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Journal of Chromatography B, 756 (2001) 141–150

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Investigations on the carbohydrate moieties of glycoprotein allergens

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Received 3 January 2000; received in revised form 1 August 2000; accepted 1 August 2000

Abstract

Many allergens are glycoproteins and their carbohydrate structure can contribute to the IgE reactivity. Therefore it is of great interest to study the carbohydrate structures of these particular antigens. Here, we present an overview of methods combining basic procedures in glycochemistry with various applications of electrophoresis that allow investigating single allergens in crude extracts. Various allergen extracts, e.g. from tomato, grass pollen and bacteria were analysed and the suitability of the tests are discussed.

Keywords: Food allergy; Carbohydrates; Glycoprotein

1. Introduction

Many proteins contain posttranslational modifications of which the carbohydrate structure reaches high amounts. Glycans often play important physiological roles e.g. in resistance to proteolytic degradation, transduction of information between cells and intercellular adhesion through ligand–receptor interactions [1–3]. They can represent strong antigens as in the case of the A, B, H blood group substances.

Indications for glycosylation can be deduced from the protein sequence. The sequence motif Asn-X (except of Pro)-Ser/Thr indicates probable *N*-glycosylation sites. (The prediction of *O*-linked glycans is much more difficult and less reliable. They are built up of only a few monosaccharide units and seem to be less important for the elucidation of allergy.) However, it must be verified, whether the presumed glycoprotein indeed bears carbohydrate chains.

Some allergens e.g. Bet v 1, Phl p 6 have no carbohydrate chains, although they bear such a motif [4,5]. Glycoproteins with a high carbohydrate content often appear as smeary, unsharp bands after electrophoretic separation. Furthermore, carbohydrate epitopes in contrast to protein epitopes are highly thermostable and thus retain their immunoreactivity even after strong denaturing conditions [6]. But these observations are only hints.

Although the relevance of the carbohydrate structure for the allergic reaction is controversially discussed [7,8], IgE reactivity to glycans was clearly shown in several cases. Weber et al. [9] and Tretter et al. [10] demonstrated that phospholipase A_2 in bee venom possesses an important IgE-reactive epitope, which is formed by an α1–3 fucosylation at the innermost *N*-acetylglucosamine residue. β1–2

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0378-4347/01/$ – see front matter
PII: S0378-4347(01)00099-8

Table 1
Concept for the detection and characterisation of carbohydrate moieties

Aim	Treatment
1. Glycoprotein detection	periodate
2. Carbohydrate amount Carbohydrate linkage (*N*-/*O*-glycosylation)	TFMS endoglycosidases
3. Specification of carbohydrate structure	lectins
4. Isolation of glycans	hydrazine

xylosylations were also identified in IgE-binding carbohydrate structures [11]. Most cross-reactivities that are observed among allergens of vegetable foods, pollen and bee venom are caused by similar carbohydrate structures between the unrelated organisms [12].

The analysis of glycans on a particular allergen even in a complex extract can be achieved by simple but efficient techniques. We combined the electrophoretic separation of extracts with the carbohydrate detection and determination. The diagram (Table 1) illustrates the stepwise characterisation of the carbohydrate moieties.

2. Experimental procedures

2.1. Preparation of the allergen extracts

(a) Tomato extract: The extract was prepared by a low-temperature 'acetone powder' method according to Vieths et al. [13]. Briefly, mature fresh tomatoes were homogenised in acetone/dry ice at −60 to −65°C and the mixture was kept at this temperature overnight. Resulting precipitates were washed twice with acetone and finally with acetone/diethylether, (1:1, v/v) at −60°C. The sediment was filtered, lyophilised and stored at −20°C. To obtain protein extracts 2 g of acetone powder was diluted in 30 ml of PBS (0.01 *M* potassium phosphate, 0.15 *M* NaCl; pH 7.4), centrifuged at 20 000 *g* for 45 min (5°C). The supernatant was then passed through a 0.2 μm filter, dialysed against double-distilled water overnight, lyophilised and stored until used at −20°C.

