ELSEVIER

Carbohydrate Research 301 (1997) 69–76

CARBOHYDRATE RESEARCH

Development of an immunoassay for larch arabinogalactan and its use in the detection of larch arabinogalactan in rat blood [1]

Ernest V. Groman *, Daming Gou

Advanced Magnetics, Inc., 61 Mooney Street, Cambridge, MA 02138, USA

Received 11 November 1996; accepted in revised form 14 February, 1997

Abstract

We describe a sensitive and convenient immunoassay for larch arabinogalactan and demonstrate its specificity for larch arabinogalactan. Anti-larch arabinogalactan antiserum is about 10^4 and 10^6 times more selective for detecting larch arabinogalactan than RCA lectin and rat-liver asialoglycoprotein receptor, respectively. Anti-larch arabinogalactan antiserum binds to branch terminal disaccharides consisting of the terminal β-D-galactosyl residue and the penultimate branch (1 → 6)-β-D-galactosyl residue. It does not bind L-arabinose. The sensitivity of the assay for larch arabinogalactan is less than 0.1 μg/mL. The application of the assay for measuring arabinogalactan pharmacokinetics in rat blood is illustrated. © 1997 Elsevier Science Ltd.

Keywords: Arabinogalactan; Immunoassay; Pharmacokinetics

1. Introduction

The use of polysaccharides as pro-drug carriers has attracted considerable attention [1–3]. Among the polysaccharide carriers tested, dextran is one of the most studied due to its extensive human clinical experience and physico-chemical properties [4–6]. Another polysaccharide which is beginning to gain recognition as a drug carrier is larch arabinogalactan [7–9].

Larch arabinogalactan is a highly branched polysaccharide consisting of D-galactose and L-arabinose residues [10–12]. The predominance of galactose residues and the high degree of branching of arabinogalactan suggests that arabinogalactan might bind to the hepatocyte asialoglycoprotein receptor [13]. Several reports have shown that arabinogalactan does bind to this receptor [7,12,14] and arabinogalactan has been used as a carrier to deliver drugs and magnetic resonance contrast agents to the hepatocyte via this receptor [7–9]. Thus, arabinogalactan appears to act as a drug carrier in a similar way to that

Abbreviations: AG, arabinogalactan; ASF, asialofetuin; BSA, bovine serum albumin; FITC, fluorescein isothiocyanate; HES, *O*-(hydroxyethyl)starch; IC_{50}, concentration of analyte necessary to inhibit 50% of the tracer bound in the absence of analyte; kDa, kilodalton; PBS, 0.01 M potassium phosphate, 0.15 M sodium chloride, pH 7; RCA, *Ricinus communis* agglutinin

* Corresponding author. Tel.: +1-617-497-2070; fax: +1-617-547-2445.

[1] Part III of the series Arabinogalactan for hepatic drug delivery.

PII S0008-6215(97)00077-3

previously reported for asialoglycoproteins and neoglycoproteins [15–17]. Arabinogalactan is also attractive for targeting drugs to hepatocytes because it is not immunogenic [18] and can be produced inexpensively [7]. Development of arabinogalactan as a carrier to deliver drugs to hepatocytes requires an assay to follow its pharmacokinetics in vivo.

Naim and van Oss previously reported that larch arabinogalactan is not immunogenic [18], and thus consideration of the idea of generating antibodies to measure arabinogalactan is likely to be dismissed. To develop this assay we have overcome the non-immunogenicity of larch arabinogalactan by conjugating it to bovine albumin. We describe a sensitive and convenient immunoassay for arabinogalactan, demonstrate its specificity for larch arabinogalactan, and illustrate its usefulness in following the pharmacokinetics of arabinogalactan after intravenous injection in rats.

2. Materials and methods

Sources.—All materials were obtained from common commercial sources unless stated otherwise. Stractan 2 was obtained from Champion Corp. (Tacoma, WA).

Purified arabinogalactan.—Purified arabinogalactan [arabinogalactan(37 kDa)] was prepared as previously described [12].

