

Cross-reactivity between the major allergen from olive pollen and unrelated glycoproteins: Evidence of an epitope in the glycan moiety of the allergen

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Ole e 1, the major allergen from olive pollen, is a glycoprotein containing a single Asn-linked glycan moiety. Rabbit antiserum against this protein has been obtained; and its immunologic cross-reactivities in Western blotting with ascorbate oxidase, horseradish peroxidase, bromelain, ovalbumin, and honeybee venom phospholipase A₂ have been studied. Ascorbate oxidase, peroxidase, and bromelain are recognized by the Ole e 1 antiserum. When these three proteins are deglycosylated by periodate treatment, such an immunologic reaction does not occur. The relative affinities of these proteins have been analyzed by direct and inhibition ELISA experiments. A commercially available antibody against horseradish peroxidase has also been considered in these studies. This antibody reacts with Ole e 1 but not with the periodate-deglycosylated allergen. Horseradish peroxidase, bromelain, and ascorbate oxidase are recognized by the IgE of sera from patients who are hypersensitive to olive tree pollen. This binding is also abolished by periodate treatment. The results are interpreted in terms of the presence of an epitope in the carbohydrate moiety of Ole e 1, which would contain a xylose involved in recognition by both IgE and IgG antibodies. (J Allergy Clin Immunol 1996; 97:1264-71.)

Key words: *Olea europaea*, major allergen, Ole e 1, glycan epitope, cross-reactivity

Pollen from olive tree (*Olea europaea*) is an important cause of allergy in countries of the Mediterranean area.^{1,2} The major allergen of this pollen is Ole e 1, an acidic glycoprotein of 20 kd that exhibits a high degree of polymorphism.³⁻⁷ The protein contains a single glycosylation site located at the Asn-111 residue.⁸ The glycosyl moiety of Ole e 1 was suggested to be involved in its allergenicity, because the single tryptic glycopeptide of the protein was recognized by the sera of persons with olive pollen hypersensitivity, and the allergen treated with the deglycosylating enzyme PNGase F exhibited, in inhibition ELISAs, a de-

Abbreviations used

HRP:	Horseradish peroxidase
PBS:	Phosphate-buffered saline
PLA ₂ :	Phospholipase A ₂ from honeybee venom
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis

creased affinity for the sera from patients allergic to olive pollen.⁸ Moreover, the molecular size of the glycan was estimated by mass spectrometry as the difference between the glycosylated and the nonglycosylated forms of the allergen.⁸ The glycosyl portions of several plant and animal glycoproteins have been related to antigenic reactions,⁹ and allergenic activity has been reported for the oligosaccharide of phospholipase A₂ (PLA₂), a major allergen from honeybee venom,^{10,11} and for the 1 kd sugar group from a sea squirt H allergen.¹² Saccharides exhibiting IgE-binding capability are of great interest because of their potential involve-

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ment in the cross-reactivity between glycosylated allergens from different sources.¹³

The study of the structure of carbohydrates from plant glycoproteins has recently received important support with the availability of specific antibodies.¹⁴ The presence in a glycoprotein of a monosaccharide residue, its linkage and configuration, and its potential involvement in antigenicity can be analyzed by studying the cross-reactivities with antibodies obtained against other unrelated proteins.^{14, 15} A short but useful pool of glycoproteins is commercially available for these purposes. Considering these approaches, we have studied antigenic and allergenic cross-reactivity properties of the glycosyl moiety of the olive pollen allergen.

METHODS

Proteins and enzymes

Horseradish peroxidase (HRP; EC 1.11.1.7), ascorbate oxidase (EC 1.10.3.3) from *Cucurbita pepo*, and PLA₂ (EC 3.1.1.4) from honeybee venom were purchased from Boehringer Mannheim (Mannheim, Germany). Pineapple stem bromelain (EC 3.4.22.4) and ovalbumin were from Sigma Chemical Co. (St. Louis, Mo.) HRP was used after reduction and carboxyamidomethylation to avoid any interference of the HRP activity in the immunodetection system described below. Reduction and alkylation were performed as described by Menéndez-Arias et al.¹⁶