(b) Grass pollen extract: 1 g of timothy grass pollen (ARTU Biologicals, Lelystad, The Netherlands) was incubated in 50 ml of 0.1 *M* NH_4HCO_3 buffer (pH 8.0) for 30 min. Afterwards the soluble fraction was isolated by centrifugation at 17,000 g for 30 min. and dialysed against double-distilled water overnight. The extract was lyophilised and stored at 4°C until used.

(c) Bacterial extracts: *Saccharopolyspora rectivirgula* (ATCC 15437) was cultured in a synthetic medium described elsewhere [14]. Briefly, shaking cultures were incubated at 55°C for 3 days. The supernatant was filtered through 0.45 μm membranes and concentrated by ultrafiltration (cut-off 10 kDa) in an Amicon stirred cell. The extract was subsequently dialysed against distilled water, lyophilised and store at −20°C.

2.2. Patient sera and monoclonal antibodies

Serum samples were drawn from patients with pollinosis and those with a clinical history of farmer's lung. The patients' sera were obtained from Hospital Borkum Riff (Dr. H. Aulepp) and the Medical Clinic in Borstel (Dr. U. Lepp).

The monoclonal antibody IG 12 was raised in BALB/c mice against crude timothy pollen extract and is specific for grass group I allergens [15].

2.3. SDS–PAGE and blotting

Proteins (6 μg per cm gel) were separated by SDS PAGE (T=12.5%, C=4%; size 120×70×0.5 mm^3) using the Laemmli buffer system [16]. The proteins were subsequently transferred onto nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) by semi-dry blotting at 0.8 mA/cm^2 for 30 min [17].

2.4. Immunological detection

For the identification of antigens and allergens the blotting membrane was incubated with supernatants of hybridomas and patients' sera, respectively. As secondary antibodies we used alkaline phosphatase-conjugated goat anti-mouse IgG/IgM (Dianova, Hamburg, FRG), goat anti-human IgE (Tago, Hamburg, FRG) or human IgG2 specific monoclonal antibody (1:2000, Unipath, Manchester, UK), respectively.

The binding patterns were visualised by a substrate solution consisting of 0.033% (w/v) nitro blue

tetrazolium chloride (NBT) and 0.017% (w/v) 5-bromo-4-chloro-3-indolyl phosphate potassium salt (BCIP) (Serva, Heidelberg, Germany) in 0.1 *M* Tris buffered saline (pH 9.5) [18].

2.5. Detection of glycoproteins

In contrast to proteins, vicinal hydroxyl groups of carbohydrate structures are altered by periodate oxidation, they become oxidised to aldehydes.

Carbohydrate detection was achieved with the Glycan detection kit (Roche, Mannheim, Germany) according to the manufacturer's description [19,20]. The periodate treatment was performed on native extract before electrophoresis or on nitrocellulose membranes after protein separation and blotting. Afterwards the steroid hapten digoxigenin was covalently bound to the aldehyde groups via a hydrazide group. Glycoproteins were visualised by an alkaline phosphatase labelled anti-digoxigenin antibody followed by incubation in NBT/BCIP substrate.

To determine whether the carbohydrate chains are involved in the immunoreactivity, unblocked nitrocellulose strips were incubated in 10 m*M* sodium acetate buffer (pH 5.0) containing 50 m*M* $NaIO_4$ [21] after SDS–PAGE blotting had been performed. The incubation was carried out in the dark for 2 h at room temperature. The reaction was stopped by the addition of sorbitol to a final concentration of 15 m*M*.

2.6. Deglycosylation procedures

Crude timothy grass pollen extract was treated with different endoglycosidases (*N*-glycosidase F, *N*-glycosidase A, *O*-glycosidase, Roche, Mannheim, Germany) and trifluoromethanesulfonic acid (TFMS; Deglycosylation Kit, Oxford Glycosystems, Oxford, UK) to destroy their carbohydrate structures [22].

(a) Recombinant *N*-glycosidase F from *Flavobacterium meningosepticum* (Peptide-N^4-(acetyl-β-glucosaminyl) asparagine amidase): 15 μg extract was diluted in 25 μl of 10 m*M* sodium phosphate buffer (pH 7.5) containing 1% (v/v) 2-mercaptoethanol and were incubated for 3 min at 95°C. Afterwards 10 μl of *N*-glycosidase F (2 U) were supplemented. Digestion was performed at 37°C for 18 h.

