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Complementary DNA cloning and expression in *Escherichia coli* of *Aln g I*, the major allergen in pollen of alder (*Alnus glutinosa*)

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Previous data showed that the major pollen allergens from trees of the order Fagales, in particular alder, birch, hazel, and hornbeam, are highly interrelated. As only the complete amino acid sequence of Bet v I, the major allergen from birch, has been known, it was of interest to obtain the primary structure of other major allergens of this group, to attribute IgE-binding properties to certain features of the amino acid sequences of those allergens. cDNA was synthesized from alder pollen mRNA, sequence-specifically amplified by polymerase chain reaction and cloned into plasmid bluescript. Comparison of the deduced amino acid sequences of Aln g I and Bet v I revealed a 86.8% homology. The Aln g I encoding cDNA was subcloned into pKK223-3 and expressed in Escherichia coli as a full-length nonfusion protein. The recombinant Aln g I bound IgE from tree pollen-allergic patients and was shown to share IgE-epitopes with Bet v I by inhibition studies with recombinant Bet v I. Computer-aided calculations predicted epitopes in both Aln g I and Bet v I at the same position; the Bet v I molecule was predicted to possess two additional epitopes near the N-terminus of the molecule. (J ALLERGY CLIN IMMUNOL 1992;90:909-17).

Key words: Alder pollen, major allergen, *Aln g I*, cDNA cloning, expression, recombinant nonfusion protein, IgE-binding, epitopes

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An apparently increasing percentage of the population of industrialized countries of the northern hemisphere suffer from type I (IgE-induced) allergies caused by airborne pollen from grasses, trees, and weeds. The prevalence of tree pollinosis in patients with allergic conjunctivitis, allergic rhinitis, and allergic asthma might be as high as 10%, or even higher. Pollens from trees, in particular from birch, alder, hazel, and hornbeam, are responsible for these allergies in the early spring in large areas of the temperate climate zone.

The exact knowledge of the structure and possibly also of the function of allergenic proteins is a prerequisite for investigating new concepts of mechanisms in the pathogenesis of atopic diseases. Molecular biology of atopic allergens has made considerable progress within the last few years.¹⁻⁶ Expression of recombinant allergens facilitates the investigation of the biochemical features of the cloned allergen, which will also be interesting tools for diagnosis and possibly even for new therapeutic approaches.^{7,8} Furthermore, information pointing at biologic functions could be gained from amino acid sequence comparisons. It is likely that the major house dust mite allergen, *Der p* I, represents a cysteine-protease.¹ *Bet v* II, a birch pollen allergen, was shown to belong to a highly conserved family of actin-binding proteins, profilins.⁶ *Bet v* I, the major allergen from birch (*Betula verrucosa*),^{9,10} was found to be highly homologous to a group of pathogenesis-related proteins described for bean, parsley, pea, and potato.^{2,11-14} The high sequence homology and recent data from our laboratory showing that *Bet v* I and a *Bet v* I homologue are induced by environmental stress factors (air pollutants, microbial pathogens)¹⁵ suggest a role for members of the *Bet v* I gene family as pathogenesis-related proteins in birch.

In central Europe, patients suffering from tree pollinosis (approximately one third of all patients with pollen allergies) show symptoms starting in February or early in March. These allergic reactions may last 2 or 3 weeks and are due to pollens of hazel and alder. With the end of March, but usually in the middle of April, pollen of birch are responsible for type I allergic reactions. Practically all patients with strong allergy to birch pollen react also with alder pollen, although the symptoms might be less pronounced. This observation might be explained by a lower count of alder pollen but also by a lower allergenicity of the responsible alder pollen allergens. Nevertheless, since the alder flowers approximately 1 month earlier than the birch, alder pollen represents a significant source for pollen allergy in early spring. For these reasons, a detailed investigation of molecular properties of alder allergens seemed of particular interest to us.

