Birch pollinosis is often accompanied by hypersensitivity to fruit as a consequence of the cross-reactivity of pollen allergen-specific IgE antibodies with homologous food proteins. To provide a basis for examining the cross-reactivity on a structural level, we used heteronuclear multidimensional NMR spectroscopy to determine the high-resolution three-dimensional structure of the major cherry allergen, Pru av 1, in solution. Based on a detailed comparison of the virtually identical structures of Pru av 1 and Bet v 1, the major birch pollen allergen, we propose an explanation for a significant aspect of the observed cross-reactivity pattern among the family of allergens under consideration. The large hydrophobic cavity expected to be important for the still unknown physiological function of Bet v 1 is conserved in Pru av 1. Structural homology to a domain of human MLN64 associated with cholesterol transport suggests phytosteroids as putative ligands for Pru av 1. NMR spectroscopy provides experimental evidence that Pru av 1 interacts with phytosteroids, and molecular modeling shows that the hydrophobic cavity is large enough to accommodate two such molecules.

Birch pollinosis is one of the prevailing allergic diseases in regions with birch trees, such as Northern and Central Europe and Northern America. Up to 70% of birch pollen allergic patients who suffer from clinical syndromes like hay fever and asthma also show hypersensitivity to fresh fruit or vegetables (1). The allergic reactions after ingestion of foodstuff are predominantly oropharyngeal, for example itching and swelling of the lips, tongue, and throat, but in rare cases even severe anaphylactic reactions are possible. The symptoms of these type I allergies are caused by an immune response that is triggered when two receptor-bound IgE antibodies on the surface of a mast cell or basophil are cross-linked by simultaneous binding of an otherwise harmless antigen, the so-called allergen (2). Pollen-associated food allergies are a consequence of the cross-reaction of pollen allergen-specific IgE antibodies with highly homologous proteins contained in foodstuff. The 17.4-kDa major birch (Betula verrucosa) pollen allergen, Bet v 1, is responsible for IgE binding in more than 95% of birch pollen allergic patients (3). A series of allergens with high sequence identity to Bet v 1 have been reported in the literature, pollen allergens from other trees belonging to the Fagales order as well as food allergens like, for example, Api g 1 from celery (Apium graveolens) (4), Mal d 1 from apple (Malus domestica) (5), Pru av 1 (formerly Pru a 1) from cherry (Prunus avium) (6), Pyr c 1 from pear (Pyrus communis) (7), and Cor a 1.401 from hazelnut (Corylus avellana) (8) (Fig. 1). In contrast to the three-dimensional structure of Bet v 1, which has been studied extensively in recent years (9–12), as yet no high-resolution structure of any of the corresponding food allergens is available. Because this is a prerequisite for a detailed understanding of the observed immune cross-reactivity on a structural level, we determined the three-dimensional structure of the major cherry allergen Pru av 1 in solution. Like Bet v 1, Pru av 1 is produced as a 160-residue precursor protein that is processed by cleavage of the NH₂-terminal methionine (13), yielding a protein with a calculated molecular mass of 17.5-kDa and a calculated isoelectric point of 5.9. The physiological function of these allergens is still unknown. They show high sequence similarity to pathogenesis-related and stress-induced proteins (14, 15) but seem to be expressed constitutively, even though the expression of several genes related to Bet v 1 has been reported to be induced upon contact with microorganisms (16). A potential ribonuclease activity of Bet v 1 was also discussed (17). Three highly conserved regions on the surface of the Bet v 1 molecule were proposed as candidates for IgE antibody binding epitopes (10); one of them, the glycine-rich P-loop around Glu45, was recently confirmed by the crystal structure of Bet v 1 in complex with an Fab fragment of a monoclonal murine IgG antibody with high capacity to inhibit binding of serum IgE from allergic patients to Bet v 1 (18). Additional information on potential epitopes is provided by biochemical data like the study of low IgE-binding isosforms or mutants for both Bet v 1 (12, 19) and Pru av 1 (20). A thorough knowledge of the IgE binding epitopes is the key to the development of hypoallergenic allergen variants that can be used as vaccines for a patient-tailored specific immunotherapy with reduced anaphylactic side effects (21).

