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Mapping and conformational analysis of IgE-binding epitopic regions on the molecular surface of the major Ara h 3 legumin allergen of peanut (*Arachis hypogaea*)

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ABSTRACT

Eight distinct sequential IgE-binding epitopes were identified along the amino acid sequence of Ara h 3 using the Spot technology. They essentially correspond to preferencially electropositive regions exposed on the molecular surface of the protein. A few IgE-binding epitopes are coalescent to create more extended IgE-binding regions exposed on the surface of the allergen. Ara h 3 contains a core region corresponding to the cupin motifs and predicted to be preserved upon the trypsin and chymotrypsin attack in the gastro-intestinal tract. Some of the identified IgE-binding epitopes should remain unaltered in the core region to subsequently interact with the local immune system. They most probably account for the strong allergenic potency of Ara h 3. Most of the identified IgE-binding epitopes of Ara h 3 readily differ from the corresponding regions of other legume and tree-nut legumin allergens except for epitope #1 and #7 which are rather conserved essentially in other allergens. These structurally related epitopes could account for some cross-reactions occurring between Ara h 3 and other legumin allergens.

1. Introduction

Nowadays, peanut allergy has developed in industrial countries to become one among the most potentially severe and life-threatening food allergies, especially in children (Sicherer et al., 2003; Sicherer and Sampson, 2007). Different peanut allergens responsible for both the sensitization and the IgE-binding reactivity of allergic patients have been identified as seed storage proteins. Essentially, three seed storage proteins corresponding to a vicilin (Ara h 1) (Burks et al., 1995), a 2S albumin (Ara h 2) (Viquez et al., 2001) and a legumin (Ara h 3) (Rabjohn et al., 1999; Restani et al., 2005), respectively, have been identified as the major peanut allergens. Other minor allergens have been described which less fequently trigger the synthesis of appreciable amounts of specific IgE in sensitized individuals (de Leon et al., 2007). In the late 1990s, continuous IgE-binding epitopes have been characterized on Ara h 1 (Burks et al., 1997; Shin et al., 1998), Ara h 2 (Stanley et al., 1997) and Ara h 3 (Rabjohn et al., 1999) by the Bannon's group, using the Spot technique performed with 15-mer synthetic peptides overlapping by eight residues. Twenty-three, ten and three distinct IgE-binding epitopes were recognized along the amino acid sequence of Ara h 1, Ara h 2 and Ara h 3, respectively. Most of these epitopic stretches contain a high proportion of both electropositively (Arg, Lys) and electronegatively (Asp, Glu) charged residues are thus expected to be nicely exposed at the molecular surface of the allergens. However, a limited number of IgE-binding epitopic regions identified on Ara h 3 is rather intriguing with respect to the high molecular mass and the strong allergenicity associated to this allergen. The Ara h 3 amino acid sequence comprises 507 residues corresponding to a calculated mass close to 58,000 (Rabjohn et al., 1999), and is predicted to occur as a homotrimer similar to that of phaseolin of French bean (Lawrence et al., 1994) and proglycinin A1aB1b of soybean (Adachi et al., 2001), like Ara h 1 (Barre et al., 2005) which also belongs to the vicilin group of seed storage proteins. In addition, native Ara h 3 would occur as a hexameric structure resulting from the stacking of two similarly oriented homotrimers like the glycinin A3B4 homohexamer (Adachi et al., 2003). Apparently, the diversity of the IgE-binding epitopes occurring on the so extended

Abbreviations: Ara h 1, *Arachis hypogaea* allergen 1 (vicilin); Ara h 2, *Arachis hypogaea* allergen 2 (2S albumin); Ara h 3, *Arachis hypogaea* allergen 3 (legumin); TBS, Tris-buffered saline (pH 7.0); TBSTw, Tris-buffered saline (pH 7.0) containing 0.1% (v/v) Tween-20.

