



## Recombinant Zea mays profilin forms multimers with pan-allergenic potential

<http://www.firstlight.cn> 2006-03-16

European studies have shown that approximately 20% of all pollen-allergic patients display IgE reactivity to various plant profilins. Profilins are ubiquitous intracellular proteins, with a role in cell signalling and morphology. Recently, functionally relevant human profilin tetramers were identified, but the characterization and allergenic roles of plant profilin multimers have not been reported. Because larger molecules are generally more antigenic, the present objectives were to: (i) determine if plant profilin forms multimers; (ii) use the allergenic property of profilin in the design of an immunoassay to detect type I allergies in the local population; and (iii) assess the allergenic potential of monomeric versus multimeric profilin. In agreement with other known profilin forms, silver-stained sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblot analyses revealed that a significant 14.8 kDa protein was purified from *Escherichia coli* transformed with the cDNA of a plant (*Zea mays*) profilin isoform (ZmPRO1). Higher molecular weight proteins (particularly 60 kDa and 30 kDa) were also observed, which became predominant and larger (> 90 kDa) in the absence of reducing agents. Human IgE reactivity to profilin was measured by enzyme-linked immunosorbent assay (ELISA) that was developed using patient serum samples classified as either negative (no type I allergies), positive (type I plant allergies) or miscellaneous (i.e. allergies other than classical type I plant allergies). The IgE-ZmPRO1 complexes were seen in three of nine patients with type I plant allergies, compared with one of eight negative controls and three of 14 from the miscellaneous category. Dot filtration immunoblots were subsequently developed to absorb profilin diluted in the presence or absence of reducing agent to yield mostly monomeric or multimeric profilin, respectively. Immunoglobulin E from positive patients displayed a greater intensity of binding to ZmPRO1 under conditions that favored profilin multimers. In summary, recombinant ZmPRO1 profilin forms multimers and is suitable for a developed ELISA. The data further suggest that profilin has pan-allergenic potential in the North American population and raise the possibility that profilin multimers have greater immunogenicity than monomers.

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