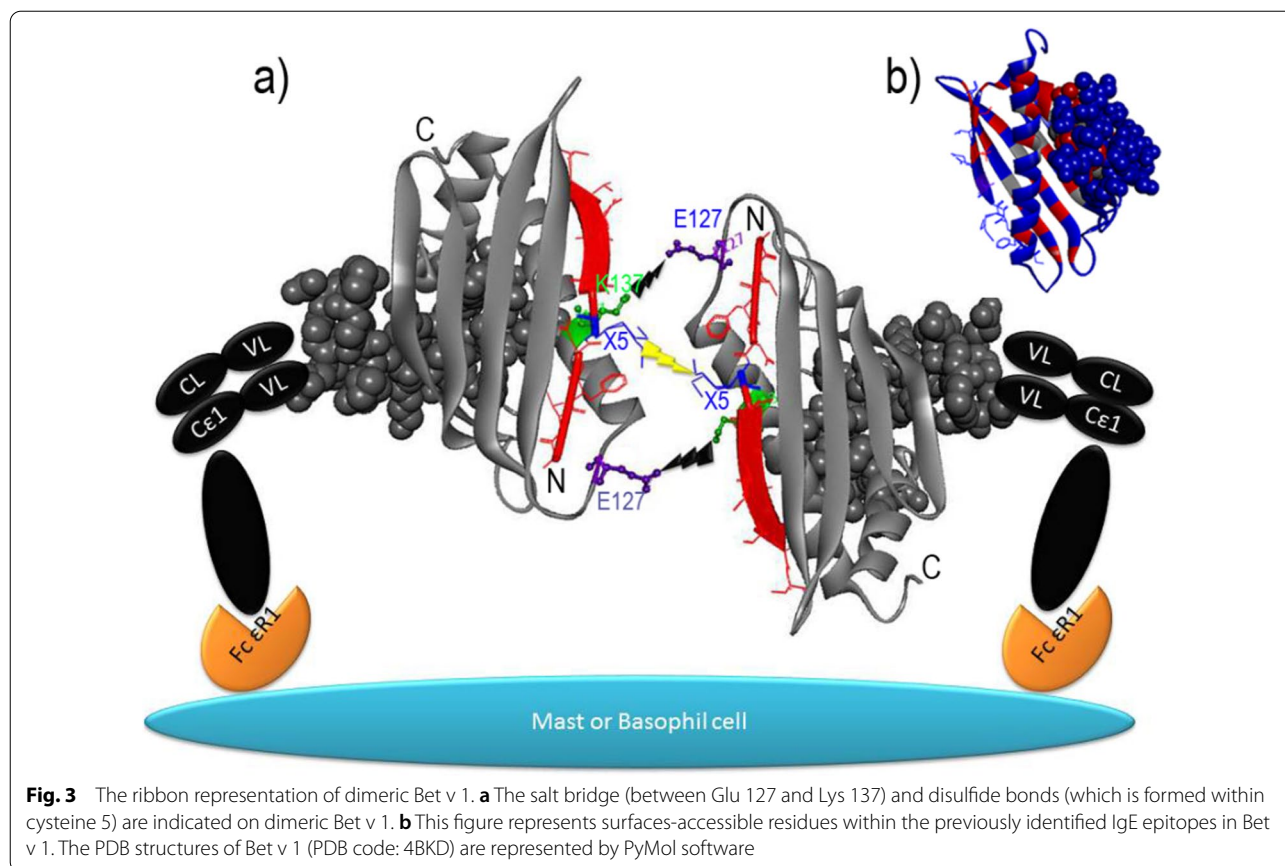
The dimer form of Bet v 1 is generated by the interaction of two monomer forms of Bet v 1 through their N-terminal β -strands. This interaction is then strengthened by disulfide bonds and the salt bridge between Glu 127 and Lys 137 (Fig. 3). Kofler et al. found that the N-terminal region of the Bet v 1 protein, especially residue 5, plays an important role in the protein homodimerization. This finding is related to the intrinsic binding properties of the N-terminal β -strand in each monomer. In addition to its impact on the Bet v 1 homodimerization, several significant roles have been proposed for β -strand, such as IgE recognition, allergen uptake and presentation by primary dendritic cells accompanied by changes in cytokine profiles [45]. Previous studies show that four identified IgE epitope are located on the Bet v 1

dimer [45–48], which allows the cross-linking of monovalent Fc ϵ RI-bound IgE (Fig. 3).

