

IgE antibodies specific for carbohydrates in a patient allergic to gum arabic (*Acacia senegal*)

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The present study deals with the detailed investigation of the IgE antibody response of a gum arabic-allergic patient. The patient showed multiple serologic and skin test sensitizations to a range of pollen, other inhalants and foods, and bee venom, and to the recombinant allergens Bet v 1 and Bet v 2. Moreover, the patient's serum reacted strongly to gum-arabic extract. The NaIO₄-treated and thus deglycosylated extract showed no binding to IgE. In contrast, removal of the protein backbone by basic hydrolysis did not deplete the IgE reactivity. Therefore, it is concluded that the gum arabic-specific IgE antibodies of this patient were mainly directed against the carbohydrate fraction of this material. In IgE-inhibition assays, cross-reactions occurred in the range of 60% between gum arabic and known immunogenic *N*-glycans containing α 1–3-linked fucose. Since the inhibition graphs were not parallel and the inhibition was not complete with heterologue antigens, the cross-reacting epitopes of gum arabic appeared to be different from the latter well-known cross-reactive carbohydrate determinants (CCD). Inhibition may have been caused by a partial immunologic identity of the investigated carbohydrate moieties. A strong IgE response to the fucose-containing glycan from bromelain was measured in a glycan ELISA that utilizes purified glycopeptides at the solid phase. This response, which may explain the multiple sensitizations without clinical significance diagnosed in the patient, could originate from inhalation of pollen, which is known to contain similar glycans, or from occupational sensitization during work as a baker and confectioner. Since the gum-arabic protein showed only very weak participation in the IgE reactivity, the clinical symptoms of the patient caused by gum arabic may be attributed to carbohydrate epitopes. Due to the repetitive polysaccharide sequence of gum arabic, several epitopes for the cross-linking of IgE should exist.

Key words: *Acacia senegal*, allergy; carbohydrate epitopes; cross-reactivity; gum arabic; IgE; immunoblotting; occupational sensitization.

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Gum arabic is a high-molecular-weight, slightly acidic, highly branched polysaccharide, consisting of a major chain of β 1–3-linked galactose units, some of which are substituted in β 1–6-linkage with side-chains composed of D-galactose, L-rhamnose, L-arabinose, and D-glucuronic acid. The carbohydrates are attached to a hydroxyproline-rich polypeptide backbone that accounts for only 2% of the molecular weight (1, 2). It is collected as dried exudate from trees belonging to the genus *Acacia*, mainly in Sudan. Gum arabic has wide and varied

industrial uses, mainly in the food industry as stabilizer, thickening agent, and emulsifier, but also in cosmetics, lithography, the manufacture of inks and adhesives, the textile industry, and the production of galactose (3). It is not known whether or how carbohydrate structures are involved in the development of allergies to gum arabic. However, in recent years, evidence has accumulated that "cross-reactive carbohydrate determinants" (CCD), not only of food and pollen glycoproteins, but also in material of

invertebrate origin, including insect venom, caddis fly, and mollusks, can cause cross-reactions of allergen-specific IgE (4–9). The key IgE-binding epitopes in most of the described cases are α 1–3-linked fucose (10–13) and β 1–2-linked xylose (14, 15), which occur in specific *N*-glycans. One or both of these carbohydrates are also present in many other newly investigated plant glycoproteins (16–19). Bromelain, a cysteine proteinase with one oligosaccharide unit that contains both monosaccharides (20), is recognized by IgE of sera from patients with very different allergies; for example, to insect venom, tree pollen, and grass pollen (12, 21, 22). In 1978, two cases of asthma caused by bromelain inhalation were described (23).

Occupational sensitizations to gum arabic have been described for patients working in the pottery and lithography industries (24, 25). Moreover, the former use of gum arabic as an additive in drugs led to hypersensitivity in a transplant recipient taking prednisolone tablets (26). The most important role of gum arabic in occupational allergy is to cause printer's asthma (27–29). However, there is a general lack of data on the offending gum-arabic allergens. Due to its high carbohydrate content, one would expect carbohydrate-specific IgE antibodies to be involved in allergies to gum arabic. To test this hypothesis, we analyzed the specificity of IgE against gum arabic in a patient with strong respiratory allergy to this plant gum. Moreover, cross-reactions between gum arabic and the known immunogenic carbohydrate structures mentioned above were investigated.

