

Immunological Studies of the Glycoprotein Allergen Ag-54 (Cla h II) in *Cladosporium herbarum* with Special Attention to the Carbohydrate and Protein Moieties

Marit Swärd-Nordmo, Berit Smestad Paulsen, Jens Kristian Wold

Institute of Pharmacy, University of Oslo, Norway

Abstract. The glycoprotein allergen Ag-54 (Cla h II) isolated from *Cladosporium herbarum* was split into its carbohydrate and protein moieties by using alkaline-borohydride treatment and deglycosylation, respectively. The native Ag-54, the deglycosylated protein and the protein-free carbohydrate moieties were tested for IgE-binding activity by radioallergosorbent test inhibition using selected sera from individuals with *C. herbarum* allergy. The deglycosylated material had a stronger allergenic activity than the native Ag-54 with all the sera tested. The Ag-54 carbohydrate moieties were not found to possess any IgE-binding activity. The galactoglucomannan part of the carbohydrate moiety was isolated and it was demonstrated to precipitate IgG rabbit antibody. Removal of the galactofuranose units by mild acid hydrolysis did not alter the IgG-binding capacity of the polysaccharide, indicating that galactofuranose was not immunodominant.

Introduction

Cladosporium is reported to be the most common fungus found in the air of temperate zones [4] and in many countries the species *Cladosporium herbarum* is a major fungal source of inhalant allergy and allergic asthma in man [2, 3]. Extracts from *C. herbarum* have been found to contain at least 60 antigens of which more than 20 components had IgE-binding activity [4]. One of the allergens, Ag-54, has been isolated and characterized [5, 6]. This allergen corresponds to Cla h II according to the IUIS allergen nomenclature [7]. The Ag-54 molecule is a glycoprotein which contains 80.2% carbohydrate made up of mannose, galactose and glucose in the proportion 1:0.6:1.3 [6]. The main carbohydrate moiety is a highly branched galactoglucomannan with an apparent molecular weight of approximately 19,000 daltons [8]. Mannose and threonine are involved in the carbohydrate protein linkage. A few oligoglycosidic chains are also linked to the protein [9].

The IgG-binding property of a large number of carbohydrate structures in nature is well established. N-acetyl-D-glucosamine has been identified as the antigenic determinant of the cell wall mannan from

the yeast *Kluyveromyces lactis* [10] and the terminal 1,3 linkage in the mannan side chain of the *Saccharomyces cerevisiae* mannan was found to be an immunodominant structure [11]. Reiss and Lehmann [12] were able to identify a circulating galactomannan antigen in human beings with invasive aspergillosis and in 1985 Bennet et al. [13] established that galactofuranosyl groups were immunodominant structures in the *Aspergillus fumigatus* galactomannan.

The significance of the carbohydrate moiety for the IgE-binding properties of glycoproteins is not clear though many allergens have been identified as glycoproteins [14-19]. In 1959 Augustin [20] treated grass pollen extracts with α -amylase, emulsin and takadiastase without detecting any loss of allergenic activity. Marsh et al. [21] reported no reduction of skin reactivity when group-I rye grass pollen allergens were digested with β -galactosidase and cellulase. The monosaccharide composition of these extracts was not known. Modification of the carbohydrate moiety of the well-characterized allergen Ag-30 from timothy pollen did not significantly affect its binding to IgE [22].

However, some findings have suggested that covalently linked carbohydrate may contribute to the al-

lergenic activity of a glycoprotein. Malley et al. [23] have indicated that glucose may constitute a part of the allergenic determinant of antigen D in timothy pollen. Periodate oxidation of a purified *C. herbarum* preparation significantly reduced the IgE-binding ability of the preparation without altering the amino acid composition [24]. Recently Weber et al. [25] were able to demonstrate that the terminal oligosaccharide of a glycosylated variant of bee venom phospholipase A₂ was an epitope recognized by human IgE antibody.

The purpose of the present study was to find out whether the carbohydrate moiety of Ag-54 contributes to the IgE- or IgG-binding property of the molecule.

Material and Methods

Allergen Material

The allergen denoted Ag-54 from *C. herbarum* Link ex. Fr. was isolated, purified and identified as described earlier [6].

Human Sera

A serum pool from 35 individuals with proven or suspected clinical allergy to *C. herbarum* was employed as described previously [4]. Individual sera were used which contained high amounts of specific IgE to Ag-54. The specific IgE concentration corresponded to crossed radioimmuno-electrophoresis (CRIE) class A and B as defined by Aukrust [4].

