

# Characterization of Api g 1.0201, a New Member of the Api g 1 Family of Celery Allergens

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## Key Words

Major celery allergen · Isoforms · Api g 1.0201 · Food allergy · IgE-binding

## Abstract

**Background:** The association of pollinosis with allergy to plant foods occurs in up to 70% of tree pollen-allergic patients. In recent years, some of the relevant cross-reacting proteins have been characterized at the molecular and immunological level. Api g 1 has been identified as the celery homologue of the major birch pollen allergen, Bet v 1. Although a number of Bet v 1 isoforms have been characterized from birch pollen, little is known about isoforms of food allergens and their allergenic features. **Methods:** Api g 1.0201, an isoform of Api g 1, was isolated from a cDNA library, cloned and sequenced. The cDNA was expressed in *Escherichia coli* and the purified recombinant protein was tested in immunoblots. **Results:** Api g 1.0201 displays 72% sequence similarity to

the previously identified Api g 1.0101 and consists of 159 amino acid residues. The sequence of Api g 1.0201 has five additional amino acid residues at the carboxy-terminus as compared to Api g 1.0101. Purified recombinant Api g 1.0201 is recognized by IgE from the sera of celery-allergic patients, as well as by the murine monoclonal anti-Bet v 1 antibody. In general, this isoform displays a weaker IgE-binding capacity than Api g 1.0101, as concluded from immunoblotting experiments. Results from inhibition assays revealed that IgE-binding to Api g 1.0201 is only slightly reduced by preincubation with either purified recombinant Api g 1.0101 or purified recombinant Bet v 1a. Total inhibition was only achieved when using purified natural Bet v 1. **Conclusions:** At present, little is known about the IgE-binding capacity of isoforms of Bet v 1 homologues of food allergens. Identification and characterization of such isoforms may help to contribute to a better understanding of food allergy and the observed cross-reactivity to pollen allergy.

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The Api g 1.0201 encoding sequence data reported in this paper have been submitted to the EMBL nucleotide sequence database and have been assigned the accession number Z75662.

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## Introduction

One fourth of American households changes their dietary habits because at least one family member is perceived to have food allergies [1]. In contrast, various clinical studies in European countries and the USA have indicated a prevalence of food allergy of only 1–2% in adults [2]. Prevalence in young children and atopic individuals is higher; for example, 8% of children up to 3 years old showed a confirmed food allergy in an American study [3]. The pathological basis of IgE-mediated reactions to food is the cross-linking of antigen-specific IgE on the surface of mast cells and basophils following ingestion of a specific allergen in an individual sensitive to that allergen. Food allergy has to be differentiated from other adverse reactions to food, such as toxic reactions and non-immune-mediated reactions, referred to as food intolerances [4].

A widespread syndrome of food allergy is the oral allergy syndrome (OAS) [1, 5], which manifests itself within seconds after food ingestion by pruritus and swelling of the lips, tongue and soft palate, often accompanied by mild laryngeal symptoms such as a sensation of tightness, itching, cough and pruritus of the ear canals. Other symptoms of food allergy include gastrointestinal symptoms, rhinitis, asthma, cutaneous reactions and, the most severe, systemic anaphylaxis.

The oral allergy syndrome is frequently observed in reactions of pollen-allergic patients to fresh fruits or vegetables. Recent studies have shown that sensitization to cross-reactive allergens present in food and inhalant sources may explain the development of food allergy [6–8]. The immunological cross-reactivity of allergens poses certain problems for a precise diagnosis. For example, multiple sensitization to legumes was described as very common and confirmed by immunoblot studies, but double-blind placebo-controlled food challenges (DBPCFC) demonstrated, in most cases, a cross-reactivity of no clinical importance. Therefore, this cross-reactivity represents a mere *in vitro* phenomenon [9].

Celery is a major elicitor of food allergy in central Europe. In a study of 402 established food-allergic patients in Switzerland, 42% of them showed IgE reactivity to celery [10]. Almost every celery-allergic patient exhibits sensitization to birch and/or mugwort pollen; therefore, the term 'birch-mugwort-celery syndrome' was proposed [7, 11–13]. Serological IgE cross-reactivity with celery is due to at least three classes of allergenic proteins: profilin, a protein with a molecular weight of 14 kD, identified as a ubiquitous plant allergen [14, 15]; the 17-kD allergen

Api g 1 [16], a homologue of the major birch pollen allergen Bet v 1 [17], and a group of yet unidentified allergens with molecular weights between 46 and 70 kD [7].