Material and methods

Human sera and antisera

Serum from the patient (PEI 39) was stored at -20°C until used. Serum from one nonatopic subject (PEI 19) with no history of gum-arabic allergy or allergies to any other inhalants or foods was used as a negative control.

Rabbit antiserum against horseradish peroxidase (HRP), which is known to recognize β 1–2-linked xylose and α 1–3-linked fucose (30, 31), was purchased from Sigma (ref. P7899, lot 115H8955).

Allergen extracts and allergens

The recombinant major cherry allergen Pru a 1 was kindly supplied by S. Scheurer (Paul-Ehrlich-Institute, Langen, Germany). The recombinant major birch-pollen allergen Bet v 1 and recombinant Api g 1, the major celery allergen, were purchased from BIOMAY (Linz, Austria).

Timothy grass-pollen extract was obtained from Dr A. Petersen (Borstel Research Center, Germany) and birch-pollen extract from MAST Diagnostica (Reinfeld, Germany). All other extracts were self-prepared, as previously described for apple and celery (32, 33), and stored freeze-dried at -20°C until used.

Gum arabic was extracted with sodium carbonate buffer, pH 9.6, at 60°C for 1 h followed by a 30-min treatment in an ultrasonic bath and centrifugation at 3000 g for 10 min at room temperature. The supernatants were then freeze-dried (extract concentration: 5 mg/ml dry weight of crude material).

Preliminary structural investigation of *N*-linked glycans in gum arabic was performed with peptide-*N*-glycosidase A to release asparagine oligosaccharides. These were analyzed by HPLC as fluorescent derivatives, as previously described (34).

For removal of the protein moiety, gum-arabic extract was subjected to alkaline hydrolysis and reduction with a mixture of 0.2 M NaOH and 0.2 M NaBH_4 at 50°C for 5 h, as described by Akiyama et al. (35). In a separate experiment, the sample was digested with pepsin (150 U/mg sample in 5% HCOOH for 20 h at 37°C).

Bromelain was inactivated as follows. An amount of 50 mg was dissolved in 0.4 M Na/K-phosphate buffer at pH 6.0 containing 8 mM DTT and 4 mM EDTA. The sample was then incubated at 37°C for 1 h in the presence of 10 mM iodoacetic acid. Upon dialysis against a volatile buffer, the bromelain was lyophilized. To obtain a preparation devoid of detectable protease activity, the procedure had to be repeated. Protease activity was measured as described (36).

Periodate oxidation of the gum arabic extract and of the inactivated bromelain was performed, as previously described (37), with 0.05 M NaIO_4 .

N-linked glycopeptides with the glycan structure $\text{Man}\alpha 1-6(\text{Xyl}\beta 1-2)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4(\text{Fuc}\alpha 1-3)\text{GlcNAc}$ that contained only two to four amino-acid residues were purified from pineapple stem bromelain by pronase digestion and a series of chromatographic steps, as described by Fu and O'Neill (38). Three types of controls were used. First, glycopeptides with the common pentasaccharide core $\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4\text{GlcNAc}$ were prepared from bovine fibrin, as previously described (38). Second, defucosylated bromelain glycopeptides were obtained by treating bromelain glycopeptides with trifluoro acetic acid (38). Finally, glycopeptides with an α 1–6-fucose attached to the proximal GlcNAc [$\text{Man}\alpha 1-6(\text{Man}\alpha 1-3)\text{Man}\beta 1-4\text{GlcNAc}\beta 1-4(\text{Fuc}1-6)\text{GlcNAc-peptide}$] were isolated from

human IgG by the same procedure. Amino-acid and monosaccharide analysis of the glycopeptides was performed as previously described (38, 39). The antigens were stored freeze-dried at -20°C until used.

Skin tests

Skin prick tests (SPT) with routine inhalants were performed with conventional glycerol skin prick solutions according to the manufacturer's protocol (Alyostal extracts, Stallergènes Laboratoires, France) and graded according to the guidelines of the European Academy of Allergology and Clinical Immunology (EAACI) (40). Intracutaneous tests (ICT) with routine allergens were performed according to the protocol of the manufacturer (Stallergènes, France).

Total and specific IgE

Diagnostic measurements of total and allergen-specific IgE were performed with the CAP System FEIA (Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions.

