Biochimica et Biophysica Acta, 658 (1981) 377—386

BBA 69245

SUBSTRATE SPECIFICITY OF α-L-ARABINOFURANOSIDASE FROM PLANT *SCOPOLIA JAPONICA* CALLUSES AND A SUGGESTION WITH REFERENCE TO THE STRUCTURE OF BEET ARABAN

MICHIO TANAKA, ATSUKO ABE and TSUNEKO UCHIDA

Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194 (Japan)

(Received August 4th, 1980)
(Revised manuscript received December 31st, 1980)

Key words α-L-*Arabinofuranosidase*, *Arabino-disaccharide*, *Araban*, *Substrate specificity*

Summary

Substrate specificity of α-L-arabinofuranosidase from plant *Scopolia japonica* was examined using three kinds of arabinodisaccharides prepared from natural sources or synthetically. This enzyme hydrolyzed arabinofuranosyl-arabinoses which had either an α-(1 → 3) or a α-(1 → 5) linkage, but hydrolyzed arabinopyranosyl-arabinose having a α-(1 → 5) linkage to a lesser degree. α-L-Arabinofuranosidase (α-L-arabinofuranoside arabinofuranohydrolase, EC 3 2.1.55), which was shown by us to be an exo-enzyme, degraded beet araban incompletely Arabinose oligomers and galactose-containing fragments, isolated following acid hydrolysis of araban, were both incompletely degraded by the enzyme The reasons for the incomplete degradation were explained by the novel finding of (1 → 2) linkages and arabinopyranosides and the inclusion of trace amounts of galactose into the carbohydrate chain of araban. This enzyme was practically non-reacting with the hydroxyprolyl-arabinose linkage of glycopeptides from plant cell walls.

Introduction

The enzyme α-L-arabinofuranosidase (α-L-arabinofuranoside arabinofuranohydrolase, EC 3.2.1.55) occurs in the plant *Scopolia japonica*. The purification of the enzyme and some of its properties have been previously described [1]. α-L-Arabinofuranosidase could be useful in studies of the structure of arabinose-containing biopolymers, such as polysaccharides and glycoproteins

Abbreviations $Ara_f(1 \rightarrow 3)Ara$, α-arabinofuranosyl-(1 → 3)-arabinose, $Ara_f(1 \rightarrow 5)Ara$, α-arabinofuranosyl-(1 → 5)-arabinose, $Ara_p(1 \rightarrow 5)Ara$, α-arabinopyranosyl-(1 → 5)-arabinose

in plant cell walls [2—7], provided its substrate specificity were known The present paper defines the specificity of the enzyme for arabinoside linkages and its activity against beet araban The incomplete degradation of beet araban is explained by our findings of (1 → 2) linkages, galactose residues within its structure, and the occurrence of arabinopyranosides

Materials and Methods

Materials

Beet pulp chips were the generous gift of Nippon Tensai Seitoh Co., Japan. α-L-Arabinofuranosidase and cell wall samples were prepared from calluses of *S japonica* according to Tanaka and Uchida [1]. α-Galactosidase from green coffee beans was purchased from Sigma Chemicals Co , U S.A. and β-galactosidase from *Charonia lampas* from Seikagaku Kogyo Co , Japan. L-Arabinose, triphenylmethyl chloride, titanium tetrachloride and other chemicals for the synthesis of arabinopyranosyl-arabinose were purchased from Wako Chemicals Co , Japan

Preparation of beet araban. Beet araban was prepared from beet pulp chips according to Jones and Tanaka [8].

