Structure
Article

Mapping the Interactions between a Major Pollen Allergen and Human IgE Antibodies

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SUMMARY

The interaction of specific IgE antibodies with allergens is a key event in the induction of allergic symptoms, thus representing an important target for therapeutic interventions in Type I allergies. We report here the solution NMR structure of Art v 1, the major mugwort pollen allergen. Art v 1 is the first protein structure with an allergenic defensin fold linked to a polyproline domain, which has not been identified in any reported allergen structure in the PDB. Moreover, the direct interaction of polyclonal IgE antibodies from an allergic patient has been mapped on the surface of an allergen for the first time. The data presented herein provide the basis for the design of tools for safe and effective vaccination against mugwort pollen allergy.

INTRODUCTION

Allergic diseases such as allergic rhinitis or hay fever, allergic asthma, food allergy, allergic skin inflammation, and anaphylaxis affect up to 25% of the population in industrialized countries, and their incidence is continuously rising, particularly in children and young adults (Finkelman and Vercelli, 2007). Type I allergy is characterized by an overwhelming expansion of allergen-specific T helper (TH)2 cells resulting in class switching of B cells to produce polyclonal IgE antibodies specific to common environmental allergens originating from various sources including pollen of grasses, weeds, or trees, spores of molds, foods, mites, cockroaches, and dander from pets and other domestic animals (Parronchi et al., 2000). Although numerous allergens have been characterized at the structural level, common features that would definitely predict the allergenic potential (allergenicity) of a protein are still unknown (Thomas et al., 2009). Therefore, the identification of IgE epitopes is crucial for better understanding the phenomenon of allergenicity and has direct implications for the improvement of allergen-specific therapeutic approaches. Bioinformatics (Stadler and Stadler, 2003) and peptide-based approaches (Lin et al., 2009) have been successfully applied to reveal linear or sequential IgE epitopes but are less suited for the identification of conformational or discontinuous epitopes, which have been studied in most cases by mutational analysis of allergens (Ferreira et al., 1998). Thus, 3D structure determination of the antigen-antibody interaction remains the most accurate method to identify IgE-binding sites on allergens. Such approaches have been successful in revealing epitopes recognized by monoclonal antibodies directed against mite (Li et al., 2008; Naik et al., 2008), bee venom (Padavattan et al., 2007), food (Niemi et al., 2007), as well as pollen (Mirza et al., 2000; Padavattan et al., 2009; Spangfort et al., 2003) allergens. However, to date no structural information on the interaction between allergens and polyclonal human IgE antibodies of allergic patients has been reported. To investigate the interaction of human IgE and allergens at the atomic level of resolution, we have focused on Art v 1, the major allergen of *Artemisia vulgaris* (mugwort) pollen. The herb mugwort can be considered one of the main causes of pollen allergy in late summer and autumn in Europe, Northern America, and parts of Asia (Dedic et al., 2009). Among the allergic population, 10%–14% are sensitized to mugwort and more than 95% of these patients display specific IgE antibodies to the major allergen Art v 1. At the primary structure level, Art v 1 appears as a modular glycoprotein composed of two domains: an N-terminal domain with high homology with plant defensins and a hydroxyproline-rich C-terminal part (Himly et al., 2003). The Art v 1 carbohydrate moiety which comprises up to 30% of the molecular weight has been characterized in detail (Leonard et al., 2005). The hydroxyproline-rich domain carries two types of glycosylation: (1) single adjacent hydroxyproline-linked β-arabinofuranoses, and (2) a large type III arabinogalactan composed of a short β1,6-galactan core substituted by a variable number (5–28) of α-arabinofuranoses. The immune response against Art v 1 is characterized by IgE antibodies that are mainly directed against the disulfide-bond stabilized defensin domain (Dedic et al., 2009; Himly et al., 2003). Interestingly, T cell recognition of Art v 1 is conspicuously restricted to a single immunodominant epitope located in the defensin domain (Art v 1_{25-36}) and is

associated with the expression of the HLA-DRB1*01 phenotype (Jahn-Schmid et al., 2005, 2008). In addition, Art v 1 was considered by Aalberse et al. (2001) as an intriguing and clinically highly relevant cross-reactive system.

In this study, we report the solution NMR structure of an allergenic defensin linked to a polyproline domain and the identification of conformational IgE-binding epitopes using antibodies isolated from the peripheral blood of a mugwort-pollen allergic patient. We identified two IgE-binding patches on the surface of the Art v 1 defensin domain that are crucial in triggering allergic reactions in mugwort pollen-sensitized patients. These epitope patches are predominantly positively charged and are highly conserved among Art v 1-homologous allergens in ragweed and sunflower pollen. Thus, the data presented herein provide a basis for the design of novel tools for safe and effective vaccination against mugwort pollen allergy.