Abbreviations used

<i>Aln g</i> I:	Major allergen from alder pollen
<i>Bet v</i> I:	Major allergen from birch pollen
PCR:	Polymerase chain reaction
mRNA:	Messenger RNA
cDNA:	Complementary DNA
r:	Recombinant
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
RAST:	Radioallergosorbent test
BSA:	Bovine serum albumin
PBS:	Phosphate-buffered saline
Mr:	Apparent molecular weight

All pollen from trees of the order *Fagales* investigated so far (alder, birch, hazel, hornbeam, oak) contain a single major allergen that is cross-reactive between the species and is responsible for an IgE response in 95% of patients allergic to tree pollen.^{9,10} From previous data it was known that these major allergens are closely interrelated in terms of partially known amino acid sequences and of nucleotide sequence similarities as shown by Northern blot hybridization techniques.^{9,16} Moreover, those allergens strongly react with IgE from patients allergic to tree pollen,^{9,17} as well as with a monoclonal antibody, BIP 1, raised against *Bet v* I as shown by immunoblot experiments.^{16,18} The knowledge of the complete amino acid sequence of a further important major tree pollen allergen will certainly facilitate the disclosure of relevant IgE-binding domains (epitopes) within the allergenic molecules. Moreover, the molecular characterization of *Aln g* I, the major allergen of alder pollen, was of particular interest to us, since the amino acid sequence deduced from the nucleotide sequence of a complete *Aln g* I-encoding cDNA would enable us to compare (and possibly to evaluate) computer-aided predictions about the antigenic index of the two closely related allergens, *Bet v* I and *Aln g* I.

Because the *N*-terminal protein sequence of *Aln g* I has been determined by Edman degradation,⁹ it was possible to directly amplify the *Aln g* I cDNA with use of a specific 5' terminal primer and a nonspecific 3' terminal primer for the polymerase chain reaction (PCR). By this method we obtained a complete cDNA sequence encoding *Aln g* I, the major allergen of alder pollen.

MATERIAL AND METHODS

Extraction and isolation of Poly(A+) RNA

RNA was extracted from 4 gm of mature nondefatted pollen from the black alder *Alnus glutinosa* (Allergon, Engelsholm, Sweden) according to the acidic guanidinium

thiocyanate method.¹⁹ The pollen grains were initially broken under liquid nitrogen with a mortar and pestle and further homogenized in extraction buffer with use of an ultraturrax (CIKA-Werke, Staufen, Germany) blender. Thirty micrograms of poly(A⁺)-enriched RNA were isolated by passing the total RNA over an oligo(dT) cellulose column following standard procedures.²⁰

Synthesis of the first cDNA strand

The first strand of cDNA was synthesized from 5 µg of the poly(A⁺) RNA with AMV reverse transcriptase (USB, Cleveland, Ohio). The cDNA synthesis was primed by 100 ng of an oligodeoxynucleotide comprising a T7 primer (underlined) at its 5' end, restriction enzyme sites for *Bgl*II, *Sma*I, and *Hind*III, and an oligo(dT) stretch of 16 nucleotides at the 3' end with the following sequence: 5'-TTTAATACGACTCACTATAGATCTCCCGGAAGCTT-TTTTTTTTTTTTTT-3'.

Polymerase chain reaction

After synthesis of the first strand, the reaction mixture was used directly for PCR. A 1:1 mixture of two oligodeoxynucleotides (5'-GTTTTCAATTACGAAGCGGAAAC-3' and 5'-GTTTTCAATTACGAAGCGGAGAC-3') was used as primers for the 5' end of the molecules. The nucleotide sequences of these oligonucleotides were derived from the partial N-terminal amino acid sequence of alder *Aln g I* as determined by Edman degradation.⁹ The codon usage of the *Bet v I* allergen of birch (*Betula verrucosa*) was followed.¹ The T7 primer sequence, which is likewise a constituent of the cDNA synthesis primer, served as a primer for the 3' end of the molecules. The following mixture was used for the PCR reaction: one microliter of the first strand reaction mixture; 5 µl 10× PCR buffer (400 mmol/L KCl, 10 mmol/L MgCl₂, 10% gelatin, 100 mmol/L Tris, pH 8.3); 20 pmol T7 primer (Pharmacia, Uppsala, Sweden); 40 pmol of the mixed primers; 2.5 µl 2 mmol/L dNTPs (Pharmacia); 1.5 µl 100 mmol/L MgCl₂ and H₂O to bring the volume to 50 µl. One unit of Taq DNA polymerase (DNA polymerase from *Thermophilus aquaticus*) (USB) was added, and the reaction mixture was incubated for 30 seconds at 93° C, for 30 seconds at 55° C, and for 1 minute at 72° C. This cycle was repeated 30 times. Finally, the reaction mixture was kept at 72° C for another 10 minutes.

