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Generation of a monoclonal antibody specific to (1→5)-α-L-arabinan

William G.T. Willats, Susan E. Marcus, J. Paul Knox*

Centre for Plant Biochemistry and Biotechnology, University of Leeds, Leeds LS2 9JT, UK

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Abstract

A neoglycoprotein (a heptasaccharide of (1→5)-α-L-linked-arabinosyl residues linked to bovine serum albumin) has been used to generate a rat monoclonal antibody specific to a linear chain of (1→5)-α-L-arabinan which is a structural feature of the side chains of pectins. The antibody, designated LM6, detected 100 ng of debranched sugar beet arabinan in an immunodot binding assay and 1 μg of commercial citrus pectin in a similar assay. Hapten inhibition studies indicated that the antibody recognized 5–6 Ara residues and 50% inhibition of antibody binding in a competitive inhibition ELISA was achieved with ca. 2 ng (21 nM) of (1→5)-α-L-Arabinohexaose. The antibody will be useful for the localization of arabinans in plant tissue and will have uses in the analyses of pectin structure. We report here on the localization of the arabinan epitope in lemon fruits using tissue printing.

Keywords: Arabinan; Monoclonal antibody; Pectin

1. Introduction

Pectin is a complex acidic polysaccharide occuring in the matrix of the primary cell walls of all land plants. The functions of pectin are diverse and include ill-defined roles in cell expansion, cell adhesion, cell wall porosity and plant development and defence [1,2]. We do not yet have a complete understanding of the structure of pectin or of its modification during plant cell development. Pectin is composed of four major acidic structural elements: homogalacturonan, rhamnoglacturonan I, rhamnogalacturonan II and xylogalacturonan [3–5], all of which may be covalently attached together in the cell wall. The Rha in some or all of these is a point of substitution with neutral side chains rich in Gal and/or Ara. The precise structures, linkages and arrangements of side chains vary considerably between species and possibly between cells and molecules within a plant. In adddition to its roles in determining cell wall properties, pectin is used extensively in the food industry to exploit its gelling properties and is most commonly extracted from citrus pulp and apple pomace. A full understanding of the structure–function relationships of pectic polysaccharides is of considerable interest and biological importance.

Highly defined monoclonal antibodies are valuable for the analysis of pectin structure and for studying the distribution of pectin within cell walls and in cells and tissues [6–11]. An anti-(1→4)-β-D-

* Corresponding author. Fax: +44-2333144; e-mail: j.p.knox@leeds.ac.uk

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galactan monoclonal antibody has been generated using a neoglycoprotein as an immunogen resulting in a highly specific antibody probe for an epitope of a galactan pectic side chain [12]. To extend the range of available anti-pectin probes we report on the generation of a monoclonal antibody recognising (1→5)-α-L-arabinan that occurs as a structural feature of pectin side chains.

2. Experimental

Preparation of (1→5)-α-L-arabinoheptaose–BSA neoglycoprotein.—(1→5)-α-L-Arabinoheptaose was obtained from Megazyme (Bray, Ireland) and coupled to BSA by Dextra Laboratories Ltd (Reading, UK). A short three-atom spacer arm was used to conjugate BSA to the arabinoheptaose, and the product (Ara7–BSA) was analyzed by matrix-assisted laser desorption time of flight mass spectroscopy which indicated an average of three heptasaccharides per mol of protein (data not shown).

Immunization procedures and hybridoma preparation.—Three male Wistar rats were immunized subcutaneously with 100 μL of an emulsion of the Ara7–BSA neoglycoprotein at 2 mg/mL in PBS with an equal volume of Freund's complete adjuvant on day 0. On days 30 and 65 the injections of the neoglycoprotein were repeated in a similar manner using Freund's incomplete adjuvant. Tail bleeds were taken on days 40 and 75, serum was prepared and the immune response assessed. The selected rat with the best response was given a pre-fusion intraperitoneal boost of 100 μg of Ara7–BSA in phosphate-buffered saline on day 114 and the spleen was removed on day 117. Lymphocytes were isolated and fused with the IR983F rat myeloma cell line [13] using standard hybridoma preparation and limiting dilution cloning procedures [14]. Hybridoma supernatants resulting from the fusion were screened for the presence of anti-arabinan antibodies on ELISA using the neoglycoprotein and sugar beet arabinans as antigens. A number of positive antibody-secreting cell lines were identified. One monoclonal antibody (LM6, immunoglobulin subclass IgG2b) was selected for characterization and use in further studies. In certain cases the previously prepared anti-galactan LM5 [12] and anti-homogalacturonan JIM7 [6] were used as control antibodies.