RESULTS

Art v 1 Consists of a Defensin Domain Linked to a Proline-Rich Segment

The solution structure of Art v 1, the major allergen from *Artemisia vulgaris* pollen, was solved by NMR. Recombinant isotopically ^{15}N- and ^{13}C-labeled Art v 1 was produced in *Escherichia coli* and used for data collection. Chemical shift assignments were deposited in the BMRB (entry 16111) (Razzera et al., 2009). An ensemble of 100 structures was calculated from 627 distance constraints using a simulated annealing method within the program CYANA (Guntert et al., 1997). All structures are in agreement with the experimental constraints showing only minor deviations from the idealized covalent geometry (Table 1). The 20 lowest energy structures had no violations of either distance restraints greater than 0.25 Å or dihedral angles greater than 3.0° and were chosen to represent the solution structure of Art v 1 (deposited in the PDB under the assigned ID code 2kpy). The ensemble of the calculated structures superposed over the backbone is illustrated in Figure 1A; a cartoon representation is shown in Figure 1B. The backbone rmsd of the 20 refined Art v 1 structures is 0.49 Å for residues involved in the secondary structure elements of the defensin domain and 9.71 Å for the full-length polypeptide chain. Considering all atoms, the defensin domain or the entire polypeptide presented a rmsd of 1.12 or 9.85 Å, respectively. The calculated structure revealed that Art v 1 displays three distinct regions: (1) a defensin domain, (2) a transitional region, and (3) a C-terminal polyproline segment. The N-terminal domain (residues 1–56) shows the typical α/β fold of plant defensins (Thomma et al., 2002) composed of an α helix from residues 20–29 and three antiparallel β strands formed by amino acid residues 4–9, 34–40, and 44–53, respectively. Moreover, this fold is stabilized by eight disulfide-linked cysteines, with disulfide bonds linking C6–C53, C17–C37, C22–C47, and C26–C49. These data are in accordance with the structural motif of the cysteine-stabilized α/β fold of other plant defensins: two consecutive cysteine residues spaced by a tripeptide sequence (C-X-X-X-C) in the α helix and by a C-X-C in the β strand (Thomma et al., 2002). A comparison of 3D structures using Dali analysis (Holm et al., 2008) showed that the Art v 1 defensin domain is closely related to a radish (*Raphanus sativus*) seed defensin (PDB: 1ayj) that has antifungal activity. In contrast, the Art v 1 proline-rich segment (residues 57–108) seems to be unique in the PDB database and has not been identified in any structure reported so far. Using PONDR, the poly-proline region was predicted to be unstructured. However, several long-range NOEs between the defensin domain and the transitional region (residues 57–70) were observed, restricting the calculated structure of this region to 1.7 Å rmsd. For the identification of long-range NOEs, each decision of the network anchoring algorithm in CYANA was manually verified when transitional NOEs were added.

Table 1. Summary of the Conformational Restraints and the Statistical Analysis

Experimental Restraints	
NOE distances[a]	627
Intraresidues (i, i)	164
Sequential (i, i ± 1)	221
Medium range (i + 2, i + 3)	63
Long range (>i + 3)	179
Hydrogen bonds	24
Dihedral angles	58
Φ (°)	29
Ψ (°)	29
Rmsd from the average structure	
Structured defensin domain (3–56)	
Backbone (Å)	0.49 ± 0.07
Heavy atoms (Å)	1.12 ± 0.12
Quality Check	
Ramachandran plot (PROCHECK)[b]	
Most favored regions (%)	89.5
Additional allowed regions (%)	10.0
Generously allowed regions (%)	0.5
Disallowed regions (%)	0.0
CYANA "target function" (Å^2)	2.97 ± 0.39

[a] Derived from 627 assigned NOESY cross-peaks, including intraresidue peaks.
[b] For secondary structure elements.

The defensin domain was the first to be calculated, excluding all NOEs from the transitional and the polyproline region. In a first step, calculations were done without restraints for disulfide bonds. Once the structure is allowed to unambiguously attribute all disulfide bonds, this pattern was included in the subsequent calculations. Next, the list containing the NOEs for the defensin domain was fixed and manually given beforehand. Following the manual inclusion of the sequential NOEs (αN(i,i+1) and NN (i,i+1)) of the transitional and C-terminal region, and those defining the stereochemistry of the prolines, i.e., $\alpha\delta$(i,i+1) for *trans* and $\alpha\alpha$(i,i+1) for the *cis* prolines, the entire protein was calculated by CYANA. In this way, we could clearly identify NOEs between the defensin and the transitional region, suggesting some degree of order in the transitional region. It should be noted that the heteronuclear NOE goes from ~0.8 in the well-ordered defensin domain to ~−0.5 in the flexible C terminus, while the transitional region displays intermediate values. In addition, we observed two residues (G66 and A67) with