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Homology of Two cDNAs Coding for Birch Pollen Allergens with Calmodulin: Protein-Bound Ca^{2+} Affects the IgE-Binding Capacity

Key Words

Birch pollen allergens
Calmodulin
Epitopes
IgE

Abstract

Birch pollen is known to contain two well-defined allergens, *Bet v I*, a protein, which is a major allergen for 95% of the birch-pollen-allergic individuals, and birch profilin, which we could identify as a novel type of plant pan allergen. Here, we report the identification of two novel birch pollen allergens with significant sequence homology to calmodulin. Although both allergens are rear targets for patients' IgE (5–10%), we consider them of particular interest because both proteins require the native protein conformation and protein-bound Ca^{2+} for IgE binding. Thus we describe for the first time an IgE epitope which is assembled by a polypeptide and a divalent cation, Ca^{2+} .

Introduction

Antigen-antibody interactions are still poorly understood, and there exists considerable disagreement about the definition of epitopes [1]. The molecular characterization of allergens by recombinant techniques contributed to our knowledge about IgE-binding structures of allergens. So far, polypeptides as well as large conformational structures assembling different portions of one molecule were described as IgE epitopes. In case of birch pollen profilin [2], we could identify a C-terminal IgE-binding polypeptide of twenty amino acid residues, whereas in case of the major birch pollen allergen *Bet v I* the complete protein seems to be required for IgE binding, because proteolytic fragments of this molecule lost their IgE-binding capacity [3]. In general, most of the allergens described so far were capable of binding patients' IgE after denaturing SDS gel electrophoresis and electroblotting to nitrocellulose [4]. Yet one example of a

house dust mite allergen was described which could be isolated as a native recombinant protein from phages, but could not be detected on immunoblots prepared from mite extracts [5]. We now describe the cloning of calmodulin-like allergens from birch pollen which require the presence of protein-bound Ca^{2+} in addition to the native protein conformation for IgE binding. This novel type of an IgE epitope illustrates the diverse requirements for allergen-IgE interactions.

Results and Discussion

We screened a birch pollen cDNA library with serum IgE from allergic patients and isolated two cDNAs encoding birch pollen allergens with significant sequence homology to calmodulin and calmodulin-like proteins. Both clones, designated c13 and c16, contained the typical Ca^{2+} -binding motif [6] in their deduced amino acid sequence

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1018–2438/92/0004–0380
\$2.75/0

but represent distinct proteins. Oligonucleotides complementary to the coding region of these cDNAs hybridized with birch pollen mRNA at 900 nucleotides but not with mRNA from leaves and female inflorescences [7]. This indicates a prominent expression of these proteins with a presumable molecular weight of about 20 kD in pollen. Although only 5–10% of pollen-allergic patients bound with their IgE to both recombinant allergens, we considered these proteins to be of particular interest because calmodulins are proteins that can be found in all eukaryotic cells including man. They play a key role in Ca^{2+} binding and in activation of phosphodiesterase, and similar to the plant pan allergen profilin, calmodulins are linked to the phosphoinositide pathway [8]. Binding of patients' IgE requires the native conformation of the protein since SDS polyacrylamide gel electrophoresis of the pollen-derived as well as of the recombinant proteins led to complete loss of IgE binding. Depletion of Ca^{2+} with EGTA from plaque lifts containing the native recombinant allergens lead to a significant and reversible loss of the binding of calmodulin-specific IgE antibodies, but did not affect IgE binding to other allergens [7]. This indicates that allergen-bound Ca^{2+} is part of the IgE epitope and that changes of the native protein conformation affect the IgE binding capacity. Further characterization of the structure of these proteins may contribute to our knowledge about the mechanisms of IgE-allergen interactions on the molecular level.

Acknowledgements

This work was supported by grant 3/8603 of the 'Fonds zur Förderung der gewerblichen Wirtschaft' and by grant S6002 Bio of the 'Fonds zur Förderung der wissenschaftlichen Forschung'.

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CLONE 13: DEDGDGYISARE
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 CLONE 16: DTDGDFISFQE

Fig. 1. Deduced amino acid sequences of the Ca^{2+} -binding motifs of clones 13 and 16. Identical amino acid residues are indicated.

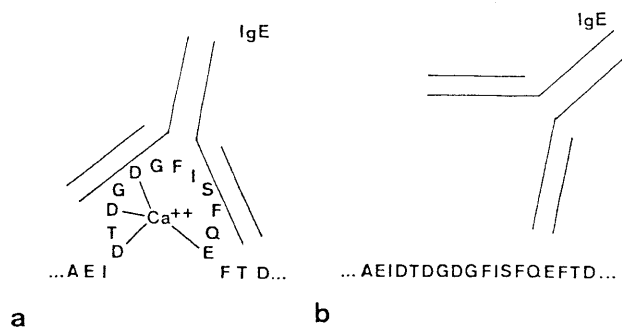


Fig. 2. Proposed mechanism for the interaction of patients' IgE with calmodulin-like allergens from birch (c13, c16). **a** Binding of patients' IgE requires protein-bound Ca^{2+} to form the epitope. **b** Depletion of Ca^{2+} by EGTA leads to a reversible change of the allergen conformation and loss of IgE binding.