Cloning and sequencing of the PCR fragment

The single DNA band synthesized by the PCR was isolated from a 1.5% agarose gel by diethylaminoethyl paper.²⁰ The isolated DNA was blunt-ended with the Klenow fragment of DNA polymerase and purified from unincorporated deoxynucleotides with a nick column (Pharmacia).²⁰ Several restriction enzyme sites had already been incorporated at the 3' end of the *Aln g I* sequence through the use of the cDNA priming oligodeoxynucleotide. A *Bgl*II site was generated at the 3' end by cleavage in the primer sequence. The fragment with a blunt 5' end and a *Bgl*II end at the 3' end was ligated into the compatible *Eco*RV and *Bam*HI sites

of the plasmid bluescript KS⁺ (Stratagene, La Jolla, Calif.) and transformed into *E. coli* XL1-Blue (Stratagene). The plasmid DNA was sequenced with T7 polymerase (Pharmacia) according to the manufacturer's instructions.

Expression of recombinant nonfusion *Aln g I* in *E. coli* and IgE immunoblot analyses

To produce a nonmodified nonfusion recombinant (r) *Aln g I*, site-specific in vitro mutagenesis was performed.²¹ This method was applied to introduce cloning sites for insertion of *Aln g I* cDNA excised from the bluescript KS⁺ vector into the expression plasmid pKK223-3 (Pharmacia). To use the ribosomal binding site of the expression plasmid pKK223-3 (Pharmacia) into which the cDNA fragment should be subcloned, the 5' end of the *Aln g I* sequence was modified to contain an *Eco*RI site (underlined below) immediately adjacent to the ATG start codon. The oligodeoxynucleotide 5'-CTTCGTAATTGAAAACACCCATGAATTCGATACCGTCGA-3' was used for in vitro mutagenesis.²¹ The *Aln g I* sequence of the mutagenized plasmid was ligated in correct orientation between the *Eco*RI and *Hind*III sites into the expression plasmid pKK223-3 (Pharmacia). *E. coli* JM105 was transformed with the resulting recombinant plasmid. The cells were grown to an OD₆₀₀ of 0.6. Isopropyl-D-thiogalactopyranoside was then added to a final concentration of 2.5 mmol/L. After 3 hours cells were harvested by centrifugation. The pellet was resuspended in 750 µl of 50 mmol/L Tris-HCl buffer, pH 7.5, containing 220 mmol/L NaCl. The cells were then disrupted by freezing in liquid nitrogen, followed by thawing at 37° C. This procedure was repeated twice. Insoluble material was removed by centrifugation at 16,500 g for 10 minutes. Proteins in the supernatant were separated by electrophoresis on 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes.^{22, 23} IgE-binding proteins were detected as previously described with use of allergic patients' sera and rabbit antihuman IgE antibodies labeled with iodine 125.¹⁰

Allergens from alder pollen

Allergenic proteins from alder pollen were extracted and subjected to immunoblotting as described in detail previously.¹⁰

Recombinant *Bet v I* (r*Bet v I*)

Bet v I encoding cDNA was inserted into pKK223-3 and expressed in *E. coli* as described for *Aln g I*.² r*Bet v I* was purified by chromatofocusing and reversed-phase high-performance liquid chromatography.

Patients and sera

Patients allergic to tree pollen were selected according to typical case history (symptoms starting with end of winter and early spring). They showed positive skin prick tests to tree pollen allergens and radioallergosorbent test (RAST) classes greater than 3.5. Twenty nonallergic individuals (case history, skin prick test, RAST) were selected (normal human serum pool). Sera were stored at -20° C.

