

Molecular characterization of Api g 1, the major allergen of celery (*Apium graveolens*), and its immunological and structural relationships to a group of 17-kDa tree pollen allergens

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Individuals suffering from immediate hypersensitivity (type-I allergy) to a particular pollen frequently display intolerance to several foods of plant origin. In this respect, individuals sensitized to birch pollen and/or mugwort pollen frequently display type-I allergic symptoms after ingestion of celery. In this study, we expressed the major allergenic protein of celery, Api g 1, which is responsible for the birch-celery syndrome, in the form of a non-fusion protein. The open reading frame of the cDNA of Api g 1 codes for a protein of 153 amino acids with a molecular mass of 16.2 kDa and 40% identity (60% similarity) to the major allergen of birch pollen, Bet v 1. Furthermore, Api g 1 exhibited similar characteristics to (a) two proteins in parsley induced by fungal infection, (b) the major tree pollen allergens and (c) pathogenesis-related and stress-induced proteins in other plant species. The reactivity of recombinant Api g 1 with IgE antibodies present in sera from celery intolerant patients was comparable to that of the natural celery allergen. Cross-reactivity with Bet v 1 was proven by cross-inhibition experiments, which provides further support for the existence of the birch-celery syndrome and for the suggestion that allergies to some vegetable foods are epiphenomena to allergies caused by inhalation of tree pollen.

Keywords: Api g 1; Bet v 1; IgE cross-reactivity; vegetables; type-I allergy.

Ingestion of celery frequently leads to intolerance reactions in man, a fact that has been known for almost 70 years [1–9]. The symptoms comprise local reactions (oral itching, inflammation of the lips and oral mucosa, angioedema of the throat, gastroenteritis, and diarrhoea), and a considerable number of patients experience systemic symptoms (urticaria, asthma, anaphylactic shock). In the light of current knowledge, these symptoms have the characteristics of IgE-mediated allergic reactions referred to as type-I allergies. Some of these patients also exhibit identical reactions after ingestion of other plant foods belonging to the taxonomic families *Rosaceae* (apple, pear, cherry, peach), *Apiaceae* (celery, carrot) and *Solanaceae* (tomato, potato) [10–14]. Moreover, it has been recently demonstrated that patients with type-I allergies to the major birch-pollen allergen, Bet v 1 [15], and to the major pollen allergens of related trees of the *Fagales* order [16–18], frequently display an intolerance, i.e. presumably an allergic reaction, to a variety of fruits and vegetables.

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Abbreviations. Bet v 1, major allergen from birch pollen; BIP 1, anti-(Bet v 1) murine mAb; PR, pathogenesis related.

Note. The nucleotide sequence reported in this paper has been submitted to the EMBL/Gen Bank/DBJ nucleotide sequence databases and is available under accession number Z48967.

The presence of proteins in celery cross-reacting immunologically with Bet v 1 was demonstrated by IgE immunoblot analysis and by inhibition experiments [9]. Furthermore, the murine mAb to Bet v 1 (referred to as BIP 1) reacted with a 15-kDa component of an aqueous protein extract from celery tuber [9]. Hence, it may be concluded that celery contains birch-pollen-related allergens which can lead to allergic symptoms in patients allergic to birch pollen after ingestion of celery. Indeed, polyclonal IgE in sera of birch-pollen-allergic patients bind to celery proteins and serological studies have led to the conclusion that celery and birch pollen allergens share several B-cell epitopes [9]. This study was designed to confirm the presumed relationship between Bet v 1 and the corresponding celery allergen at the molecular level.

In this study, the complete amino acid sequence of the major celery allergen, Api g 1, was derived on the basis of the nucleotide sequence of a cDNA clone obtained from celery tuber mRNA; the sequence is in complete agreement with a partial N-terminal protein sequence of Api g 1, which was determined by automated solid-phase Edman degradation. The IgE-binding properties of the purified recombinant Api g 1, produced in *Escherichia coli*, were compared to those of Bet v 1 and of the natural Api g 1 present in protein extracts from celery tuber.

The difficulties in extracting, purifying and standardizing natural food allergens which are present in minute amounts in vegetables make them less suited for diagnostic and therapeutic purposes than their recombinant counterparts. Therefore, standardized recombinant allergens, which can be produced in un-

limited amounts, will certainly play an increasingly important role for (a) the development of new diagnostic procedures, (b) further investigations of the molecular basis of the IgE response, and (c) development of novel therapeutic strategies [19, 20].