Arabinogalactan(9 kDa).—Arabinogalactan (9 kDa) was prepared from arabinogalactan(37 kDa) as described by Prescott et al. [14]. The resulting material was identical to arabinogalactan(37 kDa) with respect to carbohydrate composition and linkage analysis except that its molecular weight was decreased from 37 to 9 kDa [14].

[^{14}C]Arabinogalactan(9 kDa).—Arabinogalactan (9 kDa) (1 g) was dissolved in 4 mL of 2 M NaOH and cooled to −10 °C. The chilled arabinogalactan solution was transferred to the top of a breakseal flask containing 250 μCi of [^{14}C]ethylene oxide (Amersham). The lower chamber, which was under vacuum, was immersed in liquid nitrogen. The reaction vessel was sealed with parafilm and the glass septum separating the two chambers was broken, allowing the arabinogalactan solution to enter the lower chamber. The reactants were warmed to room temperature and mixed for 18 h. The ^{14}C-labeled arabinogalactan was purified with deionized water by exhaustive ultrafiltration against a YM-3 membrane (Amicon, Beverly, MA). The retained fraction was lyophilized. The specific activity of the labeled arabinogalactan was 204 μCi/g.

Arabinogalactan(9 kDa) – carboxymethyl$_{10}$.—Arabinogalactan(9 kDa) (1.5 g) and NaOH (2.3 g) dissolved in 14.4 mL of deionized water were combined with 1.1 g of bromoacetic acid dissolved in 2.85 mL of deionized water. The solution was stirred for 2 h at 25 °C, neutralized to pH 7 with 6 M HCl, and exhaustively ultrafiltered against a YM-3 membrane with deionized water. The final retain was lyophilized. The conjugate contained 10 mmol of carboxymethyl group per gram of product.

Arabinogalactan(9 kDa) – hexanoic acid$_9$.—Arabinogalactan(9 kDa) (10 g) and NaOH (16 g) dissolved in 50 mL of water were combined with 10.8 g of 6-bromohexanoic acid and 2.2 g of NaOH. The solution was stirred for 1 h at room temperature, neutralized to pH 7 with 6 M HCl, and exhaustively ultrafiltered against a YM-3 membrane with deionized water. The final retentate was lyophilized. The conjugate contained 9 mmol of hexanoic acid adduct per g of product.

Periodate oxidation of arabinogalactan.—Arabinogalactan(37 kDa) (500 mg) was dissolved in 500 mL of 0.02 M $NaIO_4$ adjusted to pH 4.5 and incubated for 18 h at 4 °C in the dark. Excess periodate was decomposed by the addition of 2 mL of ethylene glycol. The mixture was exhaustively dialyzed against deionized water and lyophilized.

Galactose oxidase treatment of arabinogalactan.—Arabinogalactan(37 kDa) (50 μg dissolved in 0.5 mL deionized water) was combined with 1.4 mL of 0.1 M sodium phosphate buffer (pH 7) containing 0.005% *o*-toluidine, 0.1% Triton X-100®, and 0.001 units of horseradish peroxidase (Sigma Chemical Co., St. Louis, MO, Catalog No. P 6782) and 0.1 mL of 0.1 M sodium phosphate buffer (pH 7) containing 5 units of galactose oxidase (EC 1.1.3.9) (Sigma Chemical Co., Catalog No. G 7907). The reactants were incubated for 3 h at room temperature. Absorbance readings at 425 nm showed that ~15 galactose C-6 hydroxyl groups per mol of arabinogalactan(37 kDa) had been oxidized to the corresponding aldehyde. A sample (0.5 mL) of the galactose oxidase-treated arabinogalactan was removed for radioimmunoassay, and the remainder of the sample was treated with $NaBH_4$ (300 μg) for 30 min at room temperature in order to reduce the C-6 aldehydes to the starting alcohol. The borohydride-reduced arabinogalactan was reassayed for immunoreactivity.