(b) *N*-glycosidase A from sweet almonds (Peptide-N^4-(*N*-acetyl-β-glucosaminyl) asparagine amidase): 15 μg extract was diluted in 25 μl of 10 m*M* sodium acetate buffer (pH 5.1), containing 0.5 *M* sodium thiocyanate and 0.1 *M* 2-mercaptoethanol. After 3 min incubation at 95°C, 10 μl of *N*-glycosidase A (0.5 mU) was added. Digestion was performed at 37°C for 18 h.

(c) *O*-glycosidase from *Diplococcus pneumoniae* (*O*-glycopeptide endo-D-galactosyl-*N*-acetyl-α-galactosamino hydrolase): 15 μg extract was diluted in 34.5 μl of 20 m*M* sodium phosphate buffer (pH 7.5), containing 1% (v/v) 2-mercaptoethanol. After 3 min incubation at 95°C, 0.5 μl of *O*-glycosidase (0.25 mU) was added. Digestion was performed at 37°C for 18 h.

(d) Neuraminidase from *Clostridium perfringens* (Acylneuraminyl hydrolase; Roche, Mannheim, Germany): 200 μg extract was diluted in 20 μl of 10 m*M* sodium acetic buffer (pH 5.0). 2 μl of neuraminidase (1 U) were added and the incubation was performed at 37°C for 15 h.

(e) TFMS treatment [23]: 100 μg of lyophilised extract was dissolved in 45 μl trifluoromethanesulfonic acid under anhydrous conditions. After incubation at −20°C for 4 h the solution was neutralised by addition of 150 μl 60% pyridine–water-solution and again stored for 15 min at −20°C. Finally, 400 μl of 0.5% (w/v) NH_4HCO_3 were added to precipitate the deglycosylated proteins. The suspension was dialysed against double-distilled water.

(f) KOH treatment [24]: 200 μg of crude pollen extract was diluted in 15 μl of 50 m*M* KOH. After incubation for 12 h at room temperature the sample was directly applied for Western blotting.

2.7. Determination of carbohydrate moieties by the use of lectins

Lectins are carbohydrate-binding reagents with a high affinity to certain carbohydrate configurations. To study the carbohydrate moieties blotted samples were incubated with several digoxigenin-labelled lectins (Roche, Mannheim, Germany) [25]. Lectin binding was visualised by alkaline phosphatase-

labelled anti-digoxigenin antibody and a NBT/BCIP substrate. A specific binding to the lectins was shown, when the immunoreactivity could be inhibited by addition of the corresponding monosaccharides.

2.8. Hydrazinolysis of glycoproteins to isolate glycans

For the analysis of the carbohydrate structures two sets of 2.5 mg pollen extract or 5 mg lyophilised *S. rectivirgula* extract were dried exhaustively by lyophilization. Subsequently the samples were resuspended in a minimum amount of anhydrous hydrazine (50 μl) and incubated for 5 h at 60°C and 95°C for recovery of *O*- and *N*-glycans, respectively (*O*- and *N*-glycan Recovery Kit, Oxford Glycosystems) [26]. For removal of the released proteins the samples were passed over a glycan binding column according to the manufacturer's description. For immunological studies of the *N*- and *O*-glycans the eluted samples were dotted onto a CNBr-activated nitrocellulose membrane [27].

3. Results

3.1. Detection of glycoproteins

We aimed at the detection of glycoproteins on the blotting membrane. This allows an easy comparison between the carbohydrate-bearing bands and those identified by patients' sera or monoclonal antibodies. Furthermore, the sensitivity of the immunodetecting system (biotin-streptavidin or digoxygenin anti-digoxygenin) is much higher than the PAS (periodic acid Schiff reagent) staining. We studied tomato extract as an example for glycoprotein detection.

