Characterization of carbohydrate moieties on grass pollen group 13 glycoprotein allergens

Chiara Giangrande[1], Pascal Poncet[2], Hélène Sénéchal[2], Joëlle Vinh [1]

[1] SMBP Spectrométrie de Masse Biologique et Protéomique, CNRS USR 3149, ESPCI ParisTech, 10 rue Vauquelin, 75231 Paris Cedex 05, France.
[2] Service de Biochimie et de Biologie Moléculaire de l'hôpital Trousseau, 26 avenue Arnold Netter, 75771 Paris cedex 12, France

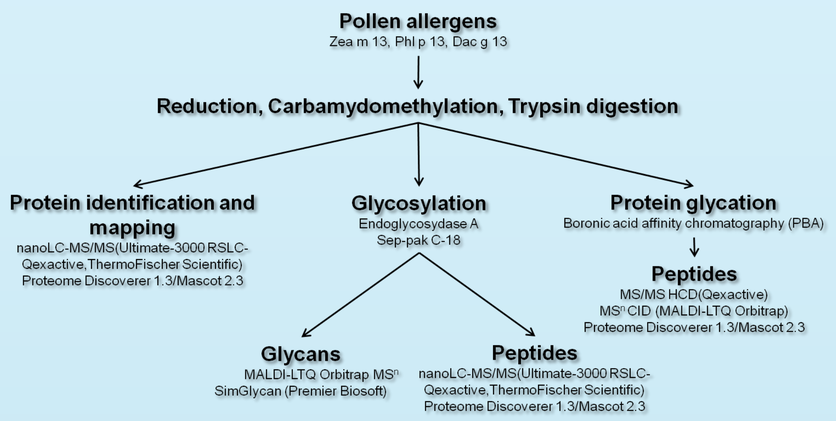
Introduction

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 allergenic 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 carbohydrates determinants (CCD). Numerous studies report on carbohydrates as polyvalent allergenic determinants in various types of allergies.

Aims

Our study aims at characterizing carbohydrates 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) et alkylation (iodoacetamide, 50mM) in 50mM ammonium bicarbonate at pH8.4
- Analyse nanoLC-MS/MS in triplicates (Ultimate 3000 RSLC - Qexactive, ThermoFisher Scientific) LC: column nano C18 Acclaim pepMap100 (Dionex) Viper 75µm d.i. x 50cm; gradient 1%-50%B in 60 min, (A: H_2O/ACN/AF 98:2:0.1, B: H_2O/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-ter)
- Treatment with endoglycosidase A (Roche) in phosphate/citrate buffer (pH 5).
- Sep-pak C-18 to separate glycans (5% acetic acid) and peptides (40% isopropanol/5% acetic acid)
- Glycans and glycated peptides analysis by MALDI-LTQ Orbitrap using DHB 30mg/ml in 70% ethanol/0.1% TFA. Full scan analyses in positive ion mode, resolution 60 000. Automatic Gain Control (AGC) on with 1 microscans/step and 5 microscans/cycle. Crystal Positioning System (CPS) on. Fragmentation experiments in the linear ion trap with ion trap detection, normalized collision energy manually chosen and using Q=0.250 and T=30 ms
- SimGycan data interpretation for glycans MS/MS: Charge State: 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 *Zea m 13* was represented by 3 different isoforms of the polygalacturonase from *Zea mays* with a high sequence coverage. The sample *Phl p 13* was identified as a polygalacturonase from the specie *Phleum pratense* with a sequence coverage of 73.1%. The sample *Dac g 13* is not identified by any entry corresponding to the specie *Dactylis glomerata*, 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.