MATERIALS AND METHODS

Extraction and isolation of poly(A)-enriched RNA. Total RNA was extracted from 25 g celery tuber (*Apium graveolens*) by the acidic guanidinium thiocyanate method [21]. The celery tuber was initially cut into small pieces, then ground to a fine powder under liquid nitrogen with a mortar and pestle. The powder was then transferred to cooled centrifugation tubes, the extraction buffer added, and the RNA was extracted as described [21]. Poly(A)-enriched RNA was isolated from total RNA by biotinylated oligo(dT) and streptavidin coupled to paramagnetic particles provided by the PolyAtract mRNA Isolation System (Promega).

Synthesis of first-strand cDNA. Poly(A)-enriched RNA (2.5 µg) was reverse transcribed in a reaction volume of 20 µl using 50 U Moloney murine leukemia virus reverse transcriptase as recommended for use with the GeneAmp RNA PCR Kit (Perkin Elmer). A synthetic oligodeoxynucleotide was used as a primer for cDNA synthesis. This cDNA primer contained a spacer sequence, a *Hind*III recognition site (underlined) and an oligo(dT) tract, 5'-GAG AGA GAG AGA AAG CTT T₁₈-3'.

Priming oligodeoxynucleotides for DNA amplification. Proteins were extracted from celery tuber as described [17] and separated on SDS/PAGE. A predominant component of approximately 15 kDa, which had been identified as a major allergen of celery [9], was purified to homogeneity following an established protocol [20]. This component was then blotted to a poly(vinylidene fluoride) membrane (Millipore Corporation). The first 17 amino acid residues of the N-terminus of this protein were determined by solid-phase Edman degradation. Degenerate oligodeoxynucleotides for DNA amplification were designed according to the N-terminal sequence MG⁺VQTHV, 5'-ATG GGN GTN CA(A/G) ACN CA(C/T) GTN (C/T)TN GA(A/G)-3' (where the slash between two bases signifies that they were added at that particular position in equimolar amounts during the synthesis of the primer). The downstream primer was complementary to the cDNA primer but lacked the oligo(dT) tract.

Amplification of Api g 1 cDNA by PCR. The products of the first-strand cDNA synthesis were directly subjected to DNA amplification by PCR using AmpliTaq® DNA Polymerase (Perkin Elmer). Five cycles were performed, each for 1 min at 94°C, 2 min at 40°C, followed by a steady increase in temperature to 72°C over a period of 4 min, and 3 min at 72°C. This was followed by 30 cycles, each of 1 min at 94°C, 2 min at 55°C, 2 min at 72°C, and 20 min at 72°C after the last cycle. PCR products were fractionated on 1.5% agarose. DNA fragments from the predominant band (650 bp) were isolated (GeneClean, Bio101) and cloned into the pCRII vector (TA cloning kit, Invitrogen). Inserts were sequenced by the dideoxy method with universal and reverse-oriented primers (Sequenase, US Biochemicals).

Expression of proteins and characterization by immunoblotting. The complete coding sequence of Api g 1 was modified by the addition of an *Nco*I site at the 5' end and an *Eco*RI site at the 3' end by PCR. The primers used were 5'-GGC CCA TGG GAG TGC AGA CAC ATG TG-3' (*Nco*I site containing the ATG is underlined) and 5'-CAA GGA ATT CTT AAT TAG CGA TGA GAT AGG-3' (*Eco*RI site is underlined). The PCR products were cut with *Nco*I and *Eco*RI and ligated to the respective sites of the expression plasmid pMW175 (provided by M. Susani of Advanced Biological Systems Inc). Competent *E.*

coli BL21(DE3) cells were transformed with the resulting recombinant plasmids. The cells were grown to an absorbance of 0.8 at 600 nm in 50 ml cultures. For large-scale protein expression, the *E. coli* cells were grown in 1% peptone, 0.5% yeast extract, 0.5% glycerol, 10 mM NaKHPO₄, pH 7.4. Isopropyl d-thiogalactopyranoside was then added to the culture to a final concentration of 1.0 mM and incubation continued for another 4 hours at 37°C. After expression of the recombinant proteins, the cells were harvested by centrifugation. The pellets were resuspended in 750 µl 50 mM Tris/HCl, pH 7.5, containing 220 mM NaCl. The cells were then disrupted by repeated cycles of freezing and thawing. Insoluble material was removed by centrifugation at 16000×g for 20 min. Proteins in the supernatant were separated by electrophoresis on a 15% SDS/polyacrylamide gel and either stained with Coomassie brilliant blue or transferred to nitrocellulose membranes. IgE-binding proteins were detected using sera from allergic patients (1:10 dilution) and ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia) [19].

