

N-terminal sequences of high molecular weight allergens from celery tuber

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Summary

Background Celery tuber is an important source of food allergens. Low molecular weight celery allergens were identified as homologues of Bet v 1 and profilin. Little is known about the relevant allergens with molecular weights between 45 and 60 kDa, which cross-react with other plant food and pollen allergens.

Objective The aim of this study was to isolate cross-reactive, high molecular weight allergens from celery and to characterize them by N-terminal sequencing.

Methods High molecular weight allergens of celery were identified by immunoglobulin (Ig) E immunoblotting with patients' sera, and the IgE-binding patterns were compared with those of the monoclonal antibirch pollen antibody BIP3, as well as of a polyclonal rabbit anti-Art v 1 antiserum. Two independent methods, elution from preparative SDS-PAGE or anion exchange chromatography, were used to purify the IgE-binding celery proteins of interest. The isolated proteins were examined by N-terminal sequencing and IgE-immunoblots.

Results Celery allergens with molecular masses of 55, 58 and 63 kDa, which were also recognized by the monoclonal BIP3 antibody and a polyclonal anti-Art v 1 antiserum, were isolated. The 63-kDa allergen was N-terminally blocked. The 55- and 58-kDa compounds yielded the same N-terminus, which showed no homology to known proteins in the databases.

Conclusion The combination of two independent protein separation techniques, immunoblotting and N-terminal sequencing, identified an N-terminus of two allergens in the 60-kDa molecular weight region. Our data will be helpful for the definite molecular characterization of these important cross-reactive molecules.

Keywords: Api g 5, birch pollen, celery, cross-reactive allergen

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Introduction

Celery tuber is an important source of food allergens in Central Europe [1,2]. When eaten raw or cooked it can induce various immunoglobulin (Ig) E-mediated reactions

ranging from local symptoms in the oral mucosa to life-threatening anaphylactic shock. Almost every celery-allergic patient exhibits sensitization to birch and/or mugwort pollen, therefore the term 'birch-mugwort-celery-syndrome' was proposed [3]. Serological IgE cross-reactivity with celery is due to at least three classes of allergenic proteins: (i) homologues of the major birch pollen allergen Bet v 1 [4,5], in celery designated Api g 1 [6] (ii) profilins [5,7,8], and (iii) allergens of 32–70 kDa [9]. Little is known about the higher molecular weight allergens, which also

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cause IgE cross-reactivity in 30–40% of celery allergic patients [8,10].

Antigenic cross-reactivity between these allergens in extracts of celery, birch pollen and mugwort pollen was observed using murine monoclonal antibodies. BIP3, a monoclonal antibody raised against birch pollen proteins between 32 and 63 kDa [11], recognized cross-reactive homologues in celery and mugwort pollen, and in extracts of the latex from *Hevea brasiliensis* (data not shown). Furthermore, monoclonal antibodies against the major mugwort pollen protein Art v 1, a glycoprotein of 60 kDa, were used to detect cross-reactive proteins in this molecular weight area in extracts of other plants, including celery [12–14].

So far, there are few data available on a definite characterization of this important class of cross-reactive allergens. To further explore them, we purified IgE-binding celery proteins in the molecular weight range of 55–63 kDa by two independent methods. The isolated proteins were analysed by N-terminal sequencing.

Materials and methods

Protein extracts, SDS-PAGE and immunoblotting

Celery (*Apium graveolens*) tuber was purchased from a local retail shop, ground in liquid nitrogen and proteins were extracted by shaking overnight at 4 °C in potassium phosphate buffer. After centrifugation (40 000 g, 60 min, 4 °C) the supernatant was filtered, dialysed and lyophilized.

Pollen from birch (*Betula verrucosa*) were obtained from Allergon AB (Engelhom, Sweden), extracted in a carbonate buffer solution at 4 °C for 20 h and centrifuged (23 000 g, 60 min, 4 °C). The extract was then dialysed and lyophilized.

Protein extracts were separated by 8% sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and transferred to Protran nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) for immunodetection.

