

Identification of a monoclonal antibody to abscission tissue that recognises xylose/fucose-containing N-linked oligosaccharides from higher plants

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Abstract. Monoclonal antibodies raised against extracts of the rachis abscission zone of *Sambucus nigra* L. were selected for high reactivity towards abscission-zone proteins. One antibody (YZ1/2.23) has been shown to cross-react, by both indirect and competition enzyme-linked immunosorbent assay and by Western blotting, with a number of plant enzymes including horseradish peroxidase, rice α -glucosidase, almond β -glucosidase and the lectins from *Phaseolus vulgaris* and *Erythrina cristagalli*.

The major N-linked oligosaccharide isolated from horseradish peroxidase has the sequence Man α 3(Man α 6)(Xyl β 2)Man β 4GlcNAc β 4(Fuc α 3)GlcNAc. This oligosaccharide was found to be a potent inhibitor of the binding of YZ1/2.23 to the intact glycoprotein. The common determinant is therefore contained within this structure.

Key words: Abscission – Glycoprotein – Monoclonal antibody – N-linked oligosaccharide – *Sambucus*

McManus 1986). In *Sambucus nigra*, for example, the cells that comprise the rachis abscission zone in a mature compound leaf are morphologically distinct from their neighbour cells and are recognisable by their smaller size. They also exhibit the specific response of separation of one cell from another linked with well-defined ultrastructural changes when treated with the plant hormone ethylene. These changes do not occur in the neighbouring non-abscission rachis tissue (Osborne and Sargent 1976). Since only the abscission-zone cells respond to ethylene in this specific way, and because they are recognisably distinct, one may predict that ethylene target cells in the abscission zone express components, including proteins, that are either qualitatively or quantitatively different from those of their non-target neighbours and that this expression is associated with abscission-cell differentiation during leaflet development.

In the present study, monoclonal antibodies were raised to an extract from the cells of the rachis abscission zone of *S. nigra*. This paper reports the identification of one monoclonal antibody, YZ1/2.23, that preferentially recognises abscission-zone proteins. This antibody is directed towards a class of N-linked oligosaccharides present on many plant enzymes and lectins.

Introduction

Abscission zones in higher plants are excellent model systems for studying the molecular mechanisms of cell differentiation in vivo (Osborne and

Material and methods

Plant material. Leaves from vegetative shoots of *Sambucus nigra* L. (elderberry), were collected from mature bushes at several sites around Oxford. *Sambucus nigra* has compound leaves usually with five or seven leaflets each having an abscission zone at its base. A complete plate of abscission-zone cells also runs across the rachis just above the point of insertion of each pair of leaflets. This rachis abscission zone contains 15–40 cell

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Abbreviations: ELISA = enzyme-linked immunosorbent assay; Fuc = fucose; GlcNAc = N-acetylglucosamine; HRP = horseradish peroxidase; Ig = immunoglobulin; Man = mannose; SDS-PAGE = Sodium dodecyl sulphate-polyacrylamide gel electrophoresis; Xyl = xylose

layers (Osborne and Sargent 1976) and on treatment with ethylene these cells enlarge, round off and separate from their neighbours. In this state they can be easily collected as a homogeneous cell type distinct from the adjoining rachis tissue. Extracts from the separated ethylene-treated abscission-zone cells were used for the production of the monoclonal antibody characterised here.

Chemicals. All chemicals, unless otherwise specified, were purchased from BDH Chemicals, Poole, Dorset, UK, and were of Analar grade or the best grade available. Acrylamide, bis-acrylamide and N,N,N',N'-tetramethylethylenediamine (TEMED) were BDH Electran grade.

Enzymes and lectins. β -Amylase (Type I-B, from sweet potato), α -glucosidase (Type V, from rice), β -glucosidase (Type I, from almonds), peroxidase (Type VI, from horseradish, HRP), concanavalin A (Type V), *Erythrina cristagalli* lectin, soybean agglutinin and *Phaseolus vulgaris* (red kidney bean) lectin were purchased from Sigma Chemical Co., Poole, Dorset, UK.

Preparation of plant tissue. Abscission zones from intact leaves (designated "0 h zone"), were obtained by excising sections of leaf rachis (approx. 2.0 mm), just distal to the insertion of the leaflets. Sections of non-abscission tissue (3.5 mm) were also excised from the mid point of the inter-leaflet region of the rachis (designated "mid-rachis"). Fully separated abscission-zone cells (designated "zone") were prepared by excising explants (cut to include both the rachis and leaflets abscission zones), mounting the basal end in 2% (w/v) agar (Agar No. 1, Oxoid, Basingstoke, Hampshire, UK), and then enclosing them in desiccators injected with ethylene to a concentration of 10 μ l/l at 25° C in darkness. After 36 h the "zone" cells were fully separated and could be collected on a spatula.