Purification of recombinant Api g 1. The pMW175 vector directs the synthesis of recombinant Api g 1 as a non-fusion protein. The recombinant protein was purified from crude bacterial cell extract according to established methods [19]. Determination of the protein concentration was performed on the purified Api g 1 following the method of Gill and von Hippel [22].

Patients and sera. Sera of celery-allergic and birch-pollen-allergic patients were selected according to the typical case histories, positive skin prick tests, and the display of symptoms after the ingestion of celery or the inhalation of birch pollen, respectively. Sera from 12 non-allergic individuals (according to case histories, skin prick tests, radioallergosorbent tests) were pooled; this normal human serum pool was stored at -20°C and used for negative control experiments.

Immunoblot analysis of tuber protein extracts. Total protein extracts from celery tuber and recombinant Api g 1 (1 µg/lane) were subjected to electrophoresis on 15% SDS/polyacrylamide gels and transferred to nitrocellulose membranes. According to Coomassie-brilliant-blue staining, equivalent amounts of the natural and of the recombinant forms of Api g 1 were used for the immunoblots. The IgE-binding ability of proteins was detected, as previously described, with sera from allergic patients in conjunction with rabbit anti-(human IgE) antibodies labeled with ¹²⁵I [19].

Cross-inhibition experiments. Total protein extracts from celery tuber and birch pollen were separated on 15% SDS/polyacrylamide gels and used for immunoblotting. For inhibition studies, a serum pool from celery-allergic patients and a serum pool from exclusively Bet v 1 allergic individuals were used. For inhibition studies, the serum pools were incubated with recombinant Api g 1 (20 µg/ml), BSA (10 µg/ml), or recombinant Bet v 1 (10 µg/ml). For controls, the normal human serum pool and a buffer control (omitting the primary antiserum) were used.

RESULTS

Amplification of Api g 1 cDNA with PCR and subcloning. PCR amplification of cDNAs complementary to Api g 1 transcripts generated products of approximately 650 bp. When subsequently cloned and sequenced, these cDNAs differed only in the length of their poly(A) tails.

Sequence analysis of Api g 1 cDNAs. The Api g 1 sequence comprised 643 bp and had a deduced open reading frame of 462 bp which encoded a polypeptide of 153 amino acids with a predicted molecular mass of 16189 Da (Fig. 1). In the 3' non-coding region, there was a putative polyadenylation signal lo-