Isolation of Ole e 1

Olive pollen was purchased from Allergon AB. Preparation of the allergen-enriched pollen extract and purification of Ole e 1 were carried out according to the method of Villalba et al.⁴ In brief, the pollen was extracted in 10 mmol/L ammonium bicarbonate, pH 8.0, containing 1 mmol/L phenylmethylsulfonyl fluoride; and the resulting clear supernatant was chromatographed on a Sephadex G-75 column (1.5 cm × 80 cm; Pharmacia, Uppsala, Sweden) equilibrated in 0.2 mol/L ammonium bicarbonate (pH 8.0). The allergen-containing fractions were lyophilized and chromatographed in the Sephadex G-75 column under conditions identical to those described previously. The allergenic fractions were pooled, lyophilized, and dissolved in 0.1% trifluoroacetic acid. After centrifugation, the soluble material was applied to a C-18 reverse-phase high-performance liquid chromatography column. The allergen was eluted with a linear gradient (0% to 60%) of acetonitrile in 0.1% trifluoroacetic acid. According to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, amino acid composition, and NH₂-terminal amino acid sequencing, an allergen sample of more than 98% purity was obtained. The Ole e 1 concentration was determined by absorbance measurements, based on the extinction coefficient of 0.9 (0.1% wt/vol, 1 cm, 280 nm) for the sequence-grade-purified protein.^{4, 6}

Antibody production and human sera

Polyclonal sera against purified Ole e 1 were prepared by immunizing a New Zealand White rabbit over a 6-week period by weekly intramuscular injection of the protein (100 µg) in complete Freund's adjuvant. Serum was collected after bleeding. HRP-specific antibody (ref. P-7899, lot: 06248975) was purchased from Sigma.

Sera from 10 untreated hypersensitive individuals with positive skin test responses to olive pollen crude extract and high RAST values (3 to 4) were pooled and used in IgE-binding immunoblotting assays.

Electrophoresis and blotting

SDS-PAGE, under reducing conditions (5% 2-mercaptoethanol and 2% SDS incubated at 80° C for 20 minutes) was performed according to the method of Laemmli¹⁷ in 15% acrylamide gels. Proteins were visualized by Coomassie Brilliant Blue (Serva, Heidelberg, Germany) staining or electrophoretically transferred onto nitrocellulose membranes (Applied Biosystems, Division of Perkin-Elmer Corp., Foster City, Calif.) for immunostaining by using an LKB Novablot 2117 electrophoretic transfer unit (Pharmacia) in 48 mmol/L Tris-HCl, pH 9.0, containing 39 mmol/L glycine, 20% methanol, and 0.036% SDS, for 1 hour at 1 mA/cm².¹⁸

Nitrocellulose sheets with the electrophoresed and transferred proteins were equilibrated in phosphate-buffered saline (PBS)-0.1% Tween-20 for 15 minutes. After blocking with 3% (wt/vol) skim milk in PBS-0.1% Tween-20 for 1 hour, the membranes were incubated at room temperature for 1 hour with rabbit polyclonal antisera raised against Ole e 1 (diluted 1:5000) or with the HRP antibody (diluted 1:5000). After washing with PBS-0.1% Tween-20, HRP-labeled goat anti-rabbit IgG (TAGO, Inc., Burlingame, Calif.), diluted 1:3000, was used as the secondary antibody. The peroxidase reaction was developed with the ECL Western blotting reagent (Amersham Corp., Arlington Heights, Ill.) over Hyperfilm sheets (Amersham Corp.).

For IgE immunostaining assays, after blocking with the skim milk solution, the membranes were incubated for 2 hours at room temperature with the pool of allergic sera, diluted 1:10. Mouse monoclonal anti-IgE (diluted 1:5000), kindly donated by Dr. Lombardero (Abello S.A., Madrid, Spain), was added after washing the membranes with PBS-0.1% Tween-20. Goat HRP-labeled anti-mouse IgG (Pierce Chemical Co., Rockford, Ill.), diluted 1:5000, was used for the final immunodetection.