The major birch pollen allergen, Bet v 1, is composed of a mixture of isoforms with similar sequences but different immunological properties with respect to their IgE-binding capacities, as well as their ability to stimulate allergen-specific T cells [18]. Knowledge of the repertoire of allergenic and nonallergenic isoforms may lead to the development of panels of well-characterized proteins to be used either for improved diagnostic assays or for patient-tailored immunotherapy [18, 19].

At present, there is only a small amount of data on isoforms of Bet v 1-homologous food allergens; isoforms of Mal d 1, the Bet v 1 homologue from apple, have been identified [20–23], and for some isoforms their IgE-binding capacity has been determined [20–23].

Much less is known about isoforms of Api g 1, the Bet v 1-homologous allergen from celery. So far, only a small number of studies concerning the possible spectrum of isoforms and their implications for accurate diagnosis have been performed [18, 23]. Here, we report the isolation and characterization of Api g 1.0201, an isoform of the major celery allergen, and its immunological properties, which are different from Api g 1.0101, formerly designated Api g 1 [16].

## Materials and Methods

### *Isolation of PolyA+ mRNA from Celery and cDNA Library Construction*

RNA was extracted from 25 g of both celery (*Apium graveolens*) tuber and stems by the acidic guanidinium thiocyanate method [24]. The celery tuber and stems were initially cut into small pieces and ground to a fine powder with a pestle and mortar under liquid nitrogen. The powder was then transferred to cooled centrifugation tubes, extraction buffer added and the RNA was extracted as previously described [24]. PolyA+ RNA was isolated from total RNA by biotinylated oligo(dT) and streptavidin coupled to paramagnetic beads from the PolyATtract mRNA Isolation System (Promega, Madison, Wisc., USA).

Complementary DNA was synthesized using a hybrid oligo dT linker primer which contains an *Xho* I site and was hemimethylated. *Eco* RI linkers were added to the blunt-ended cDNA and the cDNA ligated via the *Eco* RI and *Xho* I sites into the  $\lambda$ -ZAP (Stratagene, La Jolla, Calif., USA) expression vector. The library was constructed according to the Stratagene protocol. A primary library of  $1 \times 10^5$  plaque-forming units (PFU) was amplified to generate a higher titer of  $10^9$  PFU.

### *Immunoscreening of the Library*

The celery  $\lambda$ -ZAP library was screened with a pool of three sera from celery-allergic patients. Approximately  $3 \times 10^5$  phages were

used to infect the *E. coli* strain XL-1 Blue, plated on LB medium plus 10 mM MgSO<sub>4</sub> and incubated for 4 h at 42 °C. Then, infected colonies were transferred to nitrocellulose filters at 37 °C for 3–5 h. Afterwards, the filters were washed and dried. Nonspecific binding was blocked by incubation with bovine serum albumin (BSA) in phosphate buffer (50 mM sodium phosphate buffer, pH 7.5; 0.5% BSA, wt/vol; 0.5% Tween 20, vol/vol; 0.05% NaN<sub>3</sub>, wt/vol) for 30 min at room temperature. Filters were incubated with the serum pool (1:4 dilution in phosphate buffer) at 4 °C overnight, followed by washing, and subsequently incubated with <sup>125</sup>I-labeled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden) at room temperature overnight. Finally, the filters were washed and exposed to X-ray films. Positive plaques were picked with a Pasteur pipette and purified by repeating the above procedure. Phages obtained after a third round were treated according to the *in vivo* excision protocol to subclone them in pBK-CMV (Stratagene).

The pBK-CMV phagemid containing the recombinant DNA inserts was isolated and purified according to standard plasmid preparation protocols. Inserts were sequenced by the dideoxy method using fluorescence-labeled primers complementary to the vector sequence [25] and analyzed on an automatic sequencer (Licor, Lincoln, Nebr., USA).

#### *Expression and Purification of rApi g 1.0201*

The complete Api g 1.0201-encoding sequence was subcloned into the high-level expression plasmid, pDS56 [26]. By ligating the coding sequence into the *Bam* HI and *Pvu* II restriction sites of the plasmid, Api g 1.0201 was expressed as a hexahistidyl-tagged protein in *E. coli* M15 cells. Cells were grown in 2 × YT medium (16 g of bacto-tryptone, 10 g of yeast extract and 5 g of NaCl per liter, pH 7.4) to an optical density of 0.6 at 600 nm. Expression of the recombinant protein was induced overnight by inactivation of the *lac* repressor via addition of isopropyl thiogalactopyranoside to a final concentration of 2 mM. Subsequently, the cells were harvested and lysed and the protein fraction was processed as described elsewhere [26]. Cell lysates were resuspended in 6 M guanidinium hydrochloride and 0.1 M sodium phosphate, pH 8.0, and purified by Ni-chelate affinity chromatography. Determination of the protein concentration of purified recombinant (r)Api g 1.0201 was performed by the micro-Kjeldahl assay [27].