Aln g I

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      1   2   3   4   5   6   7   8   9  10  11  12  13  14  15  16  17  18  19
ATG GGT GTT TTC AAT TAC GAA GCG GAA ACC CCC TCC GTT ATC CCA GCG GCT CGG CTG TTC
Met Gly Val Phe Asn Tyr Glu Ala Glu Thr Pro Ser Val Ile Pro Ala Ala Arg Leu Phe

      20  21  22  23  24  25  26  27  28  29  30  31  32  33  34  35  36  37  38  39
AAG GCC TTT ATC CTT GAT GGC GAT AAG CTC CTT CCA AAG GTT GCA CCT GAA GCT GTT AGC
Lys Ala Phe Ile Leu Asp Gly Asp Lys Leu Leu Pro Lys Val Ala Pro Glu Ala Val Ser

      40  41  42  43  44  45  46  47  48  49  50  51  52  53  54  55  56  57  58  59
AGT GTT GAG AAC ATT GAA GGA AAT GGA GGG CCT GGA ACC ATC AAG AAG ATC ACC TTT CCC
Ser Val Glu Asn Ile Glu Gly Asn Gly Gly Pro Gly Thr Ile Lys Lys Ile Thr Phe Pro

      60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79
GAA GGC AGC CCT TTT AAG TAC GTA AAG GAG AGG GTT GAT GAG GTT GAT CGC GTA AAC TTC
Glu Gly Ser Pro Phe Lys Tyr Val Lys Glu Arg Val Asp Glu Val Asp Arg Val Asn Phe

      80  81  82  83  84  85  86  87  88  89  90  91  92  93  94  95  96  97  98  99
AAA TAC AGC TTC AGC GTG ATC GAG GGT GGT GCC GTG GGC GAC GCA CTG GAG AAG GTC TGT
Lys Tyr Ser Phe Ser Val Ile Glu Gly Gly Ala Val Gly Asp Ala Leu Glu Lys Val Cys

     100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119
AAC GAG ATC AAG ATA GTG GCA GCC CCT GAT GGA GGA TCC ATC TTG AAG ATC AGC AAC AAG
Asn Glu Ile Lys Ile Val Ala Ala Pro Asp Gly Gly Ser Ile Leu Lys Ile Ser Asn Lys

     120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139
TTC CAC ACC AAA GGC GAC CAT GAG ATA AAT GCA GAG CAG ATT AAG ATT GAA AAA GAA AAG
Phe His Thr Lys Gly Asp His Glu Ile Asn Ala Glu Gln Ile Lys Ile Glu Lys Glu Lys

     140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159
GCC GTG GGA CTT CTC AAG GCC GTT GAG AGC TAC CTC TTG GCA CAC TCT GAT GCC TAC AAC
Ala Val Gly Leu Leu Lys Ala Val Glu Ser Tyr Leu Leu Ala His Ser Asp Ala Tyr Asn

     160
TAA ATTCTGCCTAATTTTGATCAGCTTGCATGTGTTCTTGTCAAGCCATAAATACTGCTTAACCTCGTCTTGCTAATA
End

AATGAAGCTGTTGTAGTCGTTTATGAGTACGTAATAATGACACCAACATATGGAGCCAATTGCTTATGAATAGAAGTT

AAGTTCTTAAAAAAAAAAAAAAAAAAAAA

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FIG. 1. Nucleotide sequence and deduced amino acid sequence of *Aln g I*, the major allergen of alder pollen. 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. Bold face: *N*-terminal amino acid sequence as determined by Edman degradation.⁹

Inhibition experiments

Nine sera from patients allergic to tree pollen were pooled and mixed with an equal volume of purified *rBet v I* (10 µg/ml PBS) or, for control experiments, with bovine serum albumin (BSA) or phosphate-buffered saline (PBS). The mixture was incubated under continuous shaking overnight at 4°C. The sera were subsequently used for immunoblotting experiments.