ELISA measurements

Relative affinities of anti-Ole e 1 rabbit serum for the different glycoproteins were estimated by titrating on solid phase-fixed antigens.¹⁶ ELISA plates (96-well; Costar Corp., Cambridge, Mass.) were coated overnight at 4° C with 0.1 ml/well antigen (0.3 nmol glycan per milliliter in PBS). After washing with 0.5% Tween-20 in PBS, the plates were blocked with 200 µl 3% skim milk

TABLE I. Structure of N-linked glycans of the glycoproteins used in the study

N-linked glycan	Glycoprotein
$ \begin{array}{c} \text{Man}\alpha 1 \rightarrow 6 \\ \quad \diagdown \\ \text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow \text{N.Asn} \\ \quad \diagup \quad \diagdown \\ \text{Man}\alpha 1 \rightarrow 3 \quad \text{Xyl}\beta 1 \rightarrow 2 \end{array} $	Ascorbate oxidase (<i>D'Andrea et al.</i> ²¹)
$ \begin{array}{c} \text{Man}\alpha 1 \rightarrow 6 \\ \quad \diagdown \\ \text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow \text{N.Asn} \\ \quad \diagup \quad \diagdown \quad \diagdown \\ \text{Man}\alpha 1 \rightarrow 3 \quad \text{Xyl}\beta 1 \rightarrow 2 \quad \text{Fuc}\alpha 1 \rightarrow 3 \end{array} $	HRP (<i>McManus et al.</i> ²⁰)
$ \begin{array}{c} \text{Man}\alpha 1 \rightarrow 6 \\ \quad \diagdown \\ \text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow \text{N.Asn} \\ \quad \diagup \quad \diagdown \quad \diagdown \\ \text{Xyl}\beta 1 \rightarrow 2 \quad \text{Fuc}\alpha 1 \rightarrow 3 \end{array} $	Bromelain (<i>van Kuik et al.</i> ²²)
$ \begin{array}{c} \text{Man}\alpha 1 \rightarrow 6 \\ \quad \diagdown \\ \text{Man}\alpha 1 \rightarrow 6 \\ \quad \diagup \quad \diagdown \\ \text{Man}\alpha 1 \rightarrow 3 \quad \text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow \text{N.Asn} \\ \quad \quad \quad \diagup \\ \text{Man}\alpha 1 \rightarrow 3 \end{array} $	Ovalbumin (<i>Dua and Bush</i> ²³)
$ \begin{array}{c} \text{Man}\alpha 1 \rightarrow 6 \\ \quad \diagdown \\ \text{Man}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow 4\text{GlcNAc}\beta 1 \rightarrow \text{N.Asn} \\ \quad \diagup \quad \diagdown \quad \diagup \quad \diagdown \\ \pm \text{Man}\alpha 1 \rightarrow 3 \quad \pm \text{Fuc}\alpha 1 \rightarrow 6 \quad \pm \text{Fuc}\alpha 1 \rightarrow 3 \end{array} $	PLA ₂ * (<i>Kubelka et al.</i> ²⁴)

*The main structures of the heterogeneous PLA₂ glycan are shown.

in PBS–0.1% Tween-20 at 37° C for 2 hours and incubated with 100 μ l per well of different anti-Ole e 1 serum dilutions in PBS at 37° C for 1 hour. After rinsing, HRP-labeled goat anti-rabbit IgG, diluted 1:3000 in PBS–0.05% Tween-20 containing 1.5% skim milk, was added and incubated at 37° C for 1 hour. Once washed, 100 μ l of 0.5 mg/ml *o*-phenylenediamine (Merck & Co., Inc., Whitehouse Station, N.J.) solution in 0.1 mol/L sodium citrate, pH 5.0, containing 4% methanol and 0.03% H₂O₂ was added; and the reaction was stopped with 3 N H₂SO₄. Optical density was measured at 492 nm.

Inhibition ELISA experiments were carried out as described previously, except that the plates were coated with 1 μ g/ml Ole e 1, and the anti-Ole e 1 rabbit polyclonal antiserum (diluted 1:300,000) was preincubated overnight at 4° C with different concentrations of the considered inhibitor before the incubation in the Ole e 1-coated wells.