Preparation of α-arabinofuranosyl-(1 → 3)-arabinose The beet araban (10 g) was acetylated with a mixture of acetic anhydride (100 ml) and pyridine (100 ml) at 25°C for 24 h. The reaction mixture was then poured into ice-cold water and the acetylated products extracted with 1 l of ethyl acetate. After removal of the solvent by evaporation, the acetylated araban was partially hydrolyzed in 200 ml of 0.1 M HCl at 80°C for 3 h. The hydrolyzate was dried in vacuo and extracted with water (100 ml). The extract was concentrated and applied to a column (1.6 × 135 cm) of Sephadex G-10 (Fig. 1). Fraction II containing relatively small oligosaccharides was applied to a column (3.0 × 39 cm) of charcoal-celite (2 · 1, w/w) and eluted stepwise with increasing concentrations of ethanol. 0, 2.5, 5.0, 7.5, 10 and 15% (Fig. 2) A major fraction A-5 of the two fractions eluted with 5% ethanol was $Ara_f(1 \rightarrow 3)Ara$ as identified by paper chromatography according to Smith and Stephen [10]. The disaccharide was further purified by paper chromatography, using Whatman 3MM paper and a solvent of *n*-butanol/pyridine/water (9 2 · 2) with development for 43 h. The disaccharide was extracted with water from the paper, and dissolved and precipitated from hot 90% ethanol solution

Preparation of α-arabinofuranosyl-(1 → 5)-arabinose The beet araban (35 g) was partially hydrolyzed in 400 ml 5 mM H_2SO_4 at 95°C for 6 h. After neutralization with sodium hydroxide, 2 vol methanol were added to the hydrolyzate, followed by centrifugation to obtain the supernatant The latter was applied to a column (2.8 × 27 cm) of cellulose-celite (4 · 1, w/w), previously equilibrated with *n*-butanol which was saturated with water. The first fraction eluted with the same solvent was concentrated, applied to a column (3.2 × 27 cm) of charcoal-celite (2 . 1, w/w), and eluted stepwise with increasing concentrations of ethanol· 0, 5 and 10%. A major fraction B-5 eluted with 5% ethanol was identified as $Ara_f(1 \rightarrow 5)Ara$ and purified in the same manner as $Ara_f(1 \rightarrow 3)Ara$ The yield of $Ara_f(1 \rightarrow 5)Ara$ was 22.0 mg.

Chemical synthesis of α-arabinopyranosyl-(1 → 5)-arabinose Chemical reac-

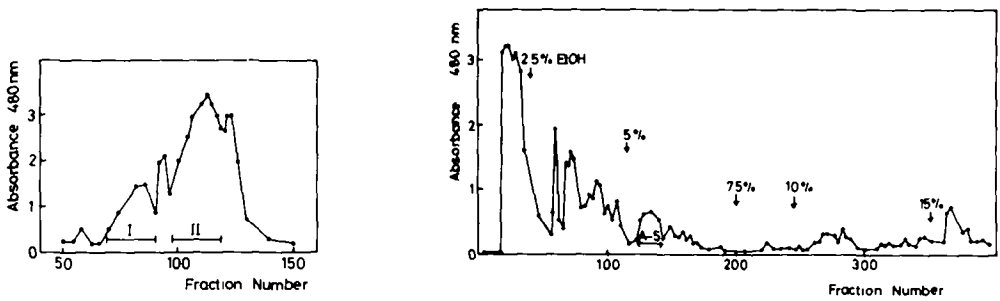


Fig 1 Gel filtration of hydrolyzate of acetylated araban on Sephadex G-10 Acetylated beet araban was partially hydrolyzed in 0 1 M HCl at 80°C for 3 h. Then the hydrolyzate was applied to a column of Sephadex G-10 Total carbohydrates in each fraction eluted with distilled water were determined at 480 nm by the method of Hodge et al [9]

Fig 2 Isolation of $Ara_f(1 \rightarrow 3)$Ara by a column chromatography on charcoal-celite The fraction II in the gel filtration of the hydrolyzate of acetylated araban (Fig 1) was applied to a column of charcoal-celite (2 1, w/w) Eluted carbohydrates were determined at 480 nm

tions were carried out at 25°C unless otherwise specified.

1-Chloro-2,3,4-tri-O-acetyl-arabinopyranoside (I) L-Arabinose (20 g) was acetylated with a mixture of acetic anhydride and pyridine under overnight stirring, followed by chlorination with $TiCl_4$ by the method of Bonner [11]. The yield of compound I was 2 1 g.

1,2,3-Tri-O-acetyl-arabinofuranoside (II). The 5-hydroxyl group of L-arabinose (10 g) was protected with triphenylmethyl chloride (23 g) in pyridine (80 ml), followed by acetylation of the other hydroxyl groups with acetic anhydride (24 ml) [12]. The triphenylmethyl group was then removed by treatment with 80% acetic acid at 100°C for 20 min. The product obtained after this treatment was then processed by the method of Reynolds and Evans [12]. The yield of compound II was 8.7 g.