#### *Patients, Sera and Antibodies*

Sera of 10 celery-allergic patients were collected according to typical case histories, positive skin prick tests and the display of symptoms after eating celery. Sera of four celery-allergic patients were pooled for inhibition experiments. Control experiments were performed with a pool of 10 sera derived from nonallergic individuals. The murine monoclonal antibody BIP1 [28] was raised against purified natural (n)Bet v 1, the major birch pollen allergen, and polyclonal rabbit antiserum was directed against purified rBet v 1a [29].

#### *Immunoblots and Inhibition Assays*

Total protein extract was prepared from celery bulb and stems as described elsewhere [6]. Recombinant Api g 1.0101 and Bet v 1a were expressed in *E. coli* and purified to homogeneity as previously described [16, 29]. Proteins of celery extracts and rApi g 1.0201 (3 µg per lane), rApi g 1.0101 (3 µg per lane) and rBet v 1a (1.5 µg per lane) were electrophoresed on 12% SDS-PAGE [30]. Subsequently, the proteins were transferred to nitrocellulose membranes [31]. The purified recombinant allergen was tested with the murine monoclonal

anti-Bet v 1 antibody, BIP1 (1:10 dilution of the antibody-containing culture medium in phosphate buffer plus 0.5% BSA wt/vol, as described above) and with a polyclonal rabbit anti-Bet v 1 serum fraction (1:1,000 dilution in phosphate buffer plus 0.5% BSA wt/vol). Detection was carried out with an alkaline phosphatase-conjugated rabbit anti-mouse IgG (1:1,000 dilution in phosphate buffer plus 0.5% BSA wt/vol; Jackson ImmunoResearch) and an alkaline phosphatase-conjugated swine anti-rabbit IgG antibody, respectively (1:1,000 dilution in phosphate buffer plus 0.5% BSA wt/vol; Dakopatts, Glostrup, Denmark) [29].

The IgE-binding ability of the proteins was detected, as previously described, by incubation of sera from allergic patients followed by detection with rabbit anti-human IgE antibodies labeled with <sup>125</sup>I (Pharmacia) [16]. A serum pool from nonallergic individuals or buffer (omitting the primary antibody) was used for controls.

A serum pool from celery-allergic patients was incubated with rApi g 1.0101, rBet v 1a, nBet v 1 and BSA (each 100 µg/ml in phosphate buffer plus 0.5% BSA) for inhibition studies. Afterwards, the remaining IgE-binding to rApi g 1.0201 was tested in an immunoblot assay, as described above.

## **Results**

### *Analysis of cDNA Inserts*

Several positive clones were identified by immunoscreening of the celery λ-ZAP library. After *in vivo* excision, plasmids were isolated and insert sizes were analyzed on an agarose gel. Sequencing of a selected insert revealed a full-length cDNA of 700 bp (fig. 1). An open reading frame of 477 bp encoding a polypeptide of 159 amino acids was identified. Forty-four base pairs of the 5' noncoding region were sequenced. A putative polyadenylation signal was located at position 605 in the 3' noncoding region. The poly(A) tail started 158 nucleotides downstream from the TGA translation termination codon. The 3' noncoding region was of exactly the same length as the 3' noncoding region of Api g 1.0101 (158 bp), and sequence comparison of this region displayed 35% identity. The full-length cDNA clone had 64% sequence identity to Api g 1.0101, as well as to Dau c 1.2, the major carrot allergen.

The polypeptide with a calculated molecular mass of 17,090 lacked tryptophan and had an overall excess of acidic residues with a pI of 4.45. Amino acid sequence comparison revealed 72% similarity and 52% identity to the previously isolated major celery allergen, Api g 1.0101 [17] (fig. 2). The cDNA clone reported in this paper was considered an isoform and designated Api g 1.0201 according to the guidelines for allergen nomenclature [32]. Three-dimensional structure predictions were performed with the SWISS MODEL program on the ExPASy