In order to determine, whether differences in the electrophoretic mobility and/or sensitivity of the glycoprotein detection arise, we compared the result obtained with periodate-oxidized extract prior to Western blotting with that of extract immobilised on nitrocellulose membrane followed by periodate treatment (Fig. 1). The positions of the IgE-reactive protein bands did not differ, indicating no changes in the molecular mass. Protein staining by AuroDye and India Ink did not work with periodate-pretreated

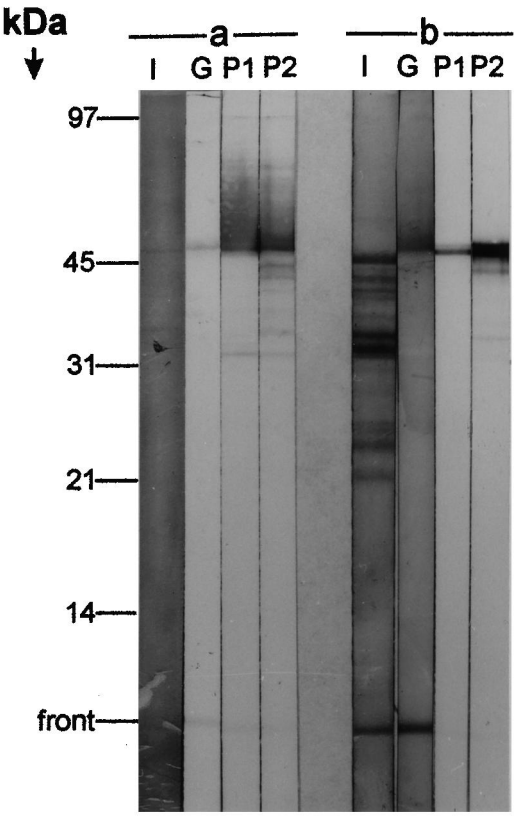

Fig. 1. SDS–PAGE combined with glycoprotein detection of tomato extract. Tomato extract was treated with periodate before (left) and after electrophoretical separation (right). I, protein staining (India ink); G, glycoprotein detection; P1 and P2, individual patients' sera.

extract. Haselbeck and Hösel [28] reported a 10 fold enhancement of sensitivity, when the glycoprotein is oxidised before electrophoresis. We observed no difference in sensitivity, but a quite dark background in the post-treated sample, which complicates the detection. Presumably it depends on the reactivity of the nitrocellulose.

To study the influence of the carbohydrate structure on the antigenicity and IgE-reactivity, periodate-oxidized and untreated samples were compared concerning their binding patterns. Fig. 2 depicts the investigations on grass pollen extract, an extract that only contains a few glycoproteins.

As shown in Fig. 2 in comparison, the carbohydrate destruction has no effect on the protein pattern and the binding of the monoclonal antibody IG 12.