α-Arabinopyranosyl-(1 → 5)-arabinose 1-Chloro-2,3,4-tri-*O*-acetyl-arabinopyranoside (I) (1 2 g) and 1,2,3-tri-*O*-acetyl-arabinofuranoside (II) (1.7 g) were coupled in the presence of silver oxide (5.6 g) and iodine (1.5 g) in benzene (30 ml) [12]. All the protecting groups of arabinodisaccharide were removed by treatment with 0.03 M barium methoxide in anhydrous methanol (18 ml) at 4°C for 16 h. The product was obtained after a treatment with Dowex 50-X8 (H^+) resin and precipitation from a 90% ethanol solution. The properties of this synthesized compound were compared with those of $Ara_p(1 \rightarrow 5)$Ara isolated from *Virgilia oroboides* gum by Smith and Stephen [10]. The yield of $Ara_p(1 \rightarrow 5)$Ara was 755 mg.

Preparation of araban fragments containing galactose Fraction (I) of the hydrolyzate of the acetylated beet araban from the Sephadex G-10 column (Fig. 1), was rechromatographed on the same column. The main fraction contained 41.4% galactose and 58.6% arabinose. The degree of polymerization was 23.4 as determined by the method of Tanaka [13].

Preparation of arabinose oligomer containing no galactose The araban fragments containing galactose were applied to a column (2.0 × 32 cm) of DEAE-cellulose (DE-52, OH^- form), equilibrated with water. The excluded fraction was subsequently applied to a column (1.2 × 100 cm) of Bio-gel P-4. The main

fraction eluted with water contained no galactose. The degree of polymerization was 12.1.

Preparation of arabino-oligosaccharides and glycopeptide containing arabinose from plant calluses The α-cellulose fraction containing glycopeptides was prepared from an alkaline hydrolyzate of cell walls of *S japonica* calluses by the method of Lamport [2,3]. The α-cellulose fraction (377 mg) was applied to a column (3.6 × 130 cm) of Sephadex G-25 and eluted with 0.1 M acetic acid (Fig 3). The fraction M (72 mg) was applied to a column (0.65 × 93 cm) of cation-exchange resin, Bio-Rad AG 50w X4 (H^+) and eluted with 3 M acetic acid/formic acid, pH 1.9, followed by elution with 1 M pyridine/acetic acid, pH 5.5. The main fraction eluted at pH 1.9 was named AOS (arabino-oligosaccharides) and the other fraction eluted at pH 5.5 was AGP (arabino-glycopeptide).

By further gel filtration on Sephadex G-25 eluted with water, AOS was separated into two fractions, AOS-1 and AOS-2. AGP was also purified with Sephadex G-25. By the gel filtration, the molecular weights of AOS-1, AOS-2 and AGP were estimated to be 2500, 1500 and 3000, respectively.

Methods

Determination of the degree of polymerization of oligosaccharides The method used was that described by Tanaka [13] and in brief involves the determination of alditols derived from reducing-end carbohydrate residues.

Specific optical rotation The specific optical rotation of arabino-disaccharides was measured at 25°C using the Automatic Digital Polarimeter PM-201, Union Giken Co., Japan.

Determination of monosaccharides To 150 μl of a solution containing about 200 μg monosaccharide, 50 μl of a standard mixture consisting of inositol (50 μg) and 2-deoxy-glucose (25 μg) were added. This mixture was reduced at room temperature for an hour after addition of 50 μl of a 5% $NaBH_4$ solution. Acetic acid (80 μl of a 2 M solution) was added to decompose the excess of the reductant The reduced sample was applied to a column (0.5 × 1.5 cm) of Dowex 50-X8 (H^+) and eluted with about 2 ml water. The eluate was evapo-