Computer analyses

The programs that were used for computer analysis were part of the UWGCG (University of Wisconsin Genetics Computer Group) program package version 4. A secondary structure analysis for the protein sequence was done according to the Chou-Fasman algorithm for the allergens.²⁴ The antigenic index was calculated according to the Jameson and Wolf algorithm.²⁵

RESULTS

Amplification of *Aln g I* with PCR

During PCR under the given conditions ("Materials and Methods") cDNA was amplified, which migrated as a single band at an approximate length of 660 nucleotides (not shown). The DNA fragments con-

tained in this band were used for the subsequent cloning procedures into the plasmid bluescript KS+.

Sequence analysis of *Aln g I* cDNA

Both strands of four of the resulting cDNA clones were sequenced. Their nucleotide sequence and the deduced amino acid sequence of the coding region were identical and are shown in Fig. 1. The insert of these clones had a length of 665 nucleotides, with an open reading frame of 477 nucleotides coding for a protein of 159 amino acid residues. The reading frame is terminated by a TAA stop codon. The consensus polyadenylation signal AATAAA is present in the 3' noncoding region (Fig. 1). The mature protein has a calculated Mr of 17,349. The apparent Mr of the *rAln g I* was estimated to be 18,000 on a denaturing SDS-PAGE (Fig. 2), which agrees with the apparent Mr of the natural protein (Fig. 2).

Comparison of the amino acid sequence of *Aln g I* to the amino acid sequence of the major birch pollen allergen *Bet v I* (Fig. 3) reveals 81.1% identity and 86.8% homology (including conservative exchanges). Therefore *Aln g I* and *Bet v I* share significant sequence

