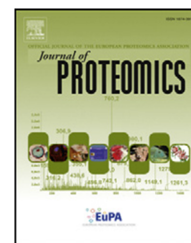
Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot



Allergomic study of cypress pollen via combinatorial peptide ligand libraries

Youcef Shahali[a], *Jean-Pierre Sutra*[a], *Elisa Fasoli*[b], *Alfonsina D'Amato*[b], *Pier Giorgio Righetti*[b], *Norihiro Futamura*[c], *Egisto Boschetti*[d], *Hélène Sénéchal*[a,e,1], *Pascal Poncet*[a,f,*,1]

[a]*Laboratoire "Sciences Analytiques, Bioanalytiques et Miniaturisation", ESPCI ParisTech, UMR 7195 CNRS, 75231 Paris cedex 05, France*
[b]*Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico di Milano, 20131 Milano, Italy*
[c]*Department of Molecular and Cell Biology, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki 305-8687, Japan*
[d]*Bio Rad Laboratories, C/o CEA-Saclay, 91181 Gif-sur-Yvette, France*
[e]*INSERM, Paris, France*
[f]*Institut Pasteur, Paris, France*

ARTICLE INFO

Article history:
Received 31 May 2012
Accepted 6 July 2012
Available online 17 July 2012

Keywords:
Peptide ligand libraries
Low-abundance proteome
Cypress pollen allergens
Cupressus sempervirens
Mass spectrometry
Proteomics

ABSTRACT

Although *Cupressus sempervirens* (*Cups*) pollen represents one of the main aeroallergens in southern Europe, only two *Cups* allergens have yet been identified and reported: Cup s 1 and Cup s 3.

The aim of this study was to identify allergens in cypress pollen using an immuno-proteomic approach. A sequential pollen protein extraction was developed and supplemented by a combinatorial peptide ligand library (CPLL) treatment to select low-abundance species. Control extracts and CPLL eluates have then been resolved by 1-DE and 2-DE gel electrophoresis, blotted and confronted with sera from cypress allergic patients. Extracted proteins including IgE-binding components were identified using nanoLC–MS/MS analysis.

A total of 108 unique gene products were identified analyzing the eluates and control loaded onto 1-DE SDS-PAGE. Forty proteins were identified in control samples and 68 supplementary species upon CPLL treatment. Out of the 12 IgE-binding proteins characterized in 2-DE gels, 9 were already reported as allergens in various sources including the two major known allergens of Cupressaceae (groups 1 and 2). Three IgE-binding proteins, not previously reported as allergens, are newly described.

The improvement in protein extraction combined with the enrichment of low-abundance species allowed us to extend the repertoire of potential cypress pollen allergens.

© 2012 Elsevier B.V. All rights reserved.

Abbreviations: 2-Me, 2-mercapto ethanol; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; CPLL, combinatorial peptide ligand library; *Cups*, *Cupressus sempervirens*; NBT, nitroblue tetrazolium; NCa, CNBr activated nitrocellulose membrane; PUN, Phosphate Urea NP40 buffer; TUC, Thiourea,Urea,CHAPS.

* *Corresponding author at:* ESPCI, LSABM, 10 rue Vauquelin, 75005 Paris, France. Tel.: +33 1 40 79 46 68, +33 1 40 79 47 81; fax: +33 1 40 79 46 54.
E-mail address: pascal.poncet@espci.fr (P. Poncet).
[1] Hélène Sénéchal and Pascal Poncet equally contributed to this study.

1874-3919/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jprot.2012.07.010

1. Introduction

The cypress pollen represents a widespread and highly invasive allergenic source worldwide. The increasing prevalence of the Italian cypress pollinosis (*Cupressus sempervirens*, *Cups*) in many of the cities surrounding the Mediterranean Basin, prompted us to determine some of the main allergens implicated in this seasonal pathology.

The analysis of cypress pollen proteins is known to be difficult due to the inherent characteristics of its matrix: high amounts of interfering compounds such as carbohydrates, phenolics and pigments [1–3]. In addition, the internal layer of the cypress pollen wall, known as intine, constitutes a highly stratified protective barrier of polysaccharides (mainly composed of cellulose and pectin) [4], impeding the proper extraction, identification and characterization of low-abundance cytoplasmic proteins. Therefore, although the characterization and standardization of *Cups* pollen extracts have been the subject of several studies, only two *Cups* allergens have yet been exhaustively characterized and unequivocally identified so far: Cup s 1 [2] belonging to the pectate lyase family and Cup s 3 a thaumatin like protein [5,6]. The low level of activity of the current non-standardized cypress pollen allergen extracts led to the selection of Mountain cedar (*Juniperus ashei*) pollen extracts as the gold standard for the diagnosis of cypress pollen allergy in Mediterranean areas [7]. However, despite the high level of cross reactivity between cypress and cedar pollen allergens, *J. ashei* is not endemic in Europe and could not be really representative of Mediterranean species as well as of the local exposure to cypress pollen allergens.