For research purposes, self-prepared solid phases were investigated with a commercial enzyme allergosorbent test (EAST), according to the instructions of the manufacturer (Allergopharma, Reinbek, Germany). The antigens for EAST were coupled to cyanogen bromide-activated filter paper disks at a concentration of $30\text{ }\mu\text{g/ml}$ ($5\text{ }\mu\text{g}$ per disk of the oxidized and nonoxidized extracts).

Dose-related EAST inhibition studies were performed as previously described (32, 33). For initial experiments, single inhibitor concentrations were applied.

IgE specific for carbohydrate structures was assayed by a glycan ELISA, as previously described (41). Results were expressed as differences of absorbances between allergic patient and nonallergic control.

ELISA inhibition was performed with patient serum, prediluted 1:5, and bromelain glycopeptide attached to the solid phase. Human serum was preincubated with either $5\text{ }\mu\text{g}$ of glycopeptide, $5\text{ }\mu\text{g}$ of the recombinant proteins (rPru a 1, rBet v 1), $100\text{ }\mu\text{g}$ inactivated bromelain, or $12.5\text{ }\mu\text{g}$ of protein extracts from different sources for 2 h at 37°C . After transfer of the mixture to the coated microtiter plate, an additional 2-h incubation at 37°C followed, and the ELISA was continued according to the standard procedure. Results were expressed as percent inhibition after subtraction of the OD of the nonallergic control.

Electrophoresis and immunoblotting

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) of the gum-arabic extracts was performed as previously described (37, 41). After transfer of the separated glycoproteins to nitrocellulose (NC) membranes by semidry blotting, the membranes were cut into 3–4-mm-wide strips and probed with sera both from the allergic patient and a nonallergic control (both diluted 1:6.7) and the HRP-specific antibody (1:5000). Bound IgE antibodies were immunostained as previously described (42).

Case report

A 50-year-old man (PEI 39) had suffered from occupational eczema on the hands and forearms since 1995. During his apprenticeship as a baker and confectioner, he also suffered from hand eczema. At that time, it was diagnosed as baker's eczema, and the symptoms disappeared after local therapy. Thereafter, the patient had no dermatologic problems. Since 1990, he had been working as a confectioner in a Swiss chocolate plant. The procedure for production of gum-arabic solution includes mixing 100 l water with 50 kg of gum-arabic powder and is associated with a moderate dust load. While working with gum arabic, he experienced episodes of cough and dyspnea. In addition, the patient had rhinoconjunctivitis caused by grass and rye pollen.

Results

SPT and intradermal tests

The patient reacted strongly in SPT to pollen of grass and rye and *Dermatophagoides pteronyssinus*, but only weakly to *D. farinae*. SPT with pollen of birch, alder, ash, hazel, and mugwort; various fruits and vegetables, cocoa; silk; epithelia from dog, cat, and horse; fungi; latex; cow's milk; and hen's egg white were negative.

ICT were positive with *D. farinae* and ash pollen, but negative with pollen of birch, alder, hazel, and mugwort.

The patient showed no reaction in SPT with gum arabic (1:1000 dilution), but reacted strongly upon ICT with gum arabic. The results of the positive skin tests are summarized in Table 1.

CAP FEIA

The total IgE level was 3520 kU/l . In the screening for specific IgE, the patient showed sensitizations to grass pollen (timothy), birch pollen, mugwort pollen, amylase, various vegetables, bee venom,

Table 1. Results of positive skin tests

Allergen extract	SPT	ICT
<i>Dermatophagoides pteronyssinus</i>	+++	nd
<i>D. farinae</i>	±	++
Grass pollen mix	+++	nd
Ash pollen	—	+
Rye pollen	++	nd
Gum arabic — 1:1000	—	nd
Gum arabic — 1:10 000	nd	+++
Gum arabic — 1:100 000	nd	+
Gum arabic — 1:1 000 000	nd	±

nd: not determined.

For additional tests (negative results), see text.

wheat and rye flour, and *Ficus* and latex, as well as to gum arabic (CAP class 5, 53 kU/l). CAP class 2 was found for Bet v 1, Bet v 2, and bovine serum albumin (BSA) (Table 2).