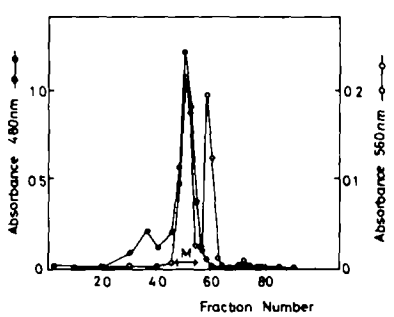


Fig 3 Gel filtration of α-cellulose fraction on Sephadex G-25. The so-called α-cellulose fraction from *S japonica* cell walls was applied to a column of Sephadex G-25 and eluted with 0 1 M acetic acid Total carbohydrates were determined at 480 nm Hydroxyproline as a marker in purification of the glycopeptide was determined at 560 nm by the method of Bondjers et al [14] •———•, *A* 480 nm, ○———○, *A* 560 nm

rated to dryness, followed by addition and evaporation of methanol (five-times) to remove the boric acid. The residue was acetylated with acetic anhydride (15 μl) plus pyridine (15 μl) at 100°C for 30 min, followed by GLC, using a Shimazu Gaschromatograph GC-4CM and a column (0.3 × 200 cm) of 3% ECNSS-M on Gaschrom Q (100—120 mesh) at 190°C [15].

Amino acid composition. Amino acid composition of the glycopeptide AGP from plant cell walls was determined after hydrolysis in 6 M HCl at 110°C for 16 h, using the Amino Acid Autoanalyzer JCL-6AH, JEOL, Japan.

Methylation analysis Arabino-disaccharides and the arabinose oligomer containing no galactose were methylated by the method of Hakomori [16]. The methylated oligosaccharides were methanolysed with 1 M HCl in anhydrous methanol at 80°C for 8 h, followed by neutralization with silver oxide. The products were applied to a gas chromatography column (0.3 × 200 cm) of 15% BDS on celite (80—100 mesh) at 175°C Each partially methylated arabinose was tentatively identified in comparison with the retention time of the following synthetic standards fully methylated glucose and galactose, and a mixture of fully and partially methylated arabinose, prepared according to Aspinall [17]

Enzymic action of α-L-arabinofuranosidase on arabinodisaccharides and glycopeptide from plant cell walls AOS-1 (200 μg), AOS-2 (200 μg) or AGP (1.0 mg) were separately incubated in the presence of α-L-arabinofuranosidase (3.3 mU) at pH 4.8 [1] and 37°C for 24 h. Each sample was applied to a column (0.5 × 3 cm) of Dowex 50-X8 (H^+) and the excluded fraction further applied to a column (0.5 × 3 cm) of Dowex 1-X8 (HCO_3^-). Free arabinose in this excluded fraction was determined as described above.

Sterilization procedure of incubation mixtures. In those cases where the enzyme treatment was for a long time period, the incubation mixtures were sterilized either by filtration with a membrane filter (pore size, 0.22 μm, Millipore), or by autoclaving. A drop of toluene was added to the mixture and the container sealed with a cap under sterile conditions.

Results

The properties of arabino-disaccharides. Three kinds of arabino-disaccharides, $Ara_f(1 \rightarrow 3)Ara$, $Ara_f(1 \rightarrow 5)Ara$ and $Ara_p(1 \rightarrow 5)Ara$, were prepared as described in Materials. The properties of these materials are summarized in Table I The optical rotation and the relative mobility of each material were close to the values reported by Smith and Stephen [10] Each degree of polymerization was approx 2. The methylation analysis confirmed the disaccharide structures $Ara_f(1 \rightarrow 3)Ara$, $Ara_f(1 \rightarrow 5)Ara$ or $Ara_p(1 \rightarrow 5)Ara$ as shown in Table I.

Enzymic action of α-L-arabinofuranosidase on the arabino-disaccharides The time course of the enzymic action on the three kinds of arabino-disaccharides is shown in Fig. 4. The relative activity at 16 h digestion was 0.592 for $Ara_f(1 \rightarrow 5)Ara$ and 0 014 for $Ara_p(1 \rightarrow 5)Ara$, assuming that the activity for $Ara_f(1 \rightarrow 3)Ara$ was 1.00. This result shows that $Ara_p(1 \rightarrow 5)Ara$ was slightly hydrolyzed by the enzyme, while $Ara_f(1 \rightarrow 3)Ara$ and $Ara_f(1 \rightarrow 5)Ara$ were good substrates.