MATERIALS AND METHODS

NMR Sample Preparation—We employed two different strategies to purify recombinant Pru av 1 from Escherichia coli lysates. The samples used for the structure determination were prepared as described previously (22, 23). For the samples used to measure [¹H]¹⁵N nuclear
Solution Structure of the Major Cherry Allergen Pru av 1

Overhauser effect (NOE) values and to investigate the interaction with homocastasterone, a completely native purification protocol of non-fusion Pru av 1 based on chromatofocusing (24) was carried out. Final purification was achieved by anion exchange chromatography. The fusion Pru av 1 based on chromatofocusing (24) was carried out. Final homocastasterone, a completely native purification protocol of non-fusion Pru av 1 and 10 mM potassium phosphate (pH 7.0) in H$_2$O/D$_2$O (9:1) or H$_2$O/Me$_2$SO-$d_6$ (9:1). The NMR samples contained 0.8–1.2 mM uniformly $^{15}$N- or $^{13}$C/$^{15}$N-labeled Pru av 1 and 10 mM potassium phosphate (pH 7.0) in D$_2$O or 4 h. $^{1}H$-$^{15}$N NOE values were measured using the pulse sequence of Dayie and Wagner (33) with a relaxation delay of 4 s. For proton saturation a train of 120° high-power pulses was applied for the final 3 s of the relaxation delay. The NMR data were processed using software written in-house and analyzed with the program packages NMRView (34) and NDEE (SpinUp Inc., Dortmund, Germany). $^{1}H$-$^{15}$N NOEs were corrected for signal decrease because of minor sample precipitation during the experiment and averaged over two independent data sets.

Structure Calculation—Based on the almost complete assignment of the $^{1}H$, $^{13}$C, and $^{15}$N resonances of Pru av 1 published previously (23), a total of 2299 distance restraints could be derived from the two- and three-dimensional NOESY spectra in an iterative procedure. NOE cross-peaks were classified manually as strong, medium, or weak according to their intensities and converted into distance restraints of less than 2.7, 3.5, or 5.0 Å, respectively. 23 of the 973 experimental restraints served as an input for the calculation of 60 distances restraints to less than 2.3 Å between the amide nitrogen and the acceptor of a slowly exchanging amide proton could be identified unambiguously from the results of initial structure calculations. For each of the 34 hydrogen bonds the distance between the amide proton and the acceptor was restrained to less than 2.3 Å and the distance between the amide nitrogen and the acceptor to less than 3.3 Å. These experimental restraints served as an input for the calculation of 60

![Structure-based sequence alignment with Pru av 1 of Pyr c 1 (83.5% sequence identity to Pru av 1), Mal d 1 (82.9%), Cor a 1.0401 (64.4%), Bet v 1 isoform a (59.1%), Api g 1 (41.2%), and the START domain of MLN64 (8.5%). The sequence positions above and below the sequences correspond to Pru av 1 and MLN64, respectively. Gaps in the alignment are indicated by dots. Residues conserved in at least four of the six allergens are highlighted by gray boxes and residues conserved in all six allergens by black boxes. The secondary structure elements of Pru av 1 are shown below the alignment. The alignment of the allergens with Pru av 1 is based on homology models created by SWISS-MODEL (55) using the lowest energy structure of Pru av 1 as a template. The alignment of the START domain of MLN64 with Pru av 1 is based on a comparison of the PDB entry of the START domain of MLN64 with the lowest energy structure of Pru av 1 by the Dali server (53). The 129 MLN64 residues used for the alignment are printed in uppercase letters and residues not used for the alignment in lowercase. Formatting was performed using ALSCRIPT (56). The secondary structure was assigned using the DSSP program (57). Residues conserved in at least four of the six allergens are highlighted by gray boxes and residues conserved in all six allergens by black boxes.
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Structures using restrained molecular dynamics with X-PLOR 3.851 (35). To this end, a three-stage simulated annealing protocol (36–38) with floating assignment of prochiral groups (39) was carried out as described previously (11, 40, 41), with the following modifications. For conformational space sampling, 160 ps with a time step of 2 fs were simulated at a temperature of 2000 K, followed by 120 ps of slow cooling to 1000 K and 90 ps of cooling to 100 K, both with a time step of 1 fs. Simulation times longer than these were tested, but no significant improvement of the results could be observed. A conformational data base term for both backbone and side-chain dihedral angles (42) was included in the target function to improve the stereochemical properties of the structures. After simulated annealing the structures were subjected to 250 steps of Powell minimization (43) of the full target function calculated, each of the sets having one of the distance restraint test sets left out. For those 16 structures of each set showing the lowest energy values, the r.m.s.d. from the distance restraints not used for their calculation were determined after assigning the restraints from the nine working sets a relative weight of 10 compared with those from their test set to prioritize them during floating assignment of prochiral groups.