Immunodot binding assays.—Immunodot binding assays were carried out to determine the binding capacity and specificity of the anti-arabinan antibody, LM6, to a range of polysaccharides and glycoproteins. The immunogen, potential antigens and test polysaccharides/glycoproteins were applied to nitrocellulose in a 10-fold dilution series starting with 10 μg (1 μL of 10 mg/mL solution) per dot. Phosphate-buffered saline containing 3% milk protein (PBS/MP) was used to block the nitrocellulose sheets for 1 h. The primary antibody was applied as a 10-fold dilution of hybridoma supernatant in PBS/MP for 2 h. The nitrocellulose sheets were then washed with water and the secondary antibody (rabbit anti-rat IgG (whole molecule) coupled to horseradish peroxidase (anti-rat-IgG–HRP), obtained from Sigma), diluted 2000-fold in PBS/MP, was added for 1 h. The nitrocellulose sheets were then washed thoroughly and antibody binding was detected by the addition of a chloronaphthol-based substrate.

Immunodot assays were performed with sugar beet arabinan (Ara:Gal:Rha:GalA 88:3:2:7) and de-branched sugar beet arabinan (Ara:Gal:Rha:-GalA 88:4:2:6) obtained from Megazyme, lemon pectin and lime pectin from Dr J.D. Mikkelson, RGI and RGII from Prof. P. Albersheim and Prof. A. Darvill, carrot extensin from Prof. L.A. Staehelin and *Chlamydomonas* 2BII glycoprotein from Prof. K. Roberts.

ELISAs.—ELISAs were performed in 96-well microtitre plates (NUNC, Maxisorb) coated with 100 μL per well of 200 μg antigen/mL PBS overnight at 4 °C. Unbound antigen was washed out of the wells with tap water and 200 μL of a blocking solution of PBS/MP was added per well. After 1 h at room temperature, plates were washed with tap water and 100 μL per well of hybridoma supernatant added at the appropriate dilution in PBS/MP. Two hours later, plates were washed again and anti-rat-IgG–HRP at a dilution of 1:2000 in PBS/MP was added at 100 μL per well. After a further 1–2 h, plates were washed thoroughly with tap water and antibody binding detected by addition of 150 μL per well of HRP substrate (0.1 M sodium acetate buffer pH 6, 1% tetramethyl benzidene, 0.006% H_2O_2). The reaction was stopped with the addition of 30 μL per well of 2 M H_2SO_4, and the absorbance read at 450 nm on a microtitre plate reader.

For the competitive inhibition ELISAs, a range of potential oligoarabinoside inhibitors of antibody binding (arabino-biose to -hexaose, purity >95%; -heptaose and -octaose, purity >90%)

were obtained from Megazyme. Oligoarabinosides (1 mg/mL solutions) were serially diluted 1:10 (in PBS/MP) in an ELISA plate coated with arabinan prior to the addition of antibody. A volume of 50 μL of the anti-(1→5)-α-L-arabinan (LM6, diluted to provide 90% of maximum binding) was then added to each well. All other steps in the ELISA were carried out as described above. Concentrations of haptens resulting in 50% inhibition of LM6 binding were determined by plotting inhibitor concentrations against absorbances. Values from controls with no inhibitor were taken as 0% inhibition of antibody binding and values from controls with no added antibody represented 100% inhibition of binding.

Tissue printing.—Cut surfaces of lemons were pressed firmly onto a nitrocellulose sheet (for approximately 10 s). The prints were air dried for 30 min and the nitrocellulose sheets were then blocked with PBS/MP for 1 h. Primary antibodies (LM6, LM5 and JIM7) were added at 10-fold dilutions in PBS/MP of hybridoma supernatants and incubated for 2 h. After washing rabbit-anti-rat-IgG–HRP was added (diluted 2000-fold). After a further 1 h the nitrocellulose was washed and antibody binding was detected as for the immunodot binding assay.

3. Results and discussion

Immunization with the Ara7–BSA neoglycoprotein was effective in inducing an immune response against the oligoarabinoside component. Selection of hybridoma supernatants using Ara7–BSA and sugar beet arabinan led to the isolation of a monoclonal antibody that reacted strongly with the neoglycoprotein, isolated pectins and plant tissue. This antibody, designated LM6, was fully characterized. In immunodot binding assays, in which potential antigens were immobilised on nitrocellulose, LM6 detected 1 ng of the Ara7–BSA neoglycoprotein and showed no reaction with 10 μg of BSA (Fig. 1). In the same assay LM6 detected 100 ng of purified sugar beet arabinans and was found to be approxiamtely 10-fold more reactive with debranched arabinans. This suggests that LM6 does not require a terminal arabinose residue for recognition but can bind to linear (1→5)-α-L-linked arabinosyl residues within an extended polymer. It was found to react with lemon pectin but not lime pectin and showed a