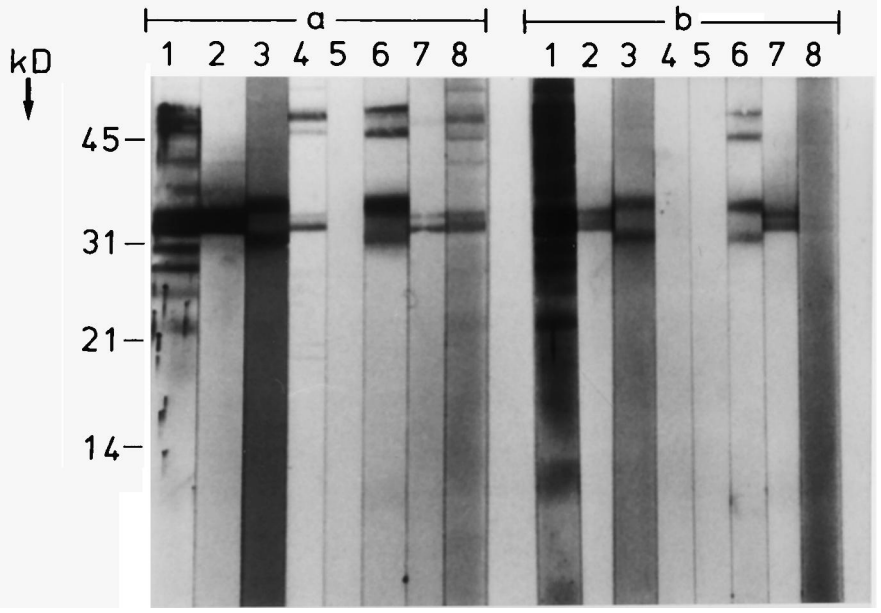

Fig. 2. Studies to identify IgE-reactive structures on the Phl p 1 protein core. After Western blotting of timothy grass pollen extract one part of the nitrocellulose membrane remained untreated (a), while the other was treated by sodium periodate (b). 1=protein staining (India ink), 2=moab IG 12, 3=moab Bo 1, 4=lectin AAA, 5=serum of a healthy donor, 6–8=individual patients' sera [22].

(The complete loss of glycans of the respective allergen was confirmed by the reactivity of the lectin AAA (data not shown).) The serum used in lane 8 only binds to the untreated components indicating that this patient recognises carbohydrates. Unfortunately, the serum in lane 7 shows a stronger binding to the periodate-treated sample. This enhancement is probably caused by alterations in the protein structure which might increase the accessibility for the IgE antibodies. According to Howlett and Clamp [29], a lot of amino acids are sensitive to periodate oxidation, e.g. serine, threonine. Therefore, it is necessary to perform additional experiments and to use control proteins and glycoproteins.

3.2. Deglycosylation experiments

The type of carbohydrate linkage to the protein backbone was determined by deglycosylation experiments. Tables 2 and 3 give an overview of the chemical reagents and enzymes and their deglycosylation products.

The deglycosylation procedure in crude extracts can only be interpreted, when monoclonal antibodies are available to identify the glycoprotein and the mixture contains just a few glycoproteins.

Fig. 3 reveals a SDS–PAGE blot of timothy grass pollen. The allergen recognised by the monoclonal antibody IG 12 (Phl p 1) shows microheterogeneity comprising two bands of 35 and 37 kDa. Prior to electrophoresis, the samples were treated with the described deglycosylating reagents. After electrophoresis and blotting the membranes were proteinstained with India ink and immunostained with the monoclonal antibody. While untreated pollen extract (lane 1, control) reveals antigens of 37 and 35 kDa, the completely deglycosylated sample (TFMS-treated, lane 5) shows protein bands of 35 and 33 kDa. Thus, there is a shift of 2 kDa in each of the components indicating a 5% carbohydrate content in the grass groups I allergens. Deglycosylation by *N*-glycosidase F (lane 2) and *O*-glycosidase (lane 4) had no detectable effect compared to the untreated control. *N*-glycosidase A treatment produced an additional protein band of 33 kDa, while the 37 and 35 kDa bands were slightly less intense (compared to the control). The 33 kDa band was not detected by lectin AAA indicating that it lacks carbohydrates (data not

Table 2
Deglycosylation by endoglycosidases showing the specific cleavage sites

Enzyme	Organism	Specificity
endoglycosidase H	Streptomyces	release of glycans of the oligomannose and oligohybride type, not of the complex type Asn-GlcNAc-⇓-GlcNAc-...
N-glycosidase F	Flavobacterium	broad specificity, no cleavage when the innermost GlcNAc is modified Asn-⇓-GlcNAc-GlcNAc-...
N-glycosidase A	Prunus dulcis	broad specificity, even cleaves glycans with substitutions Fuc/α1–3 Asn-⇓-GlcNAc-GlcNAc...
O-glycosidase	Diplococcus	Ser/Thr-⇓-GalNAc-...

Table 3
Deglycosylation by chemical reagents showing the specific cleavage sites

Reagent	Specificity
Potassium hydroxide	Ser/Thr-⇓-GalNAc, not Hyp-⇓-Ara
Trifluoromethanesulfonic acid	complete deglycosylation

shown). Thus, *N*-glycosidase A causes an incomplete deglycosylation of Phl p 1 components. The observation that *N*-glycosydase A in contrast to *N*-glycosidase F results in the cleavage of glycans assumes that the innermost *N*-acetylglucosamine bears a substitution [10]. Further experiments using defined glycopeptides confirmed a substitution by an α1–3 bound fucose [30].