Immunoblots were performed as described before, using TBS/0.05% Tween 20/1% powdered milk for blocking, washing and antibody dilutions. Blotted proteins were incubated with sera from patients with positive case histories, skin prick tests and RASTs to celery and birch pollen (diluted 1 : 4). Bound IgE was detected using ¹²⁵I-labelled rabbit antihuman IgE (RAST RIA, Pharmacia, Uppsala, Sweden) diluted 1 : 10.

Alternatively, blot strips were incubated with an undiluted BIP3 hybridoma supernatant [11]. Bound IgG was detected using rabbit antimouse Ig (Nordic Immunology, London, UK) followed by ¹²⁵I-labelled donkey antirabbit IgG (Amersham, UK), both diluted 1 : 1000.

Polyclonal rabbit anti-Art v 1 antiserum (kindly provided by Dr B. Weber, Allergopharma, Reinbek, Germany) was

diluted 1 : 1000 and bound IgG was detected by ¹²⁵I-labelled donkey antirabbit IgG.

Isolation of proteins by gel elution

Celery tuber proteins were separated by preparative 8% SDS-PAGE. Gels were stained with Coomassie Brilliant Blue R 250. Destaining was done in 10% 2-propanol and 7% acetic acid. The 63 kDa allergen was cut out on a transilluminator. Gel slices were equilibrated in running buffer containing 0.5% (w/v) bromophenol blue and loaded on a 30% supporting gel. Stacking gel was added in two layers. Running buffer was used double concentrated in contrast to normal SDS-PAGE. Electrophoresis was run with reversed poles at 150 mA constant current. Proteins were eluted into a layer of TRIS-HCl buffer (pH 6.8) containing 30% (v/v) glycerol. The eluent was dialysed and then lyophilized.

Purification of the 60-kDa allergen by chromatography

Eight milligrams celery extract were dissolved in 40 mL buffer A (20 mmol/L TRIS-HCl, pH 7.5) and loaded onto a Mono Q HR 5/5 anion exchange column (Pharmacia Biotech, Uppsala, Sweden). After elution of unbound compounds with buffer A, proteins were eluted by a linear gradient from 0 to 40% buffer B (20 mmol/L TRIS-HCl, pH 7.5 containing 1 mol/L sodium chloride) within 40 mL at a flow rate of 1 mL/min. Five hundred-microlitre fractions were collected and analysed by SDS-PAGE and immunoblotting using celery and birch pollen allergic patients' sera.

N-terminal amino acid sequencing

For N-terminal amino acid sequencing 10 µg of lyophilized eluent was separated by 15% SDS-PAGE and blotted to a polyvinylidene difluoride (PVDF) membrane (Immobilon-P, Millipore, Bedford, MA, USA). The membrane was stained with 0.1% (w/v) Coomassie Brilliant Blue R 250 in 50% methanol. For localization of the antigenic protein, strips from the PVDF blots were tested with BIP3 or serum IgE. The bands on the PVDF membrane, which bound IgE, BIP3 or polyclonal anti-Art v 1 IgG, were excised and subjected to sequence analysis using a Hewlett-Packard G1005A (Hewlett-Packard Co, Palo Alto, CA, USA) protein sequencing system. Protein samples were loaded directly onto the hydrophobic part of a biphasic sequencer column and subjected to Edman degradation using the manufacturer's Routine 3.0 chemistry.

Results and discussion

BIP3, a monoclonal antibody originally generated by immunizing mice with birch pollen extract [11], as well

