

Identification of Allergens in Apple, Pear, Celery, Carrot and Potato: Cross-Reactivity with Pollen Allergens

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Introduction

Patients with type I allergy to pollens (in particular birch- and mugwort pollen-allergic individuals) frequently suffer from hypersensitivity to a variety of fruits and vegetable additionally [1]. The aim of this work was to identify potentially dangerous foodstuffs by demonstrating the presence of pollen-related proteins in these sources. Protein extracts from apple, pear, celery, carrot and potato (all known to elicit allergic symptoms in pollinosis patients) were investigated. Bet v 1 and Bet v 2 (profilin), important birch pollen allergens, were cloned, sequenced and expressed as recombinant proteins in our laboratory. These recombinant proteins and specific antibodies were used to study cross-reactivities. IgE-binding, pollen (birch, mugwort)-related, cross-reactive proteins (allergens) could be observed in all vegetable foods investigated.

Materials and Methods

Protein extracts: Birch and mugwort pollen were obtained from Allergon AB (Engelholm, Sweden). The following plant sources were investigated: apple (Golden Delicious), pear, celery, carrot and potato. Pollens and vegetable foods were extracted as described [2].

Expression and purification of recombinant Bet v 1 and recombinant birch profilin (rBet v 2): *Escherichia coli* JM 105 harboring the recombinant pKK 223-3 plasmids, were grown and harvested as described [3]. Bet v 1 was purified by chromatofocusing and HPLC on

C8-reversed phase columns as described in detail [3]. Purification of rBet v 2 was performed by affinity chromatography to poly(L-proline) as described previously [4].

Sera: Sera from patients suffering from pollinosis (typical case history, positive skin prick tests, and RAST class 3.5–5.0 to pollens) were collected. These patients were selected because they had experienced adverse reactions to at least two of the following plant-derived foodstuffs: apple, pear, celery, carrot, potato. Sera from nonallergic individuals (negative case history, negative skin prick tests, negative tree pollen RAST) were pooled (NHS) and used for control experiments.

SDS-PAGE, protein transfer and IgE immunoblotting: SDS-PAGE (12% homogeneous gels, 140×115×1 mm, 5% stacking gels) was performed in a vertical slab gel apparatus (Hoefer, San Francisco, Calif., USA) as previously described [5]. All sera were tested for IgE binding to birch and mugwort pollens, apple, pear, celery, carrot and potato.

IgE inhibition experiments with purified recombinant allergens. Serum pool for inhibition experiments: Sera from patients with reactivity to Bet v 1 and Bet v 2 in the birch pollen extract and displaying reactivity to proteins in the same molecular weight range (13–18 kD) in mugwort, fruit and vegetable extracts investigated, were pooled. This serum pool was used in 1:10 dilution for all inhibition experiments. The serum pool was preincubated overnight at 4°C with 10 mg purified recombinant Bet v 1 or Bet v 2, respectively. Thereafter, NC-blotted food proteins were incubated with the serum samples (1 ml/NC strip) and bound IgE was detected as described [6].

Results and Discussion

Tree pollen allergy is a common disease in countries of the Northern hemisphere. In this respect, the pollens of trees of the order *Fagales* (e.g. birch, hazel, alder, hornbeam) represent an important source of allergens. The major allergens of these pollens are highly homologous on the nucleic acid and protein level, and therefore cross-react, so that virtually all tree pollen-allergic patients suffer from type I reactions during the flowering season of all the trees of this particular order in spring [7, 8]. The clinical observation that birch pollen-allergic patients frequently develop symptoms after ingestion of particular fruits and vegetables is well established [1, 2, 6, 9, 10]. Mugwort-sensitive subjects frequently display allergic symptoms to celery [11–13]. The symptoms have mostly local character, but in several cases systemic symptoms like urticaria, angioedema or anaphylaxis have been reported. For particular food sources (i.e. apples, hazelnuts) the presence of allergens, cross-reacting with allergen Bet v 1 [14], the major birch pollen allergen and for Bet v 2 (profilin [15]) have been demonstrated [1, 2, 6, 13, 16]. Moreover, the pan-allergen profilin has been shown to be present in the mugwort pollen extract [17]. This study addressed the question whether cross-reacting proteins to these two molecules could be detected in all those plant-derived foods, which are frequently not well tolerated by pollen-allergic individuals. IgE immunoblot analysis revealed IgE binding to extracts produced from