From the Lineweaver-Burk plot of the enzymic action on both arabino-

TABLE I

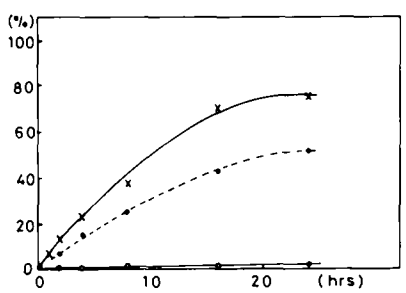
PROPERTIES OF ARABINO-DISACCHARIDES

R_{gal} Relative mobility of arabino-disaccharide to galactose on paper-chromatogram with solvent (A), *n*-butanol/pyridine/H_2O (9 2 2), or (B), ethyl acetate/acetic acid/formic acid/H_2O (18 3 1 4) $\overline{Dp}$ Average degree of polymerization

	$Ara_f(1 \rightarrow 3)Ara$	$Ara_f(1 \rightarrow 5)Ara$	$Ara_p(1 \rightarrow 5)Ara$
[α]D	−13 9	−98 4	ca 0
R_{gal} (A)	1 58	2 30	1 02
	1 16	1 22	0 94
$\overline{Dp}$	2 05	2 19	1 95
Methylation analysis	1,2,3,5-*O*-Me-Ara (1 000)	1,2,3,5-*O*-Me-Ara (1 000)	1,2,3,4-*O*-Me-Ara (1 000)
(Molar ratio)	1,2,5-*O*-Me-Ara (0 749)	1,2,3-*O*-Me-Ara (0 950)	1,2,3-*O*-Me-Ara (0 802)
	1,2,4-*O*-Me-Ara (0 279)		

furanosyl-arabinoses, the Michaelis constant (K_m) and maximum velocity (V) were determined to be 9.22 mM and 22.2 mU/mg protein for $Ara_f(1 \rightarrow 3)Ara$, and 8.06 mM and 13 0 mU/mg protein for $Ara_f(1 \rightarrow 5)Ara$, respectively. The enzyme had a slight preference for $Ara_f(1 \rightarrow 3)Ara$, although the values of K_m for the two arabinofuranosides were very similar

The mode of action of α-L-*arabinofuranosidase on araban* Beet araban partially digested by α-L-arabinofuranosidase for different time periods was applied to a column of Bio-gel P-10 (Fig. 5) Only the arabinose monomer appeared in fractions retarded with Bio-gel P-10. No oligomers smaller than 100 degrees of polymerization could be observed at any incubation time. Therefore,



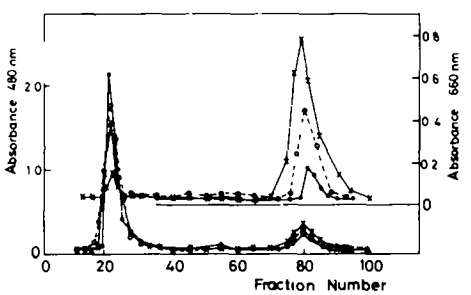


Fig 4 Time course of enzymic action of α-L-arabinofuranosidase on the arabino-disaccharides 50 μl of 18 mM $Ara_f(1 \rightarrow 3)Ara$ (X———X), $Ara_f(1 \rightarrow 5)Ara$ (● - - - - - - ●) or $Ara_p(1 \rightarrow 5)Ara$ (○———○) were incubated with 50 μl of α-L-arabinofuranosidase solution (6 03 mU) and 50 μl of 0 15 M sodium citrate/phosphate buffer, pH 4 8 at 37°C for different periods The released arabinose was determined as described in Methods

Fig 5 Enzymic action of α-L-arabinofuranosidase on beet araban 60 mg beet araban were incubated with α-L-arabinofuranosidase (43 8 mU) at pH 4 8 and 37°C After sampling at 4th (●———●), 7th (○ - - - - - - ○) and 14th (X———X) day, each sample was applied to a column (1 8 X 130 cm) of Bio-gel P-10 and eluted with water Total carbohydrate (upper left to right down) in each fraction was determined at 480 nm The reducing sugar (upper right) was determined at 660 nm by the method of Park and Johnson [18]

it was concluded that this enzyme hydrolyzed exo-enzymically the natural substrate, beet araban.