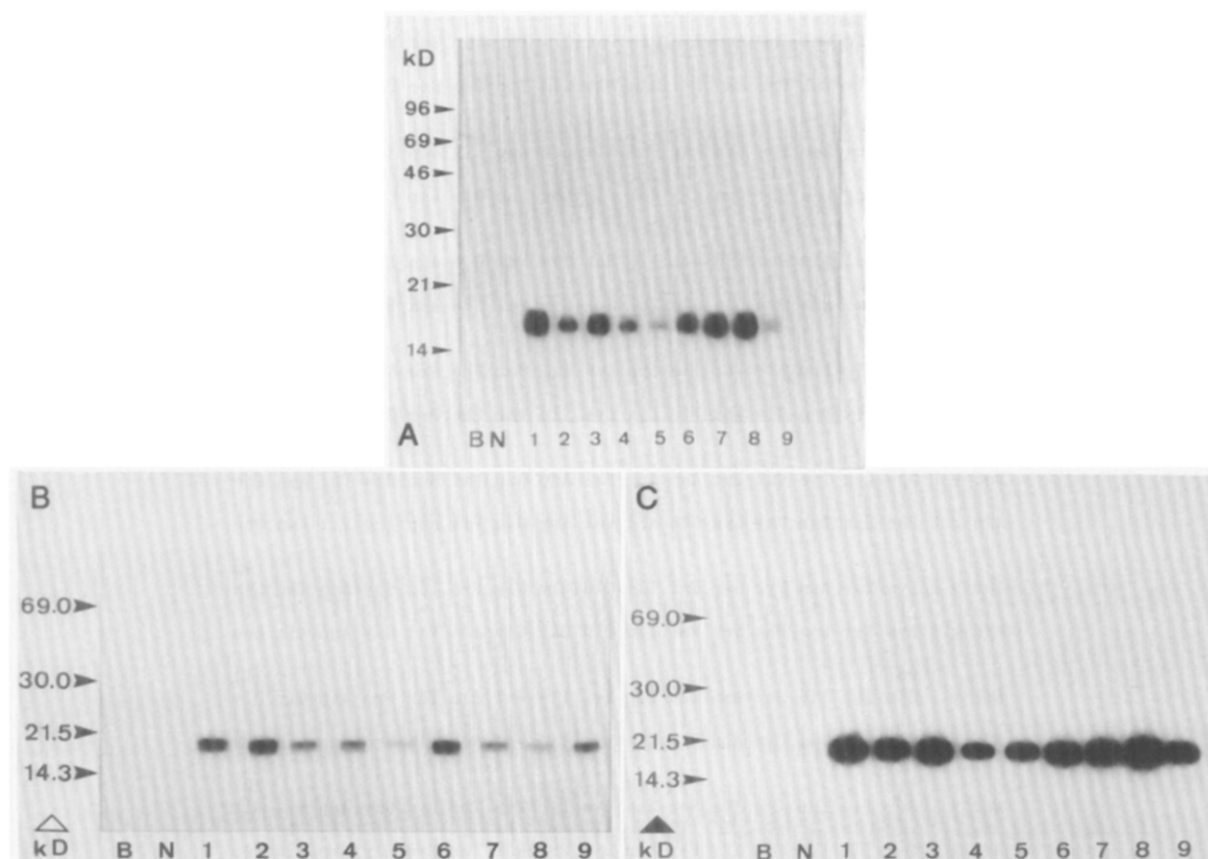


FIG. 2. Immunoblot analysis of *rAln g I*. Alder pollen extract (**A**) and soluble proteins from induced *E. coli* JM 105 transformed with pKK223-3/*Aln g I* (**B**) and pKK223-3/*Bet v I* (**C**), respectively, were separated by 15% SDS-PAGE. Proteins were blotted onto nitrocellulose and probed with different sera from patients allergic to tree pollen,¹⁻⁹ a pool of sera from nonallergic individuals (*N*), and serum-free buffer as negative control (*B*). Bound IgE detected by ¹²⁵I-labeled antihuman IgE.

homology with the group of pathogenesis-related proteins found in bean, parsley, pea, and potato.¹¹⁻¹⁴

Immunoblot analysis of alder pollen extract, *rAln g I*, and *rBet v I*

Immunoblot analyses of the natural and recombinant proteins was performed with nine sera from individuals allergic to tree pollen. Figs. 2 A, B, C show that *Aln g I* from alder pollen extract, *rAln g I* and *rBet v I* were able to bind IgE from all the sera used (Figs. 2, A, B, C, lanes 1-9). No IgE binding could be detected without addition of allergic patients' sera (Figs. 2, A, B, C, buffer control lane B) and when a serum-pool from non-allergic individuals was used (Figs. 2, A, B, C, lane N). An identical set of experiments was performed for a protein extract from *E. coli* JM105 harboring the expression plasmid pKK223-3 without a cDNA insert. No IgE binding could be detected (not shown).

Inhibition experiments

Preincubation of patients' serum pool with purified *rBet v I* inhibited IgE binding to pollen-derived *Aln g I* as well as to *rAln g I*. Preincubation with BSA or PBS did not influence IgE reactivity to *Aln g I* to *rAln g I* (Fig. 4).

Computer analyses

To evaluate the significance of the high degree of sequence homology, secondary structures were calculated for both allergens, *Aln g I* and *Bet v I*.²⁴ Fig. 5 shows this prediction for *Bet v I*, Fig. 6 for *Aln g I*. Furthermore, the antigenic index was calculated, and areas with a high antigenic index were marked along the sequence. Although the overall predicted structure differs markedly between the two allergens, most of the regions with high antigenic index are at identical positions of the sequences, with the exception of two additional regions of high anti-

FIG. 3. Comparison of the deduced amino acid sequences of the major allergens of birch and alder, *Bet v 1* and *Aln g 1*. Vertical bars represent identities, amino acid exchanges marked by asterisks. Amino acids numbered by designating the first amino acids of the native pollen protein Glv as 1.

It should be noted that the calculated Mr of *Aln g* I (17,349) differs slightly from the experimentally observed Mr (18,000) of *Aln g* I as well as *rAln g* I. It is interesting to note that the calculated Mr of *Bet v* I (17,420) differs only marginally from that of *Aln g* I, the experimental Mr of *Bet v* I, however, is closer

to 17,000 for the natural as well as the recombinant protein.

Comparison of the deduced amino acid sequence of *Aln g I* with that of *Bet v I* revealed 30 amino acid differences (Fig. 3), but it is interesting to note that both proteins have the same length of 159 amino acids. Along with the high amino acid identity (81.1%) with *Bet v I*, *Aln g I* also shares its homologies and length with a group of pathogenesis-related proteins found in somatic tissues of bean, parsley, pea, and potato.¹¹⁻¹⁴ These proteins are expressed on contact with biologic and chemical stress inducers in all of these taxonomically quite distant plants. It is tempting to speculate that *Aln g I* fulfills a similar biologic function in the pollen of alder in view of the high conservation of the amino acid sequence over such long evolutionary distances. The metabolic activity of mature pollen is rather low, which may explain why *Aln g I* is expressed constitutively in pollen in contrast to tissues of the other plants mentioned above.