Chemical deglycosylation treatment

Periodate oxidation treatment of membrane-transferred proteins was performed according to the method of Yasuda et al.¹⁹ with some modifications. After blotting, the nitrocellulose sheet was incubated in 0.26 mol/L sodium acetate, pH 4.5, containing 26 mmol/L sodium metaperiodate, for 20 hours in the dark at 4° C during constant shaking. After rinsing with deionized water for 30 minutes at room temperature, the membranes were immunostained as described previously.

Other analytic procedures

The protein concentration was determined by amino acid analysis. Proteins (1 to 5 nmol) were hydrolyzed with 0.1 ml of 5.7 N HCl containing 0.1% (wt/vol) phenol and 20 nmol/ml 2-aminoheptanoic acid as internal standard, in sealed tubes under vacuum, at 105° C for 24 hours. Hydrolysates were analyzed on a Beckman system 6300 amino acid analyzer (Beckman Instruments, Inc.,

Fullerton, Calif.) with an analog interface module of System Gold (Beckman Instruments, Inc.). Protein bands on SDS-PAGE gels and developed films were scanned on a Beckman DU-8 spectrophotometer equipped with the corresponding accessories.

RESULTS

Binding of anti-Ole e 1 rabbit polyclonal antiserum to unrelated plant glycoproteins

Ovalbumin, ascorbate oxidase, bromelain, carboxyamidomethylated HRP, PLA₂ (which have well-known glycan structures, Table I)²⁰⁻²⁴, and Ole e 1 were electrophoresed and blotted onto nitrocellulose membranes and then tested for immunodetection with a rabbit polyclonal antiserum raised against Ole e 1. Different amounts of each glycoprotein were applied to the gel to obtain similar carbohydrate concentrations. The calculations were based on the known oligosaccharide content of each protein: bromelain (22 kd), one oligosaccharide unit per polypeptide chain; HRP (45 kd), seven units per polypeptide chain; ascorbate oxidase (65 kd), four units per polypeptide chain; PLA₂ (16 kd), one unit per polypeptide chain; and ovalbumin (44 kd), one unit per polypeptide chain. The SDS-PAGE patterns obtained after Coomassie Brilliant Blue staining are shown in Fig. 1. Sequence grade-purified Ole e 1 shows the previously reported pattern.⁶ Two main bands are observed. Their apparent molecular weights are 18.5 kd (nonglycosylated allergen) and 20 kd (glycosylated allergen).⁶ Depending on the preparation, two more weak bands at 22 kd (highly glycosylated form) and around 40 kd (dimers of the previous forms) can also be detected.^{6,8}

The patterns obtained after immunostaining with the anti-Ole e 1 rabbit antiserum are shown in Fig. 2. The main bands of the olive pollen allergen were recognized by the polyclonal antiserum. In addition, the three plant glycoproteins (ascorbate oxidase, bromelain, and HRP) were also recognized by the anti-Ole e 1 antiserum. The amino acid sequences of these three proteins do not show any significant similarity to that of Ole e 1. Therefore it is unlikely that the observed cross-reactions are due to the polypeptide components. Ovalbumin and PLA₂ did not react with the anti-Ole e 1 allergen antiserum.

Membranes containing transferred carboxyamidomethylated HRP, bromelain, and ascorbate oxidase were treated with periodate before immunodetection with the anti-Ole e 1 polyclonal antiserum. The results are shown in Fig. 2. It is well known that periodate sequentially removes

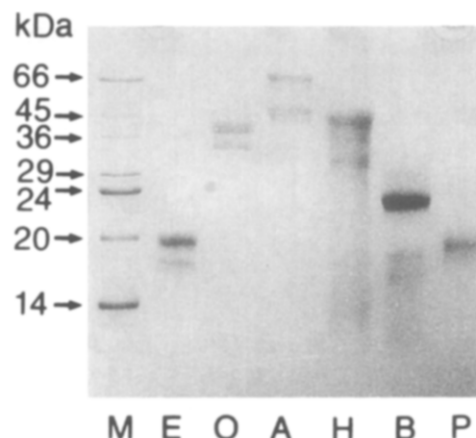


FIG. 1. SDS-PAGE analysis of the proteins used for testing cross-reactivity. Coomassie Brilliant Blue staining of Ole e 1 (E, 7 μ g); ovalbumin (O, 6 μ g); ascorbate oxidase from *Cucurbita pepo* (A, 10 μ g); HRP (H, 7 μ g); pineapple stem bromelain (B, 6 μ g); PLA₂ (P, 6 μ g). M, Molecular mass markers (Bio-Rad Laboratories, Life Science Group, Hercules, Calif.).