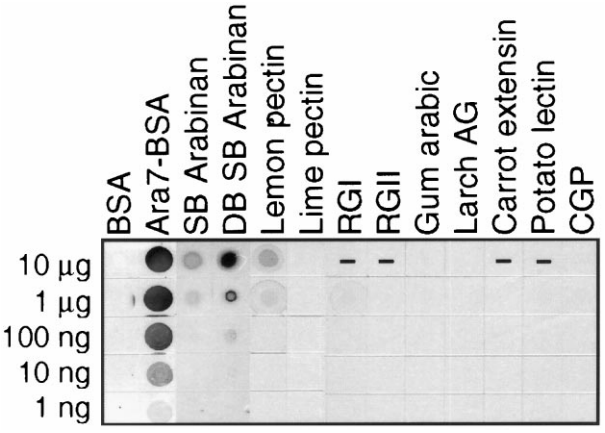

Fig. 1. Immunodot binding assay of LM6 binding with a range of potential antigens. Antigens were applied in a dilution series and amounts are indicated on the left-hand side. BSA, bovine serum albumin; Ara7–BSA, neoglycoprotein; SB, sugar beet; DB SB, debranched sugar beet; AG, arabinogalactan; CGP, *Chlamydomonas* glycoprotein. A dash in a square indicates that an antigen was not used at that particular concentration.

weak reaction with 1 μg of RGI but no reaction with RGII at this level. This can be explained by structural differences as RGI contains (1→5)-α-L-linked polyarabinose chains whereas RGII does not. LM6 showed no reaction with 10 μg of gum arabic and larch arabinogalactan and no reaction with 1 μg of carrot extensin, potato lectin and the *Chlamydomonas* 2BII glycoprotein which are plant cell surface macromolecules that contain arabinose but not (1→5)-α-L-linked polyarabinose chains.

The size of the epitope was investigated by means of competitive inhibition ELISAs using a series of defined (1→5)-α-L-linked oligoarabinosides. No inhibition of antibody binding was observed with up to 1 mg/mL arabinose (Table 1). The series of (1→5)-α-L-arabinobiose to (1→5)-α-L-arabinopentaose displayed increasing capacity to inhibit LM6 binding in the competitive inhibition assays with the arabinohexaose being the most effective inhibitor indicating that the size of the epitope is 5–6 (1→5)-α-L-linked arabinose residues (Table 1).

The usefulness of LM6 as a probe for the localization of arabinan epitopes was assessed by tissue printing of lemon fruits which are known to contain linear arabinans [15]. During tissue printing soluble material is transferred from a cut surface of the fruit onto nitrocellulose where it may then be detected using antibodies and much of the spatial information regarding distribution is maintained. LM6 reacted strongly with tissue prints of lemon

Table 1
Hapten inhibition of LM6 binding by oligoarabinosides

Hapten	Concentration for 50% inhibition (nM)
L-Arabinose	$>1.0\times10^{7}$
(1→5)-α-L-Arabinobiose	2.1×10^{4}
(1→5)-α-L-Arabinotriose	730
(1→5)-α-L-Arabinotetraose	260
(1→5)-α-L-Arabinopentaose	53
(1→5)-α-L-Arabinohexaose	21
(1→5)-α-L-Arabinoheptaose	43
(1→5)-α-L-Arabinooctaose	75

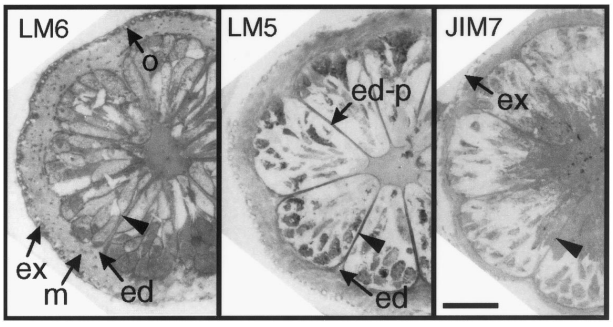

Fig. 2. Immuno-tissue prints of pectin epitope distribution within lemon fruits indicated by binding of anti-arabinan (LM6), anti-galactan (LM5) and anti-homogalacturonan (JIM7). ex, exocarp; m, mesocarp; ed, endocarp; ed-p, endocarp partition; o, oil gland. Arrow head indicates the centre of an endocarp-derived partition between locules. Scale bar = 1 cm.

fruit and revealed important information regarding the distribution of the arabinan epitope as indicated in Fig. 2. The distinctive distribution of the arabinan epitope is of particular interest when compared with tissue print patterns of pectic galactan and galacturonan epitopes as shown in Fig. 2. The arabinan epitope occurred abundantly in the exocarp surrounding the oil glands and also in the mesocarp. The LM6 epitope was also abundant in the regions of endocarp and endocarp-derived partitions that surround the locules and also in the endocarp extensions forming the juice sacs. In contrast, the galactan epitope, recognized by LM5, is absent from the endocarp extensions forming the juice sacs although it occurred in the centre of the endocarp partitions between the locules and was present within certain juice sacs towards the periphery of the locules. The galacturonan epitope recognized by JIM7 was notably absent from the exocarp and occurred throughout the pericarp and in the centre of the endocarp partitions of the locules but absent from the endocarp extensions surrounding the juice sacs.

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