# **API G 1.0201**

```

-44 gttt teagtttgat caaatcttgc aacttagatt cttaagaat
1  ATGGGTGTCC AAAAGACCGT GGTGAGGCT CCTTCTACCG TGTCTGCGGA
   M G V Q K T V V E A P S T V S A E
51  GAAAATGTAT CAGGAGTTTC TCCTTGATAT GGACACTGTT TTTCCTAAAG
   K M Y Q G F L L D M D T V F P K
101 TTCTTCTCA GCTTATCAAG AGTGTGAAA TTCTTGAGG CGATGGTGGC
   V L P Q L I K S V E I L E G D G G
151 GTTGAACCG TCAAGCTCGT CCATCTTGGC GAAGCTACTG AATATACCAC
   V G T V K L V H L G E A T E Y T T
201 CATGAAGCAA AAGGTGGATG TGATTGACAA GGCTGGATTG GCTTACACTT
   M K Q K V D V I D K A G L A Y T
251 ACACCACCAT CGGAGGCGAC ATTTTGGTGG ACGTACTCGA GTCAGTGGTG
   Y T T I G G D I L V D V L E S V V
301 AATGAATTCG TTGTTGTGCC AACCGACGGA GGTGTCATAG TGAAGAACAC
   N E F V V V P T D G G C I V K N T
351 CACCATTAC AACACCAAAG GCGACGCTGT GCTCCCGGAA GACAAGATCA
   T I Y N T K G D A V L P E D K I
401 AGGAAGCTAC TGAGAACTCT CCTCTGGCCT TCAAAGCTGT TGAAGCTTAC
   K E A T E K S A L A F K A V E A Y
451 CTCCTAGCAA ACTTGCAGTT TCTTGCTTGA taatagtatt atgagtggt
   L L A N L Q F L A *
501 ccggtacttt aatcaagttt gcttgtttgt ttacgagtta tgtatgagtg
551 aactgagggc ttcaactgct gagatatata cacctcgagt atgcatatat
601 catcaataaa acatcgaatc atccgctttt ccatttctaa aaaaaaaaaa
651 aaaaaa
  
```

**Fig. 1.** Nucleotide sequence and deduced amino acid sequence of Api g 1.0201. A putative polyadenylation signal is printed in bold.

WWW server provided by the Swiss Institute of Bioinformatics [33]. This model is based on the three-dimensional structure of one Bet v 1 isoform (ALK 2227), determined both in crystalline-state X-ray diffraction and in solution [34, 35]. Both Api g 1 isoforms displayed a fairly similar structure based on the Bet v 1 structure model. The only significant difference between the Bet v 1 structure and the model of Api g 1.0201 was the conformation of the loop between  $\beta$ -sheet six and seven (fig. 3). This loop is part of one of the three conserved B cell epitopes proposed by Gajhede et al. [35].

A search of the sequence databases revealed 75% similarity (62% identity) to PcPR1-2, a member of the group of pathogenesis-related proteins from parsley [36], and 69% similarity (51% identity) to Dau c 1.1, the major food allergen from carrot [37]. Comparison with the related group of pollen allergens showed 65% similarity (42% identity) to Bet v 1 Sc3, a Bet v 1 isoform expressed in tissue culture [38], and 62% similarity (39% identity) to Bet v 1a, the major birch pollen allergen [17].

## *Expression and Purification of rApi g 1.0201*

Api g 1.0201-encoding cDNA was expressed as a 6  $\times$  His-tagged protein in *E. coli* M15 [pREP4]. Api g 1.0201 was the most prominent band obtained from bacterial lysates when analyzed by Coomassie-stained 12% SDS-PAGE (fig. 4, lane 2). Purification by Ni-chelate chromatography resulted in a pure fraction of rApi g 1.0201 (fig. 3, lane 3). An overall yield of 80 mg of purified recombinant protein per liter was achieved.

**Fig. 2.** Sequence comparison of Api g 1.0201 (upper line, amino acid residues 1–159) and Api g 1.0101 (lower line, amino acid residues 1–154). Identities are marked by vertical lines. Dots indicate gaps in the sequence produced by the aligning program.

| API G 1.0201 : API G 1.0101 |                                                            |
|-----------------------------|------------------------------------------------------------|
| Similarity: 72%             |                                                            |
| Api g 1.0201                | 1 MGVTQTVVEAPSTVSAEKMYQGFLDMDTVFPKVLFPOLIKSVEILEGDGG 50    |
| Api g 1.0101                | 1 MGVTQTVHLELTSSVSAEKIFQGFVIDVDVLPKAAFGAYKSVEI.KGDGG 49    |
| Api g 1.0201                | 51 VGTVKLVHLGEATEYTTMKQKVDVIDKAGLAYTYTTIGGDILVDVLESVV 100  |
| Api g 1.0101                | 50 PGTLLKIITLFDGGPITMTLRIDGVNKEALTFDYSVIDGDILLGFIESIE 99   |
| Api g 1.0201                | 101 NEFVVVPT.DGGCIVKNTTIYNTKGDAVLPEDKIKEATEKSALAFKAVEA 149 |
| Api g 1.0101                | 100 NHVVLVPTADGGSICKTTAIFHTKGDAVVPEENIKYANEQNTALFKALEA 149 |
| Api g 1.0201                | 150 YLLANLQFLA* 159                                        |
| Api g 1.0101                | 150 YLIAN* 154                                             |