Computer-aided calculations predict four antigenic regions in both *Bet v I* and *Aln g I* molecules.²⁵ However, the program proposes two additional antigenic regions at the *N*-terminal portion of *Bet v I* (Figs. 5 and 6). It might be noted that in position 10 a proline residue in *Aln g I* is replaced by a threonine residue in *Bet v I*. This exchange might be responsible for the loss of the two predicted antigenic sites present at the *N*-terminus of *Bet v I* but missing from *Aln g I*. It can be observed that patients allergic to tree pollen are more reactive to *Bet v I* than to *Aln g I* with respect to both clinical and immunochemical criteria (RAST, IgE immunoblots). Furthermore, patients allergic to alder pollen are always also allergic to birch pollen (we never observed reactivity to alder without reactivity to birch pollen). Thus *Bet v I* represents the predominant allergen in tree pollen allergy. These observations may be explained by the presence of more binding sites for IgE on the *Bet v I* molecule in comparison to *Aln g I* and fit well to our results that *rBet v I* is capable of inhibiting the binding of patients' IgE to pollen-derived *Aln g I* as well as to *rAln g I*.

For immunotherapy of tree pollen allergy, a mixture of pollen extracts from birch, alder, and hazel is used to ensure treatment with all possible epitopes. The disclosure of the entire primary structure of birch,² alder (Fig. 1), and hazel will help us to decide whether this protocol (treatment with all three tree pollen extracts) is in fact necessary, or whether hyposensitization treatment with birch pollen extract alone would be sufficient (as it seems likely from the data mentioned above). Molecular biology provides the techniques to prove this hypothesis: Mutational analyses with recombinant proteins, *rAln g I* and *rBet v I*, will enable us to test which amino acid exchanges are

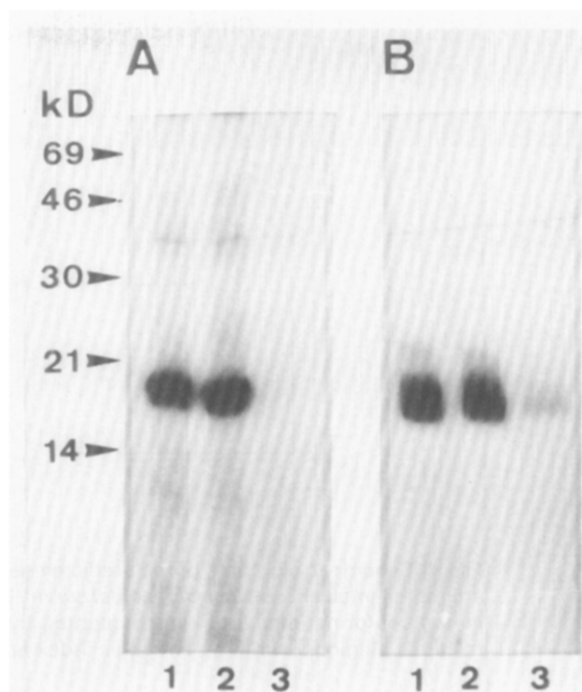


FIG. 4. Inhibition of IgE-binding to *Aln g I* and *rAln g I* with *rBet v I*. Soluble proteins from induced *E. coli* JM 105 transformed with pKK223-3/*Aln g I* (panel A) and alder pollen extract (panel B) were separated by 15% SDS-PAGE. The proteins were blotted onto nitrocellulose and probed with a serum pool consisting of patients' sera (Fig. 2) (lane 1),^{1,9} with this serum pool preincubated with 10 μ g/ml BSA (lane 2), or with 10 μ g/ml purified *rBet v I* (lane 3).