Arabinogalactan – albumin conjugate.—Arabinogalactan(9 kDa)–hexanoic acid$_9$ (1 g) and 70 mg of *N*-hydroxysuccinimide were dissolved in 5 mL of formamide. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (95 mg) was added to the solution and mixed until dissolution. The reactants were incubated for 30 min at 25 °C. This solution (4.5 mL) was next added to 1.5 g of bovine albumin dissolved in 20 mL of 0.1 M $NaHCO_3$ (pH 9.2) and incubated for 2 h at 25 °C. The resulting solution was dialyzed against three changes of 4 L of deionized water and lyophilized.

Arabinogalactan – tyramine conjugate.—Arabinogalactan was conjugated to tyramine by first activating arabinogalactan(9 kDa)–hexanoic acid$_9$ with *N*-hydroxysuccinimide as already described. This solution [0.5 mL containing 100 mg arabinogalactan(9 kDa)–hexanoic acid$_9$] was added to 50 mg of tyramine dissolved in 1 mL of formamide and the incubated solution at 25 °C, for 2h. The resulting solution was dialyzed against three changes of 4 L of deionized water and then lyophilized.

Arabinogalactan - [^{125}I]tyramine.—Arabinogalactan–tyramine conjugate (10 μg) was dissolved in 50 μL of 0.5 M sodium phosphate buffer (pH 7.4). To this solution was added in order 0.5 mCi ($\sim$ 5 μL) of ^{125}I-sodium iodide (Amersham) and 10 μg of chloramine T dissolved in 10 μL deionized water. The reactants were incubated for 3 min at 25 °C. Next, 20 μg of sodium metabisulfite dissolved in 10 μL of deionized water was added and the solution incubated for 1 min at 25 °C. The solution was transferred to a PD-10 column (Pharmacia) equilibrated with PBS, and 1-mL fractions were collected. The first peak containing radioactivity eluting at the void volume of the column was collected. The isolated arabinogalactan-[^{125}I]tyramine was diluted with PBS–0.1% BSA to a count rate of 800,000 dpm/mL. The tracer is stable for 30 days when stored at 4 °C.

Immunization of rabbits with arabinogalactan and arabinogalactan – albumin conjugate.—Four rabbits (NZW) were challenged with arabinogalactan(37 kDa), and an additional four rabbits were challenged with arabinogalactan–albumin conjugate. The immunization schedules of both antigens were the same: 1 mg of antigen mixed with Freund's complete adjuvant was administered subcutaneously by multiple injection followed by subcutaneous boosts of 250 μg of antigen mixed with Freund's incomplete adjuvant after 2, 3, and 6 weeks and every fourth week thereafter. Bleeds were obtained every four weeks beginning four weeks after initiation of the immunization protocol, and the resulting sera were stored frozen until assayed.

Immunoassay procedure.—Anti-arabinogalactan rabbit serum was diluted with PBS containing 0.1% BSA (usually 1:200) to achieve about a 30% binding of arabinogalactan-[^{125}I]tyramine. Standards were prepared in the same matrix as samples. 100 μL of standard or sample (duplicate tubes) were combined in sequence with 100 μL arabinogalactan-[^{125}I]tyramine and 100 μL of diluted rabbit anti-arabinogalactan antiserum. The solution was incubated for 60 min at room temperature. Goat anti-rabbit BioMag® particles (500 μL; PerSeptive Diagnostics, Framingham, MA) were added, and the suspension was incubated for 10 min at room temperature. The BioMag particles and associated anti-arabinogalactan antibody–tracer complex were separated magnetically, and the supernatant was aspirated. Radioactivity retained in the pellets was counted using a gamma counter. Data were analyzed by fitting standard curves to a four-parameter logistic function and interpolating unknowns from the resulting curves [19]. Samples of this antiserum may be obtained for research purposes by writing to the corresponding author.

Receptor assay.—Asialoglycoprotein receptor from rat liver was isolated following the method of Hudgin et al. [20]. Assay results are reported as the concentration of analyte necessary to inhibit 50% of binding of ^{125}I-labeled arabinogalactan (IC_{50}); the assay was performed as previously described [12].