α-L-Arabinofuranosidase released only 17.3% arabinose from the beet araban after 21 days incubation. The beet araban was not completely hydrolyzed by the enzyme under any condition.

Enzymic action of α-L-arabinofuranosidase on the araban fragments in the presence of α- and β-galactosidase As described above, the beet araban was incompletely hydrolyzed by excess amounts of α-L-arabinofuranosidase even after long periods of incubation. Addition of active enzyme after extended incubation did not result in further degradation. Therefore, other reasons than enzyme inactivation were considered. The beet araban contained 4.3% galactose and trace amounts of other monosaccharides. The possibility was considered that enzymatic degradation would proceed at the non-reducing end of the beet araban until a galactose residue was exposed. At this point no hydrolysis of arabinoside linkages would occur, since α-L-arabinofuranosidase is an exo-enzyme. This possibility was proven as follows.

Araban fragments containing galactose were obtained from the hydrolyzate of acetylated araban as described in Materials. The fragments were digested by α-L-arabinofuranosidase in the presence of both α- and β-galactosidase, followed by application to a column of Bio-gel P-4 (Fig. 6). After 12 days of digestion by α-L-arabinofuranosidase without galactosidase (Fig. 6A), only 5.9% of the arabinose was released. In the presence of both galactosidases (Fig. 6B), 28.2% of the arabinose and 24.3% of the available galactose were released, though the araban fragments were not completely hydrolyzed The result indicates that trace amounts of galactose are included in the araban chain.

Enzymic action of α-L-arabinofuranosidase on arabinose oligomer derived from beet araban. Arabinose oligomer containing no galactose was prepared from beet araban as described in Materials. The oligomer was not completely hydrolyzed by α-L-arabinofuranosidase even after long incubation periods as

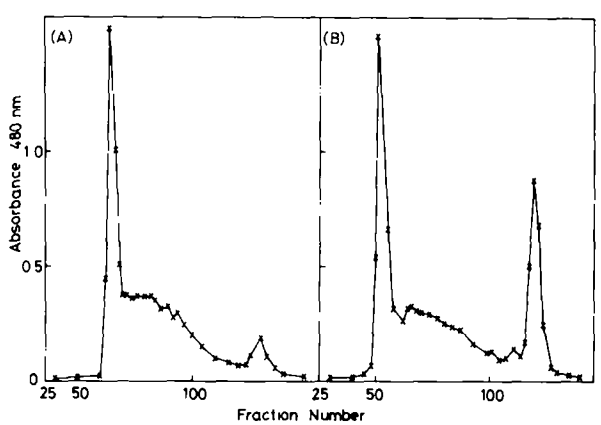


Fig 6 Enzymic action of α-L-arabinofuranosidase on the araban fragments in co-presence of galactosidases The araban fragments containing galactose (4 95 mg) were incubated in the presence of α-L-arabinofuranosidase (3 03 mU), (A), without or (B), with both α-galactosidase (25 mU) and β-galactosidase (100 mU) at pH 4 8 for 12 days The incubated mixture was applied to a column (1 2 × 100 cm) of Bio-gel P-4 and eluted with 0 02 M acetate buffer, pH 5 6. Total carbohydrates were determined at 480 nm

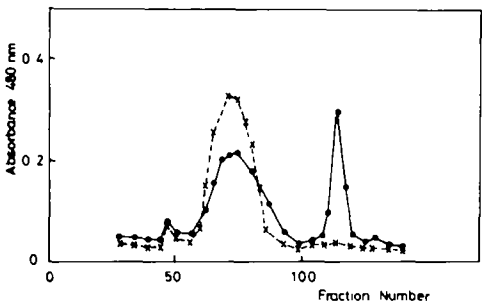


Fig 7 Enzymic action of α-L-arabinofuranosidase on the arabinose oligomer The arabinose oligomer containing no galactose (260 μg) was incubated in the presence of α-L-arabinofuranosidase (1 46 mU) at pH 4 8. After 14 days, the incubation mixture was applied to the column of Bio-gel P-4 The elution pattern (●———●) of total carbohydrates from the column was compared with that (X———X) of a control mixture at zero-time

shown in Fig. 7 In several experiments, only up to 29.3% of the arabinose were released from the oligomer