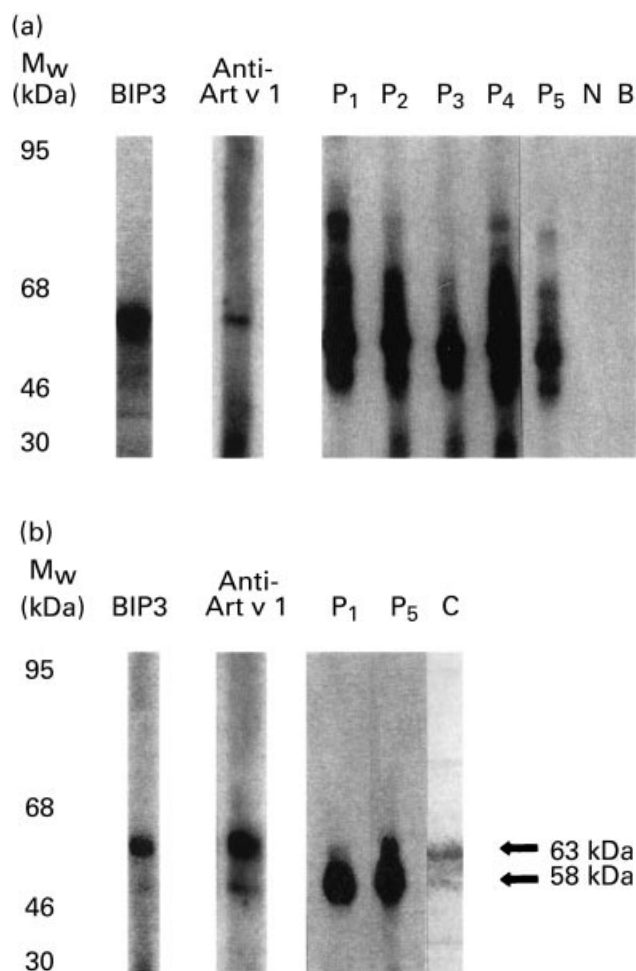


Fig. 1. Celery proteins isolated by gel elution are recognized by murine monoclonal antibody BIP3, polyclonal rabbit anti-Art v 1 antiserum and human IgE. Immunoblotting of celery extract (a) or purified celery antigens (b). Monoclonal antibirch pollen antibody BIP3 (lanes BIP3) and polyclonal rabbit anti-Art v 1 antiserum recognize antigens in celery extract and the purified celery antigens. Sera of patients allergic to birch pollen and celery (lanes P₁-P₅) show IgE binding to blotted celery extract and to isolated celery antigens. Lane N: serum pool from non allergic control persons. Lane B: buffer control. Bound Ig were detected by ¹²⁵I-labelled antibodies. The autoradiograms are shown. Lane C: Coomassie stain of the separated celery antigens blotted to a PVDF membrane for N-terminal sequence analysis

as a polyclonal rabbit anti-Art v 1 antiserum [12] directed against a major mugwort pollen allergen, recognized high molecular weight celery proteins (Fig. 1a). This observation is in concordance with previous reports describing cross-reactivity of plant allergens in this molecular weight range [9,10,12,13]. Moreover, the typical IgE reactivity of sera from patients allergic to celery and birch pollen with nitrocellulose-blotted celery proteins is shown (Fig. 1a).

Table 1. Amino acid sequences of the celery antigens purified by two independent methods

Purification method	Amino acid sequences
Gel elution	LPNPSGFVTSLSVI
Chromatography	XPNPSGFVTXLXXIXKXV
Combined sequence	LPNPSGFVTSLSVIXKXV

The IgE binding to molecules ranging from 55 to 63 kDa in extracts of celery (and in birch pollen, data not shown) was strongly reminiscent of the binding pattern of BIP3 and of polyclonal anti-Art v 1 antiserum. These Ig-binding similarities suggested that these antibodies and human IgE might recognize similar proteins.

To gain more information about these IgE-binding allergens, celery proteins were isolated by two independent methods. First, celery proteins were separated by preparative SDS-PAGE and a major protein band of 63 kDa was purified by gel elution. After a further separation step by SDS-PAGE two proteins could be detected: the original protein with a molecular weight of 63 kDa and a minor component of 58 kDa. Immunoblotting showed that the dominant protein of 63 kDa was preferentially recognized by monoclonal BIP3 antibody and polyclonal anti-Art v 1 antiserum, but to a lesser extent by human IgE (Fig. 1b). In contrast, the 58-kDa protein showed more intense IgE-binding but less binding of murine and rabbit IgG. The isolated proteins were subjected to sequence analysis. The N-terminus of the 63-kDa celery antigen was blocked. Analysis of the 58-kDa allergen yielded the sequence LPNPSGFVTSLSVI (Table 1). However, both antigens were recognized by IgE as well as by antibodies BIP3 and polyclonal anti-Art v 1 antiserum, although to a different extent. These antigens might therefore harbour similar epitopes. On the other hand, the 63-kDa celery antigen, which was N-terminally blocked, might be degraded during the separation procedure producing a smaller protein fragment. Thereby the N-terminus might be unblocked, and conformational changes might occur, resulting in better accessibility of IgE epitopes.