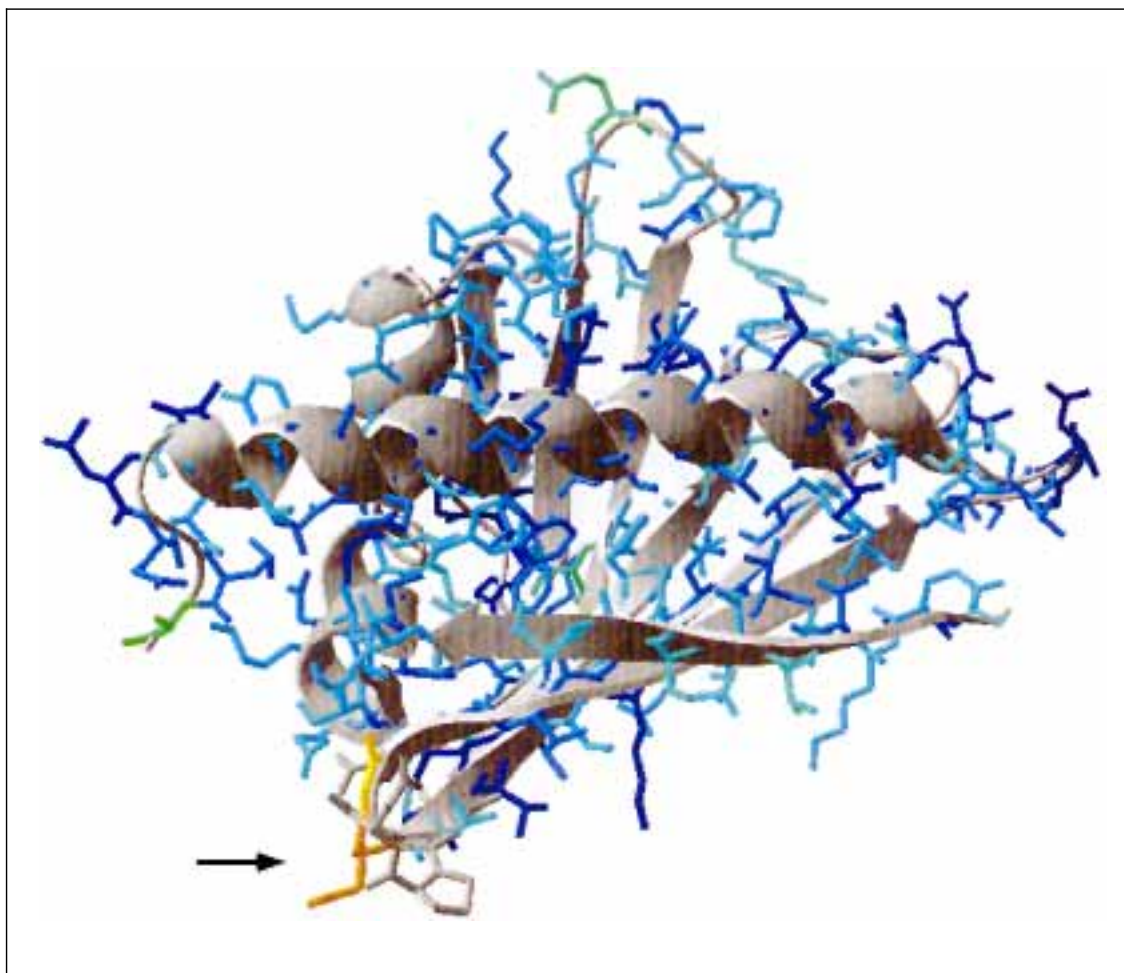


**Fig. 3.** Model structure of Api g 1.0201. The structure was calculated by homology modelling based on the structure of Bet v 1 (ALK 2227). Deviations from the Bet v 1 structure are color coded, ranging from blue (highest correspondence) to red (lowest correspondence). In the loop with the most significant differences to Bet v 1 (marked by an arrow), the Bet v 1 chain is displayed in gray.