Methylation analysis of the arabinose oligomer Partially methylated arabinoses were isolated from a methylated product of the arabinose oligomer containing no galactose (Table II) The isolation of 1,3,5-tri-*O*-methyl-arabinose and 1,3-di-*O*-methyl-arabinose indicates the presence of arabino-(1 → 2) linkages in the beet araban, and 1,2,4-tri-*O*-methyl-arabinose indicates the presence of arabinopyranosides This is the first evidence for the presence of (1 → 2) linkages and arabinopyranoside in beet araban, which may explain the incomplete digestion by α-L-arabinofuranosidase

Enzymic action of α-L-arabinofuranosidase on the arabino-oligosaccharides and the glycopeptide from plant cell walls The arabino-oligosaccharides and the glycopeptide containing arabinose were prepared from the cell walls of *S japonica* calluses as described in Materials.

AOS-1 and AOS-2 were regarded as arabino-oligosaccharides contaminated with a trace amount of hydroxyproline (Table III) The degree of polymerization of AOS-1 and AOS-2 was presumed to be 17 or 10, respectively, from the elution pattern on Sephadex G-25 column On the other hand, AGP was regarded as a glycopeptide with a molecular weight of about 3000 Thus, this glycopeptide was presumed to contain an average of 0.5 hydroxyprolyl-arabinose linkages per single molecule.

TABLE II

METHYLATION ANALYSIS OF ARABINOSE OLIGOMER DERIVED FROM BEET ARABAN

	Molar ratio
1,2,3,5-*O*-Me-Ara	4 73
1,2,3-*O*-Me-Ara	2 81
1,2,5-*O*-Me-Ara	2 73
1,2,4-*O*-Me-Ara	1 39
1,3,5-*O*-Me-Ara	1 00
1,3-*O*-Me-Ara	1 16
1,2-*O*-Me-Ara	n d

n d Not detected

TABLE III

RELATIVE COMPOSITION OF ARABINO-OLIGOSACCHARIDES AND GLYCOPEPTIDE FROM *SCOPOLIA JAPONICA* CELL WALLS

Relative composition is expressed by molar ratio

	AOS-1	AOS-2	AGP
Arabinose	64 93	464 9	0 122
Hyp	1 00	1 00	1 00
Asp	—	—	0 346
Thr	—	—	0 142
Ser	—	—	0 099
Glu	—	—	0 450
Pro	—	—	0 471
Ala	—	—	0 773
Cys	—	—	—
Val	—	—	0 668
Met	—	—	0 226
Ile	—	—	0 354
Leu	—	—	1 007
Phe	—	—	0 075
Lys	—	—	0 456
His	—	—	0 044

When these oligomers containing arabinose were used as substrates, α-L-arabinofuranosidase released 36.9, 41 7 and 5.9% of arabinose from AOS-1, AOS-2 and AGP, respectively. The two arabino-oligosaccharides were relatively good substrates, while a trace amount of arabinose was released from AGP It appears unlikely that the hydroxyprolyl-arabinose linkage of glycopeptide from plant cell walls could be split by this arabinofuranosidase

Discussion

It has been reported that araban of *Pinus pinaster* was composed of long linear chains of arabinose in (1 → 5) linkages with numerous arabinose monomers attached through (1 → 3) linkages [19]. Beet araban was also presumed to have a similar structure [20]. Arabinofuranosidase was considered to have a preference for (1 → 3) linkages attached to an arabinose residue of the main chain [20]. The preference of the enzyme for (1 → 3) linkages was suspected to account for the incomplete degradation of araban. However, our results indicated a very small difference in the rates of the enzymic hydrolysis of (1 → 3) linkages compared with those of (1 → 5) linkages. Therefore, other reasons for the incomplete degradation of araban by α-L-arabinofuranosidase must be considered.