RCA lectin assay.—Sample (100 μL), arabinogalactan-[^{125}I]tyramine (100 μL; 80,000 dpm), and FITC-labeled RCA lectin (100 μL containing 20 μg; Sigma, L-4638), were combined in order. All components were dissolved in PBS containing 0.1% BSA. The mixture was incubated for 60 min at 37 °C. Sheep anti-FITC BioMag® particles (500 μL; PerSeptive Diagnostics) obtained were added and incubated for 10 min at room temperature. The BioMag particles and associated FITC-labeled lectin-tracer were separated magnetically, and the supernatant was aspirated. Radioactivity retained in the pellets was counted using a gamma counter. Results are reported as the concentration of analyte necessary to inhibit 50% of binding of arabinogalactan-[^{125}I]tyramine (IC_{50}).

Clearance of [^{14}C]arabinogalactan from rat blood.—Male Sprague Dawley rats ($\sim$ 300 g) were anaesthetized by intraperitoneal injection with Inacton (70 mg/kg). The femoral vein was exposed and [^{14}C]arabinogalactan (25 mg/kg) was injected. The

rats were kept anaesthetized for the duration of the experiment. Heparinized blood samples (0.75 mL) were collected at selected intervals up to 60 min. The plasma was isolated and stored frozen. Radioactivity in plasma samples was determined by liquid scintillation counting.

3. Results

Arabinogalactan RIA.—Rabbits were immunized with either arabinogalactan(37 kDa) or arabinogalactan–albumin conjugate. None of the rabbits immunized with arabinogalactan produced any detectable level of antiserum over a six-month period. Serum volumes tested were 10 and 100 μL. All rabbits immunized with arabinogalactan–albumin conjugate produced antibodies to arabinogalactan within three months with titers between 500 and 2000 tests per mL of serum. Serum from the highest titer rabbit was used for all subsequent studies reported herein. Using the assay procedure described in Methods, a dose–response curve between 0.1 and 10 μg/mL arabinogalactan(9 kDa) was obtained (Fig. 1). The sensitivity of the assay was < 0.1 μg/mL and the concentration required to inhibit 50% of the tracer binding (IC_{50}) was about 1 μg/mL. Reproducibility measurements for arabinogalactan between 0.3 and 5 μg/mL had a coefficient of variation (CV) of less than 12%. Spike and recovery experiments performed in rat serum gave recovery values between 85 and 112%.

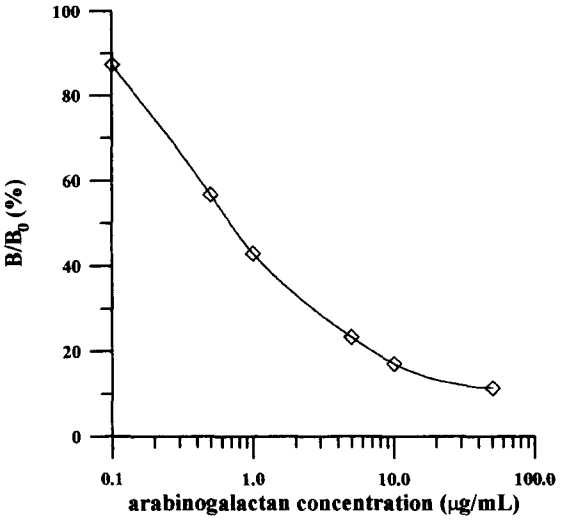

Fig. 1. Representative standard curve for measuring arabinogalactan(9 kDa) obtained with rabbit anti-arabinogalactan antiserum and arabinogalactan-[^{125}I]tyramine.

Characterization of the anti-arabinogalactan antibody - binding site.—Inhibition of ^{125}I-labeled arabinogalactan antibody binding with saccharide inhibitors. Anti-arabinogalactan serum was characterized initially by studying the ability of various mono-, di-, and tri-saccharides to inhibit binding of this antiserum to ^{125}I-labeled arabinogalactan. The saccharide concentrations required for 50% inhibition of the binding between antibody and tracer are shown in Table 1. Of the saccharides examined, the disaccharide 6-*O*-β-D-galactopyranosyl-D-galactose was the most potent inhibitor, approximately 60 times more effective on a molar basis than the next most effective inhibitor, methyl β-D-galactopyranoside, and 100 times more effective than D-galactose. The antiserum showed preference for the galactosides in the β-configuration as the β-D-galactosides were all considerably better inhibitors than the α-D-galactosides tested. A range of other related monosaccharides