monosaccharide residues from the nonreducing end of the sugar moiety of glycoproteins. Treatment with this reagent under the conditions used produces completely deglycosylated forms of these glycoproteins, as deduced from the absence of binding to anti-HRP antibody (Fig. 3), which recognizes β 1 $\rightarrow$ 2-linked xylose and α 1 $\rightarrow$ 3-linked fucose.^{11,15} As shown in Fig. 2, the three bands corresponding to the deglycosylated derivatives of Ole e 1 reacted with the antiserum, indicating that Ole e 1 contains polypeptide epitopes, which are also bound by IgG of the serum under these conditions. No immunostaining was observed for the remaining deglycosylated proteins (Fig. 2). This indicates that the oligosaccharide moieties of these glycoproteins must be responsible for their immune recognition by this antiserum. Interestingly, the glycoproteins immunostained share a β 1 $\rightarrow$ 2-linked xylose on their glycan structures, which is not present in either PLA₂ or ovalbumin.

The binding of the anti-Ole e 1 polyclonal serum to solid phase-fixed carboxyamidomethylated HRP, bromelain, ascorbate oxidase, ovalbumin, and PLA₂ was also measured. The obtained plots are given in Fig. 4, where the relative affinity of these proteins for the rabbit antiserum can be observed. Ovalbumin and PLA₂ did not show any binding to the IgG antibodies, as expected from the results shown in Fig. 2. The other three proteins exhibited more than 90% of the total binding obtained for the olive pollen allergen. The relative affinities of these three interacting proteins de-

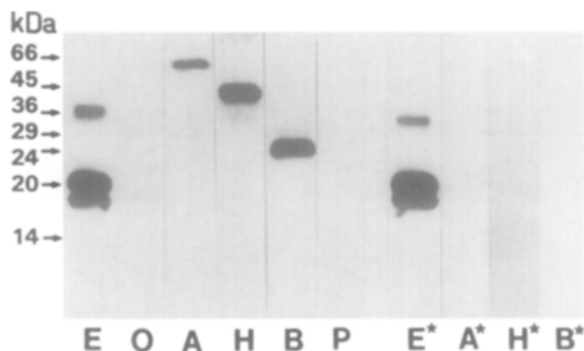


FIG. 2. Western blot of glycoproteins and immunostaining with anti-Ole e 1 rabbit antiserum. Abbreviations as in Fig. 1. The applied amounts of the proteins were the equivalent of around 50 pmol of glycan, except for Ole e 1: *E*, 0.2 μ g; *O*, 2 μ g; *A*, 0.8 μ g; *H*, 0.3 μ g; *B*, 1 μ g; *P*, 0.8 μ g. **Left panel**, Membrane not treated with periodate. **Right panel**, Proteins treated with the deglycosylating reagent (marked by an asterisk) before incubation with rabbit antiserum. Molecular mass marker positions are indicated.

creased in this order: (1) HRP, (2) bromelain, (3) ascorbate oxidase. These results were corroborated by IgG-binding inhibition assays (Fig. 5). These plant glycoproteins were able, only to some extent, to inhibit the binding of Ole e 1 to its antiserum in the concentration range studied. This reveals a dominance of the polypeptide epitopes of the allergen versus the glycan determinant in the recognition by the polyclonal serum.

Ole e 1 recognition by anti-HRP antibody

Commercially available anti-HRP antibody has been used for immunodetection of both native and deglycosylated Ole e 1. Periodate-treated and untreated membranes, containing the transferred allergen, were immunostained with the anti-HRP antibody. The results are shown in Fig. 6. Native Ole e 1 (glycosylated forms: bands at 20, 22, and 40 kd) was recognized by anti-HRP but was not the chemically deglycosylated allergen. Moreover, ELISA titration of the olive allergen with the anti-HRP antibody reaches up to 100% of binding (data not shown). These data corroborate the antigenicity of the Ole e 1 glycan and its similarity to that of HRP.