Patients’ Sera—Sera from patients allergic to birch pollen and with an oral allergy syndrome after ingestion of fresh fruits (cherry, apple, pear, hazelnut) and vegetables (celery) were selected for this study. Most of the sera showed positive CAP or EAST (enzyme allergo-sorbent test) classes (greater than class 2) to the major allergens of birch pollen (Bet v 1, celery (Api g 1), cherry (Pru av 1), apple (Mal d 1), and pear (Pyr c 1)). Sera were taken from the serum collection of the Paul-Ehrlich-Institut or kindly supplied by Dr. H. Aulepp (Hospital Borkum Riff, Borkum, Germany).

Recombinant Allergens for Immunoblot Experiments—The recombinant major allergens from birch pollen, Bet v 1 isofrom a, apple, Mal d 1, and celery tuber, Api g 1, were obtained from BIOMAY, Länz, Austria. The recombinant major allergen from sweet cherry, Pru av 1, was purified as described elsewhere (6). The major allergens from pear, Pyr c 1, were purified from 1 g of Bet v 1a, 10 µg of Pru av 1, 10 µg of Api g 1, and buffer as control for 5 h. Thereafter, samples were diluted to 0.6 ml (1 ml for the samples with Bet v 1a as an inhibitor) and added to the blot strips (3 mm width). After overnight incubation, bound IgE was detected with a rabbit anti-human IgE antiserum (1:4000, 1 h; DAKO, Glosstrup, Denmark) followed by a biotin-labeled goat anti-rabbit immunoglobulin antibody (1:6000, 1 h; DAKO) as a secondary antibody and a streptavidin-horseradish peroxidase (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) incubation (1:10000, 30 min). Visualization was performed with the ECLTM Western blotting detection reagents (Amersham Pharmacia Biotech).

Modeling of Allergen-Steroid Complexes—Cavities were examined with SURFNET 1.5 (48) using a grid separation of 1.0 Å and a minimum and maximum gap sphere radius of 1.4 and 3.5 Å, respectively. Topology and parameter files for cholesterol were generated by the HIC-Up server (49) based on the cholesterol linoleate moiety of a crystal structure (50) from which the linoleate atoms were removed and subsequently modified to obtain topology and parameter files for castasterone. The simulated annealing protocol described above was also used to model Bet v 1 complexes with castasterone. To this end the atom positions of Bet v 1 as given by its crystal structure (10) were kept fixed, whereas a set of 33 distance restraints between the C-8 atom of castasterone and those Co atoms of Bet v 1 lining the cavity was introduced for each castasterone molecule, which effectively restrained C-8 to within 0.5 Å of a point near the center of the cavity. The resulting models were refined by 1000 steps of Powell minimization (43) of a modified target function where the van der Waals interaction was represented by a Lennard-Jones potential. The results were transferred to Pru av 1 by placing the castasterone molecules into equivalent positions of the lowest energy structure of Pru av 1, which was followed by 1000 steps of Powell minimization of the original target function (including all experimental restraints and the modeling distance restraints) to remove steric clashes and subsequent refinement by 1000 steps of Powell minimization of the modified target function (again including all restraints).
Solution Structure of the Major Cherry Allergen Pru av 1

**Table 1**

Summary of the structure calculation

Except for the experimental restraints, all values are average values over the 22 accepted structures in the form average value ± standard deviation.

| Experimental restraints used for the structure calculation |
|----------------------------------------------------------|
| Intra-residual NOEs                                      | 658 |
| Inter-residual NOEs                                      |     |
| Sequential                                               | 729 |
| Medium range                                             | 330 |
| Long range                                               | 592 |
| Dihedral angle restraints                                | 71  |
| Hydrogen bonds                                           | 34  |

| Molecular dynamics simulation statistics                  |
|----------------------------------------------------------|
| Energies/kcal/mol                                         |
| Total                                                    | 244 ± 7 |
| Bond lengths                                             | 0.5 ± 3 |
| Improper angles                                          | 0.7 ± 0.7 |
| van der Waals repulsion                                  | 0.6 ± 0.6 |
| Distance restraints                                      | 0.6 ± 0.6 |
| Dihedral angle restraints                                | 0.6 ± 0.6 |

| Atomic r.m.s.d.s from the average structure               |
|----------------------------------------------------------|
| Backbone                                                |
| Heavy atoms                                             |
| Overall                                                 | 0.69 ± 0.09 | 0.93 ± 0.09 |
| Regular secondary structure                             | 0.41 ± 0.08 | 0.72 ± 0.06 |
| β-Strands                                               | 0.29 ± 0.06 | 0.61 ± 0.07 |
| COOH-terminal α-helix                                   | 0.39 ± 0.12 | 0.85 ± 0.12 |