Rabbit Antiserum

A polyvalent antiserum (anti-Ch-77) was used as previously described [4]. The rabbits had been immunized using aqueous extracts of *C. herbarum* mycelium and conidia available from ALK (Allergologisk Laboratorium A/S, Copenhagen).

The rabbit antiserum contained precipitating antibodies to Ag-54 detectable in CRIE.

Isolation of the Protein Moiety

The carbohydrate moiety was removed from the glycoprotein by deglycosylation basically as described by Edge et al. [26]. A sample of Ag-54 (7 mg) was dissolved in 2 ml of a mixture of anisole and trifluoromethanesulfonic acid (1:1) precooled to 0°C and flushed with nitrogen. After stirring at 0°C for 3.5 h, 50 ml of diethyl ether containing 10% petrol ether and precooled to -40°C was added to the mixture. The solution was kept at -40°C for 20 min before addition of 10 drops of pyridine to facilitate the precipitation of the deglycosylated protein moiety. After totally 1 h, the precipitate was centrifuged and washed repeatedly with cold ether before dissolution in ethanol and rotary evaporation. The protein was separated from the remaining reagents and the low molecular weight carbohydrate material by gel filtration. The mixture was dissolved in water and loaded onto a Bio-Gel P-4 (Bio-Rad Laboratories, Calif.) column (1.5 × 15 cm) and eluted with 0.075 mol/l ammonium bicarbonate buffer (pH 7.9). The elution profiles were estimated for

carbohydrate material by the phenol-sulfuric acid method of Dubois et al. [27] and for protein material by the method of Waddell and Chapel Hill [28]. The protein fraction was freeze-dried before radioallergosorbent test (RAST) inhibition experiments as described below.

Isolation of the Carbohydrate Moiety

The carbohydrate moiety was split off from the protein by alkaline-borohydride treatment. The glycoprotein (3 mg) was heated at 50°C for 6 h in 1 ml containing 0.125 mol/l NaOH and 1 mol/l sodium borohydride. Excess borohydride was decomposed by addition of 3 mol/l HCl and removed as methyl borate by repeated evaporation with methanol. The free carbohydrate moiety was separated from the protein moiety by anion exchange chromatography using an equilibrated column (1.5 × 10 cm) of DEAE Sephadex A-25 (Pharmacia, Sweden) eluted with 0.025 mol/l ammonium bicarbonate buffer (pH 7.9). The carbohydrate fraction was desalted by gel filtration using Bio-Gel P-4 (Bio-Rad Laboratories, Calif.) column (1.5 × 15 cm) eluted with 0.075 mol/l ammonium bicarbonate buffer (pH 7.9). The carbohydrate elution profile was detected by the phenol-sulfuric acid method of Dubois et al. [27]. The carbohydrate moiety was freeze-dried before further examination by RAST inhibition and amino acid analysis as described below.

Isolation of the Galactoglucomannan

The Ag-54 carbohydrate moiety isolated by alkaline-borohydride treatment as described above was fractionated on a Bio-Gel P-4 (Bio-Rad Laboratories, Calif.) column (1.5 × 15 cm) which had been calibrated with dextrans (7,600 and 2,360 daltons). The column was eluted with 0.075 mol/l ammonium bicarbonate buffer (pH 7.9). The high molecular weight fractions corresponding to a molecular weight > 4,000 daltons (void) were pooled and freeze-dried before carbohydrate analysis, immunoprecipitation and mild acid hydrolysis as described below.

Pronase Digestion

Ag-54 was dissolved at a concentration of 17 mg/ml in Tris-HCl buffer 0.05 mol/l (pH 7.8) containing 0.002 mol/l CaCl₂ and incubated with 10 mg/ml pronase (120,000 PUK/g, Calbiochem, Calif.) at 37°C for 113 h before RAST inhibition as described below.

