

NOTE

Identification of cyclophilin as a novel allergen from *Platanus orientalis* pollens by mass spectrometry

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Oriental plane trees are an important source of airborne allergens in cities of southwest Asia. In spite of extensive studies on *Platanus acerifolia* allergy, there are no reports on the molecular characterization of pollen allergens from *Platanus orientalis* trees. In this study, a newly recognized member of cyclophilin family with a molecular weight of 18 kDa was identified as being partly responsible for IgE reactivity of *P. orientalis* pollen extract.

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The prevalence of asthma and allergy has been increasing in developed and developing countries. It was estimated that over 20% of the world's population suffers from IgE-mediated allergic diseases (1). Airborne allergens derived from pollens are considered to be one of the most important causes of asthma and rhinitis (2). Currently, allergy diagnosis and specific immunotherapy are performed using allergenic extracts containing a variety of allergenic and non-allergenic components that make them too complex to be standardized (3). Identification and characterization of proteins causing IgE-mediated allergies are the main research objectives in the area of molecular allergy in order to establish component-resolved diagnosis and immunotherapy. *Platanus orientalis* (Oriental plane) is the dominant tree species in parks and streets in most cities of Iran (4). Previous research has indicated that *Platanus* pollen is a major contributor to pollinosis symptoms during March and April (5, 6). A high prevalence of positive skin prick tests for *P. orientalis* pollen extract (43%) has been recorded in Mashhad, Iran. Highest concentrations of its pollens have been detected during the flowering season, reaching up to 15% of the total pollens in Mashhad (A. Varasteh et al, unpublished data, 2008). Until now, three major allergens have been identified in *P. acerifolia* pollen extract, which have been characterized as Pla a 1, a nonglycosylated protein of 18 kDa belonging to a family of invertase inhibitors (7, 8); Pla a 2, a polygalacturonase protein with a molecular weight of 43 kDa (9);

and Pla a 3, a lipid transfer protein (LTP) with a molecular weight of 12 kDa (10). In spite of extensive studies on *P. acerifolia* allergy, there are no reports on the molecular characterization of pollen allergens from *P. orientalis* trees, which are rarely found in Western Europe and America.

Some of the patients showed IgE reactivity against an 18 kDa allergen in *P. orientalis* pollen extract, while no reactivity was detected against 18 kDa recombinant invertase inhibitors of *P. orientalis* pollens. In the present study, we aimed at identifying and characterizing the 18 kDa allergen from *P. orientalis* pollen extract by means of matrix-assisted laser desorption-ionization-time of flight mass spectrometry (MALDI-TOF-MS) analysis. Seven patients (four males and three females with a mean age of 25±10.5 years), who showed adverse reactions to *P. orientalis* pollens and reactivity to the 18 kDa allergen of *P. orientalis* pollen extract, were included in this study. All subjects underwent skin prick test (SPT) with *P. orientalis* pollen extract. The SPT was performed according to guidelines from the European Academy of Allergology and Clinical Immunology (11). Pollens from *P. orientalis* were purchased from Greer Laboratories, Inc (Lenoir, NC, USA). Diffusates of the pollens were prepared, as described previously (12). To use this extract for SPT, glycerol was added to a final concentration of 50% (v/v). After obtaining informed consent, sera were taken from all patients and stored at -20 °C until used in the assays. A serum pool from volunteers with no clinical history of allergy and with negative SPT to *P. orientalis* pollen extract was used as a negative control.

To assess the reactivity of patients' sera with the 18 kDa allergen of *P. orientalis* pollen extract, the protein content of *P. orientalis* pollen extract was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), according to Laemmli (13), in 12.5%

Abbreviations: MALDI-TOF-MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; PVDF, polyvinylidene difluoride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SPT, skin prick test.

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TABLE 1. Table shows all the proteins identified with acceptable scores (>50) and mass matched peptides

Protein name	Protein MW(kDa)/pI	Protein score	Species	Accession key	Observ. mass	Sequence
Cyclophilin	18.16/8.7	103	<i>Glycine max</i>	gi 17981611	1097.57 1406.77 2721.31	⁹⁰ FADENFVKK ⁹⁸ ²⁰ IVMELYADVTPR ³ ⁶³ VIPSMCQGGDFTAGNGTGGE SIYGA ⁸⁹
Cyclophilin	18.14/8.3	77	<i>Phaseolus vulgaris</i>	gi 829119	1097.57 1422.75 2723.35	⁹⁰ FADENFVKK ⁹⁸ ²⁰ IVFELYADVTPR ³¹ "HTGPGILSMANAGPCTNGSQFFICTTK ²
Cyclophilin	18.2/8.9	51	<i>Populus tremuloides</i>	gi 29124975	1097.57 1392.73 1454.73	⁹⁰ FADENFVKK ⁹⁸ ²⁰ IVMELFADTTTPR ³ ⁶ VYFDMITGAPAGR ⁹

mass tolerance of 1 Da. Search results were tabulated and scores were assigned, allowing assignment of likely proteins.

The 18 kDa allergen of *P. orientalis* pollens was identified by immunoblotting with individual patient's serum (Fig. 1B). Four out of seven patients' sera showed IgE reactivity against recombinant invertase inhibitors of *P. orientalis* pollens, Pla a 1 homolog (Lanes 2, 4, 5, and 6, Fig. 1C). In contrast, there are two sera that showed strong IgE reactivity against the 18 kDa component of *P. orientalis* pollens, but no reactivity against rPla or 1 (Lanes 1 and 3, Fig. 1B, C). The protein band at 18 kDa corresponding to the IgE-reactive protein of *P. orientalis* pollens was excised from the gel and analyzed by MALDI-TOF-MS. The Ms profile of the protein band is shown in Fig. 2. By comparison with the established database, a protein score >50 with more than two matched peptides was considered to be significant for identification. In the peptide mass fingerprint analysis, the masses obtained from the excised protein band showed the highest correlation with cyclophilin from *Glycine max*, *Phaseolus vulgaris*, and *Populus tremuloides* (Table 1), with a peptide sequence coverage of 43%. As shown in Fig. 2, four out of seven matched peaks belonged to prominent mass peaks.

During the last decade, purified native or recombinant allergens have shown promise as a safe and reliable diagnosis and therapy of allergic disorders (16). For this propose, identification of relevant allergens with appropriate techniques is a crucial step (17). Peptide mass fingerprinting is a standard method for allergen identification by MALDI-TOF-MS (18, 19). In this study, we evaluated a proteomic strategy for characterizing a novel 18 kDa allergen, which did not seem to be homologous to Pla a 1. Peptide mass fingerprinting revealed that the allergenic protein component at 18 kDa belongs to the cyclophilin family.

Cyclophilins have also been identified as allergens in *Betula verrucosa* (20), carrot (21), *Psilocybe cubensis* (22), and *Aspergillus fumigatus* (23).

In summary, some sera from the plane tree pollen-sensitive patients showed specific IgE against an 18 kDa protein component. Two allergens, a Pla a 1 homolog and a newly recognized member of cyclophilin family, were identified as being responsible for IgE reactivity to *P. orientalis* pollen extract at the molecular weight of 18 kDa.

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