

cDNA Cloning and Characterization of a Cross-Reactive Birch Pollen Allergen: Identification as a Pectin Esterase

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Key Words

Birch pollen allergen · Pectin esterase · Cross-reactivity · cDNA cloning

Introduction

Birch pollen represents a predominant allergen source in middle and northern Europe, North America and Australia. In addition to Bet v 1, the major birch pollen allergen which is recognized by more than 95% of birch pollen-allergic patients, several other birch pollen allergens have been described [1–7] (table 1). These allergens are recognized at a much lower frequency than Bet v 1 but are important because they share IgE epitopes with homologous allergens from unrelated allergen sources and thus can be responsible for clinically relevant cross-reactivities. Cross-reactive homologs of Bet v 1 are present in pollen of trees of the *Fagales* order and plant-derived food (fruits, vegetables, spices) [8, 9]. Bet v 2, birch pollen profilin, represents one of the most cross-reactive allergens described so far. It is a cytoskeleton protein which also participates in the signal transduction cascade and therefore occurs as cross-reactive allergen in eukaryotic organisms from plant up to man [2, 3, 10]. Bet v 3 and 4 are

calcium-binding proteins which are highly expressed in mature pollen and thus represent cross-reactive allergens in pollen of trees, grasses and weeds [4, 5]. Recently, an isoflavone reductase (Bet v 5) and a cyclophilin (Bet v 7) were reported as minor allergens in birch pollen and plant food [6, 7]. Here we report the molecular characterization of a novel birch pollen allergen which shares sequence homology with plant pectin esterases.

Material and Methods

Serum IgE from a patient allergic to tree, grass and weed pollen as well as to plant-derived food was used to screen a birch pollen cDNA library as previously described [1]. A partial cDNA clone (clone 49) which coded for a C-terminal fragment of the birch pollen allergen was isolated. By oligonucleotide screening of the original and newly constructed libraries additional cDNAs were isolated. A full length clone was obtained by assembly PCR of overlapping cDNAs coding for the N-terminal and C-terminal portion of the allergen. DNA and deduced amino acid sequence were compared with the NIH databases (BlastN, BlastP). The cDNA was subcloned into plasmid pET23b (Novagen, Bad Soden, Germany) and the corresponding protein was expressed in *Escherichia coli* and purified by Ni²⁺ affinity chromatography. IgE-ELISA was performed as described [11] using sera from birch pollen-allergic patients. A rabbit antiserum raised against the C-terminal portion of the protein was used to detect cross-reacting moieties in nitrocellulose-blotted pollen, vegetable and fruit extracts as described [3].

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Fig. 1. IgE binding to ELISA plate-bound rBet v 8. ELISA plate-coated rBet v 1 and rBet v 8 were exposed to serum from a non-allergic individual, serum from a patient allergic to Bet v 1 (patient 1) and sera from two polysensitized patients allergic to various types of pollen and plant food (patients 2 and 3). Levels of bound IgE antibodies correspond to the optical density (OD) levels on the y-axis.

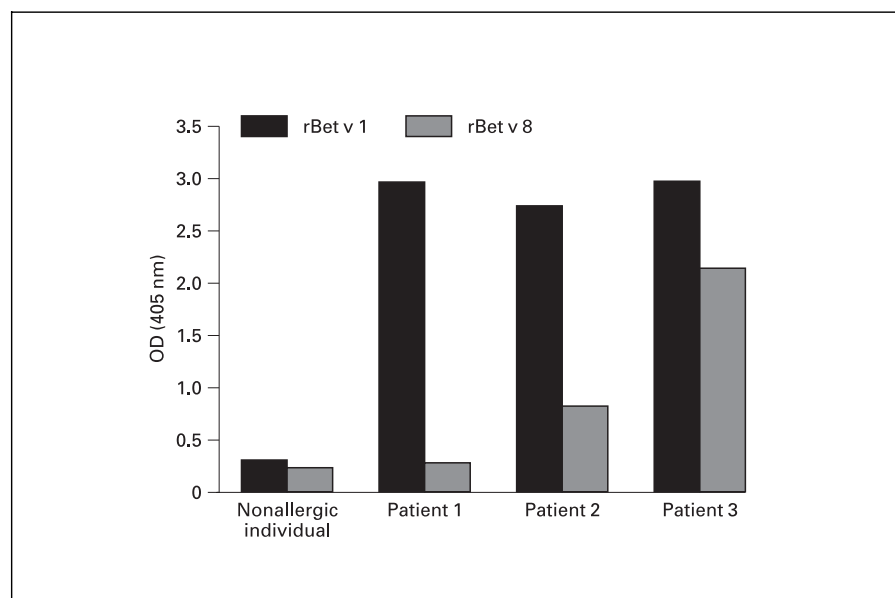


Table 1. Characterized birch pollen allergens [1–7]

	MW kD	Cross- reactivity
Bet v 1	17	+
Bet v 2	14	+++
Bet v 3	21	++
Bet v 4	9.3	++
Bet v 5	33.5	++
Bet v 7	18	?

Results

A complete cDNA coding for a novel birch pollen allergen with a predicted molecular mass of 65.3 kD was obtained. The deduced amino acid sequence of the newly isolated birch pollen allergen, tentatively designated Bet v 8, showed significant end to end sequence homology with pectin esterases from various plant species. A rabbit antiserum raised against the C-terminal portion of Bet v 8 crossreacted with 40–60 kDa moieties present in pollen from trees, timothy grass and mugwort, as well as in peanut, celery and apple. ELISA testing of the recombinant his-tagged Bet v 8 protein showed that it reacted in partic-

ular with sera from birch pollen-allergic patients who were also sensitized against pollen from various other plants and types of plant food (fig. 1; patients 2 and 3).

Discussion

A cDNA coding for a novel birch pollen allergen of high molecular weight (65 kD) was obtained by IgE immunoscreening and oligonucleotide screening of birch pollen cDNA libraries. The cDNA and deduced amino acid sequence of this allergen were highly homologous to plant pectin esterases that are ubiquitously distributed in monocotyledonic and dicotyledonic plants and are mainly known as ripening enzymes that cleave methoxylated pectins [12]. Our data indicate that the newly characterized birch pollen allergen, Bet v 8, represents a novel cross-reactive allergen which may be used as a marker allergen to define a group of patients allergic to pollen, fruits, vegetables and spices.

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