RAST Inhibition

RAST inhibition experiments were performed basically as described by Yman et al. [29]. A partly purified allergen extract derived from the same *C. herbarum* source as the purified Ag-54 was coupled to CNBr-activated paper discs (Whatman No. 20). Preliminary dose-response experiments indicated 5 mg/ml allergen extract to be the optimal concentration for coupling. 20 µl of patient serum or serum pool was added to serial dilutions of the allergen dissolved in 0.1 mol/l phosphate buffer (pH 7.4; 30 µl). After 1 h of incubation at room temperature, an allergosorbent paper disc was added to each tube and incubated over night at room temperature. The supernatant was aspirated and the discs rinsed 3 times for 10 min with 0.154 mol/l NaCl containing 0.5% tween 20 before incubation with 50 µl ¹²⁵I-anti-IgE (Phadebas® RAST, Pharmacia Diagnostics, Sweden) over night at room temperature. After rinsing the discs 4 times as described above, the radioactivity of the discs was determined. Maximum uptake was determined by performing the RAST-inhibition procedure as described above, exchanging the

allergen solution to be tested with phosphate buffer during preincubation with serum. Unspecific radioactive uptake was estimated by using activated paper discs prepared in the same way as the allergosorbents, replacing the allergen solution during the coupling step by phosphate buffer used for coupling.

Amino Acid Analysis

The sample was dissolved in 6 mol/l HCl (suprapure grade, Merck). Hydrolysis was carried out at 110°C for 20 h. After removal of the HCl, the sample was subjected to amino acid analysis using a Biotronic LC 5000 automatic amino acid analyzer.

Mild Acid Hydrolysis

The galactoglucomannan isolated as described above was heated at 100°C with 0.005 mol/l H₂SO₄ to remove galactofuranosidic residues. Samples were withdrawn after 30 min, 1, 2.5 and 5 h before removal of monomeric material by desalting on a Bio-Gel P-4 (Bio-Rad Laboratories, Calif.) column. The high molecular weight fraction was freeze-dried before carbohydrate analysis and immunoprecipitation as described below.

Quantitative Carbohydrate Analysis

Monosaccharide composition was quantified by methanolysis (1 mol/l methanolic HCl, 24 h, 80°C) and gas liquid chromatography as described previously [30]. The trimethylsilylated methylglycosides were chromatographed on a DB-5 fused silica column (0.2 cm × 15 m) with H₂ used as carrier gas (1.2 ml/min). The chromatograph was equipped with a flame-ionization detector.

Immunoprecipitation

The galactoglucomannan moiety of Ag-54 isolated as described above was made in serial dilution with 0.154 mol/l NaCl and 0.5 ml was incubated with 0.5 ml of anti-Ch-77 rabbit antiserum at 4°C for 20 h. Material hydrolysed with mild acid was used in one single concentration. The samples were centrifuged (10,000 rpm) and the pellets were washed five times with 2 ml of 0.154 mol/l NaCl. 0.4 ml of 0.5 mol/l NaOH was added and the samples were heated in a boiling water bath until the material was dissolved. The solutions were neutralized with 0.4 ml of 0.5 mol/l HCl before the protein content was estimated by the method of Bradford [31] as described in the instruction manual of the microassay procedure of Bio-Rad Laboratories [32] using bovine serum albumin as reference.

Results

The native glycoprotein Ag-54 was separated into its protein and carbohydrate moieties by deglycosylation and alkaline-borohydride treatment, respectively. The carbohydrate content of these products is given in table 1. Pronase digestion was also used as an alternative method for enriching the carbohydrate moieties.

The various samples were tested for their IgE-binding activity by RAST inhibition analysis using allergosorbents coupled with *C. herbarum* extract. The RAST inhibition curves with different patient sera are

Table 1. Carbohydrate content in Ag-54 and the modified material

	Carbohydrate content	
	% of dry weight	ratio mannose:galactose:glucose
Native Ag-54	80.2	1:0.6:1.3
Deglycosylated Ag-54	5.9	1:0:0.3
Carbohydrate moieties	100	1:0.6:1.3

presented in figure 1. The concentrations needed for 50% inhibition of the uptake are listed in table 2. The inhibition curves of the deglycosylated and native material were nearly parallel and the deglycosylated material was more active than the native glycoprotein in all cases. This was demonstrated most clearly by the inhibition of the HES serum (table 2), where 100 times more native Ag-54 than deglycosylated material was required to reach the same inhibition level. The protein-free carbohydrate moieties did not inhibit any of the sera tested, including the serum pool. Carbohydrate-rich glycopeptides resulting from pronase digestion of Ag-54 possessed no allergenic activity in the systems used (fig. 1).