Der p 1 protein

Der p 1, a major allergen from *Dermatophagoides pteronyssinus*, is a 25-kDa glycoprotein, which approximately 70% of individuals with rhinitis, asthma, and dermatitis show an allergic reaction to this allergen [49, 50]. The crystallography studies have demonstrated that an extended interface, including three peptide segments (i.e., 69–75, 146–148, and 163–168) with polar and aromatic amino acids is constructed between each monomer and thereby leads to the dimer form of Der p 1. It is revealed that histidine 69 (His 69) and histidine 72 (His 72) have indispensable roles in the dimerization of the Der p 1. Having considered that disulfide bond, which participates in the dimerization of this allergen, is unstable in low acidic conditions, the allergenic properties of Der p 1 are largely dependent on pH, monomeric form of Der p 1 present at acidic pH while dimeric form is most stable in higher pH [51, 52] (Fig. 4). Therefore, Der p 1 shows greater allergenic properties under neutral and alkaline conditions.

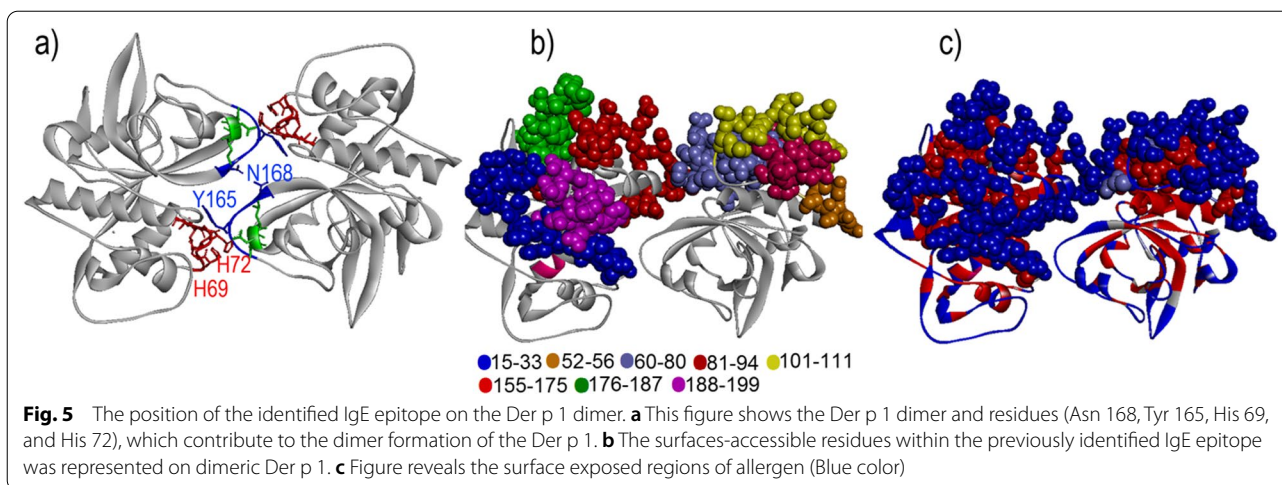
In addition to the disulfide bond, other interactions contribute to the dimer formation and stability of the Der p 1 dimer. It is reported that a bifurcated hydrogen bond is formed by the interaction of the side chain of His 72 with Asp 146 and Asp 148 from another monomer (Fig. 4). Moreover, carbonyl oxygen hydrogen from His 72 interacts with the nitrogen of Tyr 165 from another monomer (Fig. 5). Asn 168 from each monomer interacts with another monomer in the center of interface and thereby participates in the dimerization of Der p 1 (Fig. 5).



Magnesium binds to Der p 1 via several residues, such as Asp 56, Glu 59, and Glu 91, which produces a site binding for magnesium. Furthermore, a hydrogen bond between the carbonyl oxygen of Leu 57 and magnesium ion has an important role in stabilizing magnesium binding to Der p 1 [52]. As shown in Fig. 5, the analysis of the surface accessibility of previously described IgE epitopes on the Der p 1 dimer revealed that peptide 15–33 is well accessible on the surface of Der p 1 and near to peptide 188–199 [53–55]. Therefore, these two peptides can create a discontinuous epitope (Fig. 5). Moreover, four well-accessible peptide segments, including 52–56, 60–80, 81–94, and 101–111, make linear or continuous epitopes. Peptide 155–175 is relatively buried within the dimer interface but can form a discontinuous epitope with peptide 176–187 or 60–80 (Fig. 5) [52].