Extraction of plant tissue. Freshly excised or collected tissue was ground in a cooled mortar, together with a little acid-washed sand, in freshly prepared phosphate-buffered saline (PBS; 0.2 g KH_2PO_4 , 2.9 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.2 g KCl, 8.0 g NaCl/l; buffer (ml):fresh weight (g) ratio=1:1.5). The resultant slurry was filtered through Miracloth (Calbiochem, Cambridge, UK), centrifuged at 10000 $\cdot$ g for 15 min and the supernatant either kept on ice until injection into laboratory rats or stored at -20° C for use in hybridoma screening.

Production of monoclonal antibodies. The "zone" extract was emulsified with an equal volume of Freund's complete adjuvant (Difco, East Molesey, Surrey, UK) and 1 ml of the mixture injected at multiple sites into each of three strains of laboratory rats, AO, DA and LOU. The rats were boosted four times over a nine-month period with similarly prepared extracts but emulsified with Freund's incomplete adjuvant. Bleeds were taken 7-10 d after each boost, and the antibody titre monitored using enzyme-linked immunosorbent assay (ELISA). After the fifth bleed the antibody titre of the AO rat against "zone" extract exceeded 1:100000. Four days before sacrificing, this rat received an intravenous booster of normally prepared "zone" extract without adjuvant.

Cell fusions were performed from the single AO spleen cell preparation using the method essentially as described by Galfre and Milstein (1981). Hybridoma colonies were cloned by serial dilution and the culture supernatants were assayed for reactivity against "zone", "0 h zone" and "mid-rachis" extracts using ELISA.

Larger-scale production of one cell line secreting YZ1/2.23 was achieved by inducing ascitic tumours in laboratory rats by intraperitoneal injection of the hybridoma cells, after injection with Pristane (Galfre and Milstein 1981).

Isolation of immunoglobulin fraction from ascitic fluid. YZ1/2.23 ascitic fluid was diluted 1:1 with PBS and an equal volume of saturated $(\text{NH}_4)_2\text{SO}_4$ (76%, w/v), adjusted to pH 6.5-7.5, added drop-wise with continuous stirring. The mixture was stirred slowly overnight at 4° C, the precipitate collected by centrifugation at 12000 $\cdot$ g for 30 min, then resuspended in PBS and dialysed, with several changes, against the same buffer for 18 h at 4° C. The immunoglobulin content was determined from the absorbance of solution at 280 nm ($A_{280}1.4 = 1$ mg/ml). This partially purified preparation was used for ELISA and Western blotting.

Procedures for ELISA. *Sambucus nigra* tissue extracts diluted in 50 mM sodium carbonate, pH 9.6 (coating buffer) to give a final protein concentration of 20 μ g/ml, were used to coat the wells of Micro-ELISA plates (Dynatech Laboratories, Billingshurst, Sussex, UK). The plates were incubated with the antigen at 4° C for at least 8 h before washing off any unbound extract with PBS containing 0.05% (v/v) polyoxyethylene sorbitan monolaurate (Tween 20) (PBS-Tween). Hybridoma supernatants diluted 1:10 with distilled water were added to the wells and the plates were incubated and washed as before. As second antibody, HRP-conjugated rabbit anti-rat immunoglobulin 9 (IgG) (Nordic Immunological Laboratories, Maidenhead, Berks., UK) diluted 1:1000 with PBS-Tween, containing 0.2% (w/v) ovalbumin and 2% (w/v) polyvinylpyrrolidone, was then added to each well and the plates incubated for at least 4 h at room temperature before excess conjugate was washed off. The HRP bound was quantified by addition of 0.05% (w/v) *o*-phenylenediamine, 0.03% (v/v) H_2O_2 in 0.02 M sodium acetate, pH 5.0 and the reaction stopped with 50 μ l of 3 M H_2SO_4 . Developed colour was measured at 495 nm using a Dynatech Micro-ELISA MR 580 plate reader.