In previous studies, we revealed that the very poor protein concentration of conventional cypress pollen extracts is not related to the low protein content of the pollen grain itself but, instead, to important physical barriers imposed by the pollen sporoderm [3,8]. Indeed, the use of detergents and chaotropic agents, giving access to sporoderm recalcitrant fractions, increased about 17-fold the quantity of extracted *Cups* pollen proteins and allowed the detection of several yet-undescribed allergens. Therefore, the study of the allergenic potency of whole cypress pollen proteome requires the use of a series of pollen extractions yielding a number of fractions according to the relative solubility of different proteins. This approach allows the optimization of experimental conditions according to the specific properties of cypress pollen.

The recent development of new proteomic protocols led to more efficient protein extraction, precipitation and concentration procedures to remove non-proteinaceous compounds present in the starting material and to increase the concentration of underrepresented proteins. Besides, combinatorial peptide ligand libraries (CPLL) [9] were recently applied to plant tissues [10] for capturing the low- and very low-abundance proteins constituting a significant part in any proteome. Several IgE binding proteins which were previously absent or poorly represented and thus not detected by using conventional 1-DE and 2-DE gel sample preparation protocols have been recently detected and characterized in maize, milk, latex, peanut and almond syrup using a CPLL-based approach [11–15]. Therefore, despite their very low concentration in the allergenic source, these components are able to induce IgE sensitization in allergic patients that could not be detected because of overrepresentation of major proteins.

In the present survey, these techniques combining improved protein extraction and enrichment of very low concentration proteins have been adapted and applied to *Cups* pollen in order to provide a proteomic analysis including "hidden proteins" as well as to unravel novel potential allergen candidates.

2. Material and methods

2.1. Chemicals and biologicals

C. sempervirens pollen was supplied by Allergon AB (Ängelholm, Sweden). The solid-phase combinatorial peptide library known under the trade name of ProteoMiner™ beads as well as materials for electrophoresis such as gel supports, IPG and reagents were from Bio-Rad Laboratories (Hercules, CA, USA). N-ethylmaleimide, urea, thiourea, 3-[3-cholamidopropyl dimethylammonio]-1-propansulfonate (CHAPS), Nonidet™ 40 (NP-40), tris(2-carboxyethyl)phosphine hydrochloride, bis-(2-hydroxyethyl)disulfide, isopropanol, acetonitrile, trifluoroacetic acid, 2-mercaptoethanol (2-Me), polyoxyethylenesorbitan monolaurate (Tween® 20) and sodium dodecyl sulfate (SDS) were all from Sigma-Aldrich (St Louis, MO, USA). The test used to determine serum-specific IgE (ImmunoCAP®) was from Phadia AB (Uppsala, Sweden). Optitran BA-S 83 nitrocellulose membrane was from Schleicher & Schuell (Dassel, Germany). Both phenylmethanesulfonyl fluoride (PMSF) and ethylenediaminetetraacetic acid (EDTA) as protease inhibitor cocktail were from Sigma-Aldrich. Sequencing grade bovine trypsin was from Promega (Madison, WI, USA). Polyacrylamide gel (ExcelGel gradient 8–18%) and molecular masses (Mr) standard protein mixture were from GE Healthcare (Uppsala, Sweden). 3-μm ReproSil 100C18 chromatography support was from Dr. Maisch GmbH (Ammerbuch-Entringen, Germany). Alkaline phosphatase-conjugated goat anti-human IgE, 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT) were purchased from Sigma-Aldrich. All other chemicals were also from Sigma-Aldrich and were of analytical grade.

2.2. Equipment and software

Multiphor II chamber and semi-dry blotting apparatus were from GE Healthcare (Uppsala, Sweden). Versa-Doc image system, Protean device, semi-dry blotting apparatus and PDQuest software were from Bio-Rad Laboratories (Hercules, CA, USA). StageTip devices, EasyLC chromatography system, nanoelectrospray ion source for mass spectrometer and fused silica capillaries with 75 μm ID and 360 μm OD were from Proxeon Biosystems (Odense, Denmark). Speed Vac system was from SVPT s.r.l., Italy. LTQ-Orbitrap mass spectrometer was from ThermoScientific, (Bremen, Germany). A second hybrid quadrupole time of flight mass spectrometer API Qstar PULSAR was from PE-Sciex (Toronto, Canada). The associated capillary chromatographic system Agilent 1100 Series equipped with a Nano Pump, Iso Pump, and Degaser was from Agilent (Santa Clara, CA, USA). Analyst QS 1.1 software was from Applied

Download English Version:

https://daneshyari.com/en/article/1226587

Download Persian Version:

https://daneshyari.com/article/1226587

Daneshyari.com