Per a 3 allergen

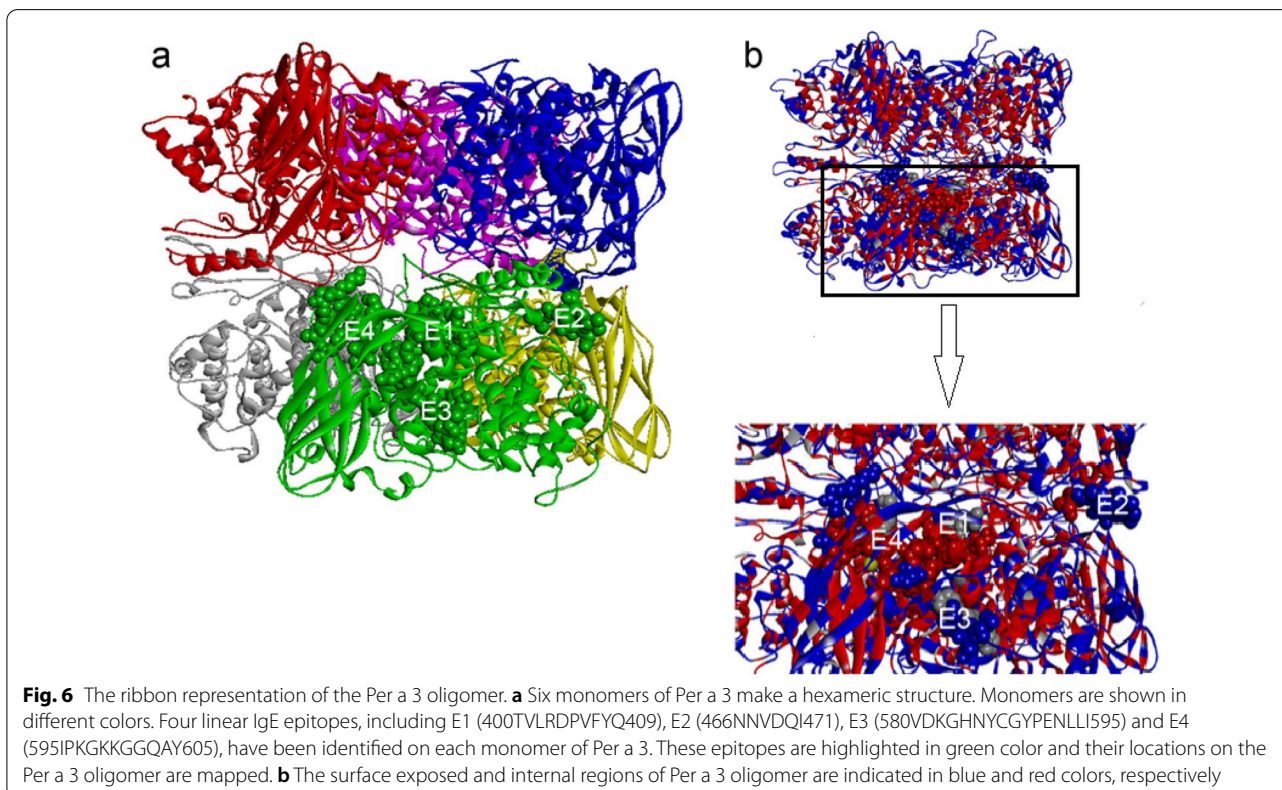
Per a 3, a hemocyanin in the hemolymph of cockroaches, possesses high identity in the amino acid level with storage proteins (hexamerins and arylphorins from the hemolymph in insects and arthropods). Per a 3 is



identified as a major allergen from the American cockroach, which was recognized by the serum IgE from cockroach-allergic patients [56]. Mindykowsk et al. showed that Per a 3 makes a hexameric form similar to other hemocyanins. Mapping of IgE epitopes on a hexameric three-dimensional model revealed that all reported IgE epitopes located on the surface of the hexamer are freely accessible to Per a 3-specific IgE (Fig. 6) [57, 58]. The hexameric form of Per a 3 has highly thermos-stable properties, which allows it to remain for a long time as a high allergenic compound in house dust [56].