3.3. Determination of the carbohydrate moiety by use of lectins

Lectins are suitable tools for the determination of specific carbohydrate structures. Because of their binding specificity and the structural characteristics of *N*- and *O*-glycans, some lectins are indicative of the type of glycosylation: AAA, GNA, MAA, SNA

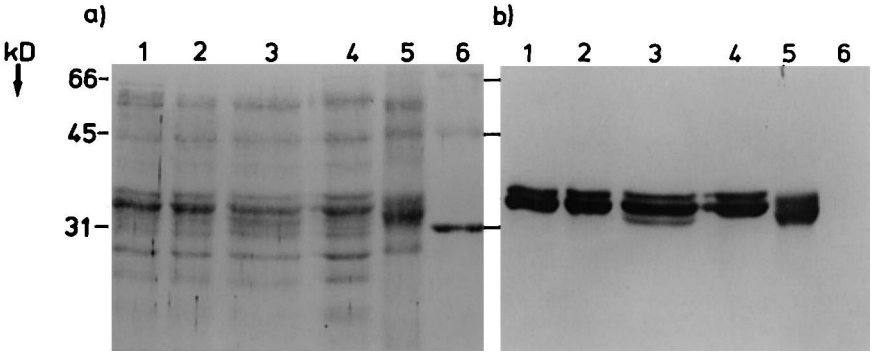

Fig. 3. Influence of different deglycosylation procedures on the immunoreactivity of Phl p 1. After Western blotting the membranes were proteinstained by India ink (a) and immunostained by moab IG 12 (b). Timothy grass pollen extract was treated in the following ways: 1=untreated sample (control), 2=*N*-glycosidase F treatment, 3=*N*-glycosidase A treatment, 4=*O*-glycosidase digestion, 5=TFMS treatment, 6=molecular mass marker [22].

Table 4
Lectins used to specify the carbohydrate structures of tomato glycoproteins

Lectin	Organism	Binding specificity for terminal sugar	Reactivity
AAA	Aleuria aurantia	α-fucose	++
Con A	Canavalia ensiformis	α-mannose, α-glucose, α-*N*-acetylglucosamine	++
DSA	Datura stramonium	galactose linked β(1–4) *N*-acetylglucosamine	−
GNA	Galanthus nivalis	terminal α-mannose linked to mannose	++
MAA	Maackia amurensis	sialic acid linked α(2–3) to galactose	+
PHA-L	Phaseolus vulgaris	α-galactose, β-galactose	−
PNA	Arachis hypogaea	galactose linked β(1–3) *N*-acetylgalactosamine	−
RCA	Ricinus communis	α-galactose, β-galactose	+
SNA	Sambucus nigra	sialic acid linked α(2–6) to galactose or *N*-acetylgalactosamine	−
WGA	Triticum vulgaris	β-*N*-acetylglucosamine	(+)

and PHA-L identify *N*-glycans, PNA and ACA indicate *O*-glycans (abbreviations are specified in Table 4).

To determine the carbohydrate structures in tomato extract, we used the glycan differentiation kit with digoxygenin labelled lectins, which detects a wide variety of glycan structures (Fig. 4). In order to get an overview of the glycoproteins and their specificity in tomato extract, we have performed this test after SDS–PAGE blotting.

Since tomato extract contains several glycoproteins, a specification of each protein band concerning its carbohydrate moiety can be performed. Table 4 only sums up the reactivity of the complete extract. IgE reactivities were determined at about 65, 35–45, 31 and 25 kDa. Lectins AAA, Con A and GNA also recognise these protein bands, indicating that these allergens contain *N*-glycans (Fig. 4). Since fucose is present in all these components, the clearly defined IgE-reactive carbohydrate structure composed of an α1–3 bound fucose to the innermost *N*-acetylglucosamine might also cause the reactivity in these components. Indeed, testing the patients' sera on isolated glycopeptides [29] or glycoproteins containing this carbohydrate structure (horseradish peroxidase, phospholipase A2) [31] confirmed this assumption. Unfortunately, no lectins exist to determine xylose (e.g. β1–2 bound xylose) in glycans which also contributes to an IgE-reactive structure [11]) and arabinose residues in glycans. The existence of xylose residues must therefore be determined from the comparison of the reactivities to glycopeptides or glycoproteins with defined carbohydrate structures.