The carbohydrate moiety was fractionated by gel filtration according to its molecular weight and the high molecular weight galactoglucomannan was isolated. This structure represented the main part of the Ag-54 carbohydrate moiety. The protein-free galactoglucomannan was incubated with rabbit antiserum and the ability to form precipitate was expressed as micrograms of protein. Figure 2 shows that precipitate formation increased over a range of 0–0.2 µmol anhydrohexose. When exceeding a polysaccharide content equivalent to 0.2 µmol anhydrohexose, an inhibition of the precipitin formation could be observed.

In order to determine whether the galactofuranose residues contributed to the antigenic specificity of the reaction, the galactofuranose was released from the galactoglucomannan by mild acid hydrolysis and removed by gel filtration before the reaction with rabbit antiserum. Polysaccharide equivalent to 0.1 µmol anhydrohexose was used in this experiment. This was below the galactoglucomannan concentration necessary for maximum precipitate formation. However, the concentration was considered high enough to ob-

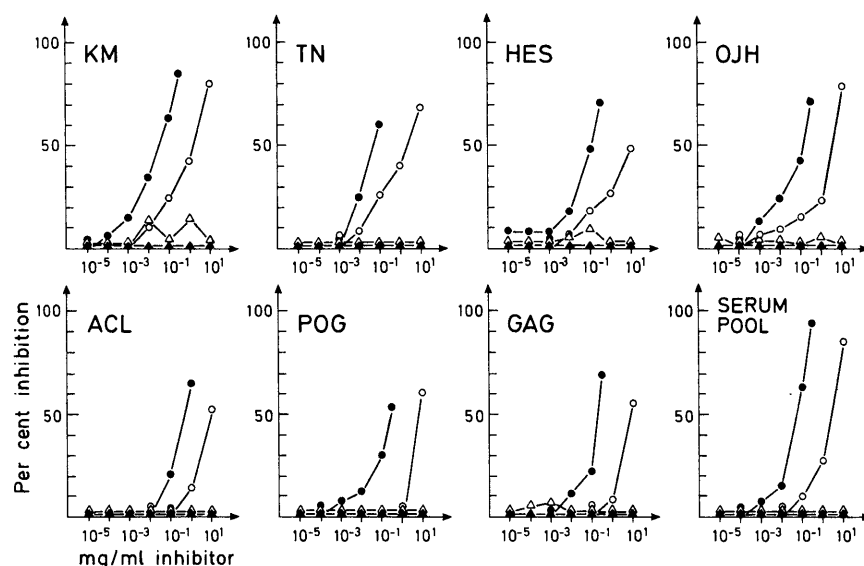


Fig. 1. The inhibition of IgE binding to *C. herbarum* allergosorbent discs in RAST inhibition analysis by native Ag-54 (○), deglycosylated Ag-54 (●), Ag-54 carbohydrate moieties (△) and pronase digested Ag-54 (▲). Seven individual patient sera and one serum pool were used. The percentage of inhibition is plotted against inhibitor concentration (see bottom left).

Table 2. Concentration (mg dry weight/ml) of Ag-54 and deglycosylated Ag-54 inhibiting RAST by 50%

Serum	Concentration, mg dry weight/ml, at 50% inhibition	
	Ag-54	deglycosylated Ag-54
KM	3	0.06
TN	4	0.09
HES	10	0.1
OJH	5	0.2
ACL	10	0.7
POG	9	0.5
GAG	9	0.3
Serum pool	4	0.07

Table 3. The carbohydrate composition of the galactoglucomannan moiety released from Ag-54

Time of hydrolysis h	Ratio mannose:galactose:glucose	Galactose content, %
0	1:0.7:0.4	33
0.5	1:0.6:0.4	30
1	1:0.5:0.4	26
2.5	1:0.3:0.4	18
5	1:0.04:0.3	3

The galactoglucomannan was treated with 0.005 mol/l H_2SO_4 at 100°C, and the monomeric material released from the polymer was removed by gel filtration.

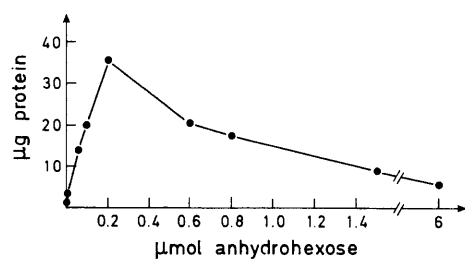


Fig. 2. Precipitate formation as a function of the quantity of galactoglucomannan. The carbohydrate dissolved in 0.5 ml of 0.154 mol/l NaCl was mixed with 0.5 ml of undiluted rabbit antiserum and incubated at 4°C for 20 h. The precipitate was isolated by centrifugation and washed with 0.154 mol/l NaCl. The protein was estimated by the Bradford method [30].