Fel d 1 allergen

Fel d 1, a 35-kDa tetrameric glycoprotein, has been reported as the main allergen in cat-allergic patients, which reacts with 90% of the serum IgE from these patients. Allergens from domestic cats cause approximately 10% of mild rhinitis but also life-threatening asthmatic reaction in the western world [59]. Heterodimer form of Fel d 1 is formed by two subunits, including chains 1 and 2 linked by three disulfide bonds among cysteine residues, such as Cys3, Cys73, Cys44, Cys48, Cys70, and Cys7 [59, 60]. The chains 1 and 2 consist of four helices, including H1 to H4



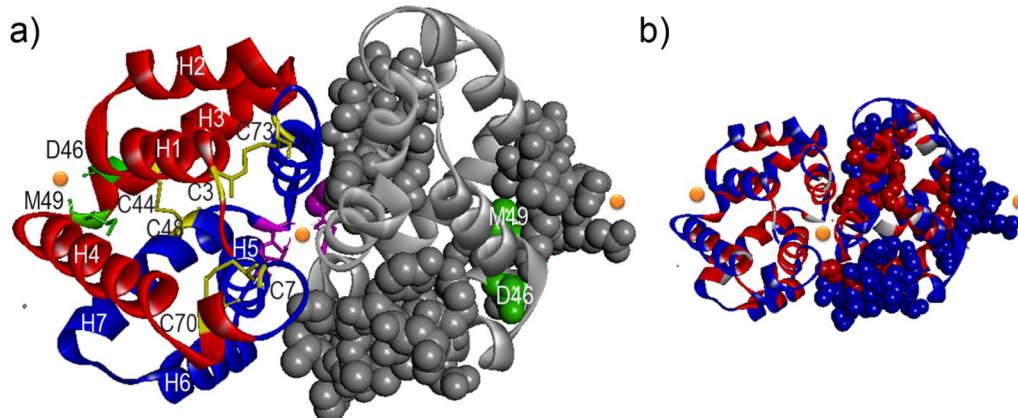


Fig. 7 The ribbon representation of the Fel d 1 oligomer. **a** Chains 1 and 2 are shown in blue and red colors, respectively. Each chain contains four helices (H) linked to each other by three disulfide bonds (C3–C73, C44–C48, and C7–C70). **b** The surface-accessible residues within the previously identified IgE epitope are shown on oligomer Fel d 1. Orange sphere reveals the calcium ion. The PDB structures of the Fel d 1 oligomer (PDB code: 2ejn) are indicated by PyMol software

and H5 to H8, respectively. H5 and H8 helices participate in dimerization of Fel d 1 [60]. Tetrameric Fel d 1 made from two heterodimers (Fig. 7).

The interface generated between two monomer forms of Fel d 1 is constructed of a central hydrophobic core surrounded by a hydrogen bond made by charged residues. Three calcium-binding sites exist in tetrameric Fel d 1; one site is within the dimerization interface, and other sites are located on the side surface of each dimer in tetrameric Fel d 1. Three linear IgE epitopes were

identified on the Fel d 1 allergen. These epitopes are located on the first and the end segments of H1–H2, H3–H4, and H5–H6, respectively [61].

Effect of allergen concentrations on dimerization

In spite of amino acid-base interactions and peptide bonds, allergen concentration is a key factor in dimerization of monomeric allergens. Previous studies have shown that many allergens are able to make dimers or oligomer structures. The accumulations of monomeric

