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X-Ray Crystal Structures of Birch Pollen Profilin and Phl p 2

Abstract

Background: Type 1 allergy affects 20% of industrialized populations and thus represents a major health care issue. The symptoms of type 1 allergy, which include rhinitis, conjunctivitis, dermatitis and asthma, are elicited by the cross-linking of IgE receptors through polyvalent allergens. A detailed understanding of the cell surface phenomena and the rational development of new therapies require high-resolution structural information. **Methods:** The structures of two widespread allergens, birch pollen profilin (BPP) and Phl p 2 have been solved by multiple isomorphous replacement. Refinements are underway to 2.4 and 2.0 Å, respectively. In addition, the IgE-reactive epitopes of BPP were identified by screening an epitope expression library with the serum IgE of an allergic individual. **Results:** BPP exhibits an α/β -fold which is similar to the mammalian and amoeba profilins. The structure of Phl p 2 is a compact eight-stranded β -barrel. Screening an epitope library of BPP identified three major epitopic regions involved in IgE binding, including the amino and carboxy-terminal α -helices. These regions also interact with the physiologically relevant ligands of profilin, actin and proline-rich peptides. **Conclusions:** The distribution of IgE-binding sites on BPP allows for the productive interaction with IgE antibodies of different epitope specificities required for efficient signal transduction. These epitopes correspond to the most highly conserved regions of the profilin molecule and thus provide the molecular basis for allergen cross-sensitivity. Due to steric considerations, the involvement of these epitopic regions in the binding of physiologically relevant profilin ligands indicates that the native profilin is the species responsible for eliciting the allergic response. A comparison of the BPP and Phl p 2 structures shows that there is no preference for secondary structural elements in the allergic response. The detailed chemical and physical description of the major reactive epitopes provides a data base for the design of tight-binding monovalent ligands which can prevent receptor aggregation and thereby reduce the allergic response.

Key Words

Allergen
Profilin
Phl p 2
Epitope
Crystallography
Structure

Introduction

Type 1 allergy affects up to 20% of the population in industrialized countries and is associated with symptoms of asthma, rhinitis and conjunctivitis. An allergic response is initiated by the binding of polyvalent allergens to multiple

IgE molecules causing an aggregation of high-affinity IgE receptors (Fc ϵ RI). This is accompanied by the activation of one or more tyrosine kinases and subsequent release of histamine, prostaglandins, leukotrienes and cytokines. To address the structural constraints associated with these cell surface phenomena, we have solved the X-ray crystal struc-

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Table 1. IgE-binding sites: the full-length BPP sequence is displayed on the top line, with the experimentally determined IgE-reactive epitopes shown below

1	10	20	30	40	50	60	70	80
MSWQTYVDEHLMCDIDGQGEELAASAIVGHDG	SVWAQSSSFPQFKPQEITGIMKDFEGPGHLAPTGLHLGGIKY	MVIQGEAGAVIRGKK						
SWQTYVDEHLMCDIDGQGE								
MSWQTYVDEHLMCDIDGQGEELAASAIVG								
MSWQTYVDEHLMCDIDGQGEELAASAIVGHDG								
	MCDIDGQGEEL							
		ELAASAIVG						
			VGHDG	SVWAQSSSFPQFKPQ				
				VWAQSSSFPQFKPQE				
					AQSSSFPQFKPQEITGI			

tures of two widespread allergens, birch pollen profilin (BPP) [1, 2], an actin and poly-*L*-proline binding protein, and Phl p 2 [3, 4], a major grass pollen allergen.

Materials and Methods

Protein Purification

BPP was expressed in *Escherichia coli* using a T7 expression system as previously described [5]. The cDNA coding for Phl p 2 was ligated into the polylinker of pMW175 and this construct was used to transform *E. coli* strain BL21(DE3) and the recombinant Phl p 2 purified by chromatography [3, 4].