Table 1
Comparison of saccharides as inhibitors of ^{125}I-labeled arabinogalactan binding to anti-arabinogalactan antiserum

Inhibitor	IC_{50} (mM)
6-*O*-β-D-Galactopyranosyl-D-galactose	0.515
Methyl-β-D-galactopyranoside	31.8
Galactose	49.2
4-*O*-β-D-Galactopyranosyl β-D-glucose (β-Lactose)	156
Methyl-α-D-galactopyranoside	722
Methyl-β-D-glucopyranoside	965
4-*O*-(4-*O*-β-D-galactopyranosyl-D-galactopyranosyl)-D-glucose	12,600
3-*O*-β-D-Galactopyranosyl-D-arabinose	16,100
6-*O*-α-D-Galactopyranosyl-D-glucose	57,250
4-*O*-α-D-galactopyranosyl-D-galactose	No inhibition
L-Arabinose	No inhibition
D-Galactal	No inhibition
Galactitol	No inhibition
D-Galactonic acid	No inhibition

Table 2
Inhibition of ^{125}I-labeled arabinogalactan binding to anti-arabinogalactan antiserum by chemically and enzymatically modified arabinogalactan

Inhibitor	Molecular weight [a] (kDa)	Relative IC_{50} (%)
Arabinogalactan (AG 37 kDa)	37	100
Reduced arabinogalactan	37	118
Sodium hydroxide-treated arabinogalactan (AG 9 kDa)	9	121
Carboxymethylated arabinogalactan	9	44
Periodate-treated arabinogalactan	15	0.1
Galactan	Not determined	0.2
Galactose oxidase-treated arabinogalactan	37	3
Galactose oxidase-treated arabinogalactan followed by sodium borohydride treatment	37	62

[a] Molecular weight is reported as a weight average.

(*N*-acetyl-D-galactosamine, D-glucose, D-mannose, L-rhamnose, L-fucose, and D-xylose) were all poor inhibitors, similar to L-arabinose. Although 6-*O*-β-D-galactopyranosyl-D-galactose was the most potent saccharide inhibitor (IC_{50} 176 μg/mL), it was a substantially weaker inhibitor than arabinogalactan (IC_{50} ~ 1 μg/mL).

Inhibition of ^{125}I-labeled arabinogalactan binding by enzymatically and chemically modified arabinogalactan. The antigenic features of arabinogalactan were investigated by examining the consequence of modifying arabinogalactan both enzymatically and chemically. This was assessed by assaying the ability of the modified arabinogalactan to inhibit binding between ^{125}I-labeled arabinogalactan and anti-arabinogalactan antiserum. The results are summarized in Table 2. Comparisons of IC_{50} values are made on a weight basis. Modification of arabinogalactan by reduction of its terminal aldehyde or cleavage into its 9 kDa fragment had minimal effect on IC_{50} values compared to arabinogalactan(37 kDa).

Reaction of arabinogalactan(37 kDa) with galactose oxidase results in the oxidation of the C-6 hydroxyl of terminal branches of galactose. This treatment lowered the IC_{50} to 3%. Subsequent borohydride reduction of the galactose oxidase-treated arabinogalactan reduces the C-6 aldehydes back to alcohols. The reduction appears to be nearly complete as the IC_{50} of the galactose oxidase–sodium borohydride-treated arabinogalactan has been substantially restored to that of the untreated arabinogalactan(37 kDa). A control solution of arabinogalactan containing all reactants except galactose oxidase had the same immunoreactivity as arabinogalactan(37 kDa). Periodate oxidation destroyed almost all the antigenic determinants of arabinogalactan (IC_{50} 0.1%).

Reaction of arabinogalactan(9 kDa) with bromoacetic acid introduces about 1 mmol of carboxymethyl group per gram of arabinogalactan. This degree of modification is tolerated by the antibody, as shown by a decrease in the relative IC_{50} of only 44%. Galactan representing the β-(1 → 3)-linked galactose backbone was a weak inhibitor.