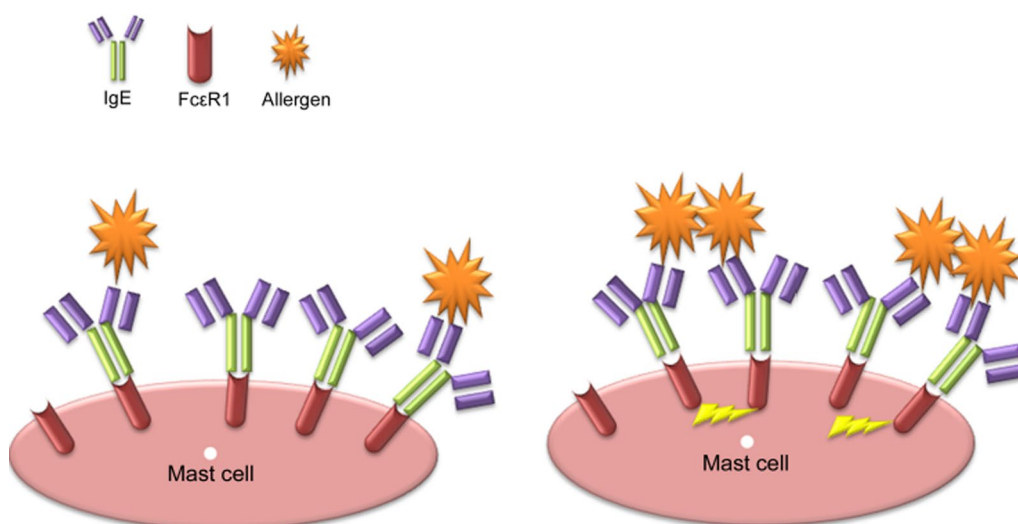


Fig. 8 The dimer formation of the allergen on a mast cell. Individual allergens that present only one epitope, disable to cross-link the FcεRI-bound IgE antibodies (left). The increase of the local concentration of the allergen on a mast cell can increase the dimerization of allergens, leading to the cross-linking of FcεRI-bound IgE antibodies (right)

antigens at higher concentrations provide a chance to increase their dimerization and thereby participate in enhancing protein immunogenicity [19, 62]. Although oligomerization and specific structural characteristics play indispensable roles in allergenicity of an allergen, these features are largely dependent on allergen concentrations. It is shown that allergens tend to have a monomeric form at low concentrations, while they shift to have dimer or oligomer forms at higher concentrations [57]. For example, the dimerization of the rBos d 2 is dependent on its concentration. The increasing concentration of this allergen progressively induces its dimer structure, resulting in a monomer-dimer equilibrium in the solution [19].

Rouvinen et al. demonstrated that approximately 80% of allergens are able to produce symmetric dimers or oligomers in crystals. Allergens frequently create a transient dimer structure at high concentrations; this observation is confirmed by the fact that their hypoallergenic variants have a monomer structure [19]. Kuriyan et al. reported that an increase in the colocalization of allergen molecules can significantly enhance their local concentrations [63]. In this regard, the local increase of monomer allergens bounding to the FcεRI-bound IgE antibodies enhance the interaction between allergen that leads to dimer formation and subsequently the cross-linking of FcεRI-bound IgE antibodies on the effector cells (Fig. 8) [19].

Conclusions

Oligomerization of allergens play undeniable roles in the allergenic features and stability of allergens. In the present study, the protein–protein interactions were reviewed, which are responsible for allergen oligomerization (including amino acid-base interactions and peptide bonds). There are numerous studies pointing to at least two epitopes on the surface of allergens are necessary for the successful and effective cross-linking, however, some reports showing small proteins with only one IgE epitope (which form homo-oligomerization) can also cross-link FcεRI-bound IgE by providing two same IgE epitopes.

In conclusion, homo-oligomer structure in an allergen, which is mainly mediated by protein–protein interactions, has a key role in allergenicity of an allergen through providing at least two epitopes and enhancing the cross-linking of the BCRs on IgE-B cells and FcεRI-bound IgE on effector cells, but is not necessary. In addition, there are some reports revealing many important allergens are protein monomers.

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Author details

¹Autoimmune Diseases Research Center, Shahid Beheshti Hospital, Kashan University of Medical Sciences, Kashan, Iran. ²Persian Gulf Marine Biotechnology Research Center, The Persian Gulf Biomedical Sciences Research Institute, Bushehr University of Medical Sciences, Bushehr, Iran. ³Research Center for Biochemistry and Nutrition in Metabolic Diseases, Institute for Basic Sciences, Kashan University of Medical Sciences, Kashan, Iran. ⁴Department of Medical Nanotechnology, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran. ⁵Department of Anatomy, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.

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