1	ATG	GGA	GTG	CAG	ACA	CAT	GTG	TTG	GAG	CTC	ACC	TCC	TCA	GTC	TCA	45
0	M	<u>G</u>	<u>V</u>	<u>Q</u>	<u>T</u>	<u>H</u>	<u>V</u>	<u>L</u>	<u>E</u>	<u>L</u>	<u>T</u>	<u>S</u>	<u>S</u>	<u>V</u>	<u>S</u>	14
46	GCT	GAG	AAA	ATA	TTC	CAG	GGC	TTT	GTC	ATT	GAT	GTT	GAC	ACA	GTC	90
15	<u>A</u>	E	K	I	F	Q	G	F	V	I	D	V	D	T	V	29
91	CTT	CCC	AAG	GCT	GCG	CCT	GGA	GCT	TAC	AAG	AGT	GTC	GAA	ATC	AAG	135
30	L	P	K	A	A	P	G	A	Y	K	S	V	E	I	K	44
136	GGA	GAT	GGT	GGA	CCT	GGA	ACC	CTC	AAA	ATT	ATT	ACT	CTT	CCC	GAT	180
45	G	D	G	G	P	G	T	L	K	I	I	T	L	P	D	59
181	GGT	GGC	CCG	ATT	ACC	ACA	ATG	ACC	CTT	AGA	ATT	GAT	GGA	GTT	AAC	225
60	G	G	P	I	T	T	M	T	L	R	I	D	G	V	N	74
226	AAG	GAG	GCA	TTG	ACA	TTT	GAT	TAC	AGC	GTG	ATC	GAC	GGA	GAC	ATC	270
75	K	E	A	L	T	F	D	Y	S	V	I	D	G	D	I	89
271	CTC	TTG	GGA	TTT	ATC	GAA	TCC	ATT	GAA	AAC	CAT	GTT	GTA	CTA	GTG	315
90	L	L	G	F	I	E	S	I	E	N	H	V	V	L	V	104
316	CCA	ACT	GCT	GAT	GGA	GGA	AGC	ATT	TGC	AAG	ACC	ACT	GCC	ATC	TTT	360
105	P	T	A	D	G	G	S	I	G	K	T	T	A	I	F	119
361	CAC	ACC	AAA	GGT	GAT	GCT	GTG	GTT	CCA	GAA	GAG	AAT	ATC	AAG	TAT	405
120	H	T	K	G	D	A	V	V	P	E	E	N	I	K	Y	134
406	GCC	AAT	GAG	CAG	AAC	ACT	GCT	CTT	TTC	AAG	GCT	CTC	GAG	GCC	TAT	450
135	A	N	E	Q	N	T	A	L	F	K	A	L	E	A	Y	149
451	CTC	ATC	GCT	AAT	TAA		tctgaatgt	cttgtgtgtaa	tgctcaatggt							495
150	L	I	A	N	END											
496	tggtttttgga	agtaggctac				aaatgccttc		gcttcaaaat		atttgtgttt						545
546	tgtatttttgt	ttccatgtac				cttcgtttggg		taatgagttt		gatgtttccc						595
596	atgacaaaata	aagcqaatgt				gatattgtta		aaaaaaaaaaa		aaaaaaaaa						643

Fig. 1. Nucleotide sequence and deduced amino acid sequence of Api g 1, the major allergen of celery. Deduced amino acid residues are numbered, starting with the glycine residue which is the N-terminus of the natural protein. A putative polyadenylation signal is underlined. The N-terminal amino acid sequence, as determined by Edman degradation, is underlined.

cated at position 602. The poly(A) tail was located 236 nucleotides downstream from the TAA translation termination codon. The deduced amino acid sequence of the Api g 1 cDNA lacked tryptophan and had an overall excess of six acidic residues in relation to basic amino acids (20 acidic versus 14 basic amino acids).

Relation to published sequences. A search of the nucleotide and protein databases revealed significant similarities between the sequences of Api g 1 and a group of intracellular pathogenesis-related (PR) proteins described previously, i.e. two parsley PR proteins, PcPR1-3 (79.1% similarity, 61.4% identity; [23]) and PcPR1-1 (78.4% similarity, 61.4% identity; [23]); a wound-induced and stress-induced protein from soybean, SAM22 (62.7% similarity, 38.6% identity; [24]); two potato PR proteins, pSTH-21 (62.7% similarity, 38.0% identity; [25]) and pSTH-2 (60.7% similarity, 35.3% identity; [25]); the bean elicitor-induced transcripts, PvPR2 (60.8% similarity, 36.6% identity; [26]) and PvPR1 (59.7% similarity, 37.0% identity; [26]); a pea-disease-resistance protein, pI49 (59.5% similarity, 34.6% identity; [27]) and an asparagus-wound-induced transcript, AoPR1 (55.6% similarity, 28.5% identity; [28]). Furthermore, significant similarities were revealed with respect to a group of major tree pollen allergens, including Aln g 1 of alder (66.2% similarity, 41.6% identity; [16]), Bet v 1 of birch (60% similarity, 41.6% identity; [15]), Cor a 1 of hazel (64.3% similarity, 38.3% identity; [18]) and Car b 1 of hornbeam (64.3% similarity, 36.4% identity; [17]). Two abscisic-acid-responsive proteins of pea also showed significant sequence similarities to Api g 1; ABR18 (60.7% similarity, 35.3% identity; [29]) and ABR17 (57.1% similarity, 30.6% identity; [29]).