Comparison with other structures

| Backbone atomic r.m.s.d.                                |
|----------------------------------------------------------|
| Bet v 1 X-ray                                          | 1.94 ± 0.15 |
| Bet v 1 NMR                                            | 2.31 ± 0.13 |
| MLN64                                                  | 2.93 ± 0.07 |

a residues 1–159.

b residues 2–58, 65–85, 97–104, 112–122, 130–153.

b residues 2–11, 41–49, 65–85, 97–104, 112–122.

d residues 130–153.

f from Ref. 10.

(11); average structure; residues 1–154.

(52); residues 2–29, 31–35, 37–47, 50–60, 63–71, 73–77, 78–86, 98–110, 111–120, 129–156 (Pru av 1) with 280–307, 308–312, 313–323, 326–336, 343–351, 352–356, 359–367, 384–396, 399–408, 416–443 (MLN64), respectively.

**RESULTS AND DISCUSSION**

**Structure Determination**—Analysis of the NMR spectra of Pru av 1 yielded a total of 2438 experimental restraints for the structure calculation. In particular, the good dispersion of the amide proton resonances (23) allowed the identification of 1908 backbone atomic r.m.s.d.s from the average structure of 0.60 Å for the backbone and 0.93 Å for all heavy atoms. A schematic representation of the solution structure of Pru av 1 is shown in Fig. 3. The NH$_2$ terminus on the left is hidden by the loop from Ile$^{96}$ to Glu$^{96}$, and the COOH terminus can be seen on the right. Except for the loop from Glu$^{60}$ to Tyr$^{64}$, which is indicated by an arrow, the structures are in excellent agreement, especially as far as the β-strands are concerned. The side-chain of Glu$^{60}$ shown at the bottom is clearly solvent-exposed in all structures. The overlay was performed using Sybyl 6.5 (Tripos Inc., St. Louis, MO).

**Description of the Structure**—Pru av 1 shows a well defined structure in solution (Fig. 3) with average atomic r.m.s.d. from the average structure of 0.60 Å for the backbone and 0.93 Å for all heavy atoms. A schematic representation of the solution structure of Pru av 1 (Fig. 4) reveals that a folded seven-stranded antiparallel β-sheet (residues 2–11, 41–49, 53–58, 65–75 with a kink at Asp$^{72}$, 80–85, 97–104, and 112–122) and two short α-helices arranged in a V-shaped manner (residues 15–22 and 26–33) wrap around a long COOH-terminal α-helix (residues 130–153) to form a basket-like structure with the long helix resembling a handle, thus creating a large hydrophobic cavity. In contrast to the precision of the overall structure, however, the loop from Glu$^{60}$ to Tyr$^{64}$ is experimentally less well defined because of the missing resonance assignments for the amide protons of Ser$^{52}$ and Glu$^{63}$ (23), leading to a marked increase in atomic r.m.s.d. (Fig. 2). This lack of experimental data might in fact reflect an actually existing increased
flexibility. One indication for this is the rapid solvent exchange of the amide protons in this region, resulting in exceptionally weak NMR signals. In addition, this loop is poorly defined in both sets of solution structures of Bet v 1 (10, 11), and the determination of the crystal structure of Bet v 1 yielded two conformers for this loop (i.e. Asp60 to Lys65), which still do not fit the electron density well (10). To obtain initial experimental data concerning the dynamic behavior of Pru av 1 in solution we measured \( {^1H}^{15}N \) NOE values (Fig. 2). The low \( {^1H}^{15}N \) NOEs of 0.489 ± 0.018, 0.359 ± 0.017, and 0.501 ± 0.018 for the amide protons of Glu8, Gly61, and Tyr64, respectively, strongly support the notion that this loop shows significantly increased internal flexibility. Surprisingly, one of the lowest values (0.414 ± 0.017) was measured for the amide proton of Glu\(^6\), which is located in a slight bend in the middle of the first \( \beta \)-strand.