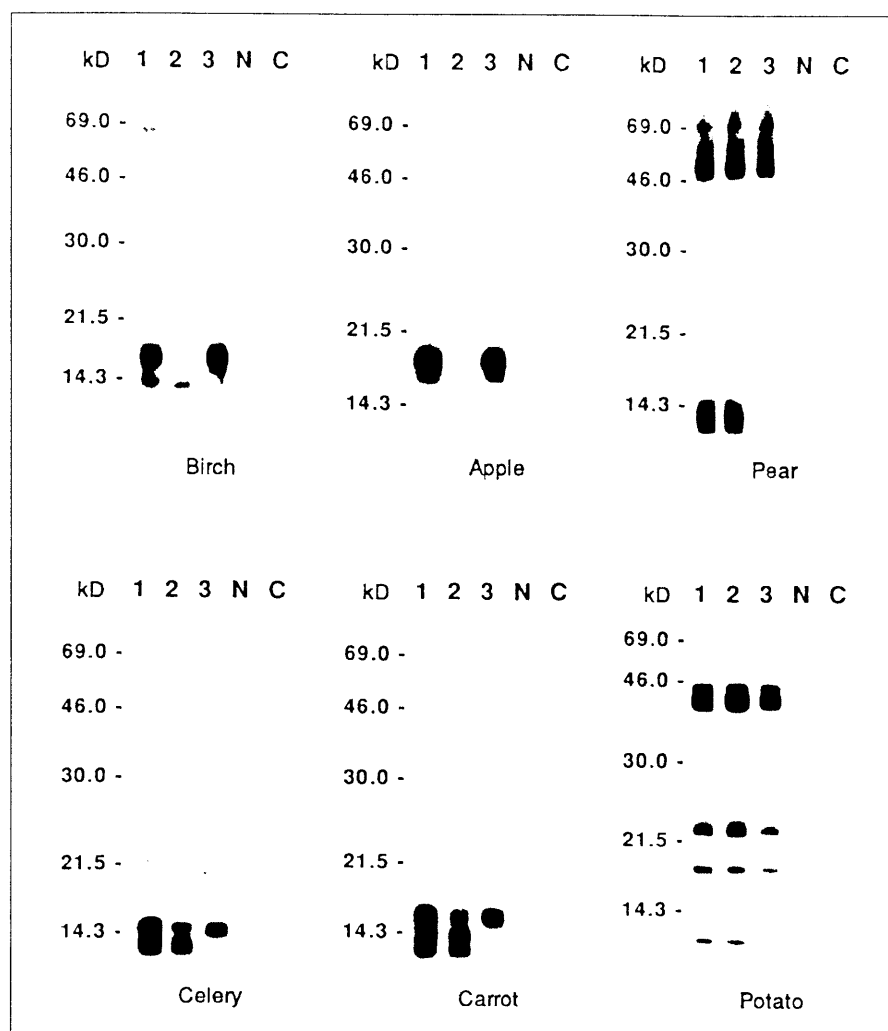


Fig. 1. Inhibition experiment. A serum pool of patients displaying IgE binding to Bet v 1 and Bet v 2 was tested for IgE reactivity with birch, apple, pear, celery and potato extract. Lane 1: no preincubation of the serum pool; lane 2: preincubation with rBet v 1; lane 3: preincubation with rBet v 2; N,C: normal human serum pool and buffer controls.

the plant sources in almost every birch pollen-allergic patient, proteins of similar molecular weight were recognized by patients' IgE in all extracts (not shown). Cross-reactivity of homologous proteins with Bet v 1 was proved by inhibition experiments. By preincubation with recombinant Bet v 1 it was possible to block or markedly reduce IgE binding to homologous proteins in apple, pear, celery and carrot, no significant inhibition could be demonstrated in potato (fig. 1). BIP 1 [18], a monoclonal antibody raised to Bet v 1, displayed a positive signal in birch apple, celery and pear but did not bind to proteins present in carrot or potato extracts, indicating shared epitopes, but also providing evidence for differences in the epitope composition of the different allergens. 25% of the sera from our food-allergic patients collective displayed IgE binding to Bet v 2 in the birch pollen extract and to the mugwort profilin in the respective preparation. In all cases, reactivity of patients' sera proved the presence of a profilin homologue in all plant extracts tested. Also in this case, cross-reactivity was proven by cross-inhibition (positive for all extracts including potato; fig. 1) and by cross-reactivity of a profilin-specific antibody [16]; positive signal in every extract tested).

It can be concluded that cross-reactivity between vegetable foods and birch pollen is due to Bet v 1-homologous allergens (Bet v 1-related allergens from apple, Mal d 1, and celery, Api g 1, have recently been cloned and sequenced [19–21]) and birch profilin (Bet v 2). Mugwort pollen cross-reacts via profilin with those foods. Moreover, a 40- to 60-kD allergen, shared by birch, mugwort, celery and other vegetables seems to play an important role in pollen-related food allergies [L. Bauer, unpubl. results]. Many fruits and vegetables contain pollen-related allergens which can lead to symptoms in atopic patients. We consider these foods as potentially harmful for pollen-allergic individuals with elevated levels of specific IgE to either Bet v 1 or to the pan-allergen profilin.

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