Amino acid sequence alignments of Api g 1 to the parsley PR protein PcPR1-3 [23] and to the major birch pollen allergen Bet v 1 [15] are shown (Fig. 2A and B, respectively).

A

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1  GVQTHVLELTSSVSAEKIFQGFVIDVDITLPLKAAPGAYKSVE.IKGDGGP 49
   ||| :| ||||| |:| :|:|:|:| ||| | | :| ||
1  GVQKSEVEATSSVSAEKIFKGLCLDITLPLRVLPGAIKSSSETLEGDDGV 50

50 GTLKIITLPDGGPITMTLRIDGVNKEALTFDYSVIDGDIILGFIESIEN 99
   ||:|:| | |:| :| || :|:|:| | :|:| ||:||||| ||||| |
51 GTVKLVHLGDASPFKTMKQKVDAIDKATFTYSYSIIDGDIILGFIESINN 100

100 HVVLVPTADGGSICKTTAIFHTKGDVAVPEENIKYANEQNTALFKALEAY 149
   |||:| |||:| |||:| |||:| |||:| |||:| |||:| |||:|
101 HFTAVPNADGGCTVKSTIIFNTKGDVAVPEENIKFANDQNLTIFKAVEAY 150

150 LIAN 153
   ||||
151 LIAN 154

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B

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1  GVQTHVLELTSSVSAEKIFQGFVIDVDTVLPKAAPGAYKSVE. IKGDDGP 49
  || | | | | : | : | : | : | | | | | | | |
1  GVFNJETETTSVIPAARLFKAFLDGDNLFPKVPAPAISSVENIEGNGGP 50

50 GTLKIITLPDGGPITMTLRLIDGVNKEALTFDYSVIDGDILLGFIESIEN 99
  ||:| | : |:| | : : | : | | : : |||:| | : : | | |
51 GTIKKISFPEGGFPKYVKDRDVEDHTNFKYNSYVIEGGPIGDTLEKISN 100

100 HVVLVPTADGGSICKTTAIFHTKGDVAVPEENIKYANEQNTALFKALEY 149
   : | : | | | : | : | | | | | | : | : | : | : |
101 EIKIVATPDGGFILKISNKYHTKGDHEVKAEQVKASKEMGETLLRAVESY 150

150 LIAN 153
   | : :
151 LLAHSDAYN 159

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Fig. 2. Sequence comparisons of Api g 1 to parsley pathogenesis-related protein (A) and the major birch pollen antigen Bet v 1 (B). (A) Sequence comparison of Api g 1 (upper line, amino acid residues 1–153) to the parsley pathogenesis-related protein PcPR1-3 (lower line, amino acid residues 1–154). Identities are marked by vertical bars and conservative exchanges by colons. Dots indicate gaps in the sequence produced by the aligning program. (B) Sequence comparison of Api g 1 (upper line, amino acid residues 1–153) to the major birch pollen allergen Bet v 1 (lower line, amino acid residues 1–159). Identities and conservative exchanges are marked as in (A).

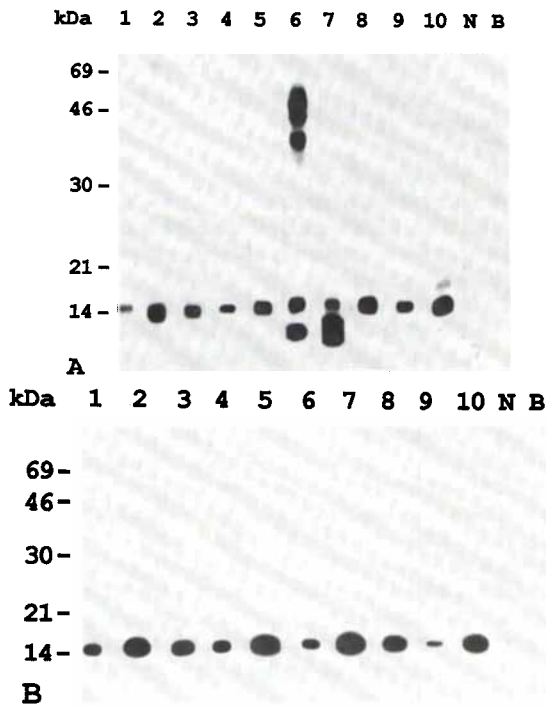


Fig. 3. Analysis of natural (A) and purified recombinant (B) celery proteins by SDS/PAGE and immunoblotting. For immunoblot analysis, proteins were electrophoresed and blotted onto nitrocellulose membranes. Blotted proteins were probed with sera from celery-allergic patients (lanes 1–10), or with normal human serum (lanes N). Buffer control (lanes B). Bound IgE was detected with ^{125} I-rabbit anti-human IgE.