Crystallization, Data Collection and Structure Solution

Recombinant BPP crystallized from 2.0 *M* ammonium sulfate, 100 mM MES, 5% *t*-BuOH pH = 6.0, at 4°C. Diffraction from these crystals is consistent with the space group P4₁2₁2 (unit cell dimensions: *a* = 59.90 *c* = 85.37 Å) with one molecule of BPP in the asymmetric unit. One heavy-atom derivative was prepared by soaking native crystals in 0.001 *M* ethyl mercury phosphate for 48 h at pH = 6.0. Phl p 2 was crystallized from 23% PEG 4000, 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate, pH 4.6 at 18°C. Diffraction from these crystals is consistent with the space group P2₁2₁2 (unit cell dimensions: *a* = 39.3, *b* = 64.6, *c* = 36.1), with a single molecule in the asymmetric unit. Isomorphous derivatives of uranyl acetate, mercuric acetate, samarium acetate and platinum tetrachloride were obtained by soaking crystals in 10, 5, 10 and 1 μ M heavy-atom solutions, respectively. Data were collected on a Siemens multiwire area detector and reduced using the program package XDS [6].

The structure of BPP was determined using a combination of single isomorphous replacement and molecular replacement techniques. The resulting 3.5-ångström resolution electron density map clearly showed the central six-stranded antiparallel β -sheet, 3 helices and some loops. The current refined BPP model has an R factor of 17.8% and includes 936 nonhydrogen protein atoms, and 48 solvent molecules. Phl p 2 was solved by the method of multiple isomorphous replacement employing four different derivatives. An MIR map calculated to 3.0 Å clearly showed an eight-stranded antiparallel β -barrel motif. Refinement of the structure at 2.0-ångström resolution is in progress.

Mapping of IgE-Binding Sites

The cDNA coding for BPP was randomly digested with DNase I and fragments shorter than 300 bp isolated by preparative agarose gel electrophoresis. The fragments were ligated into λ gt11, and 50,000 phage of the profilin epitope library were used to infect *E. coli* Y1090 at a density of 1,000 phage per plate (140 mm diameter). Synthesis of recombinant protein was induced by overlaying the plates with nitrocellulose filters soaked in 10 mM IPTG. Forty-eight profilin epitope clones were isolated using serum IgE from a profilin-allergic patient and ¹²⁵I-labeled antihuman IgE antibodies (Pharmacia, Uppsala, Sweden). Forty-two independent clones were sequenced and the shortest sequences are presented for clarity in table 1.

Results

Allergen Structures

The BPP has an α/β -structure built around a central six-stranded antiparallel β -sheet (fig. 1a). The BPP molecule contains two hydrophobic cores which are separated by the central six-stranded β -sheet. The structure of Phl p 2 consists of a β -sandwich formed by two layers of twisted β -sheets that enclose a hydrophobic core. The overall shape of the molecule is ellipsoidal with approximate dimensions 39 Å $\times$ 32 Å $\times$ 29 Å. The amino and carboxy termini are located at the same end of the molecule. The strands are joined by loop segments and no α -helical structure is present.

IgE-Binding Sites

The distribution of the positive clones shows a clustering of reactive epitopes at the amino- and carboxy-terminal α -helices and a two-stranded segment roughly composed of residues 30–50 (table 1, fig. 1c). The location of the prominent epitopes at these sites affords sufficient separation to allow the simultaneous binding of multiple IgE antibodies required for efficient receptor aggregation.

90	100	110	120	130
GSGGITIKKTGQALVFGITEEPVTPGQCNMVVERLGDYLIDQGL				
ITEEPVTPGQCNMVVERLGDYLIDQGL				
TEEPVTPGQCNMVVERLGDYLI				
TEEPVTPGQCNMVVERLGDYLIDQG				
GDYLIDQGL				
EEPVTPGQCNMVVERLGDYLIDQGL				
TEEPVTPGQCNMVVERLGDYLIDQGL				
GQCNMVVERLGDYLID				
VERLGDYLIDQGL				
RLGDYLIDQGL				