responsible for different antigenicity, allergenicity, and IgE binding properties of the molecules and parts thereof (epitopes). In this context it should be mentioned that the predicted antigenic sites in both major allergens (with one exception) are located in highly conserved areas of the proteins. This makes members of the group of plant pathogenesis-related proteins,¹¹⁻¹⁴ to which *Aln g I* and *Bet v I* belong, potential allergens. The conserved areas—possibly representing epitopes—within the pathogenesis-related proteins might, at least in part, provide an explanation for allergies to food items, in particular those allergies that are accompanied with birch pollen allergy.^{29, 30}

In summary, we cloned, sequenced and expressed in *E. coli* as a nonfusion protein the complete major allergen of alder pollen, *Aln g I*. The recombinant protein was shown to bind IgE from patients allergic to tree pollen. Computer-aided calculations of potential B-cell epitopes predicted four epitopes common to *Aln g I* and *Bet v I*, the latter possessing two additional predicted epitopes. This is in good agreement with immunochemical as well as clinical observations.

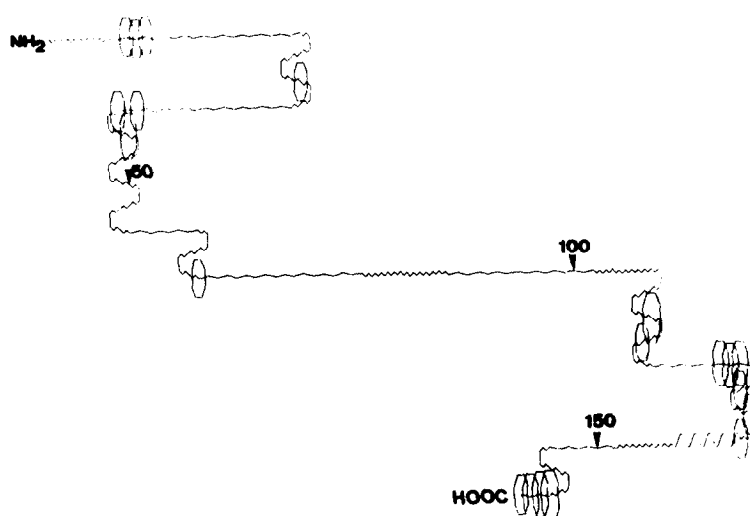


FIG. 5. Schematic diagrams of the predicted secondary structure in *Bet v I*. Amino acid residues are represented as α -helical (*sinusoidal curve*), β -sheet (*saw tooth curve*), and coil (*flat sinusoidal curve*) conformations. β -turns are denoted by chain reversals. Numbers indicate amino acid residues. Regions with high antigenic index are marked (*octagons*).

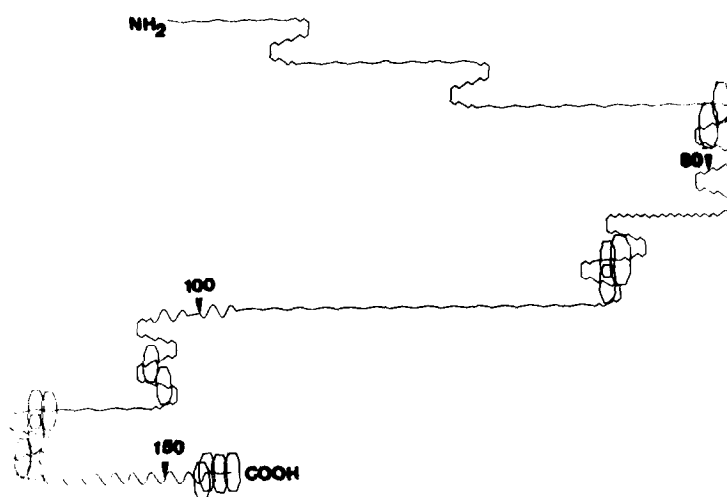


FIG. 6. Schematic diagrams of the predicted secondary structure *Aln g I*. Amino acid residues are represented as α -helical (*sinusoidal curve*), β -sheet (*saw tooth curve*), and coil (*flat sinusoidal curve*) conformations. β -turns are denoted by chain reversals. Numbers indicate amino acid residues. Regions with high antigenic index are marked (*octagons*).

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