To test cross-reactivity, proteins were diluted in coating buffer, plated and incubated as before. After washing with 50 mM sodium phosphate buffer pH 7.4, containing 250 mM NaCl (PBsalt) and 0.05% (v/v) Tween 20, the plates were blocked with 3% (w/v) BSA (low immunoglobulin; Sigma) in PBsalt for 3 h at room temperature. This was then removed to be followed by the monoclonal antibody diluted 1:10000 with PBsalt. Alkaline-phosphatase-conjugated goat anti-rat IgG (affinity purified; Sigma), diluted to 1:1000 with PBsalt, was used as the second antibody, with 4-nitrophenyl phosphate (1 mg/ml) in 0.1 M diethylamine-HCl buffer, pH 9.8, as substrate. Absorbance was read at 405 nm using a Titertek Multiskan MCC340 plate reader.

For competition ELISA, microtitre plates were treated with HRP (20 μ g/ml in coating buffer) washed and blocked with BSA as before. Each competitor compound tested was prepared as five-fold serial dilutions in PBsalt containing 0.05% Tween 20 and 1% BSA (PBsalt-Tween-BSA) and the monoclonal antibody was added to give a final antibody dilution of 1:100000 and a total volume of 100 μ l. The mixtures were incubated at room temperature for 2 h then transferred to the HRP-treated plates and the incubation was continued for a further 1 h. The mixtures were removed, the plates washed with PBsalt-Tween-BSA and the bound antibody quantified with alkaline-phosphatase-conjugated goat anti-rat IgG as described before. Values of 50% inhibition were obtained from logit-log plots of the inhibition versus concentration. Inhibition curves were fitted to unweighted data by non-linear regression using a modification of the iterative least-squares computer program of Duggleby (1981).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. The discontinuous SDS-PAGE method of Laemmli (1970) was used, essentially as described by Studier (1973).

Proteins were dissolved in 50 mM sodium phosphate buffer, pH 6.0, to a final concentration of 2 mg/ml, and aliquots denatured in sample buffer (60 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HCl, pH 6.8, containing 10% (v/v) glycerol, 2.5% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.005% (w/v) bromophenol blue) to give a final concentration of 2 µg/µl. Samples were heated at 100° C for 3 min, 10 µg of each loaded as appropriate and fractionated by electrophoresis through 12.5% (c=2.7%) acrylamide gel, containing 0.1% (w/v) SDS, at 20 mA constant current at room temperature.

Western blotting was carried out as described by Towbin et al. (1979) with electro-elution of the fractionated proteins onto nitrocellulose (Schleicher and Schull, BA 85; Anderman and Co., London, UK) at 25 V for 16 h at 4° C. After transfer, the nitrocellulose sheet was blocked with a solution of 13.5% (w/v) milk powder (Co-op, UK Ltd.) in PBsalt, washed with PBsalt, and then blocked with 10% (v/v) chicken serum (Sera-lab, Crawley Down, Sussex, UK) in PBsalt and washed as before. Monoclonal antibody, diluted 1:1000 with PBsalt containing 1% BSA (low immunoglobulin; Sigma), was incubated with the nitrocellulose for 4 h at room temperature and after washing the incubation was continued for 2 h in 1% (w/v) BSA in PBsalt containing 92.5 kBq of ¹²⁵I-labelled F(ab')₂ fragment of sheep anti-rat Ig (Amersham International, Amersham, Bucks., UK). Following exhaustive washing in PBsalt, containing 0.05% (v/v) Tween 20, the sheet was air-dried and antibody recognition detected by autoradiography using Fuji (Tokyo, Japan) RX X-Ray film. Identical nitrocellulose sheets, were stained for 2 min with 0.1% (w/v) Coomassie blue (PAGE blue 83; BDH) in 10% (v/v) acetic acid and 50% (v/v) methanol, and destained in a solution of 10% (v/v) acetic acid and 30% (v/v) ethanol.

Isolation and separation of oligosaccharides. The N-linked oligosaccharides from glycoproteins were released by hydrazinolysis, subsequently reduced with NaBH₄ and separated by Bio-Gel P-4 column chromatography as previously described (Ashford et al. 1987). Large amounts of unreduced oligosaccharides from HRP were prepared by large-scale hydrazinolysis (Homans et al. 1984). The major oligosaccharide purified by Bio-Gel P-4 chromatography was confirmed as Man₃(Man₆)(Xyl₂)β2 Man₄GlcNAc₄(Fuc₃)GlcNAc by compositional analysis and high-resolution ¹H nuclear-magnetic-resonance spectroscopy.

Determination of protein content. Protein concentrations were determined as described by Bradford (1976), with bovine serum γ-globulin (Bio-Rad, Watford, Herts., UK) as a standard.