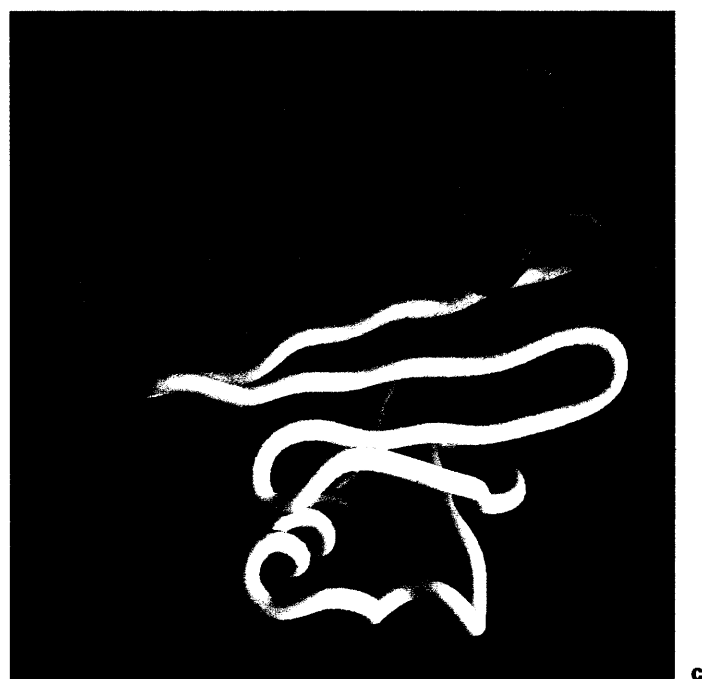
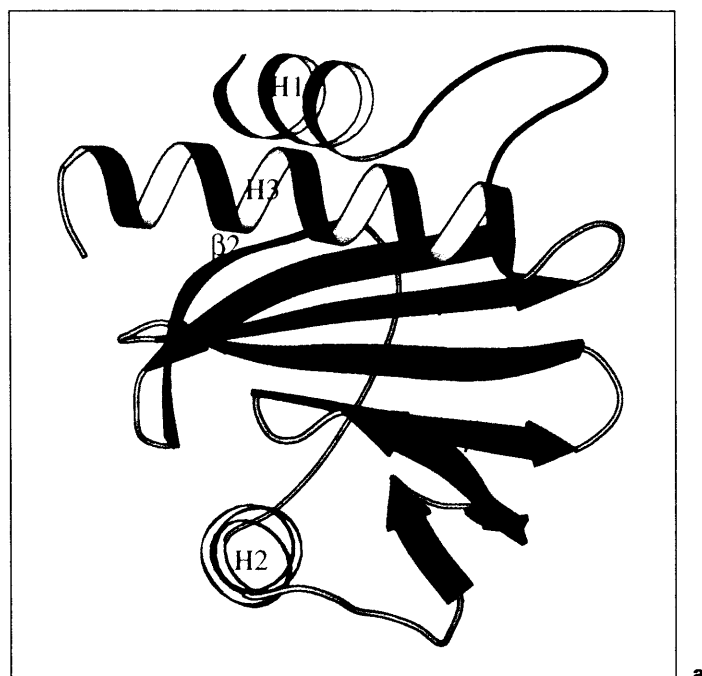
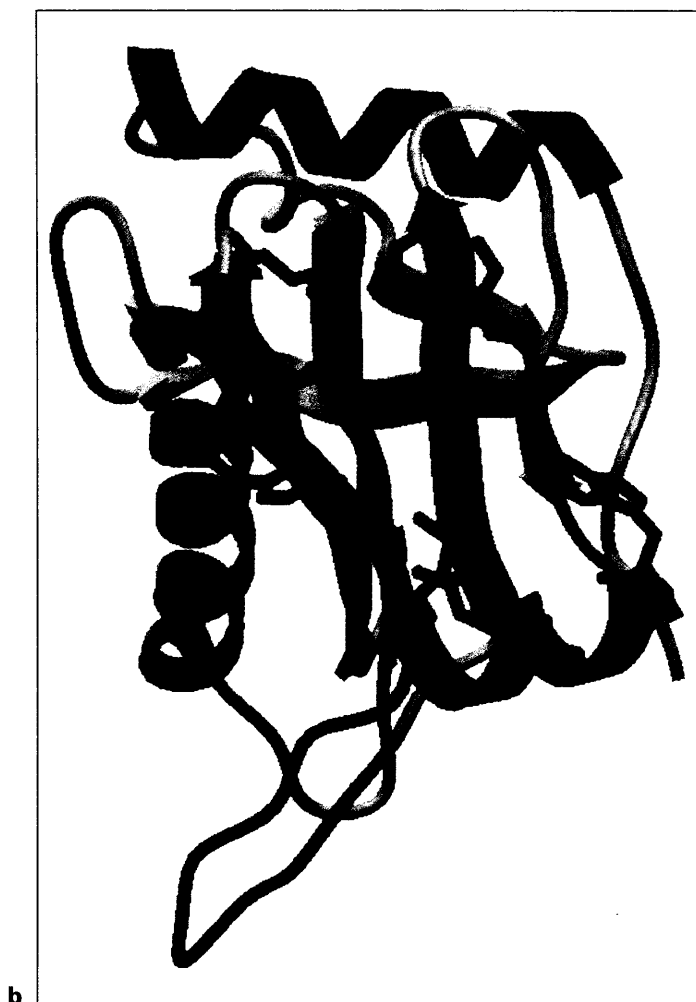