Expression and purification of rApi1. A non-fusion form of the full-length Api g 1 cDNA was expressed in *E. coli* as an intracellular protein. Coomassie-brilliant-blue stained SDS/PAGE showed that the recombinant protein could be extracted in sufficient quantity from the bacterial cells by repeated cycles of freezing and thawing. After purification from total bacterial lysates, the recombinant Api g 1 protein appeared to be homogeneous, as determined by SDS/PAGE (data not shown). The purified recombinant Api g 1 was totally soluble in aqueous solutions and its yield was calculated to be 300 mg/l bacterial culture.

Immunoblot analysis of natural and recombinant Api g 1. Immunoblot analyses of total protein extracts from celery tuber and of the recombinant protein were performed with 10 sera from individuals allergic to celery. Api g 1 from celery tuber extract and recombinant Api g 1 were able to bind IgE of all the sera used (Fig. 3A and B, lanes 1–10). No IgE binding could be detected with the pool of sera from non-allergic individuals (Fig. 3A and B, lanes N) or without the addition of sera from allergic patients (Fig. 3A and B, lanes B). IgE from different patients bound to the allergens with different intensities, but they were comparable for natural and recombinant Api g 1 from the same patient. Sera from patients 6, 7 and 10 recognized additional proteins in the celery tuber extract, i.e. the lower molecular-mass component corresponding to Bet v 2, which is birch profilin (Fig. 3A, lanes 6 and 7) [30].

Cross-inhibition experiments. For cross-inhibition experiments, a serum pool of celery-allergic individuals (Fig. 4A and B, lanes 1–3) and a serum pool of exclusively Bet v 1 allergic

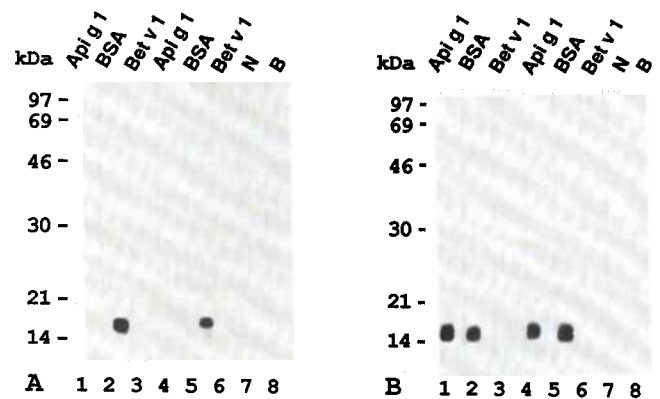


Fig. 4. Cross-inhibition experiments. Protein extracts from celery tuber (A) and from birch pollen (B) were separated by SDS/PAGE and blotted onto nitrocellulose. IgE binding was studied using a serum pool from celery-allergic patients (lanes 1–3) and a serum pool from birch-pollen-allergic patients (lanes 4–6). Inhibition was by incubating the serum pools with recombinant Api g 1 (lanes 1 and 4), BSA (lanes 2 and 5), and recombinant Bet v 1 (lanes 3 and 6). The normal human serum pool (N) and the buffer control (B) were used untreated.

individuals (Fig. 4A and B, lanes 4–6) were applied to SDS/PAGE-separated total protein extracts from celery tuber (Fig. 4A) and from birch pollen (Fig. 4B). Treatment with recombinant Api g 1 or recombinant Bet v 1 blocked IgE binding to the natural celery allergen for both serum pools (Fig. 4A, lanes 1, 3, 4 and 6). BSA had no effect on the IgE binding to natural Api g 1 (Fig. 4A, lanes 2 and 5). However, incubation of the serum pools with recombinant Api g 1 did not block IgE binding to natural Bet v 1 (Fig. 4B, lanes 1 and 4), nor did BSA (Fig. 4B, lanes 2 and 5). In contrast, recombinant Bet v 1 was able to inhibit all IgE binding to natural Bet v 1 from both serum pools (Fig. 4B, lanes 3 and 6). No signals were obtained when the primary antiserum was replaced by normal human serum (Fig. 4A and B, lanes 7), or when it was omitted from the reaction procedure (Fig. 4A and B, lanes 8), i.e. these negative results confirm the specificity of the reactions observed with sera containing IgE antibodies.