By anion exchange chromatography, two IgE-binding proteins with molecular masses of 55 and 58 kDa co-eluted (Fig. 2) when separating celery extract. N-terminal sequence analysis of both compounds yielded the N-terminus XPNPSGFVTXLXXIXKXV, which matched the sequence obtained from the protein purified by preparative SDS-PAGE.

The sequences resulting from the two independent methods form the consensus sequence LPNPSGFVTSLSVIXKXV (Table 1). No homologous sequences were found in

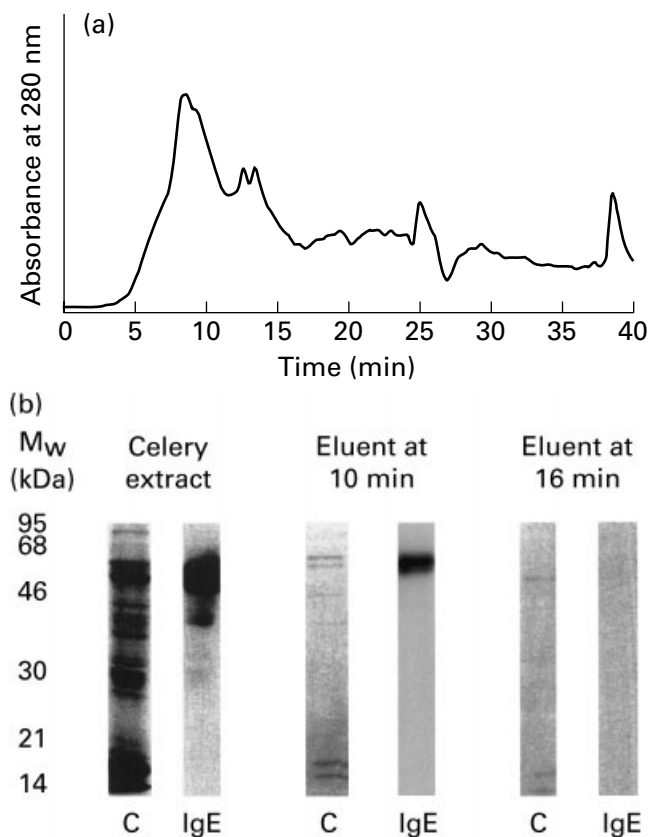


Fig. 2. Purification of high molecular weight celery antigens from celery tuber extract by anion exchange chromatography. (a) Chromatogram: elution was performed at 1 mL/min using a linear salt gradient of 10 mmol/L/min. Proteins were tracked by UV detection at 280 nm. (b) SDS-PAGE analysis/IgE immunoblotting of two eluent-fractions (at 10 and 16 min) containing high molecular weight proteins in comparison with whole celery extract. Lanes C: Coomassie stain. Lanes IgE: Blots incubated with serum of patient P₁. Bound IgE was detected by ¹²⁵I-labelled antihuman IgE. The autoradiogram is shown

the databases (SWISS-PROT, GenBank and EMBL) indicating that the 55 and 58 kDa antigens represent members of a protein family not described so far. However, these data are a starting point for cloning experiments by PCR using degenerate primers. These proteins are obviously size variants of a new allergen, tentatively designated Api g 5. Besides Bet v 1 and profilin [5,6] this allergen seems to be involved as a third cross-reactive protein in the celery-birch-mugwort-spice syndrome [3,9,15–17] and is especially important for patients with primary sensitization to mugwort pollen [10].

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