In methylation analysis, the recovery of partially methylated arabinoses derived from branching points should be close to that of the fully methylated arabinose from the non-reducing ends. However, the recovery of 1,3-di-*O*-methyl-arabinose was considerably less than 1,2,3,5-tetra-*O*-methyl-arabinose. Therefore, the presence of other branching structures in the arabinose oligomer must be supposed. The methylation analysis also indicated that (1 → 2) linkages and arabinopyranosides were present in the pure arabinose oligomer of beet

araban. Degradation experiments in the presence of α- and β-galactosidase also indicated that galactose was in the araban structure Those latter findings may explain the incomplete degradation of beet araban by α-L-arabinofuranosidase. Thus, it is suggested that beet araban has a more complex structure than that of araban from *P pinaster* reported by Aspinall [17], though the whole structure of beet araban can not as yet be presented.

Smith and Stephen have reported that $Ara_f(1 \rightarrow 3)Ara$, $Ara_f(1 \rightarrow 5)Ara$ and $Ara_p(1 \rightarrow 5)Ara$ were obtained following hydrolysis of beet araban with 5 mM H_2SO_4 at 95°C for 6 h [10]. We were unable to recover these disaccharides except $Ara_f(1 \rightarrow 5)Ara$. However, by changing the hydrolysis condition to 0.1 M HCl at 80°C for 3 h, $Ara_f(1 \rightarrow 3)Ara$ was obtained from acetylated araban. We are successful in synthesizing $Ara_p(1 \rightarrow 5)Ara$. Synthesis of this disaccharide has not been reported previously. Despite extensive efforts, arabinofuranosyl-disaccharides could not be synthesized.

Glycopeptide AGP from *S japonica* contained arabinose covalently attached to hydroxyproline as suggested by Lamport [2,3]. This hydroxyprolyl-arabinose linkage was first expected to be split by α-L-arabinofuranosidase. However, only a small amount of arabinose was released from AGP, whereas the arabino-oligosaccharides from the same source served as a good substrate. Therefore, it was unlikely that α-L-arabinofuranosidase has a specificity for the hydroxyprolyl-arabinose linkage.

References

1 Tanaka, M and Uchida, T (1978) Biochim Biophys Acta 522, 531—540
2 Lamport, D T A (1967) Nature 216, 1322—1324
3 Lamport, D T A (1969) Biochemistry 8, 1155—1163
4 Heath, M F and Northcote, D H (1971) Biochem J 125, 953—961
5 Talmadge, K W , Keegstra, K , Bauer, W.D and Albersheim, P (1973) Plant Physiol 51, 158—173
6 Gardiner, M and Chrispeels, M J (1975) Plant Physiol 55, 536—541
7 Tanaka, M and Uchida, T (1979) Plant Cell Physiol 20, 1295—1306
8 Jones, J K N. and Tanaka, Y (1965) in Methods in Carbohydrate Chemistry (Whistler, R L , ed), 5, pp 74—75, Academic Press, New York
9 Hodge, J E and Hofreiter, B T (1962) in Methods in Carbohydrate Chemistry (Whistler, R L and Wolfrom, M L , eds), Vol 1, pp 388—389, Academic Press, New York
10 Smith, F and Stephen, A M (1961) J Chem Soc 4893—4903
11 Bonner, W A (1958) J Am Chem. Soc 80, 3372—3379
12 Reynolds, D D and Evans, W L (1938) J Am Chem Soc 60, 2559—2561
13 Tanaka, M (1980) Carbohydr Res 87, in the press
14 Bondjers, G and Bjorkerud, S. (1973) Anal Biochem 52, 496—504
15 Sawardeker, J S , Sloneker, J H and Jeanes, A R (1965) Anal Chem 37, 1602—1604
16 Hakomori, S (1964) J Biochem. 55, 205—208
17 Aspinall, G O (1963) J Chem Soc 1676—1680
18 Park , J T and Johnson, M J (1949) J Biol Chem 181, 149—151
19 Aspinall, G O (1970) in The Carbohydrates, Chemistry and Biochemistry (Pigman, W and Horton, D , eds), 2nd edn , Vol IIB, pp 515—536, Academic Press, New York
20 Dekker, R F H and Richards, G N (1976) in Advances in Carbohydrate Chemistry and Biochemistry (Tipson, R S and Horton, D , eds), Vol 32, pp 277—352, Academic Press, New York