Table 4. The relationship between precipitate formation and the time of hydrolysis

Time of hydrolysis h	Anhydrohexose µmol	Precipitate µg protein	% Reduction of galactose
0	0.1	20.0	0
0.5	0.1	22.4	9
1	0.1	20.5	21
2.5	0.1	21.3	45
5	0.1	21.0	91

The galactoglucomannan moiety released from Ag-54 was treated with 0.005 mol/l H_2SO_4 at 100°C and the released monomeric material was removed by gel filtration. The polymer was tested for its ability to react with rabbit antiserum.

tain a significant amount of precipitate. Table 3 shows how the galactose content decreased with increasing time of hydrolysis. As demonstrated in table 4, the ability of the carbohydrate to precipitate rabbit antiserum was not affected when galactofuranosidic residues were removed. After 5 h of hydrolysis the galactose content was reduced by 91%. At these conditions some mannose and glucose were also released from the molecule. More extensive acid treatment to remove galactofuranose completely would certainly lead to damage of the remaining polymer.

Discussion

In order the study different parts of the glycoprotein, the molecule was separated into its protein moiety and a protein-free carbohydrate moiety which contained all the different carbohydrate structures of the Ag-54 molecule. RAST inhibition was performed using *C. herbarum* allergosorbents instead of coupling the individual allergens to the paper discs due to shortage of material. Total inhibition of the sera could therefore not be expected.

The glycosylated material used in the RAST inhibition experiments contained only small amounts of carbohydrate material in comparison with the native Ag-54. The galactofuranose branches had been removed during the deglycosylation, and the material could be expected to carry a few small mannan or glucomannan units. Compared to the native Ag-54 which has a large highly branched galactoglucomannan attached in addition to a few oligoglycosidic units [8], the deglycosylated protein moiety is less shielded by carbohydrate material.

In the RAST-inhibition experiments the deglycosylated material showed an increased IgE-binding capacity. The protein concentrations necessary to inhibit the sera by 50% were lower in the deglycosylated samples than in the native glycoprotein material, indicating that the protein moiety is the most important part of the Ag-54 molecule when considering the IgE-binding activity. This was in contrast to the rocket immunoelectrophoresis experiments previously reported [9], which showed that the deglycosylated material had to be increased 20 times by weight compared to the native glycoprotein in order to precipitate rabbit antibody to the same extent. This corresponded to a 100-fold increase in the protein content.

The importance of the protein moiety for IgE binding was further underlined by the inability of the protein-free carbohydrate moiety of Ag-54 to react with IgE antibody from several *C. herbarum*-allergic individuals. To ensure that the allergenic activity of the carbohydrate moiety had not been destroyed as a result of the chemical treatment during the isolation process, carbohydrate-rich glycopeptides resulting from extensive proteolytic treatment of the Ag-54 molecule were also tested for allergenic activity, but specific IgE antibodies to the carbohydrate could not be found. Though several patient sera were tested, the existence of *C. herbarum*-allergic patients who react to the Ag-54 carbohydrate moiety cannot be excluded since individuals with mould allergy seem to be a very heterogeneous group in their reaction towards the different allergenic components [4, 33, 34]. However, the data presented in this study indicate that the carbohydrate moiety of Ag-54 is not responsible in general for the IgE-binding ability of the allergen molecule. On the contrary, the material appears to be more allergenic active when the carbohydrate moiety has been removed.

The reaction of the Ag-54 carbohydrate moiety with rabbit antibody was not similar to the human response. Only very low concentrations of the galactoglucomannan part of the Ag-54 carbohydrate moiety was necessary to precipitate antibody from rabbits immunized with aqueous extracts of *C. herbarum*. Other investigators have reported serological reactivity of fungal galactomannans, peptidogalactomannans and mannans [35–37]. Some of these investigators observed that the galactofuranosyl residues had a major effect on the precipitate formation [13, 36–38]. Our data suggest that the galactofuranosyl residues of the Ag-54 galactoglucomannan are not of primary importance in directing antigenic specificity.