Binding of plant glycoproteins to olive pollen-hypersensitive human sera

The five glycoproteins used in these experiments were assayed for IgE reactivity with a pool of sera from patients allergic to olive pollen. Only ascorbate oxidase, bromelain, and HRP were immunostained (Fig. 7), and the binding was abolished

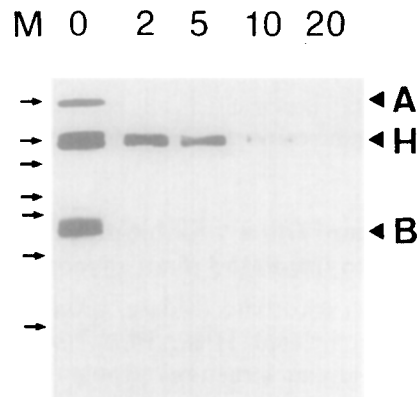


FIG. 3. Analysis of the chemical deglycosylation of ascorbate oxidase (*A*), HRP (*H*), and bromelain (*B*). Staining of the membranes with anti-HRP antibody, after different lengths of time (0, 2, 5, 10, 20 hours) of treatment with sodium metaperiodate, is shown. Anti-HRP antibody recognizes α 1 $\rightarrow$ 3-linked fucose and β 1 $\rightarrow$ 2-linked xylose. *M*, Molecular weight markers as in Fig. 1.

after treatment with periodate. These results indicate that the hypersensitive sera contain IgE antibodies, which recognize the glycan moiety of these glycoproteins, suggesting a role for the carbohydrate of Ole e 1 in the induction of type I allergy in hypersensitive patients. As for the IgG binding, the allergenic glycan epitope would involve a β 1 $\rightarrow$ 2-linked xylose. This can be deduced from the comparison of the sugar structures of the glycoproteins recognized by these human sera.

DISCUSSION

Ole e 1, the major allergen from olive tree pollen, is a glycoprotein that shows a carbohydrate linked at the Asn-111 of the polypeptide chain.⁸ The results of this study demonstrate that the carbohydrate portion of the olive allergen contains an IgG epitope. When Ole e 1 is used as immunogen in rabbits, it elicits the synthesis of antibodies directed against the sugar moiety, because they are able to bind the glycans of HRP, bromelain, and ascorbate oxidase. This is in agreement with other findings describing N-linked glycans from plant glycoproteins as very immunogenic structures, mainly when they contain β 1 $\rightarrow$ 2 xylose and α 1 $\rightarrow$ 3 fucose not found in mammalian glycoproteins.^{14, 25, 26} According to this result, the glycosyl moiety of the olive allergen must share some common structural feature with those of HRP, bromelain, and ascorbate oxidase. Most of the N-linked oligosaccharides from plant glycoproteins exhibit a complex glycan with a consensus "vacuole type" structure,²⁷ which can be represented by that