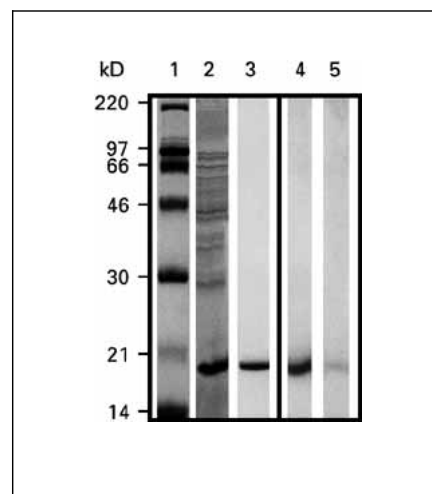


**Fig. 4.** SDS-PAGE and immunoblot of rApi g 1.0201. Bacterial extract of M15 harboring pDS-Api g 1.0201 (lane 2) and purified rApi g 1.0201 (lane 3) were separated by 12% SDS-PAGE and subsequently stained with Coomassie. Purified rApi g 1.0201 (3 µg per lane) was electrophoresed, blotted to nitrocellulose membranes and tested with murine monoclonal anti-Bet v 1 IgG (BIP 1, lane 4) and rabbit polyclonal anti-Bet v 1 IgG (lane 5); marker proteins were run in parallel (lane 1). Detection was performed with alkaline phosphatase-conjugated rabbit anti-mouse IgG and  $^{125}$ I-labeled donkey anti-rabbit IgG, respectively.

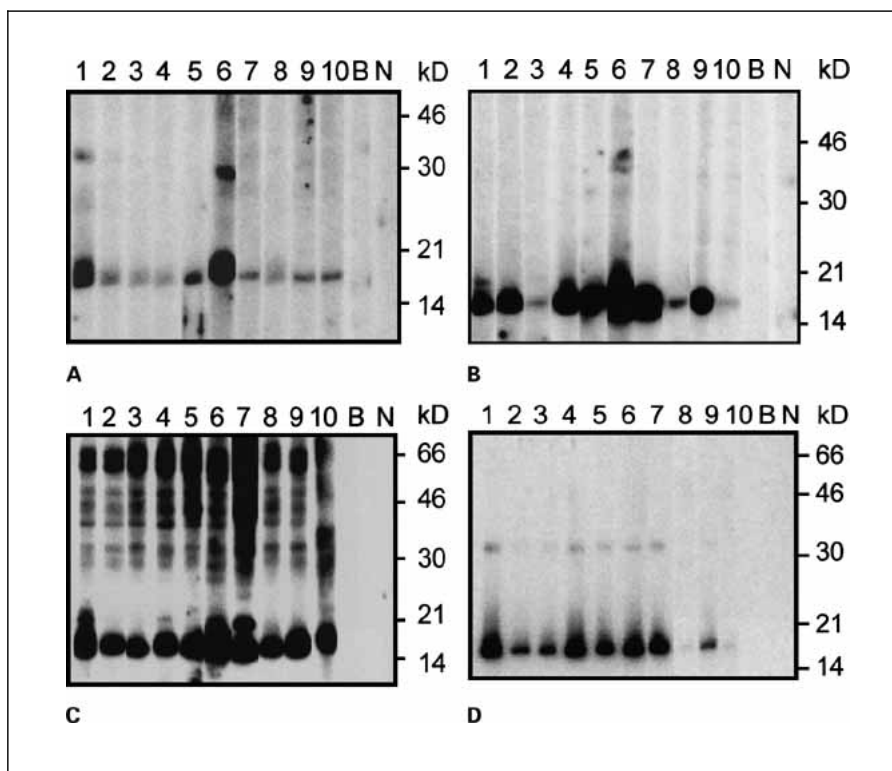


**Fig. 5.** Immunoblot of sera of 10 patients (lanes 1–10) tested with rApi g 1.0201 (**A**), rApi g 1.0101 (**B**), celery extract (**C**) and rBet v 1a (**D**). Controls were performed in parallel with nonallergic human serum (N) and buffer control (B).

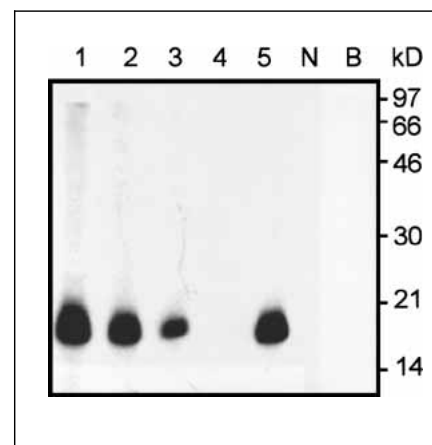


**Fig. 6.** Inhibition analyses. A serum pool from patients with celery allergy was tested for IgE-binding to rApi g 1.0201 without pretreatment (lane 1) and after preincubation with rApi g 1.0101 (lane 2), purified rBet v 1a (lane 3), purified nBet v 1 (lane 4) and BSA (lane 5). Controls were performed in parallel with nonallergic human serum (N) and buffer control (B).