EAST and EAST inhibition

Since CAP FEIA tests for NaIO₄-treated gum arabic and bromelain are not commercially available, we used self-prepared solid phases in EAST to measure specific IgE against gum arabic and bromelain in the patient's serum. Gum arabic resulted in an EAST class 2 (2.92 U/ml), whereas the EAST class for bromelain was 4 (>17.5 U/ml). In contrast, no specific IgE was found against the NaIO₄-treated extracts and against the recombi-

Table 2. Results of specific IgE determinations (CAP)

Allergen	CAP class	kU/l
Timothy pollen	6	>100
Gum arabic	5	53.0
Amylase	5	50.4
Bee venom	4	26.8
Birch pollen	4	25.9
Mugwort pollen	4	20.7
Tomato	4	23.3
Peanut	4	30.6
Soybean	4	22.5
White bean	4	26.5
Pea	4	22.3
Tragacanth	4	24.7
Latex	4	20.6
Wheat flour	4	25.9
Rye flour	4	31.2
<i>Ficus benjamini</i>	3	11.5
Bovine serum albumin	2	1.2
Bet v 1	2	2.3
Bet v 2	2	2.0
Chicken meat	2	0.9
Hen's egg white	1	0.7
Hen's egg yolk	1	0.6
Cow's milk protein	1	0.4

Table 3. Specific IgE to gum arabic and bromelain (EAST)

Allergen extract/allergen	EAST class	kU/l
Gum-arabic extract	2	2.9
Gum arabic, NaIO ₄ -treated	0	0.2
Bromelain, inactivated	4	>17.5
Bromelain, NaIO ₄ -treated	0	0.1
recApi g 1	0	0.1

nant major celery allergen Api g 1, which was applied as an unglycosylated control. No IgE binding to all allergen disks occurred with the negative control serum (PEI 19) (Table 3).

Cross-reactivity measurements were done by EAST-inhibition assays. Gum-arabic and bromelain disks were simultaneously incubated with 1:5 prediluted patient's serum and various inhibitor solutions. Since we wanted to apply comparable amounts of carbohydrate epitopes, bromelain as inhibitor had to be used on an enlarged scale: its carbohydrate content was estimated to be only 5% (20, 43), corresponding to one glycan per molecule. Strong inhibitions could be established for both solid phases with all of the extracts from different kinds of pollen and from vegetables (up to 93%), whereas recombinant allergens and ovalbumin showed less or no inhibition. Interestingly, two different soybean extracts, one prepared from native soybeans and one from soybean after 2 h of cooking, both caused significant inhibitions.

Inverse inhibition patterns were observed with the two solid phases. Competitive EAST with bromelain-coupled disks resulted in very strong inhibitions with bromelain and with the bromelain glycopeptide MXF3 (around 90%) in comparison with a weaker inhibition by gum-arabic extract (63.6%). In contrast, competitive EAST with gum arabic-coupled disks showed stronger inhibition by gum arabic (>85%) and weaker inhibition by bromelain (54.3%) and the glycopeptide MXF3 (45.5%), respectively. A considerably weaker inhibition occurred with the MM glycopeptide without fucose and xylose (17% inhibition in the case of gum arabic-coupled disks and no inhibition with the bromelain-coupled disks). The NaIO₄-treated gum-arabic extract led to no inhibition in all experiments (Table 4).

After these initial experiments, the cross-reactions were further investigated by dose-related inhibitions with gum-arabic disks and chemically modified gum-arabic extracts. Since digestion with proteinase K led only to partially degraded gum-arabic proteins, we destroyed the protein backbone by alkaline hydrolysis and reduction, and, sepa-

Table 4. Results of EAST-inhibition experiments using gum arabic and bromelain as solid-phase antigens

Inhibitor	Inhibitor concentration ($\mu\text{g/ml}$)	East inhibition (%) Coating antigen	
		Gum arabic	Bromelain
MXF ³ glycopeptide	100	45.5	95
MM glycopeptide	100	17	0
recPru a 1	100	0	0
recBet v 1	100	7.7	0
Birch pollen	250	64.5	91
Gum arabic	250	88.5	63.6
Gum arabic, NaIO ₄ -treated	250	0	0
Gum arabic, NaOH/NaBH ₄ -treated	250	68	nd
Gum arabic, HCOOH/pepsin-treated	250	45	nd
Bromelain	2000	54.3	88.4
Bromelain, NaIO ₄ -treated	2000	2.9	nd
Timothy pollen	250	43.6	89.3
Mugwort pollen	250	77.7	64.5
Tomato	250	84.5	92.6
Soybean, raw	250	70.9	74.4
Soybean, cooked	250	47.3	60.7
Wheat flour	250	75.9	73.1
Ovalbumin	250	3.2	0

nd: not determined.