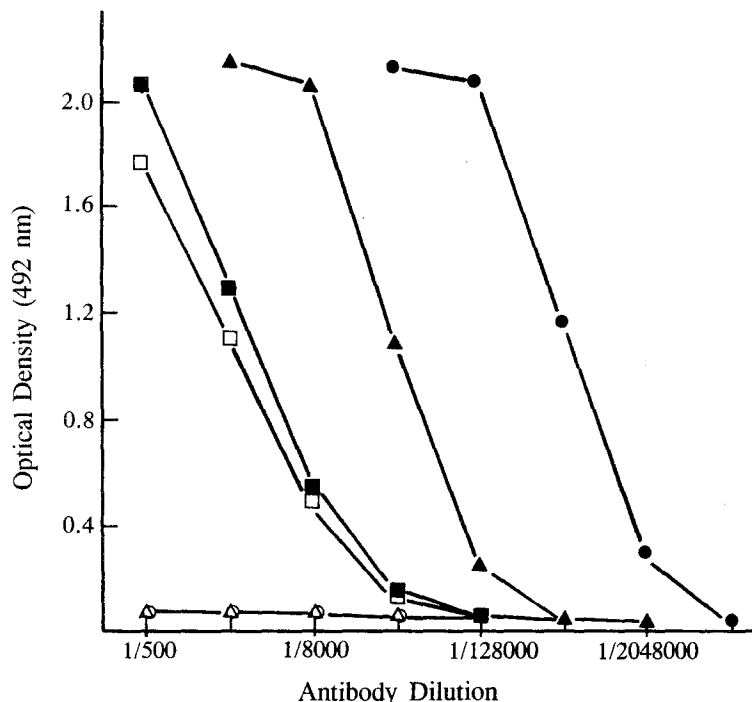


FIG. 4. Binding curves of anti-Ole e 1 antiserum to solid phase-fixed antigens. Polystyrene wells were coated with Ole e 1 (filled circles), HRP (filled triangles), bromelain (filled squares), ascorbate oxidase (open squares), ovalbumin (open triangles), and PLA₂ (open circles). Standard deviations were under 5% for triplicate samples.

from HRP (Table I). Ole e 1 N-linked carbohydrate could belong to this kind of glycans. Comparison of the structures of the carbohydrate components of these glycoproteins (Table I) reveals the presence of a common $\beta 1 \rightarrow 2$ xylose, which is not present in either PLA₂ or ovalbumin. Therefore the absence of cross-reactivities of anti-Ole e 1 with these two latter proteins strongly suggests that, at least, a xylose would be involved in the production of antibodies against the carbohydrate moiety of the allergen. Additionally, antibodies may be directed to other groups of the glycan. Therefore structural differences among the glycans of HRP, bromelain, and ascorbate oxidase would explain the differences observed in the relative affinities against the anti-Ole e 1 antiserum. On the other hand, $\alpha 1 \rightarrow 3$ fucose and $\beta 1 \rightarrow 2$ xylose residues of the oligosaccharide of HRP (Table I) have been reported to be involved in recognition by the anti-HRP antibody.^{11, 15, 28} Thus one or both monosaccharide residues should be exposed in the structure of the Ole e 1 glycan moiety because it is bound by this antibody.

Although the existence of a glycan epitope has been demonstrated for the olive allergen, inhibition assays show a dominance of the polypeptide

epitopes of Ole e 1 in IgG binding, because none of the three plant glycoproteins (HRP, bromelain, and ascorbate oxidase) were able to completely inhibit the binding of the rabbit polyclonal antiserum at reasonable concentrations. This result supports the generally accepted view about the predominant immunogenic character of the polypeptide structures.

Allergenicity of carbohydrate side chains from glycoproteins has been scarcely studied. Recently, the existence of IgE specific for carbohydrate epitopes has been demonstrated in patients with allergy.^{11, 12, 29} These antibodies have been suggested to be involved in cross-reactivity to a wide spectrum of biologic sources of allergens.³⁰ This would be remarkable for allergens from pollen and vegetable foods, in which the glycan components could explain the presence of IgE antibodies specific to an allergen in individuals not previously sensitized.¹³ Patients who are hypersensitive to olive pollen allergen have IgE antibodies specific for complex-type carbohydrate determinants, which are present in a diversity of plant glycoproteins such as HRP, bromelain, and ascorbate oxidase; and these glycoproteins bear $\beta 1 \rightarrow 2$ -linked xylose. Therefore the Ole e 1 glycan would contain an exposed xylose, which would be involved in the