### Immunoblot and Inhibition Analyses

**Monoclonal and Polyclonal IgG.** Purified rApi g 1.0201 was tested in immunoblots with the murine monoclonal anti-Bet v 1 IgG, BIP1, and polyclonal rabbit anti-Bet v 1 IgG (fig. 4, lanes 4 and 5). Both antibody preparations, the polyclonal as well as the monoclonal, cross-reacted with rApi g 1.0201. The buffer control performed in parallel was negative (data not shown).

**IgE.** A panel of 10 sera from celery-allergic patients was tested for IgE-binding to Api g 1.0201 (fig. 5A, lanes 1–10). All sera displayed IgE-binding to purified rApi g 1.0201, with varying binding intensities, as well as to rApi g 1.0101 (fig. 5B, lanes 1–10). In the case of patients 1 and 6, even the oligomers of rApi g 1.0201 and rApi g 1.0101 were weakly recognized by their IgE antibodies. In general, rApi g 1.0101 seems to exhibit a stronger IgE-binding capacity as compared to Api g 1.0201. The same set of sera was also tested with natural celery extract (fig. 5C, lanes 1–10) and purified rBet v 1a (fig. 5D, lanes 1–10). All sera displayed IgE-binding to nApi g 1 as well as rBet v 1a. Sera 8 and 10 displayed very weak IgE-binding to rBet

v 1a, whereas sera 1 and 4–7 showed IgE-binding to a dimer already observed [8, 29]. Buffer control and a serum pool of nonallergic individuals tested negative in all cases (fig. 5A–D, lanes B and N). The same results were obtained when Api g 1.0201 was cloned into the pMW-175 plasmid and expressed as an unmodified recombinant protein in *E. coli* (data not shown).

**Inhibition.** A serum pool from celery-allergic patients was tested for IgE-binding to rApi g 1.0201 in inhibition studies (fig. 6). Compared to the positive control (fig. 6, lane 1), preincubation with purified rApi g 1.0101 did not affect the IgE-binding intensity to rApi g 1.0201 (fig. 6, lane 2), and only a weak signal reduction was detectable after preincubation with rBet v 1a (fig. 6, lane 3). Total blocking of IgE-binding to Api g 1.0201 was only observed after preincubation with purified nBet v 1 (fig. 6, lane 4). Preincubation with the same amount of BSA did not affect IgE-binding to Api g 1.0201 (fig. 6, lane 5).

## Discussion

In this study, we present the cDNA sequence of a celery allergen which has been isolated and identified from a celery stem and tuber cDNA library by its IgE-binding capacity. The 159-amino acid polypeptide displays 72% sequence similarity (52% sequence identity) to the previously characterized major celery allergen, Api g 1.0101 [16], and 39% sequence identity to Bet v 1a, the major birch pollen allergen [17]. The Api g 1.0201 encoding sequence is five amino acid residues longer at the carboxy-terminus than Api g 1.0101. According to the rules for allergen nomenclature [32], isoallergens are defined as several highly similar molecules with identical biological function and sequence identities of more than 67%. Api g 1.0201 has only 52% sequence identity to Api g 1.0101 and is more comparable to PcPR1-2, a homologous protein from parsley. The same divergence can be observed among the PcPR proteins from parsley. PcPR1-3 shares 56% amino acid sequence identity with PcPR2.

The 3' noncoding region is of exactly the same length as in Api g 1.0101. Purified recombinant Api g 1.0201 displays IgE-binding to the same set of sera as does purified recombinant Api g 1.0101, but with lower binding intensity. The same results were obtained whether Api g 1.0201 was expressed as a 6-His-tagged protein or an unmodified protein in *E. coli*. Furthermore, Api g 1.0201 is recognized by the murine monoclonal anti-Bet v 1 antibody, BIP1. Although BIP1 recognizes Bet v 1 homologues present in apple and celery extract, neither purified rMal d 1 [20] nor rApi g 1.0101 [16] are detected by this monoclonal antibody. Therefore, rApi g 1.0201 seems to be the only Bet v 1-related food allergen cloned so far that possesses this epitope. It is tempting to speculate that the five additional amino acid residues located at the carboxy-terminus might be crucial for the BIP1 epitope. Nevertheless, recent data on the identification of the mimotope of BIP1 on the Bet v 1 molecule are not in agreement with this hypothesis [39]. It is known that BIP1 does not recognize an IgE epitope on the Bet v 1 molecule. Nevertheless, it has been shown by Laffer et al. [40] that the binding of Bet v 1 to BIP1 does modulate the IgE-binding of this allergen and homologues thereof. This fact may be of relevance if diagnostic kits include a monoclonal antibody which has already been tested with Bet v 1 dipsticks [41].