Allergen	Accession	Description	ΣCoverage	Σ# Proteins	Σ# Unique Peptides	Σ# Peptides
Zea m 13	P35338	Exopolygalacturonase OS=Zea mays GN=PG9 PE=2 SV=1 - [PGLR2_MAIZE]	90,73	1	6	48
Zea m 13	P26216	Exopolygalacturonase OS=Zea mays GN=PG1 PE=1 SV=1 - [PGLR1_MAIZE]	89,02	1	3	47
Zea m 13	P35339	Exopolygalacturonase OS=Zea mays GN=PG2C PE=2 SV=1 - [PGLR3_MAIZE]	81,71	1	25	41
Phl p 13	Q9XG86	Polygalacturonase (Fragment) OS=Phleum pratense PE=2 SV=1 - [Q9XG86_PHLPR]	73,10	2	20	38
Dac g 13	G9I6F6	Polygalacturonase R2-7 OS=Secale cereale x Triticum durum PE=2 SV=1 - [G9I6F6_9POAL]	33,49	1	7	17
Dac g 13	Q9XG86	Polygalacturonase (Fragment) OS=Phleum pratense PE=2 SV=1 - [Q9XG86_PHLPR]	32,23	4	4	13
Dac g 13	Q1ZYQ5	Zea m 13 allergen (Fragment) OS=Zea mays PE=2 SV=1 - [Q1ZYQ5_MAIZE]	20,19	2	3	6
Dac g 13	Q41837	Polygalacturonase (Precursor) OS=Zea mays PE=4 SV=1 - [Q41837_MAIZE]	31,98	2	1	4
Dac g 13	B6T4T5	Exopolygalacturonase OS=Zea mays PE=2 SV=1 - [B6T4T5_MAIZE]	16,14	2	2	3

Table 1

Allergen	Sequence	Proteins group accessions	MH+
Zea m 13	VNITGVTCGPGHGISIGSLGR	P26216;P35338;P35339	1996,02886
Zea m 13	DVTFKNITGTSSTPEAISLLCTAK	P35338	2660,32805
Zea m 13	GDFLVGQLNFTGPCK	P26216;P35338	1596,77202
Zea m 13	GTSLIPKGDFLVGQLNFTGPCK	P26216;P35338	2535,30869
Zea m 13	VPCTGATMDDVNVEYSGTNNK	P35338	2273,95566
Zea m 13	AVQEAVASACGGTGKGTILIPKGDFLVGQLNFTGPCK	P26216;P35338	4190,03431
Zea m 13	VPCTGATMDDVNVEYSGTNNK	P35338	2274,95707
Zea m 13	DVTFKNITGTSSTPEAVSLLCTAK	P26216	2646,31198
Zea m 13	VPCTGVTMDDVNVEYSGTNNK	P26216	2318,97344
Zea m 13	IHYENIKMEDSANPIFIDMKYCPNK	P26216;P35338	3339,48337
Zea m 13	VTVKDVTFKNITGTSSTPEAVNLLCTAK	P35339	3263,64292
Zea m 13	NITGTSSTPEAVNLLCTAK	P35339	1921,94682
Zea m 13	DVTFKNITGTSSTPEAVNLLCTAK	P35339	2674,30313
Zea m 13	GDFLVGPLNFTGPCK	P35339	1565,77355
Zea m 13	DVTFKNITGTSSTPEAVNLLCTAK	P35339	2731,31595
Phl p 13	GDFLTGPLNFTGPCK	Q9XG86	1567,74675
Phl p 13	GDFLTGPLNFTGPCKGDSVTIK	Q9XG86	2268,1243
Phl p 13	IEYSGTNNK	Q9XG86;G9I6F6	1026,47234
Phl p 13	NITGTSSTPEAVSLLCSDK	K4AL02;I1GXC2;Q9XG86;K3Y1E6;F2EHP3;F2ECB0	1923,92192
Dac g 13	NITGTSSTPEAVSLLCSDK	F2EHP3;K4AL02;I1GXC2;Q9XG86;F2ECB0	1923,92278
Dac g 13	GDFLTGPLNFTGPCK	Q9XG86	1567,74809

Table 2

Determination of the N-glycosylation sites.

The deglycosylation by the endoglycosidase A allowed to separate glycans from peptides by RP chromatography on Sep-pak C-18. 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 glycosydase treatment. The polygalacturonases are characterized by 4 N-glycosylations sites annotated as potential in SwissProt data bank. The detected N-glycosylation sites are summarized in Table 2.

Glycans analysis

Glycans released from PNGase A treatment were analysed by MALDI-LTQ Orbitrap. Full scan spectra were carried out and precursor ions selected for CID fragmentation. Data were managed by the software SimGlycan to assign correct structures (Fig. 1). As shown in Table 3, the three allergens were modified with a common oligosaccharidic structure, namely a *paucimannosidic type* glycan, but the allergen *Zea m 13* displayed a higher heterogeneity, presenting even *high mannose* type structures.