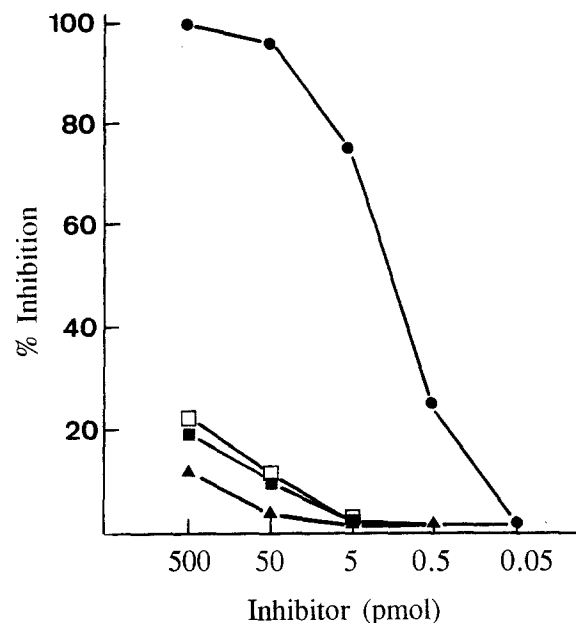


FIG. 5. Inhibition assays of anti-Ole e 1 polyclonal antiserum binding to Ole e 1-coated wells by: Ole e 1 (filled circles), HRP (filled triangles), bromelain (filled squares), and ascorbate oxidase (open squares). The inhibitor concentrations refer to the glycan concentration value of each glycoprotein assayed. Standard deviations were under 4% for triplicate samples in all the assays performed.

protein antigenicity and allergenicity, indicating that this carbohydrate is immunogenic not only for rabbits, but also for human beings.

Cross-reactions among olive tree, privet, and grass pollen proteins have been detected.³¹ Olive and privet belong to the Oleaceae family and show a similar spectrum of IgE-binding proteins by immunostaining of electrophoresed extracts with olive-allergic human sera.^{32,33} Thus the cross-reactivity between them could be explained by the sequence similarity between their very related major allergens. However, the allergens from Bermuda grass and couch grass are unlikely to be homologous to Ole e 1. Therefore many explanations exist for the observed cross-reactions among olive tree and grass pollen proteins (e.g., multi-allergic response or existence of pan-allergens such as profilin). However, the observed cross-reactions could also be due to the presence of similar carbohydrates in the corresponding glycoproteins, as discussed for plant, arthropod, and mollusk allergens.^{13,30,34} Thus cross-reactions caused by shared oligosaccharide structures could play a role in the response of patients allergic to olive pollen against allergens to which they have never been

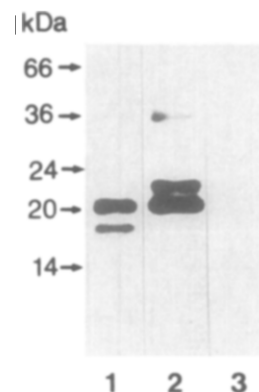


FIG. 6. Immunostaining of electrophoresed and blotted olive allergen with anti-HRP antibody. Lane 2, Ole e 1; lane 3, periodate-treated Ole e 1. Coomassie Blue staining of Ole e 1 is shown for comparison (lane 1).

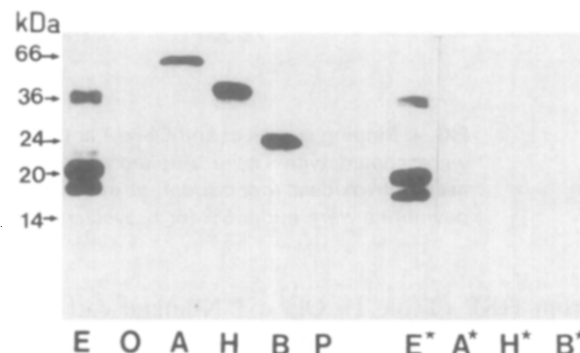


FIG. 7. Immunodetection of glycoproteins with sera of patients allergic to olive pollen. Abbreviations as in Fig. 2. The equivalent amount of glycan (100 pmol) of each protein was applied, except for Ole e 1: E, 0.2 μ g; O, 4 μ g; A, 1.6 μ g; H, 0.6 μ g; B, 2 μ g; P, 1.6 μ g. **Right panel,** Membrane immunostained after treatment with periodate. **Left panel,** Membrane not treated with the deglycosylating reagent.

exposed. Conversely, the glycan epitopes could also be responsible for allergenic responsiveness against olive pollen in patients allergic to glycoproteins from other sources. The isolation and availability of the rabbit polyclonal IgG antibodies directed to the glycan moiety of Ole e 1 would contribute to the characterization of the oligosaccharide, because sugar-specific antibodies have been useful in the elucidation of the structures of antigenic glycans.¹⁴

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