Characterization of carbohydrate moieties on grass pollen group 13 glycoprotein allergens

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Introduction

Polygalacturonases are already reported as Group 13 of grass pollen allergens and known to be cross reactive allergens [1]. They are considered as pectin-degrading enzymes whose allergic power has been already demonstrated (2). Carbohydrate determinants on glycoproteins can induce serological reactions. Cross-reactivities among allergens can be due to the homology in protein sequences or domains, but also to cross-reactive carbohydrate determinants (CCD). Numerous studies report on carbohydrates as polyvalent allergic determinants in various types of allergies.

Aims

Our study aims at characterizing carbohydrate moieties in three different group 13 allergens in order, firstly, to refine allergy diagnosis and secondly to clarify the clinical role of carbohydrate as IgE epitopes for new immunotherapeutic treatment strategies. Immunotherapy relies on the induction of specific IgG-mediated responses, thus inhibiting the IgE-mediated response in allergic patients. As a consequence the activation of the mast cells is abolished, thus suppressing the subsequent release of proinflammatory cytokines and mediators.

Materials and Methods

• Purified allergens were trypsin digested (Roche) after reduction (DTT 10 mM) and alkylation (iodoacetamide, 50mM) in 50mM ammonium bicarbonate at pH 8.4.
• Analyse nanoLC-MS/MS in triplicates [Ultimate 3000 RSLC - Q Exactive, Thermo Scientific(R)] LC: column nano C18 Acclaim PepMap300 (300 nM) 75 μm i.d, 5 μm; gradient 1%-50% in 60 min. (A: H2O/ACN/AF 98:2:0.1, B: H2O/ACN/AF 10:90:0.1). MS: FTMS (Resolution 70 000) + top 10 HCD MS/MS
• Identification by Proteome discoverer 1.4/Mascot 2.3 using Uniprot/Sprot databank, taxonomy “Other plants”, 2 missed cleavages allowed, variable modifications: CAM (C), Ox (M), Deamidation (N) and HexN (K, N-term).
• Treatment with endoglycosidase A (Roche) in phosphate/citrate buffer (pH 5).
• Sep-pak C18 to separate glycans (5% acetic acid) and peptides (40% isopropanol/5% acetic acid).
• Glycans and glycated peptides analysis by MALDI-LTQ Orbitrap using CH3OH 30%v/v in 70% ethanol/0.1% TFA. Full scan analyses in positive ion mode, resolution 60 000. Automatic Gain Control (AGC) on with 2 microscans/step and 5 microscans/cycle. Crystal Positioning System (CPS) on. Fragmentation experiments in the linear ion trap with ion trap normalisation, normalized collision energy manually chosen and using Q=0.250 and T>30 ms.
• SimGlycan data interpretation for glycans MS/MS: Charge Status -1, Error Tolerance: Precursor m/z: 1 Da Fragment m/z: 0.5 Da, Adduct: Na, Ion Mode: -ve, Chemical Derivatization: Underivatized, Reducing End Modification (Delta Mass): Free (0.0 Da).

Results

Protein identification and mapping

Peptides resulting from trypsin digestion (before and after deglycosylation) were analysed by LC-MS/MS. The allergen Zoe m 13 was represented by 3 different isoforms of the polygalacturonase from Zoe mays with a high sequence coverage. The sample PNI p 13 was identified as a polygalacturonase from the species Phleum pratense with a sequence coverage of 73.1%. The sample Dec g p 13 is not identified by any entry corresponding to the species Elizabethi, because no genome sequence is currently available. Yet, some other polygalacturonases from other species are matched with a low sequence coverage. Identifications were summarized in Table 1.

Table 1

Determination of the N-glycosylation sites.

The deglycosylation by the endoglycosidase A allowed to separate glycans from peptides by RP chromatography on Sep-pak C18. The peptides bearing the N-glycosylation site in the consensus sequence N-X-(S/T), displayed an increment of 0.98 Da, due to the conversion of Asn into Asp and they were not detected before deglycosylation treatment. The polygalacturonases are characterized by 4 N-glycosylation sites annotated as potential in SwissProt database. The detected N-glycosylation sites are summarized in Table 2.

Table 2

Conclusions & Perspectives

This approach allowed the characterization of 3 allergens of the pollen group 13. To the best of our knowledge, for the first time the 4 potential N-glycosylation sites have been confirmed as well as the determination of the oligosaccharides modifying these sites. The difference among the glycans released from the allergen Zoe m 13 and the other two allergens is under investigation and requires an accurate study at the glycopeptide level to determine the site occupancy. Moreover the enrichment by PBA affinity chromatography has led to the detection of numerous glycation sites in the three samples. Their determination was possible thanks to the use of an advanced mass spectrometry approach based on multi-stage mass spectrometry, coupling CID and HCD fragmentation. It is not clear for the moment whether the glycation is a process-induced allergen modification or naturally occurs in pollen allergens to elicit specific IgE production.

Overall, these data represent preliminary insights in the understanding of pollen group 13 species-specific allergenicity.

References


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