Results

Screening of cell fusions. When hybridoma supernatants were screened against *S. nigra* extracts, four hybridomas gave a good response to "zone". Supernatants from several cloned lines of the most active hybridoma (YZ1/2) continued to give a good response to "zone", and the best of these, secreting YZ1/2.23, was used to induce tumours in laboratory rats for the production of ascites fluid.

Proteins reacting with YZ1/2.23. The binding of a range of enzymes was determined using ELISA

Table 1A, B. Recognition by YZ1/2.23 detected using ELISA. Enzymes (A) and lectins (B) were coated onto 96-well Micro-ELISA plates at a concentration of 20 µg/µl, and YZ1/2.23 assayed at 1:10000 dilution. Recognition was quantified by detection of bound alkaline-phosphate-conjugated to goat anti-rat IgG using 4-nitrophenyl phosphate as substrate and measuring absorbance at 405 nm

Enzyme or lectin (and source)	Absorbance (405 nm)*
A.	
<i>Higher plants</i>	
Peroxidase (horseradish)	0.878
Acid phosphatase (potato)	0.705
Pectin methylesterase (tomato)	0.826
β-Amylase (sweet potato)	1.063
β-Glucosidase (almond)	0.892
Ribulose-1,5-bisphosphate carboxylase (spinach)	0.121
α-Glucosidase (rice)	1.280
<i>Mammals</i>	
Carbonic anhydrase (bovine erythrocyte)	0.003
Catalase (bovine liver)	0.045
Alkaline phosphatase (calf intestine)	0.021
<i>Fungi, bacteria</i>	
Invertase (<i>Candida utilis</i>)	0.009
Chitinase (<i>Streptomyces griseus</i>)	0.006
Cellulase (<i>Trichoderma viride</i>)	0.009
β-Galactosidase (<i>Escherichia coli</i>)	0.006
Pectinase (<i>Aspergillus niger</i>)	0.005
B.	
<i>Phaseolus vulgaris</i> agglutinin (PHA)	0.666
Concanavalin A	0.011
Soybean agglutinin	0.042
<i>Erythrina cristagalli</i> lectin	1.124

* Values are the mean of triplicates

(Table 1A). All of the enzymes from higher plants, with the exception of ribulose-1,5-bisphosphate carboxylase, reacted strongly with YZ1/2.23. None of the enzymes from either animal tissue or from lower organisms (fungi, bacteria) were recognised. The monoclonal antibody also bound the lectins from *Erythrina cristagalli* and *Phaseolus vulgaris*, but not soybean agglutinin or concanavalin A (Table 1B).

Inhibition of binding of YZ1/2.23 to immobilised HRP was used to confirm the indirect ELISA results. Competition with HRP resulted in a sigmoidal curve with 0.22 µg/100 µl giving 50% inhibition. The enzymes α-glucosidase (rice), β-glucosidase (almond) and pectin methylesterase (tomato) were all strongly inhibitory with 50%-inhibition values of 0.30, 0.08 and 0.29 µg/100 µl, respectively. When these were converted to molar concentration of subunits they gave 5.0, 1.3 and 5.7 pmol/