ately, by digestion with pepsin at a strongly acidic pH. In both preparations, no protein bands were detected on SDS-PAGE after silver staining. Analysis of the mono- and oligosaccharides in these preparations by high-performance thin-layer chromatography (HPTLC) showed no influence of the treatment on the polysaccharides in the case of alkaline hydrolysis but partial release of galactose- and arabinose-containing oligosaccharides in the

pepsin-treated extract (results not shown). Inhibition curves with gum arabic, modified gum extracts, bromelain, NaIO₄-treated bromelain, and birch-pollen extract are depicted in Fig. 1. The slopes of the fitted regression curves of both modified gum-arabic extracts and native gum arabic were found to be nearly identical (slopes of gum and NaOH/NaBH₄-treated gum 40.23 [$r=0.985$] and 40.03 [$r=0.998$], respectively, and 38.62 [$r=0.998$] with HCOOH/pepsin-treated gum). The slope of the inhibition curve obtained with bromelain was approximately 25% lower but almost identical to the inhibition obtained with birch-pollen extract (slopes 27.7 [$r=1$] and 31.3 [$r=0.986$], respectively). The reduction of the inhibitor effect of the NaOH/NaBH₄-treated gum was around 20% and around 40% for the HCOOH/pepsin-treated gum, when compared to the native sample. NaIO₄-treated bromelain showed no inhibition even at the highest concentration.

IgE binding to glycopeptides

The patient's serum was subjected to ELISA with glycopeptides isolated from bromelain, chemically defucosylated bromelain, bovine fibrin, and human IgG covalently attached to the solid phase (41). The patient's IgE showed strong reactivity to the bromelain glycopeptide (MXF3), but no reaction to any of the other glycopeptides (Table 5).

The specificity of the glycan ELISA and the cross-reactivity of the CCD-specific IgE to gum arabic and other carbohydrate-containing plant extracts was roughly checked by inhibition experiments. Bromelain glycopeptide was covalently linked to the solid phase, and the 1:5 prediluted patient's serum was incubated with 5 μg of the glycopeptides or recombinant proteins, or with 12.5 μg of gum arabic and different plant extracts. Bromelain was applied at higher inhibitor concentrations for the reasons described earlier. As already observed in the EAST inhibition experiments, strong inhibitions occurred with all plant extracts, with bromelain, with the bromelain glycopeptide alone, and with gum arabic. In contrast, less or no inhibition was measured with the other glycopeptides, with recombinant proteins as unglycosylated controls, with ovalbumin, and with the NaIO₄-treated gum-arabic extract (Table 6).

To quantify the differences, dose-dependent inhibitions were carried out with a series of fourfold dilutions of gum arabic, bromelain, and the bromelain glycopeptide as inhibitors. The results showed closely related inhibition curves for bromelain and its glycopeptide (slopes 21.16 and 17.52)

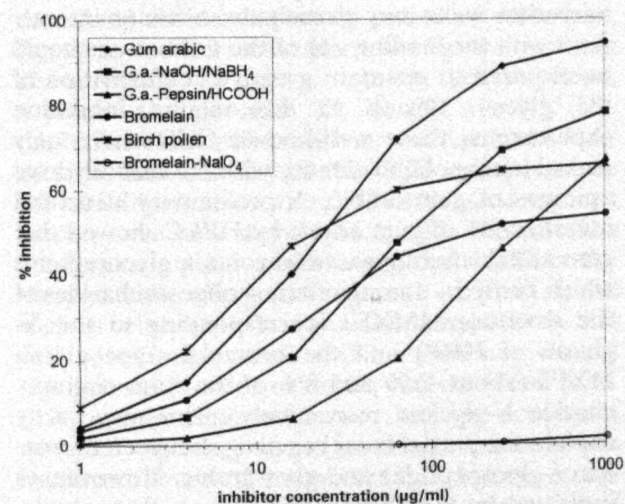


Fig. 1. Dose-related EAST inhibition. Serum dilution: 1:10. Solid phase: gum-arabic allergen disk. Inhibitors: gum-arabic extract, NaOH/NaBH₄-treated gum-arabic extract, pepsin/HCOOH-treated gum-arabic extract, birch-pollen extract, bromelain, and NaIO₄-treated bromelain.