Specificity of anti-arabinogalactan antibodies.—Cross reactivity of polysaccharides and glycoproteins with anti-arabinogalactan. The cross reactivity of a number of polysaccharides and glycoproteins with anti-arabinogalactan antiserum was assessed (Table 3). None of the compounds tested showed appreciable inhibition of tracer binding. The specificity of the antiserum was illustrated by the low cross reactivity of gum arabic, a closely related polysaccharide to larch arabinogalactan whose IC_{50} was < 1%.

Table 3
Cross reactivity of polysaccharides and glycoproteins with antiserum to larch arabinogalactan

Compound	Relative cross reactivity (%)
Polysaccharides	
Arabinogalactan(37 kDa)	100
Gum arabic	0.68
Galactomannan	0
Pectin	0.04
Mannan	< 0.003
Dextran	0
HES	0.01
Galactan	0.2
Heparin	0.03
Glycoproteins	
Asialofetuin	< 0.03
Asialomucin	< 0.002
Ovalbumin	< 0.002
Lactosylated albumin	< 0.002

Table 4
Comparison of specificity of galactose-binding proteins [a]

Galactose-binding protein	IC_{50}, μg/mL		Relative specificity IC_{50} AG/IC_{50} ASF
	Arabinogalactan	Asialofetuin	
Rabbit anti-arabinogalactan	2.6	> 10,000	< 0.0003
Hepatocyte asialoglycoprotein receptor	20	6.3	3.2
RCA lectin	223	0.237	940

[a] Standard curves were constructed for the rabbit anti-larch arabinogalactan, rat hepatocyte asialoglycoprotein receptor, and RCA lectin using larch arabinogalactan or asialofetuin as standards. The IC_{50} values were obtained from the standard curves.

Comparison of the specificity of rabbit anti-arabinogalactan antibody with hepatocyte asialoglycoprotein receptor and RCA lectin. The relative effectiveness of arabinogalactan and asialofetuin to block binding of arabinogalactan-[^{125}I]tyramine to either the rabbit anti-arabinogalactan antibody, hepatocyte asialoglycoprotein receptor, or RCA lectin are compared in Table 4. Anti-arabinogalactan antiserum is about 10^4 and 10^6 times more specific for detecting arabinogalactan relative to asialofetuin then hepatocyte asialoglycoprotein receptor and RCA lectin, respectively.

Application of anti-arabinogalactan antibody to measure arabinogalactan in rat plasma..—The correspondence of pharmacokinetic behavior of arabinogalactan following intravenous injection in rats using immunoassay and ^{14}C measurements was demonstrated. ^{14}C-labeled arabinogalactan was injected into rats, and samples of blood were collected at various times and assayed for arabinogalactan concentration by immunoassay and residual levels of radioactivity. The disappearance of radioactivity and immunoreactive arabinogalactan from the blood followed an identical time course and exhibited biphasic kinetics (Fig. 2). The blood half-life of the slower phase of arabinogalactan removal determined by immunoassay and ^{14}C-counting was 26.1 and 27.9 min, respectively.

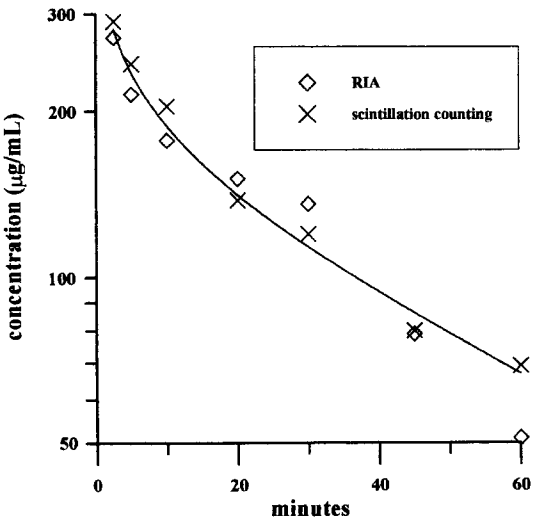

Fig. 2. Clearance of arabinogalactan(9 kDa) from rat plasma as a function of time. The concentration of arabinogalactan (ordinate) was determined at various times (abscissa) either by radioimmunoassay as described under Methods or by radioactive counting using the specific activity of [^{14}C]arabinogalactan. The solid line represents the best fit of the average of the two determinations using a multi-exponential fitting routine.