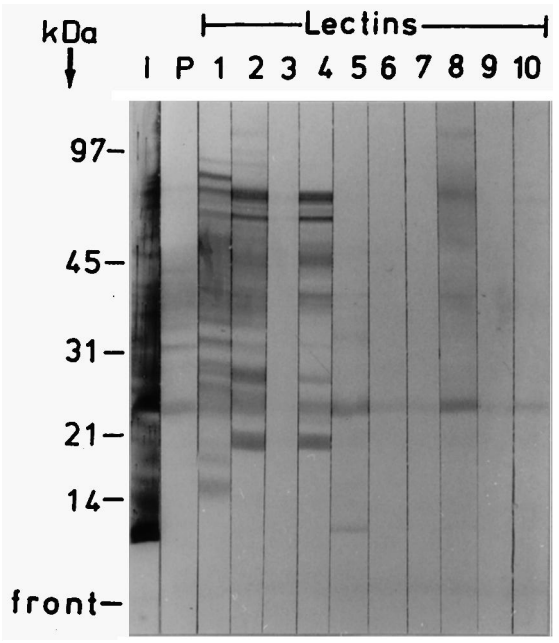

Fig. 4. Western blotting of tomato extract using the lectins described in Table 4.

3.4. Analysis of glycans isolated from extract

In order to find out, whether the carbohydrate chains possess binding sites for IgE antibodies, we split off the glycans from the extract. In the case of timothy grass pollen extract, no prior separation was necessary, since it contains only very few carbohydrate-bearing allergens. The peptide/amino acid hydrazide mixture was dotted onto CNBr-activated

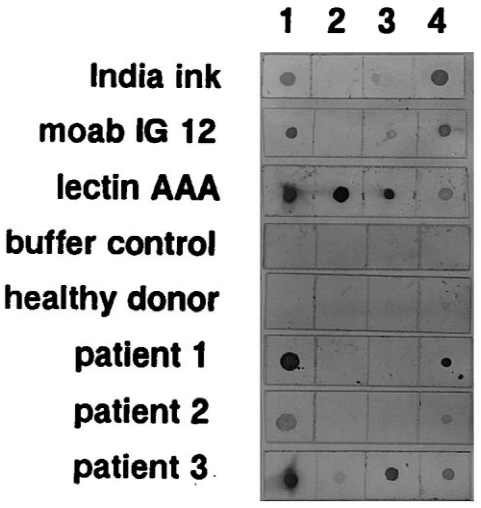

Fig. 5. Studies on the isolated *N*- and *O*-glycans obtained after hydrazinolysis of timothy pollen extract. Crude timothy pollen extract (1), extract treated by hydrazine at 95°C (2) and 60°C (3) (to split off *N*- and *O*-glycans, respectively), TFMS-deglycosylated pollen extract (4) [22].

nitrocellulose membrane and immunological tests were performed.

Fig. 5 demonstrates the dot blot results. The hydrazinolysis was performed at 60°C and 90°C according to the optimal releasing conditions of the *O*- and *N*-glycans, respectively. Reactivities of the carbohydrate chains, even after hydrazinolysis are detected with lectin AAA and with the serum of patient 3. The lectin AAA showed a faint reactivity to the TFMS-treated extract. The monoclonal antibody IG 12 directed against the Phl p 1 protein moiety also bound weakly to the fraction obtained after hydrazine treatment at 60°C. These results indicate that residual peptides are left in the glycan fractions and that the TFMS fraction contains minimal amounts of carbohydrates. However, the sera of patient 1 and 2 only bind to crude and to TFMS-deglycosylated extract, whereas the serum of patient 3 revealed additional IgE reactivities against carbohydrate structures. It is impossible to get pure glycans by this method without further isolation from peptide fragments and degraded carbohydrate chains. Nevertheless, circumstantial information can be obtained from these experiments. In type-III-allergy IgG-2 subclass reactivity is hypothesised as disease associated marker [32]. This was tested on carbohydrate residues gained from *S. rectivirgula* extract by the hydrazide technique. The obtained glyco-hydrazone chains were bound to CNBr-activated nitrocellulose and incubated with patients' pooled sera. The signal obtained was already very intensive with the amount of carbohydrates cleaved from 10 μg of complete bacterial extract (Fig. 6). No proteins and peptides could be detected in the carbohydrate fraction (data not shown). Control reactions of exposed farmers' pooled sera did not show binding with the glyco-residues of *S. rectivirgula*. This result indicated that carbohydrate structures on their own are antigenic for patients' IgG2.