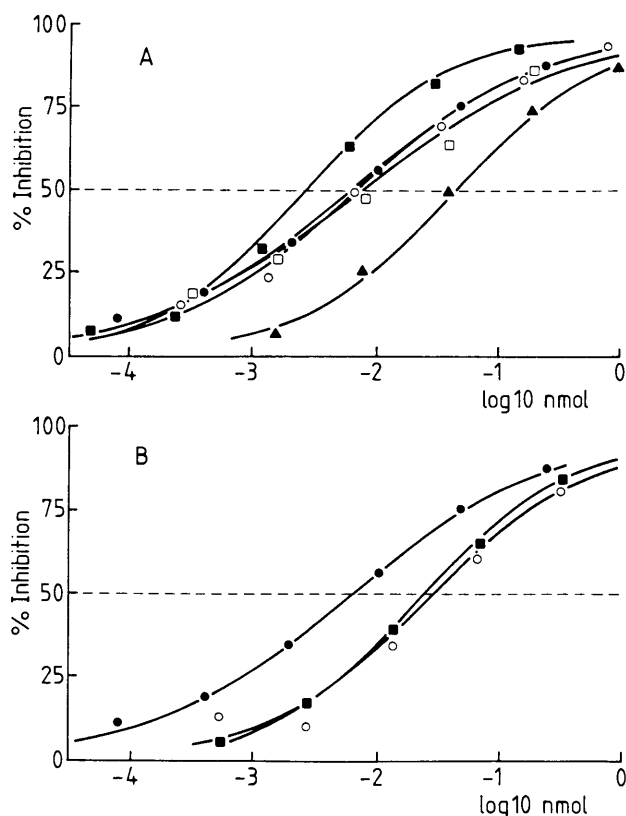


Fig. 1 A, B. Inhibition of binding of YZ1/2.23, an antibody to *Sambucus nigra* abscission-zone protein, to immobilised horseradish peroxidase by glycoprotein enzymes and lectins. **A** Inhibition curves for horseradish peroxidase (●), rice α -glucosidase (○), almond β -glucosidase (■), tomato pectin methylesterase (□) and sweet-potato β -amylase (▲). **B** Inhibition curves for horseradish peroxidase (●), *Phaseolus vulgaris* lectin (○) and *Erythrina cristagalli* lectin (■). Data were determined by competition ELISA, on horseradish-peroxidase-coated microtitre plates. Detection of bound antibody was with alkaline-phosphatase-conjugated goat anti-rat IgG. The individual points are the mean of five determinations and the curves are the best fit to the raw data

100 μl as 50%-inhibition values (HRP gave 5.6 pmol/100 μl) (see Fig. 1A). Sweet-potato β -amylase was slightly less inhibitory (50% inhibition at 0.73 $\mu\text{g}/100 \mu\text{l}$ which converts to 14.1 pmol/100 μl). No inhibition at concentrations up to 10 $\mu\text{g}/100 \mu\text{l}$ was observed with the following animal, bacterial or fungal enzymes and glycoproteins; bovine carbonic anhydrase, porcine or *Escherichia coli* alkaline phosphatase, fungal amyloglucosidase, yeast α -glucosidase, hen egg ovalbumin, human transferrin and bovine thyroglobulin, or with ribulose-1,5-bisphosphate carboxylase.

The lectins gave results that confirmed the findings of indirect ELISA (Fig. 1B). *Phaseolus vulgaris* and *Erythrina cristagalli* lectins were good inhibitors (50%-inhibition values of 16.7 and 15.6 pmol/

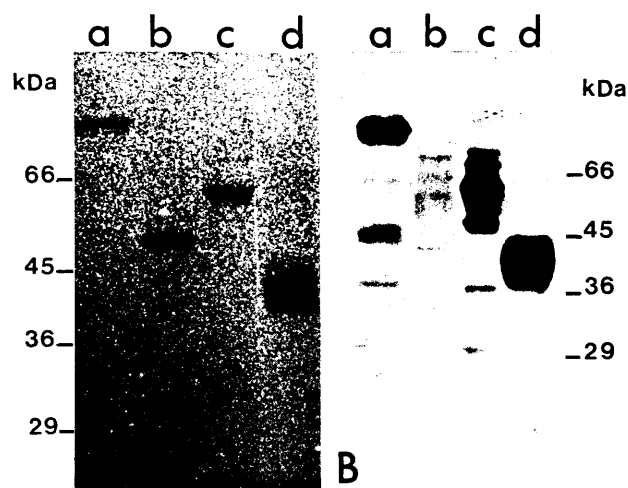


Fig. 2 A, B. Binding of YZ1/2.23 to plant enzymes after separation by SDS-PAGE and Western blotting. **A** Coomassie-blue staining of enzymes after Western blotting onto nitrocellulose. **B** Autoradiography after Western blotting of enzymes onto nitrocellulose and treatment with YZ1/2.23 and ^{125}I -labelled F(ab')₂ fragment of sheep anti-rat Ig. Lane a, rice α -glucosidase; lane b, sweet-potato β -amylase; lane c, almond β -glucosidase; lane d, horseradish peroxidase. kDa = kilodalton

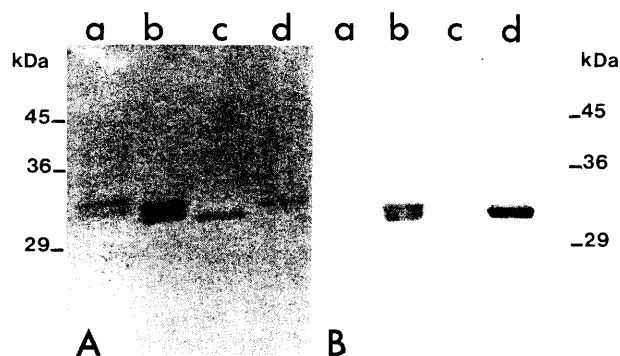


Fig. 3 A, B. Binding of YZ1/2.23 to lectins after separation by SDS-PAGE and Western blotting. **A** Coomassie-blue staining of lectins after Western blotting onto nitrocellulose. **B** Autoradiography after Western blotting of lectins onto nitrocellulose and treatment with YZ1/2.23 and ^{125}I -labelled F(ab')₂ fragment of sheep anti-rat Ig. Lane a, soybean agglutinin; lane b, *Phaseolus vulgaris* lectin; lane c, concanavalin A; lane d, *Erythrina cristagalli* lectin. kDa = kilodalton

100 μl , respectively). Concanavalin A and soybean agglutinin did not inhibit at concentrations up to 10 $\mu\text{g}/100 \mu\text{l}$.