**Comparison with Bet v 1**—The folding topology of Pru av 1 has already been observed for the major birch pollen allergen Bet v 1, and a backbone overlay of the lowest energy structure of Pru av 1 with the crystal structure of Bet v 1 (Fig. 5; steroid molecules are modeled into these structures as discussed below) confirms that indeed both the secondary structure elements and the tertiary fold of these two allergens are virtually identical. More precisely, a comparison of the average solution structure of Pru av 1 with the crystal structure of Bet v 1 (10) and the average solution structure of Bet v 1 (Ref. 11; only residues 1–154 are taken into account, because the COOH terminus is less well defined experimentally) yields backbone atomic r.m.s.d. of 1.85 and 2.23 Å, respectively, which is of the same order as the difference of 2.06 Å found upon comparing these two Bet v 1 structures with each other. Together with the considerable sequence identity between Pru av 1 and Bet v 1, the conserved backbone conformation leads to a very similar molecular surface as far as shape and charge distribution are concerned, rendering the existence of cross-reactive IgE-binding epitopes most likely. In particular, the glycine-rich P-loop around Glu\(^6\) is structurally conserved in Pru av 1 (Fig. 4). For Bet v 1, this region was recently identified as the binding epitope of a monoclonal murine IgG antibody (15) whose high capacity to inhibit binding of serum IgE from allergic patients to Bet v 1 strongly suggests that this loop is also one of the IgE binding epitopes. The introduction of four point mutations including the substitution of Glu\(^6\) by serine indeed resulted in a Bet v 1 mutant with severalfold reduced IgE binding capacity (12). In the crystal structure of the complex of Bet v 1 with the IgG Fab fragment, the negatively charged side-chain of Glu\(^6\) is located in a binding pocket of the antibody with a positive electrostatic potential, where it forms two hydrogen bonds. In addition to Glu\(^6\), which is found to be solvent-exposed in all 22 accepted structures of Pru av 1 (Fig. 3), 14 of the remaining 15 residues forming the interaction surface between Bet v 1 and the IgG Fab fragment are either conserved (Glu\(^45\), Gly\(^46\), Gly\(^48\), Gly\(^49\), Pro\(^50\), Gly\(^51\), Thr\(^52\), Asp\(^72\), Ile\(^86\), and Lys\(^97\)) or substituted conservatively (Ile\(^44\) by Leu, Asn\(^47\) by Asp, Arg\(^70\) by Lys, and His\(^76\) by Lys) in Pru av 1 (Fig. 1), which strongly suggests that this region is a cross-reactive IgE binding epitope. This proposal is supported by the significantly decreased binding of serum IgE to the mutants Pru av 1 G46P and Pru av 1 Δ52 observed for some patients (20).

**Immunoblot Inhibition Experiments**—For IgE immunoblot inhibition experiments a serum pool of seven patients was tested with Bet v 1a, Mal d 1, Api g 1, Pru av 1, Cor a 1.0401, and Pyr c 1 transferred to nitrocellulose. Preincubation of the serum pool with Bet v 1a showed complete inhibition of IgE binding to the related major food allergens (Fig. 6). Hence, all of the IgE binding epitopes presented by these food allergens exist on the molecular surface of Bet v 1a as well. This finding is consistent with the experience that sensitization usually occurs to birch pollen, whereas the related food allergies are a consequence of the cross-reaction of the resulting pollen-specific IgE antibodies. To investigate the IgE cross-reactivity with the two major food allergens from cherry and celery, preincubation of the serum pool was performed with Pru av 1a and Api g 1. Complete inhibition of IgE binding to the major cherry allergen was obtained with Pru av 1a as the positive control. By contrast, only a small reduction of IgE binding to Pru av 1a on the solid phase resulted from using Api g 1 as an inhibitor (Fig.
7). No IgE inhibition was detected with buffer as control. Serum from a nonallergic donor was used as negative control. In other words, Pru av 1 must contain at least one IgE binding epitope that is not presented by Api g 1. Sequence alignment of Pru av 1 with Api g 1 (Fig. 1) shows that the P-loop region is not conserved in Api g 1; the P-loop is not only shorter by one residue, but also the negatively charged Glu45 is substituted by a positively charged lysine. The proposal that the P-loop region forms one of the cross-reactive epitopes can therefore provide a simple explanation of why preincubation with Api g 1 fails to efficiently inhibit IgE binding to Pru av 1. To verify this hypothesis the preparation and subsequent immunological as well as structural characterization of several allergen mutants is currently under way in our laboratories.