The present work made it possible to conclude that deglycosylated Ag-54 possessed higher IgE-binding capacity than native Ag-54 while the protein-free carbohydrate moiety lacked the ability to bind IgE. The galactoglucomannan part of the Ag-54 carbohydrate moiety precipitated rabbit antiserum, but there was no evidence that the galactofuranose residues were immunodominant. The present investigation did not consider IgG-mediated hypersensitivity in human beings. Neither has skin reactivity been evaluated. This would further contribute to a better understanding of the role of the Ag-54 carbohydrate moiety in hypersensitivity reactions.

Acknowledgments

This work was supported by grants from the Norwegian Research Council for Science and the Humanities. The authors are indebted to Stig Morten Borch for supplying CNBr-activated paper discs. Dr. Knut Sletten is acknowledged for performing the amino acid analysis.

References

- Solomon WR: Aerobiology and inhalant allergens. I. Pollens and fungi; in Middleton E, Reed CE, Ellis EF (eds): Allergy: Principles and Practice. St Louis, Mosby, 1978.
- Nilsson D, Aas K: Immunological specificity and correlation of diagnostic tests for bronchial allergy to *Cladosporium herbarum*. Acta Paediatr Scand 1976;65:33-38.
- Tarlo SM, Fradkin A, Tobin RS: Skin testing with extracts of fungal species derived from the homes of allergy clinic patients in Toronto, Canada. Clin Allergy 1988;18:45-52.
- Aukrust L: Crossed radioimmuno-electrophoretic studies of distinct allergens in two extracts of *Cladosporium herbarum*. Int Arch Allergy Appl Immunol 1979;58:371-386.
- Aukrust L, Borch SM: Partial purification and characterization of two *Cladosporium herbarum* allergens. Int Arch Allergy Appl Immunol 1979;60:68-79.
- Swärd-Nordmo M, Wold JK, Smestad Paulsen B, et al: Purification and partial characterization of the allergen Ag-54 from *Cladosporium herbarum*. Int Arch Allergy Appl Immunol 1985;78:249-255.
- WHO: Allergen nomenclature. Bull WHO 1986;64:767-770.
- Swärd-Nordmo M, Smestad Paulsen B, Wold JK: The glycoprotein Ag-54 (Cla h II) from *Cladosporium herbarum*. Structural studies of the carbohydrate moiety. Int Arch Allergy Appl Immunol 1988;85:288-294.
- Swärd-Nordmo M, Smestad Paulsen B, Wold JK: The glycoprotein allergen Ag-54 (Cla h II) from *Cladosporium herbarum*. Further biochemical characterization. Int Arch Allergy Appl Immunol 1988;85:295-301.
- Raschke WC, Ballou CE: Characterization of a Yeast mannan containing N-acetyl-D-glucosamine as an immunochemical determinant. Biochemistry 1972;11:3807-3816.
- Suzuki S, Sunayama H, Saito T: Studies on the antigenic activity of yeasts. I. Analysis of the determinant groups of the mannan of *Saccharomyces cerevisiae*. Jap J Microbiol 1968;12:19-24.
- Reiss E, Lehmann PF: Galactomannan antigenemia in invasive aspergillosis. Infect Immun 1979;25:357-365.
- Bennet JE, Bhattacharjee AK, Glaudemans CPJ: Galactofuranosyl groups are immunodominant in *Aspergillus fumigatus* galactomannan. Mol Immunol 1985;22:251-254.
- Klapper DG, Goodfriend L, Capra JD: Amino acid sequence of ragweed allergen Ra3. Biochemistry 1980;19:5729-5734.
- Haavik S, Smestad Paulsen B, Wold JK: Glycoprotein allergens in pollen of timothy. II. Isolation and characterization of a basic glycoprotein allergen. Int Arch Allergy Appl Immunol 1985;78:260-268.
- Nilsen BM, Smestad Paulsen B: Allergens in pollen from mugwort (*Artemisia vulgaris* L.). II. Characterization of a crude and partly purified extract of Mugwort pollen with particular emphasis on the glycoprotein allergen Ag-7. Int Arch Allergy Appl Immunol 1986;80:113-121.
- Howlett BJ, Clarke AE: Isolation and partial characterization of two antigenic glycoproteins from ryegrass (*Lolium perenne*) pollen. Biochem J 1981;197:695-706.
- Hoffmann DR, Shipman WH: Allergens in bee venom. I. Separation and identification of the major allergens. J Allergy Clin Immunol 1976;58:551-562.
- Shipolini RA, Callewaert GL, Cottrell RC, et al: The amino acid sequence and carbohydrate content of phospholipase A₂ from bee venom. Eur J Biochem 1974;48:465-476.
- Augustin R: Grass pollen allergens. III. Their differentiation from the other pollen antigens by immunoelectrophoretic studies in relation to skin reactivity, enzymic digestions, heat and pH stabilities. Immunology 1959;2:230-251.
- Marsh DG, Milner FH, Johnson P: The allergenic activity and stability of purified allergens from the pollen of common rye grass (*Lolium perenne*). Int Arch Allergy Appl Immunol 1966;29:521-535.
- Haavik S, Smestad Paulsen B, Wold JK: Glycoprotein allergens in pollen of timothy. V. Significance of the carbohydrate moiety for the immunological activity of a basic glycoprotein allergen. Int Arch Allergy Appl Immunol 1987;83:231-237.
- Malley A, Baecher L, Begley D: Further characterization of the antigenic determinant of allergen B from timothy grass pollen. Excerpta Med Int Congr Ser 1977;414:156-160.
- Swärd-Nordmo M, Smestad Paulsen B, Wold JK, et al: Characterization of the carbohydrate moiety in a partly purified allergen preparation from the mould *Cladosporium herbarum* and its possible importance for the allergenic activity as tested by RAST-inhibition. Int Arch Allergy Appl Immunol 1984;75:149-156.
- Weber A, Schröder H, Thalberg K, et al: Specific interaction of IgE antibodies with a carbohydrate epitope from honey bee venom phospholipase A₂. Allergy 1987;42:464-470.
- Edge ASB, Faltynek CR, Hof L, et al: Deglycosylation of glycoproteins by trifluoromethanesulfonic acid. Anal Biochem 1981;118:131-137.
- Dubois M, Gilles KA, Hamilton JK, et al: Colorimetric method for determination of sugars and related substances. Anal Chem 1956;28:350-357.
- Waddell WJ, Chapel Hill NC: A simple ultraviolet spectrophotometric method for the determination of protein. J Lab Clin Med 1956;48:311-314.
- Yman L, Ponterius G, Brandt R: RAST-based allergen assay methods; in: International WHO-IABS Symposium on Standardization and Control of Allergens Administered to Man, Geneva 1974. Dev Biol Stand. Basel, Karger, 1975, vol 29, pp 151-165.
- Swärd-Nordmo M, Almeland TL, Aukrust L: Variability in different strains of *Cladosporium herbarum* with special attention to carbohydrates and contents of two important allergens (Ag-32 and Ag-54). Allergy 1984;39:387-394.
- Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-254.
- Bio-Rad Laboratories: Bio-Rad Protein Assay: Instruction Manual. Richmond, Bio-Rad Laboratories, 1977.
- Steringer I, Aukrust L, Einarsson R: Variability of antigenicity/