Certain plant enzymes recognised by the monoclonal antibody were selected for immuno-Western blotting with YZ1/2.23 and ^{125}I anti-rat Ig. Successful electrophoretic transfer of each enzyme onto nitrocellulose, was verified by protein staining (Fig. 2A). Autoradiography (Fig. 2B), in general, resulted in strong bands which corresponded to

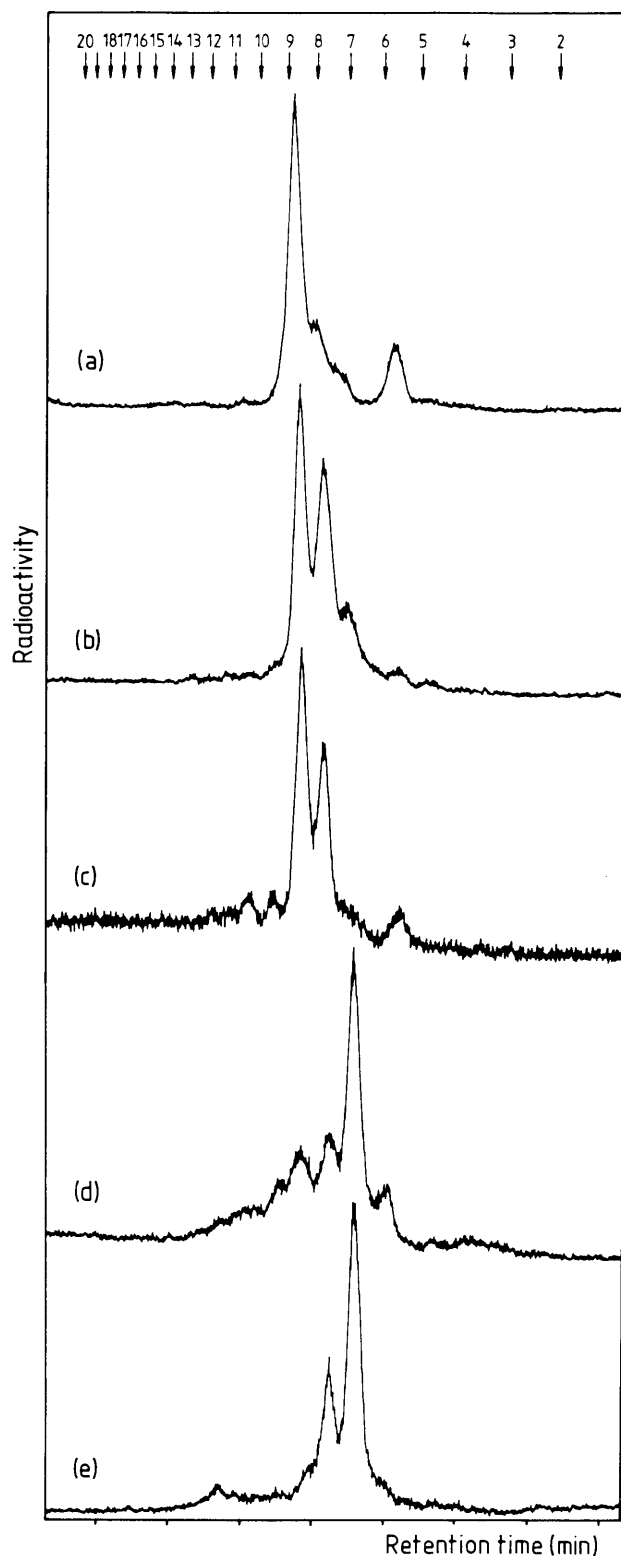


Fig. 4a–e. Bio-Gel P-4 elution profiles of the neutral oligosaccharides from glycoprotein enzymes. The enzymes **a** horseradish peroxidase, **b** almond β -glucosidase **c** tomato pectin methylesterase, **d** rice α -glucosidase and **e** sweet-potato β -amylase were subjected to hydrazinolysis and the isolated oligosaccharides were reduced, purified and separated on Bio-Gel P-4. The