**Implications for the Physiological Function—Bet v 1 and Pru av 1 form a large internal hydrophobic cavity with a volume of ~1600 Å³ (Fig. 8). This forked cavity has three openings to the protein surface, one at the P-loop, one between α5 and β1, and one between α5 and the loop from Glu60 to Tyr64. The latter is the largest opening, but its size depends strongly on the conformation of the flexible loop acting as a flap. Such a large cavity constitutes a very unusual feature for a protein structure and can therefore be expected to be important for its physiological function. An obvious possibility for the physiological purpose of the cavity is the binding of a hydrophobic ligand. An indication of what this hypothetical ligand might be was provided by the recently determined crystal structure of the START domain of the human protein MLN64 (52), part of which revealed a striking structural homology to Bet v 1 and Pru av 1 (Fig. 9). Based on a comparison of the PDB entry of the START domain of MLN64 with the lowest energy structure of Pru av 1 by the Dali server (53), an alignment of the average solution structure of Pru av 1 with the crystal structure of the START domain of MLN64 yielded a backbone atomic r.m.s.d. of 2.89 Å over as many as 129 residues, even though the sequence identity over these 129 residues is only 8.5% (Fig. 1). Bet v 1 and the START domain of MLN64 are the only proteins with
significant structural homology to the lowest energy structure of Pru av 1 that were found by a Dali server database search. The fact that START domains are associated with the transfer of lipids, especially of steroids, suggests phytosteroids as possible ligands for Bet v 1 and Pru av 1. It should be noted, however, that there are also significant structural differences between Bet v 1 and Pru av 1 on one hand and the START domain of MLN64 on the other hand. In addition to the existence of an additional α-helix and two additional β-strands at the NH₂ terminus, the cavity of the START domain of MLN64 with a volume of about 1000 Å, which is approximately the volume required for the accommodation of a single steroid molecule, is much smaller than that of the allergens. Furthermore, the cavity of the START domain of MLN64 resembles a tunnel, with only two openings to the protein surface, which correspond to the opening at the P-loop and to the opening between α3 and the loop from Glu60 to Tyr64 of Pru av 1. Unfortunately, a quantitative investigation of the binding of phytosteroids to Pru av 1 by means of NMR titration experiments is very difficult because of the hydrophobicity of virtually all the physiologically relevant steroids, but we were able to gather first qualitative experimental evidence that Pru av 1 does indeed interact with a particular phytosteroid. Upon the addition of homocastasterone, a brassinosteroid that is different from the most widely distributed brassinosteroid, castasterone, only by the replacement of the methyl group at C-24 with an ethyl group (54), several amide proton resonances of Pru av 1 disappeared from the [1H,15N] HSQC spectrum (Fig. 10). This is probably because of severe line broadening as a consequence of exchange processes that are intermediate on the NMR time scale. Interestingly, the affected residues (Leu18, Lys20, Ala21, Phe22, Val23, Leu24, Asp25, Ala26, Asn28, Val30, Ile38, Lys54, Lys55, Ile56, Lys68, Lys70, Ile71, Tyr81, Leu85, Asp89, Lys103, and Ile128) surround the lower part of the cavity like a funnel (Fig. 8), thus supporting the expectation that homocastasterone binding takes place inside this cavity. Molecular modeling was used to investigate the steric constraints that are imposed on the orientation and the position of bound steroid molecules by the size and shape of the cavity. Because the opening between α3 and the loop from Glu60 to Tyr64 of the lowest energy solution structure of Pru av 1 is larger than the corresponding opening of the crystal structure of Bet v 1, we decided to model the more constrained Bet v 1 complexes first and then transfer the results to Pru av 1. The cavity of Bet v 1 and Pru av 1 is so large that it can accommodate one or two castasterone molecules in several different positions and orientations without significant structural changes (Figs. 5 and 8). In conclusion, in light of the above evidence, the physiological function of Bet v 1 and Pru av 1 most likely involves phytosteroid binding. The striking structural homology observed between the plant proteins Bet v 1 and Pru av 1 on one hand and the corresponding domain of the human protein MLN64 on the other hand, despite a low sequence identity and despite considerable structural differences, indicates that we might be dealing with a widely distributed tertiary fold designed to bind steroids or other lipids for a variety of purposes. Although our results mark a first step toward the elucidation of the physiological function of these proteins, a series of questions remains to be answered by future investigations; for example, is the binding specific for particular steroids, and if so, what features determine the specificity? Do these allergens bind two steroid molecules simultaneously, or is there any additional ligand to occupy the extra space in the cavity? What exactly is the physiological purpose of their interaction with steroids?

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