Table 5. Blank-corrected absorbances measured by specific IgE ELISA with various immobilized *N*-linked glycopeptides

Coating antigen	A ₄₀₅
MXF ^a glycopeptide	1.66
MX glycopeptide	0
MM glycopeptide	0
MMF ^b glycopeptide	0

but a different course of the inhibition curve with gum arabic (slope 38.72) (graphs not shown).

Immunoblotting

The immunoblot results with gum-arabic extracts are presented in Fig. 2. As described earlier (37), India-ink staining of the electrophoretically separated gum-arabic extract detected only some diffuse bands. In the investigated samples, these occurred at masses above 30 kDa (Fig. 1). The IgE antibodies of the allergic patient bound to bands at approximately 40 and 67 kDa (Fig. 2, lane 1A). Bands in the same range were recognized by a rabbit antiserum (Fig. 2, lane 2A) against HRP that is known to recognize β 1-2-linked xylose and α 1-3-linked fucose (30, 31). After NaIO₄-treatment of gum arabic, binding to these bands disappeared with both sera (Fig. 2, lanes 1B and 2B).

Table 6. Results of ELISA-inhibition experiments using bromelain glycopeptide at solid phase

Inhibitor	Inhibitor concentration (μg/ml)	ELISA inhibition (%) Coating antigen MXF ^a -GP
MXF ^a glycopeptide	100	93.8
MX glycopeptide	100	0.4
MM glycopeptide	100	0
MMF ^b glycopeptide	100	0
recPru a 1	100	0
recBet v 1	100	8.2
Birch pollen	250	91.1
Gum arabic	250	84.8
Gum arabic, NaOH/NaBH ₄ -treated	250	66.8
Gum arabic, HCOOH/pepsin-treated	250	22.6
Gum arabic, NaIO ₄ -treated	250	0
Bromelain	2000	78.9
Bromelain, NaIO ₄ -treated	2000	0
Timothy pollen	250	96.2
Mugwort pollen	250	96.8
Tomato	250	98.6
Soybean, raw	250	95.2
Soybean, cooked	250	89.2
Wheat flour	250	96.2
Ovalbumin	250	1.1
Apple (Golden Delicious)	250	91.1
Sweet cherry	250	91.5
Potato	250	95.2

Discussion

Allergy caused by gum arabic has been the subject of many papers dealing with occupational asthma in offset-printing workers (27–29). However, besides the notion that gum arabic is responsible for the development of this disease, none of these studies investigated whether the protein or the carbohydrate component was involved in the elicitation of the IgE response to gum arabic. This is the first detailed investigation of a patient working in the food industry and allergic to gum arabic. The results of EAST, ELISA, and immunoblot strongly suggest that the patient's IgE bound almost exclusively to carbohydrate epitopes of gum arabic. This is underlined by an unaffected inhibition potency of gum arabic after removal of the protein backbone with NaOH/NaBH₄. After pepsin treatment, the protein was also completely degraded, but partial release of oligosaccharides caused by the acidic pH was accompanied by a reduction of inhibition potency of about 40%. In contrast, NaIO₄-treated and thus deglycosylated extracts showed no binding to IgE in all experiments.

Moreover, in the direct ELISA with purified CCD-containing glycopeptides of known carbohydrate and amino-acid composition, the patient's IgE reacted strongly to a specific glycopeptide containing α 1-3-linked fucose. The participation of the Asn-linked peptide backbone in the epitope can largely be excluded, since control glycopeptides that carry the same Asn-linked peptides did not react after defucosylation of the oligosaccharide. However, the three-residue peptide backbone which was covalently bound to the surface of the microtiter wells may participate in minor interactions with the binding site of the antibody or could be required to maintain a native conformation of the glycan. Owing to dose-related inhibition experiments, these well-known CCD share only partial immunologic identity with the carbohydrate epitopes of gum arabic. A preliminary structural investigation of gum arabic by HPLC showed that gum arabic also appeared to contain glycoproteins which carried – among others – oligosaccharides of the structure MMXF3 (corresponding to the *N*-glycan of HRP) and the bromelain-type glycan MXF3 (about 15% and 8% of the total oligosaccharide *N*-glycans, respectively). This may partly explain the partial cross-reactivity between bromelain (-glycopeptide) and gum arabic. However, as indicated by the nonidentical slopes, the polysaccharide fraction of gum arabic appears to be also involved in the observed cross-reactions.