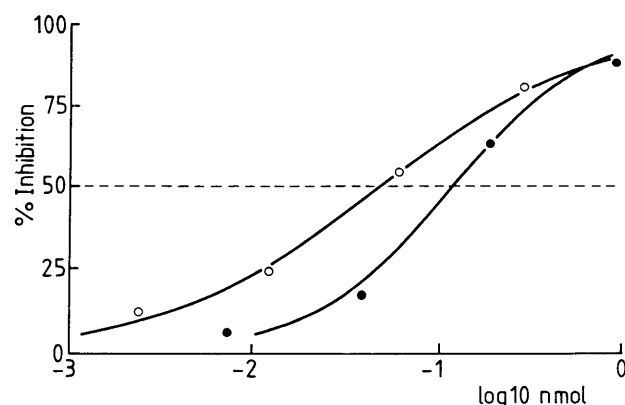


Fig. 5. Inhibition of binding of YZ1/2.23 to immobilised horseradish peroxidase by isolated oligosaccharides from horseradish peroxidase. Inhibition curves for the total hydrazone-released oligosaccharide mixture (●) and purified $\text{Man}\alpha 3(\text{Man}\alpha 6)(\text{Xyl}\beta 2)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 3)\text{GlcNAc}$ (○) were determined by competition ELISA. The individual points are the mean of two determinations and the curves are the best fit to the raw data

the molecular weight of the major protein fraction. Exceptionally with β -amylase, there was no strong recognition of any protein band though several weak bands were consistently detected (Fig. 2B, lane b). Subsidiary bands were also present in the α - and β -glucosidase samples (Fig. 2B, lanes a, c).

It should be noted that the enzymes used were obtained from commercial sources and were not purified further. It is possible therefore that YZ1/2.23 reacted to minor contaminants of β -amylase. Peroxidase is a common contaminant of plant proteins and peroxidase activity at low levels was detected in the β -amylase sample. In similar experiments (Fig. 3A, B), autoradiography of the lectins from *Erythrina cristalli* and *Phaseolus vulgaris* showed binding to the YZ1/2.23 probe while soybean agglutinin and concanavalin A did not.

Bio-Gel P-4 column chromatography of the hydrazone-released, NaB^3H_4 -reduced neutral oligosaccharides of HRP showed a major peak at 8.8 glucose units (g.u.) (Fig. 4a). The oligosaccharide eluting at this position has been characterised in *E. cristagalli* lectin as $\text{Man}\alpha 3(\text{Man}\alpha 6)(\text{Xyl}\beta 2)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 3)\text{GlcNAc}$ (Ashford et al. 1987). The Bio-Gel P-4 elution profiles of β -glucosidase and pectin methylesterase are similar (Fig. 4b, c) and both contain a major peak at 8.8 g.u. In α -glucosidase (Fig. 4d), the major component was eluted at 7.3 g.u. but the profile con-

solid arrows indicate the elution positions of isomalto-oligosaccharide standards containing the corresponding number of glucose units. The time axis is marked at 100-min intervals

tained an appreciable peak at 8.8 g.u. (15.5% of total radioactivity). The major peak in the β -amylase (Fig. 4e) was also eluted at 7.3 g.u. and had only a shoulder eluting at approx. 9 g.u. (8.8% of total radioactivity).

Inhibition of YZ1/2.23 by isolated HRP oligosaccharides. The total N-linked oligosaccharides and the major oligosaccharide of HRP were isolated and tested by competition ELISA for inhibition of YZ1/2.23. As can be seen in Fig. 5 the heptasaccharide was a slightly more effective inhibitor than the total oligosaccharide mixture (50% inhibition values of 47.5 and 119 pmol respectively). These results indicate that the epitope recognised by YZ1/2.23 is contained within the structure $\text{Man}\alpha 3(\text{Man}\alpha 6)(\text{Xyl}\beta 2)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 3)\text{GlcNAc}$.

Discussion

In the search for abscission-cell-specific determinants, antibodies in the supernatants of several hybridoma cell lines were selected for a highly positive response to extracts from separated abscission-zone cells. The antibodies also recognised extracts of unseparated abscission-zone cells ("0 h zone") and, to a much lesser degree, those from neighbouring "mid-rachis" tissue (data not shown). The most active monoclonal antibody, YZ1/2.23, was produced in quantity.