4. Discussion

Naim and van Oss [18] previously reported that larch arabinogalactan is not immunogenic, and thus consideration of the idea of using antibodies to measure arabinogalactan is likely to be dismissed. We have shown that this impediment may be overcome by conjugating arabinogalactan to albumin which results in a useful and reproducible immune response. To the authors' knowledge there is no simple fast procedure to assay for larch arabinogalactan in complex mixtures such as cell culture media, plant cell homogenates, larch wood extracts, or animal tissue. We have developed such an assay using a specific antiserum for measuring arabinogalactan from the larch tree. Thus this assay represents a significant technical advance, allowing studies of the biosynthesis and degradation of larch arabinogalactan without recourse to radiotracers. Similarly, the pharmacokinetic studies required in animals and humans for the pharmaceutical development of arabinogalactan for targeted drug delivery to hepatocytes [7,9,12] are now possible.

We reconfirm, using more animals and more stringent conditions than Naim and van Oss [18], that larch arabinogalactan is not immunogenic. This result

is important for the further development of arabinogalactan as a pharmaceutical agent.

A remarkable amount of structural information about larch arabinogalactan is found by combining immunoassay and simple chemical treatment of arabinogalactan. This information supports that previously determined using more standard methods of polysaccharide analysis (see ref. [12] and references cited therein), but can be obtained less expensively and with smaller quantities of material. On the basis of model studies with mono-, di-, and tri-saccharides the antiserum is directed to the binding of branch terminal galactose residues in the β-(1 → 6) connection mode. The next monosaccharide down the branch (galactose) probably also plays a role in binding affinity. Comparison of the binding affinities of the disaccharide, 6-*O*-β-D-galactopyranosyl-D-galactopyranose, and arabinogalactan suggests that the antiserum binds multiple sets of branch terminal galactose residues. Studies with galactose oxidase-treated arabinogalactan showed that the C-6 hydroxyl of galactose is important to antibody binding.

Periodate oxidation of the arabinogalactan causes extensive degradation of side-branches containing (1 → 6)-linked galactose residues, terminal galactose, and arabinose residues, but causes no oxidation of the (1 → 3)-linked galactan backbone. The loss of antigenic determinants as indicated by the poor inhibition of periodate-treated arabinogalactan supports the contention that the antigenic determinants of arabinogalactan reside in its side-chains. The molecular weight of the arabinogalactan seems to play little role in binding to antiserum as the 37 and 9 kDa forms of arabinogalactan exhibit similar binding affinities.

This antiserum appears to be highly specific. Little inhibition by a variety of polysaccharides, asialoglycoproteins, and neoglycoproteins is observed. Gum arabic, a polysaccharide closely related to arabinogalactan and a member of the arabinogalactan family, shows less than 1% reactivity compared to arabinogalactan(37 kDa).

The specificity of this antiserum is further illustrated by comparing its relative response to arabinogalactan and asialofetuin with rat hepatocyte asialoglycoprotein receptor and RCA lectin. The asialoglycoprotein receptor and the RCA lectin like anti-arabinogalactan exhibit a specificity for terminal galactose residues similar to that found in both arabinogalactan and asialoproteins such as asialofetuin. Thus it would not be surprising to observe similar specificities for these two classes of ligands among the three binding proteins. As shown in Table 4, however, anti-arabinogalactan antiserum is over 10,000 times more selective towards arabinogalactan than either of the two lectins.

All of these features offer a highly specific antiserum for detecting arabinogalactan in low amounts in animal extracts, as shown by the correspondence of kinetic results obtained using ^{14}C-labeled arabinogalactan. The measured clearance of ^{14}C-labeled arabinogalactan from rat blood determined by immunoassay or radioactive counting was identical.