EAST inhibition and ELISA inhibition prove that there are CCD in almost all plant extracts

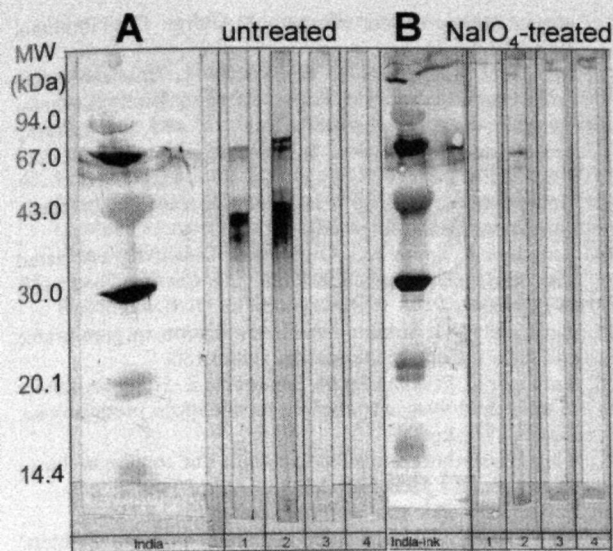


Fig. 2. Binding of IgE from human sera and IgG from rabbit anti-HRP to gum-arabic extracts separated by SDS-PAGE and transferred to NC. Molecular weight corresponds to marker proteins that were stained by India ink. Diffuse bands located on right side of marker are due to proteoglycans from gum-arabic extract. A) Gum-arabic extract; B) gum-arabic extract, NaIO_4 -treated. Lane 1) patient (PEI 39); lane 2) rabbit anti-HRP; lane 3) nonallergic control serum (PEI 19); lane 4) buffer control.

which were investigated in the present study. The specificity of these inhibitions was shown by control tests with nonglycosylated recombinant proteins and BSA that did not influence the binding of the patient's IgE to carbohydrates. The ubiquitous occurrence of CCD-containing fucose and/or xylose was shown by several recent studies describing their existence in pollen of olive tree, Japanese cedar, and Bermuda grass, and in glycoproteins of celery, tomato, potato, bean, soybean, and pea (13, 17, 19, 21, 41, 44). The inhibition experiments with soybean extracts indicate the heat stability of the CCD epitopes, since the inhibition values with the heat-treated soybean extract were only slightly decreased. These results correspond to our observation that CCD is the thermostable allergenic material in plant foods such as celery (41). It is a common hypothesis that IgE specific for these CCD is without clinical significance (9). For example, van der Veen et al. (45) recently reported a poor biologic activity of anti-CCD IgE in grass pollen-allergic patients with significant levels of anti-peanut IgE. Hence, recognition of fucose-containing CCD by a proportion of IgE from our patient may explain the wide range of clinically insignificant sensitizations determined in the CAP (Table 2). As shown by the positive CAP with Bet v 2, cross-reactive plant profilins may also

explain positive CAP results to materials derived from plants.

IgE against the bromelain-type glycan could originate from inhalation of pollen that contains this epitope. Another possibility is occupational sensitization by work as baker and confectioner. Moreover, the CAP test shows a high class against tragacanth, which is used in the confectionary industry, and bee venom, to which the patient who worked as a baker had an increased risk of sensitization. Both allergens are known to contain carbohydrates with the specifically linked fucose (3, 11, 12). Wheat, barley, and rye flour are associated with baker's asthma (46, 47), and a recently published paper may prove that xylose and fucose are present in the Asn-linked complex glycans of members of the α -amylase inhibitory family of wheat and barley, and are largely responsible for the allergenicity of these proteins (18). All these materials could have initiated the antibody response to fucose residues.

Nevertheless, we think that the clinical symptoms of the patient elicited by gum arabic are attributable to carbohydrate epitopes of this plant gum that are different from the ubiquitous CCD. As discussed above, there is little evidence that the protein moiety of gum arabic contributes to the IgE reactivity observed in the allergic patient. In addition, the repetitive polysaccharide sequences of gum arabic may explain the existence of multiple epitopes for the cross-linking of IgE. Histamine-release studies on blood leukocytes with glycopeptides and neoglycoproteins may further aid the identification of the epitopes responsible for eliciting allergic symptoms.

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