DISCUSSION

In this study, we present the cDNA sequence and the deduced amino acid sequence of the major allergen of celery, Api g 1. On the amino acid level, Api g 1 showed a significant similarity to proteins of the group of major tree pollen allergens, including Bet v 1 from birch pollen (64% similarity, 42% identity). Cross-inhibition experiments showed that Api g 1 shared some epitopes with Bet v 1, since the addition of recombinant Bet v 1 to the serum of celery-allergic patients abrogated all the binding of IgE antibodies to natural Api g 1. In contrast, the addition of recombinant Api g 1 to the sera of birch-pollen-allergic patients did not abolish all the IgE binding to natural Bet v 1. From these results, we concluded that the major celery allergen possessed some, but not all, of the epitopes of the major birch-pollen allergen; clearly, this finding provides a molecular basis for the clinically observed birch-celery cross-allergy syndrome.

Both Api g 1 and Bet v 1 belong to a class of intracellular PR proteins, as revealed by sequences in the protein databases. These proteins are known to be strongly upregulated in plants by pathogens or by treatment of cell cultures with microbial elicitors. These proteins are present in dicots from various taxonomically distantly related plant families [15–18, 23–27, 29]

and have even been found in the monocot, asparagus [28]. In particular, the deduced amino acid sequence of Api g 1 showed a striking similarity of 79.1% (61.4% identity) to the two parsley PR proteins, PcPR1-1 and PcPR1-3. *In situ* RNA hybridization in fungus-infected parsley leaf tissue demonstrated rapid and massive PR mRNA accumulation around infection sites [23]. This finding is considered to indicate a close correlation between PR mRNA accumulation and the disease-resistance response of the plant and the restriction of fungal growth.

Celery and parsley belong to the same plant family, the *Apiaceae*. Despite the fact that the function of the Api g 1 protein is still unknown, the close taxonomical relationship of celery to parsley and the high similarity between Api g 1 and the two parsley PR proteins is taken to indicate that Api g 1 may play a role in the response of the plant to pathogens.

Certain members of the widely distributed class of PR proteins, namely the tree pollen major allergens, are capable of inducing allergen-specific IgE synthesis in predisposed (atopic) individuals, probably because of their easy entry route, i.e. by inhalation. Sensitization to potential inhalant allergens occurs readily via the mucosa of the respiratory tract. In contrast, immune responses to dietary protein antigens generally lead to oral tolerance [31]. It is, therefore, tempting to speculate that allergy to celery proteins is an epiphenomenon and that allergy to these proteins is due to sensitization by inhalation of the cross-reacting pollen allergens in the first place. This hypothesis is supported by the observation that respiratory allergies subsequently lead to food hypersensitivities via cross-reacting IgE antibodies [32, 33]. As stated earlier, other members of this conserved class of proteins share epitopes with their pollen counterparts and are, therefore, able to bind IgE antibodies induced by the latter, thus leading to type-I allergic symptoms. Conservation of these proteins during evolution hints at their important protective function for a great variety of plants, and may also explain the occurrence of tree-pollen-related food allergies in terms of molecular parameters.

Birch pollen allergy is often associated with hypersensitivity to homologous allergenic proteins present in vegetables, such as celery and carrot, and in fresh fruits, such as apple, cherry, plum and peach [10, 34]. It has been argued that allergic reactions to tree pollen and these vegetable foods are due to the binding of IgE antibodies to epitopes present on the allergenic molecules of these different plant sources. Recently published N-terminal sequences of Bet v 1-related allergens from apple, cherry, celery and carrot support this view [10].

Similarly, profilins of different plant species share IgE epitopes and release histamine from blood basophils of allergic patients [30]. Profilins are characterized by their ubiquitous occurrence in distantly related plants and may, therefore, be implicated as important cross-sensitizing allergens in pollen and plant-derived foods.

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