Gleeson and Clark [21] have previously reported developing an antiserum to the carbohydrate portion of the arabinogalactan–protein from *Gladiolus* style. Their antiserum showed a qualitatively similar specificity with respect to saccharide inhibition compared to anti-larch arabinogalactan. Notable differences were observed, however, in that the antiserum reported by Gleeson and Clark showed equal reactivity to D-galactose and L-arabinose, whereas anti-larch arabinogalactan antiserum showed reactivity only to D-galactose. Another difference was that the arabinogalactan–protein from *Gladiolus* style was intrinsically immunogenic while larch arabinogalactan required conjugation to albumin to become immunogenic.

Acknowledgements

The authors are indebted to Drs. Lee Josephson and Angelo Lamola for helpful discussions and criticism of the manuscript. The authors recognize the technical assistance of Mr. Edward Menz. The authors thank Dr. James H. Prescott for his help with the graphic presentations.

References

[1] E. Schacht, L. Ruys, J. Vermeersch, J.P. Remon, and R. Duncan, *Ann. N.Y. Acad. Sci.*, 446 (1985) 199–212.
[2] L. Molteni, *Methods Enzymol.*, 112 (1985) 285–298.
[3] W.R. Grombotz and D.K. Pettit, *Bioconjugate Chem.*, 6 (1995) 332–351.
[4] L. Strebel and P.E. Siegler, *Arch. Surg.*, 96 (1968) 471–475.
[5] C. Larsen, *Adv. Drug Deliv. Rev.*, 3 (1989) 103–154.
[6] M. Hashida, R. Atsumi, K. Nishida, S. Nakane, Y. Takakura, and H. Sezaki, *J. Pharmacobio-Dyn.*, 13 (1990) 441–447
[7] L. Josephson, E.V. Groman, E. Menz, J.M. Lewis, and H. Bengele, *Magn. Reson. Imaging*, 8 (1990) 637–646.

[8] B. Gallez, V. Lacour, R. Demeure, R. Debuyst, F. Dejehet, J.-L. De Keyser, and P. Dumont, *Magn. Reson. Imaging*, 12 (1994) 61–69.
[9] P.M. Enriquez, C. Jung, L. Josephson, and B.C. Tennant, *Bioconjugate Chem.*, 6 (1995) 195–202
[10] G. Lystad-Borgin, *J. Am. Chem. Soc.*, 71 (1949) 2247–2248.
[11] H.O. Bouveng, *Acta Chem. Scand.*, 13 (1959) 1877–1883.
[12] E.V. Groman, P.M. Enriquez, C. Jung, L. Josephson, *Bioconjugate Chem.*, 5 (1994) 547–556.
[13] R.T Lee, In G.Y. Wu and C.H. Wu (Eds), *Liver Diseases*, Marcel Dekker, New York, 1991, pp 65–86.
[14] J.H. Prescott, P. Enriquez, C. Jung, E., Menz, and E.V. Groman, *Carbohydr. Res.*, 278 (1995) 113–128.
[15] M. Monsigny, A.-C. Roche, P. Midoux, and R. Mayer, *Adv. Drug Deliv. Rev.*, 14 (1994) 1–24.
[16] G. Molema and D.K F. Meijer, *Adv. Drug Deliv. Rev.*, 14 (1994) 25–50.
[17] M.T. Cerenzia, L. Fiume, W. Vernon, V. Lavezzo, M.R. Brunetto, A. Ponetto, G. Di Stefano, C. Busi, A. Mattioli, G.B. Gervasi, F. Bonino, and G. Verme, *Hepatology*, 23 (1996) 657–661.
[18] J.O. Naim and C.J. van Oss, *Immunol. Invest.*, 2 (1992) 649–662.
[19] D. Rodbard, V.B. Faden, S. Knisley, and D.M. Hunt, *National Technical Information Service PB24622*, (1975) 1–70.
[20] R.L.E. Hudgin, J. Pricer, G. Ashwell, R.L. Stockert, and A.G. Morell, *J. Biol. Chem.*, 249 (1974) 5536–5543.
[21] P.A. Gleeson and A.E Clarke, *Biochem. J.*, 191 (1980) 437–447.