Allergen	m/z	N-glycans
Zea m13	1065.37	
	1211.43	
	1419.49	
	1581.54	
	1743.60	
	1905.65	
Phl p13	1211.43	
Dac g13	1211.43	

Table 3

Glycation analysis

In the attempt to enrich for glycopeptides present in the protein digest, an affinity chromatography on a resin derivatized with phenylboronic acid was performed using in-house packed zip-tips. The MALDI-LTQ Orbitrap full scan spectrum of the peptides from *Zea m 13* before and after the enrichment is shown in Fig. 2A-B.

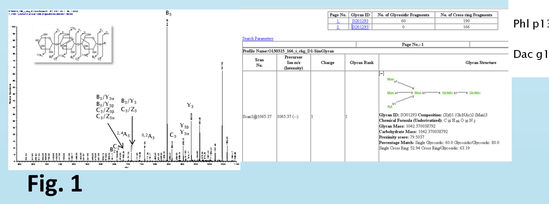


Fig. 1

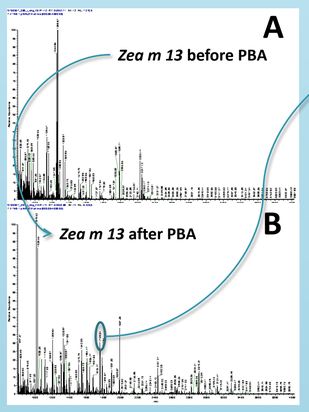


Fig. 2

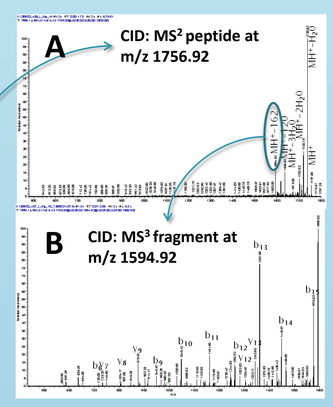


Fig. 3

Fig.3A displays the MALDI-LTQ Orbitrap MS^2 spectrum of the signal at m/z 1756.92, which is dominated by some typical neutral losses in the high mass region. These losses were interpreted as the presence of a glycation (+162.05) on the K2 of the peptide 291-305 from the allergen Zea m 13 with sequence IKAYEDAASVLTVSK. The neutral loss of 120 Da is due to a cleavage in the sugar ring, whereas the loss of 162 Da is due to the loss of the entire sugar moiety. To confirm and validate the sequence of the modified peptides MS^3 experiments were carried out. Fig. 3B shows the MS^3 spectrum at m/z 1594.92 allowing the validation of the modification on the peptide 291-305.

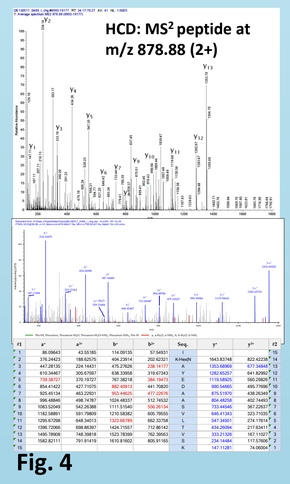


Fig. 4

Fig.4 shows the MS^2 spectrum of the same peptide obtained by HCD fragmentation on the QExactive and the relative interpretation by Proteome Discoverer. It is possible to notice that fragments carrying the sugar moiety are not visible on the spectrum, but the presence of low mass peaks allowed a better coverage of the sequence and the determination of the modification site..

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 Zea 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

1. Chardin H, Sénéchal H, Wal JM, Desvaux FX, Godfrin D, Peltre G. Characterization of peptidic and carbohydrate cross-reactive determinants in pollen polysensitization. Clin Exp Allergy. 2008 Apr;38(4):680-5.
2. Petersen A, Suck R, Hagen S, Cromwell O, Fiebig H, Becker WM. Group 13 grass allergens: structural variability between different grass species and analysis of proteolytic stability. J Allergy Clin Immunol. 2001 May;107(5):856-62.

Contact: chiara.giangrande@espci.fr