Fig. 1. a Ribbon diagram showing the fold of the BPP. The molecule is built around a central six-stranded antiparallel β -sheet (green). The black segment represents the nine amino acid loop which is not visible in electron density. This orientation shows the actin-binding surface of profilin [16], which is predominately composed of the carboxy-terminal helix (H3) and the bottom three strands (β 4, β 5 and β 6) of the central sheet. **b** Ribbon diagram of birch pollen profilin looking into the face of the six stranded β -sheet showing the amino (right) and

carboxy (left) terminal helices running approximately perpendicular and parallel to the sheet direction, respectively. In blue are the conserved hydrophobic residues (Trp3, Tyr6, Trp35, Tyr127, Leu128 and Leu133) implicated as part of the proline-rich ligand binding site. **c** Backbone representation of BPP showing the clustering of continuous IgE-reactive epitopes. The sequenced epitopes are displayed in green with the remainder of the backbone in white. This is the same view as **a** displaying the actin-binding surface.

Discussion

Profilin Function: Ligand-Binding Sites

Figure 1a shows the actin-binding surface for BPP. The basic architecture of the actin-binding surface is conserved throughout the family, indicating that all members of the divergent profilin family make similar interactions with the highly conserved actin molecule, including extensive contacts involving the carboxy-terminal α -helix of profilin. The solvent accessibility of the hydrophobic cluster (fig. 1b) suggests a binding site for hydrophobic molecules such as proline-rich ligands. This is consistent with the results of fluorescence [7], mutagenesis [8, 9] and qualitative NMR [10, 11] studies that have identified this region as the site of poly-L-proline binding.

Allergenicity: Structure-Function of Allergens

The IgE-binding sites on the native BPP molecule are represented by elements of α -helical and β -strand secondary structure. Phl p 2 adopts a compact β -barrel, and thus the IgE-binding sites on the native molecule must include β -strand and/or loop segments. When taken in combination, all of these structural data indicate that there is no common structural motif involved in the elicitation of allergic responses in general.

Two of the three major regions of IgE-reactive epitopes, the amino- and carboxy-terminal α -helices, directly overlap with the binding sites for proline-rich peptides and actin, respectively. Steric considerations would seem to preclude the simultaneous binding of these ligands and IgE antibodies. These observations indicate that the functional properties of this particular allergen are not necessary for allergenicity, and that native (ligand free) profilin is the species responsible for sensitization and subsequent stimulation of the allergic response. This is consistent with the observations that during pollen hydration and germination, the pro-

filin-actin complex dissociates, and that profilin, in contrast to actin, is easily extracted from pollen by aqueous solution, a situation which mimics contact with mucosal surfaces [12].

The IgE-binding sites in BPP epitopes provide an explanation for the clinical observation that 20% of all pollen-allergic patients are cross-sensitized towards pollen profilins from distantly related trees, grasses and weeds [1, 2]. The terminal α -helical segments display the highest degree of sequence homology observed throughout the entire profilin family. Sensitization against these particular regions readily affords cross-sensitivity against homologous proteins from very distant species due to the conserved structure and sequence of these epitopes. This may also provide the structural basis for cross-reactivity of allergic individuals to a wide variety of foods and fruits [13, 14].

In conclusion, the first high resolution X-ray structures of widespread allergens are reported. The availability of these structures, in combination with the known sequences of the IgE-binding sites, allows for the construction of a data base describing the detailed chemical, physical and conformational properties of IgE-reactive epitopes. This information will allow for the design of conformationally restricted monovalent allergen fragments (haptens) which contain the appropriate functional groups and the correct placement of these groups for optimal antibody binding. Monovalent IgE-binding haptens which do not cross-link effector-cell-bound IgE could be used for local blocking therapy of type I allergy as well as for active immunization [15]. Due to the lack of anaphylactic activity, high doses of these monovalent IgE-binding haptens could be utilized for the induction of blocking IgG antibodies that interfere with the allergen-IgE interaction. This hapten therapy has the potential to be tailored to the specific allergic spectrum of individual patients and represents a novel alternative to traditional approaches.

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