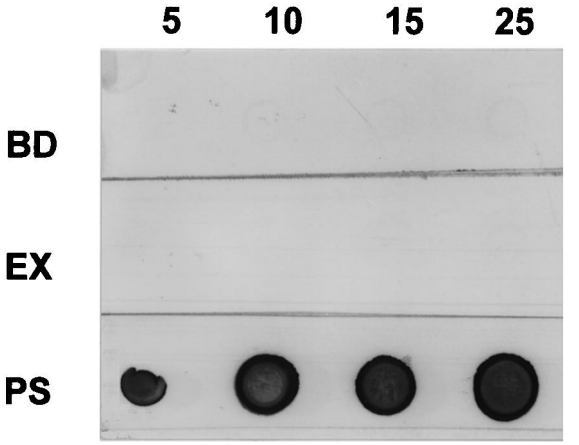

Fig. 6. Dot blot analysis of *S. rectivirgula* carbohydrate residues produced by hydrazinolysis. IgG-2 reactivity of patients'sera (PS), exposed persons' sera (EX), and blood donors' sera (BD). 5, 10, 15, 25 μg of bacterial extract were used for hydrazinolysis [32].

4. Discussion and prospects

The detection and characterisation of carbohydrate structures of glycoproteins can be achieved with the described techniques on every biological sample. The tests are easily to perform in a biochemical laboratory.

The described hydrazinolysis is a starting point for a detailed analysis of the carbohydrate structure. Generally the purification of the particular glycoprotein is necessary. This is compelling, since even proteins of identical amino acid sequence reveal microheterogeneity concerning their carbohydrate

structure. After release of the carbohydrate chains by hydrazinolysis the different glycans are separated by HPLC. Gas chromatography in combination with mass spectrometry evaluates the monosaccharide composition. The exact carbohydrate structure can then be identified by NMR analysis. Oxford Glycosystems determines the carbohydrate moieties by glycan sequencing, a combinatorial, selective enzymatic degradation of the glycoprotein and the subsequent gel permeation of the released glycans. By comparison with a glycan database it should be possible to identify the particular structure.

Concerning allergy, it has been shown that the most relevant structure for an IgE reactivity is the α1–3 fucosylation to the innermost *N*-acetylglucosamine and probably also the β1–2 xylosylation to the trimannosyl core residue [11]. In many glycoproteins e.g. phospholipase A2 from bee venom, Phl p 1 of timothy grass pollen, Ole e 1 of olive tree pollen, only one *N*-glycosylation site was determined that caused IgE binding. But these structures are not able to cross-link IgE antibodies on mast cells, a prerequisite for the elucidation of type I allergy responses. For peanut allergy, van der Veen et al. [7] demonstrated only a very low effect of the glycans. Even for glycoproteins with several *N*-glycans such as horseradish peroxidase, no clinical symptoms have been shown. On the other hand, we were able to elucidate a histamine release by a carrier molecule to which several *N*-glycans had been coupled [33]. It is inconceivable, why carbohydrate structures should be much less appropriate to bind IgE than proteins. Furthermore, *O*-glycans and their influence of IgE reactivity have hardly been studied. It is possible that the IgE reactivity depends upon the size of the carbohydrate chains [34]. However, even if the carbohydrate structure is unimportant for allergic reactions, about 20% of the allergic individuals reveal IgE antibodies directed against glycans. In diagnostics performed with natural extracts this would cause many false positive results. Thus, a determination of probable IgE-binding carbohydrate structures is mandatory.

5. Nomenclature

AAA	*Aleuria aurantia* agglutinin
Moab	monoclonal antibody
TFMS	trifluoromethanesulfonic acid

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