Conformational Epitope Mapping of Pru du 6, a Major Allergen from Almond Nut

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Results and Discussion

Although enjoyed safely by most individuals, widely consumed tree nuts, including almond, frequently trigger allergic reactions. Almond prunin (Pru du 6), an 11S globulin (legumin), is an abundant nut seed protein and a major allergen. Conformational epitope mapping studies of prunin have been performed with a murine monoclonal antibody (mAb) 4C10. That mAb reacts with non-reduced but not reduced prunin in immunoblotting assays, indicating the recognition of a conformational epitope. 4C10 competes with patient IgE, as assessed by ELISA, indicating clinical significance of the epitope. To characterize the 4C10 epitope, hydrogen/deuterium exchange (HDX) monitored by 14.5 T Fourier transform ion cyclotron resonance mass spectrometry (MS) was performed for the native prunin-4C10 complex and uncomplexed native prunin. Several epitope candidate peptides that differ in deuterium uptake between the complexed and uncomplexed forms were identified. The epitope was further mapped by analyzing chimeric molecules incorporating segments of the homologous soybean allergen, Gly m6, in immunoassays. Those data indicate that the 4C10 epitope overlaps with a subset of patient IgE-binding epitopes on almond prunin and further supports HDX-MS as a valid technique for mapping conformational epitopes.

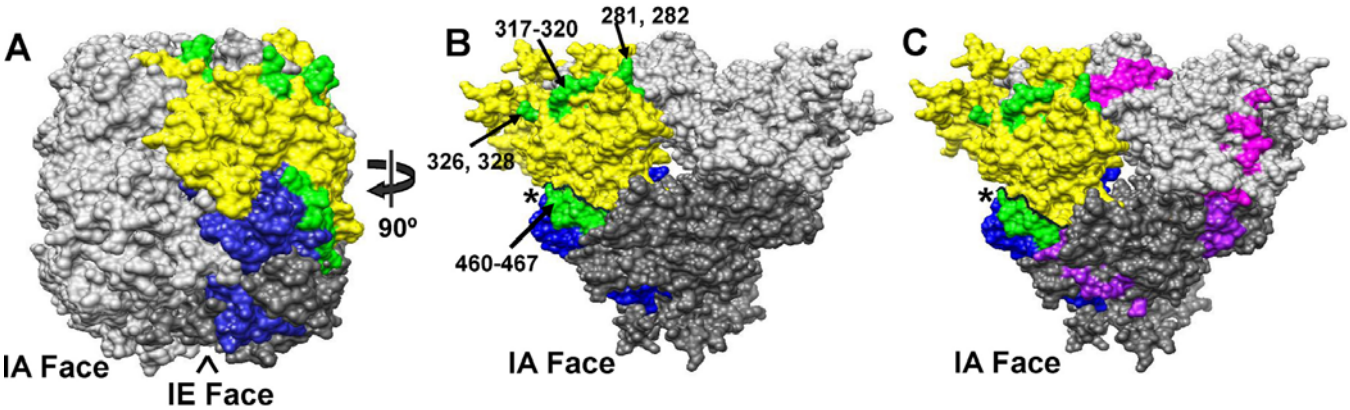

Figure 1. Structural models of prunin (PDB# 3FZ3). (A) Side view of the prunin hexamer showing the lateral IA faces and the IE face-to-face association of two trimers. One of the prunin monomers is color-coded (large subunit, yellow; small subunit, blue) and the additional monomers are represented in light gray and dark gray. Peptides identified by HDX are shown in green on one monomer. (B) Top view of the prunin trimer. The asterisk indicates the black line separating the large and small subunit in the color-coded monomer. The peptides identified by HDX are shown in green. Surface exposed residues of the HDX-identified peptides are indicated by arrows. (C) HDX-identified peptides are shown on each of three monomers in green, purple, and magenta.

Acknowledgments

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References

[1] Willison, L.N., Zhang, Q., Molecular Immunology, **55**, 253-263 (2013).