The antibody was found to recognise several enzyme proteins isolated from higher plants (Table 1A). All of the enzymes recognised are glycoproteins. Ribulose-1,5-bisphosphate carboxylase which is not glycosylated gave a weak reaction by this method but failed to show activity in competition ELISA or Western blotting.

Examination of the oligosaccharide profile from Bio-Gel P-4 (Fig. 4a) showed that the major structure present in horseradish peroxidase was eluted at 8.8 glucose units (g.u.). This structure has been characterised as $\text{Man}\alpha 3(\text{Man}\alpha 6)(\text{Xyl}\beta 2)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 3)\text{GlcNAc}$ and is identical to the major oligosaccharide present on *Erythrina cristagalli* lectin (Ashford et al. 1987). This structure is shared by two of the enzymes that react strongly with YZ1/2.23, almond β -glucosidase and tomato pectin esterase (see Figs. 1A, 2B and 4). These results indicated that this heptasaccharide contained the common epitope recognised by YZ1/2.23. This was confirmed by direct inhibition of the antibody by this oligosaccharide in the competition-ELISA system (Fig. 5).

Four lectins with known glycosylation states were tested against YZ1/2.23. The monoclonal recognised those from *Erythrina cristagalli* and *Phaseolus vulgaris* (containing xylose/fucose-type structures, Ashford et al. 1987; Vitale et al. 1984), but did not recognise soybean agglutinin (containing an oligomannose sugar, Dorland et al. 1981) or concanavalin A (non-glycosylated, Goldstein and Poretz 1986; see Table 1B, Figs. 1B and 3B).

We therefore conclude that YZ1/2.23 recognises an oligosaccharide epitope contained within the sequence $\text{Man}\alpha 3(\text{Man}\alpha 6)(\text{Xyl}\beta 2)\text{Man}\beta 4\text{GlcNAc}\beta 4(\text{Fuc}\alpha 3)\text{GlcNAc}$, and further that binding to the epitope does not require the presence of the protein.

The apparent anomalies of the reactivity of the enzyme glycoproteins that lack the 8.8 g.u. heptasaccharide may be because rice α -glucosidase possesses the epitope on other oligosaccharides with equal or greater affinity for YZ1/2.23 than the heptasaccharide, whereas β -amylase carries a partial epitope with reduced affinity for the monoclonal. These questions cannot be answered until the precise specificity of the monoclonal and the structure of the oligosaccharides on these enzymes has been determined. The production of a rat monoclonal antibody to the plant-specific xylose/fucose oligosaccharide class indicates that these structures are highly immunogenic in animals. If this is so they are likely to be major determinants in antisera raised against plant extracts. Other highly antigenic sugar residue on glycoproteins in plant extracts typed by monoclonal antibodies have been reported (Smith et al. 1984; Anderson et al. 1984). Additionally, polyclonal antibodies which recognise the carbohydrate moiety of a mitogen from *Wisteria floribunda* seeds cross-react with the glycosylated lectins from *Sophora japonica* and *W. floribunda* and the glyco-enzyme bromelain (Kaldas et al. 1983). We have also recently found that almond β -glucosidase can inhibit binding of rabbit anti-HRP serum to immobilised HRP by approx. 40%. This indicates that a considerable proportion of the polyclonal antibodies to HRP must be directed against a common oligosaccharide moiety. Reactivity to the carbohydrate portion may also explain the immunological cross-reactivity between horseradish peroxidase isoenzymes and between peroxidases extracted from other plant species (Conroy et al. 1982).

The wide distribution of oligosaccharides of the xylose/fucose type in plant glycoproteins includes the S-glycoprotein products of the self-incompatibility locus in *Nicotiana glauca* (Anderson et al. 1986; Woodward et al. 1987) and *Brassica campe-*

tris (Takayama et al. 1987). This indicates an important role for these structures in higher plants. The monoclonal antibody reported here is therefore a timely tool with which to study this role since it allows the detection of the xylose/fucose type oligosaccharide structure, without the need for lengthy extractions and purification procedures. We have used YZ1/2.23 to detect the cell-specific distribution of the epitope in the abscission zones of *S. nigra*, *P. vulgaris* and *Elaeis guineensis* by either the direct tissue-blotting method of Spruce et al. (1987) using immunogold-linked second antibodies or by SDS-PAGE and Western blotting (data not shown). Such oligosaccharides may be important in targeting proteins to specific organelles, or to vesicles for export. For instance, isoenzymes of peroxidase and β -glucosidase are both present in the cell wall (see McNeil et al. 1984) and there is evidence that some lectins are bound at the cell-surface (Etzler 1986).

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