- allergenicity in different strains of *Alternaria alternata*. Int Arch Allergy Appl Immunol 1987;84:190-197.
- 34 Wallenbeck I, Aukrust L, Einarsson R: Antigenic variability of different strains of *Aspergillus fumigatus*. Int Arch Allergy Appl Immunol 1984;73:166-172.
- 35 Grappel SF, Blank F, Bishop CT: Immunological studies on dermatophytes. I. Serological reactivities of neutral polysaccharides with rabbit antiserum to *Microsporum quinckeanum*. J Bacteriol. 1967;93:1001-1008.
- 36 Suzuki S, Takeda N: Immunochemical studies on the galactomannans isolated from mycelia and culture broths of three *Horodendrum* strains. Infect Immun 1977;17:483-490.
- 37 Preston JF III, Lapis E, Gander JE: Immunological investigations of *Penicillium*. I. Serological reactivities of exocellular polysaccharides produced by six *Penicillium* species. Can J Microbiol 1970;16:687-694.
- 38 Grappel SF, Blank F, Bishop CT: Immunological studies on dermatophytes. II. Serological reactivities of mannans prepared from galactomannans I and II of *Microsporum quinckeanum*, *Trichophyton granulosum*, *Trichophyton interdigitale*, *Trichophyton rubrum* and *Trichophyton schoenleinii*. J Bacteriol 1968; 95:1238-1242.

Received: December 23, 1988

Correspondence to: Dr. Marit Swärd-Nordmo
Research and Development Division
NYCOMED AS
P.O. Box 4220, Torshov
N-0401 Oslo 4 (Norway)