Patients with tree pollen allergy frequently display allergic symptoms after eating apples, hazelnuts, cherries, celery, carrots and various spices. These allergic reactions are in part due to the presence of proteins that are homol-

ogous to Bet v 1, the major birch pollen allergen. Several of these cross-reacting allergens have been characterized, and their cDNAs have been cloned from apple (Mal d 1) [20], cherry (Pru av 1) [42], carrot (Dau c 1) [37], celery (Api g 1) [16], and pear (Pyr c 1) [43].

It is generally accepted that primary sensitization is caused by pollen proteins in the upper airways and, subsequently, food-allergic reactions arise in response to related proteins from plant-derived food. In the case of Bet v 1 and its related proteins, sequence similarities range from 59 to 93%, which is reflected by similar structural characteristics and the presence of cross-reactive B cell epitopes. Despite this well-known clinical phenomenon, in some cases monosensitization to Bet v 1 homologues from vegetables, like Dau c 1, have been reported without cross-reactivity to Bet v 1 [44].

So far, inhibition studies have showed that IgE-binding to individual homologous food allergens was inhibited by preincubating the sera with rBet v 1a [16, 20, 37], but not vice versa, indicating that these allergens contain IgE epitopes in common with Bet v 1a. In addition, data gained from cross-inhibition assays indicate that on each Bet v 1-related food allergen, a different and individual set of IgE epitopes is present. In the case of rApi g 1.0201, preincubation with rBet v 1a was not able to block IgE-binding, whereas purified nBet v 1, consisting of a number of highly similar isoforms, prevented IgE-binding to rApi g 1.0201.

Based on the concept of cross-reactivity, a specific immunotherapy directed against pollen allergens should also lead to a reduction in pollen-related food-allergic reactions. Asero [45], in his prospective study, observed a significant reduction in food-allergic symptoms after specific immunotherapy with birch pollen extract in 84% of birch pollen-allergic patients with oral allergy syndrome to apple [45]. Nevertheless, these effects are not accompanied by a decrease in apple-specific IgE.

A number of Bet v 1 isoforms have already been identified in birch pollen by cDNA cloning by PCR, as well as by mass spectroscopy analyses. Although the sequence similarities were found to be considerably high (84.4–99.4% amino acid identities) [46], individual isoforms displayed remarkable differences in their IgE-binding capacities [18, 47]. For some of these low-IgE-binding isoforms, it has been shown that the T cell recognition and thus T cell-activating capacity was equivalent or higher than for Bet v 1a. Therefore, these hypoallergenic molecules may represent safer tools for immunotherapy, since they bear a lower risk of IgE-mediated anaphylactic side effects [18].

Recently, mutants of Mal d 1 and Api g 1 with reduced IgE-binding capacity compared to the naturally occurring isoforms have been cloned and expressed in *E. coli* [23, 48]. In both cases, several amino acid residues critical for IgE-binding to Bet v 1, as well as to homologous proteins, were defined. Son et al. [23] concluded that a serine at position 111 in Mal d 1 was essential for IgE-binding. Ferreira et al. [48] determined amino acid residues 10, 30, 57, 112, 113 and 125 to be critical for IgE-binding. Api g 1.0201 displays the features of a low IgE-binder in two of these positions. There is a proline at position 11 as in the low-IgE-binding Bet v 1a(1–6) mutant [49]. Additionally, there is a cysteine instead of a serine at position 113. Together with a missing proline after threonine 108 in the loop between  $\beta$ -sheet six and seven, this exchange may largely influence the conformation of this loop, as suggested by homology modelling. This region is part of one of the conserved B cell epitopes suggested by Gajhede et al. [35]. Changes in this structure may therefore account for the reduced IgE-binding capacity of Api g 1.0201 when

compared to Api g 1.0101. These hypoallergenic molecules, regardless of whether they are derived from natural sources or engineered according to mutational analyses, could represent valuable tools for immunotherapy and may remarkably decrease the risk of anaphylactic side effects.

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