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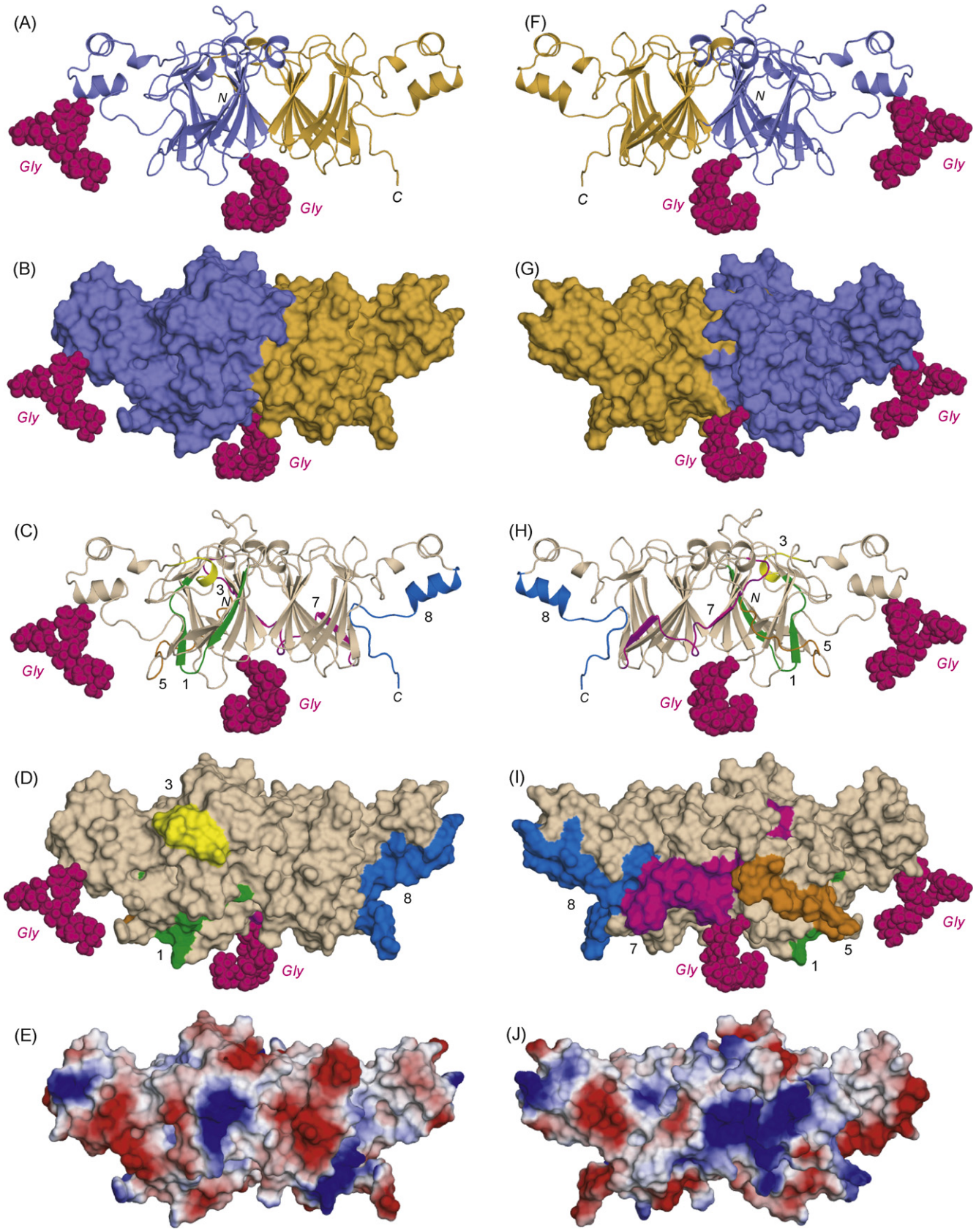

Fig. 1. (A and F) The two faces of the ribbon diagram of the Ara h 3 monomer showing the two cupin motifs colored orange and violet, respectively. The *N*-glycan chain attached to the monomer is in pink colored CPK. N and C correspond to the N- and C-termini of the polepeptide. (B and G) The two faces of the molecular surface of the Ara h 3 monomer showing the two cupin motifs (colored orange and violet) and the *N*-glycan chain (colored pink). (C and H) IgE-binding epitopes delineated on the two faces of the ribbon diagram of Ara h 3. Epitopes are numbred and colored green (#1), yellow (#3), orange (#5), magenta (#7) and blue (#8). Regions containing epitopes #2, #4 and