

Characterization of Recombinant Bet v 4, a Birch Pollen Allergen with Two EF-Hand Calcium-Binding Domains

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Key Words

Birch pollen · Allergen · EF-hand domain · Calcium binding · IgE binding

Aqueous extracts of birch pollen and related species contain a complex mixture of proteins. Approximately 40 distinct proteins are easily released from the pollen grains upon hydration, but several studies showed that only some of these proteins (approximately 10–12) are recognized by IgE from allergic individuals [1]. cDNA clones coding for three of these birch pollen allergens have been isolated and characterized, all showing sequence similarities to known proteins [2–4].

We have now isolated a complete cDNA encoding a novel birch pollen allergen designated Bet v 4. The deduced amino acid sequence indicated that Bet v 4 contains two potential Ca²⁺-binding sites of the EF-hand type. Bet v 4 shows high sequence similarity to a group of calcium-binding pollen allergens described in Bermuda grass [5] and *Brassica* [6]. To evaluate whether both EF-hand domains of Bet v 4 are able to bind calcium, we introduced point mutations to convert Asp to Ala at the first coordination position in each site (Asp19 and Asp54, respectively). This replacement is known to disrupt calcium binding to EF-hand domains. Nonfusion forms of the full-length cDNA coding for Bet v 4 and three Bet v 4 mutants were expressed in *Es-*

cherichia coli. Recombinant proteins were purified from crude bacterial lysates, as previously described [5]. ⁴⁵Ca²⁺ blot overlays of purified wild type rBet v 4 and Bet v 4 mutants clearly showed that both EF-hand domains in the Bet v 4 sequence constitute functional calcium binding sites.

The ability of rBet v 4 to bind IgE was evaluated by immunoblots using sera from pollen-allergic patients. Approximately 5% of birch pollen-allergic patients and 10–15% of timothy grass and mugwort pollen-allergic patients displayed IgE antibodies reacting with rBet v 4. Furthermore, IgE cross-inhibition experiments indicated that proteins with similar molecular weight in extracts from pollen of timothy grass and mugwort share IgE epitopes with Bet v 4. These results indicate that Bet v 4 represents a cross-reactive pollen allergen.

To determine the influence of protein-bound Ca²⁺ on IgE recognition, immunoblot experiments using sera from allergic patients were performed either in the presence of CaCl₂ or EGTA, as described [4]. Our experiments using EGTA to deplete protein-bound calcium indicated that for some patients, IgE recognition of Bet v 4 required calcium. But in most cases, substantial IgE binding was still detected after depletion of bound calcium. We have also tested IgE reactivity of calcium-binding deficient mutants of Bet v 4. Interestingly, our results indicated that most Bet v 4-specific IgE antibodies from birch pollen-allergic patients are directed to the carboxy-terminal EF-hand domain. Using syn-

thetic peptides (12-mer) based on the amino acid sequences of the two calcium-binding domains, similar results were obtained for Cyn d 7, the Bet v 4 homologue in Bermuda grass pollen [5]. Taken together, the results support the idea that the carboxy-terminal calcium-binding domain of Bet v 4 represents a major continuous (linear) IgE epitope. But we cannot exclude the possibility that this linear epitope is part of a discontinuous (conformation-dependent) epitope(s) on the folded protein.

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