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Polysaccharide components from the scape of *Musa paradisiaca*: main structural features of water-soluble polysaccharide component

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Polysaccharide components present in the pseudo-stem (scape) of *M. paradisiaca* were purified from acetone powder of the scape by delignification followed by extraction with aqueous solvents into water soluble polysaccharide (WSP), EDTA-soluble polysaccharide (EDTA-SP), alkali-soluble polysaccharide (ASP) and alkali-insoluble polysaccharide (AISP) fractions. Sugar compositional analysis showed that WSP and EDTA-SP contained only D-Glc whereas ASP contained D-Glc, L-Ara and D-Xyl in ~1:1:10 ratio, respectively, and AISP 'contained D-Glc, L-Ara and D-Xyl in ~10:1:2 ratio, respectively. WSP was further purified by complexation with iso-amylalcohol and characterized by specific rotation, IR spectroscopy, Iodine affinity, ferricyanide number, blue value, hydrolysis with α-amylase and glucoamylase, and methylation linkage analysis, and shown to be a amylopectin type α-D-glucan.

***Keywords*: polysaccharide components, pseudo-stem (scape), *Musa paradisiaca*, carbohydrates, structure**

Introduction

In continuation of our studies on structure-function relationship of plant polysaccharides [1], structural investigations on the nature of polysaccharide components present in the scape of *M. paradisiaca* was undertaken. The exotic variety of *M. paradisiaca* (called rasabale in local language) is cultivated in southern parts of India. The stalk of the rhizome (scape) of *M. paradisiaca*, available after harvesting the bananas, is used as vegetable. The prevalent belief is that consumption of the succulent scape removes toxic materials from the alimentary canal, probably because of its high fibre content. The presence of easily extractable starch in the scape, and its similarity to amylose content of potato starch has been reported [2]. In order to obtain further insights on the branching pattern of amyloid polysaccharides and also to extend our studies on plant derived polysaccharides of medicinal importance [3], the polysaccharides present in the scape of mature *M. paradisiaca* was studied. This paper describes the purification of polysaccharide components present in the scape of *M. paradisiaca* and the structural studies on water-soluble polysaccharide fraction.

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Materials and methods

General

Fresh samples of the scape of *M. paradisiaca* were collected from the locally available plants (Manasagangotri campus, Mysore, India).

All evaporations were performed under reduced pressure at below 40 °C. Dialysis was carried out against three changes of glass distilled water with continuous stirring at 4 °C. Ultrafiltration was carried out at 4 °C using an Amicon series 8000 10 ml stirred cell with a membrane with a mol wt cut off of 500. All chemicals used were of analytical grade.

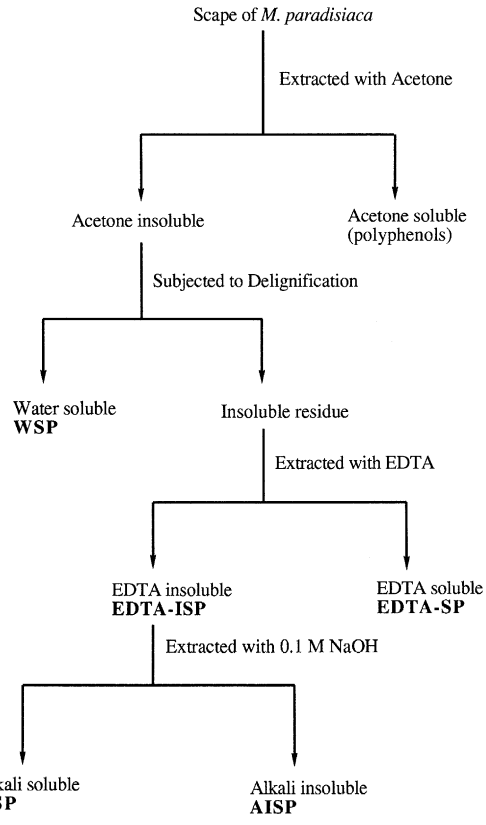
Analytical procedures

Quantitative colorimetric methods used were the phenol-sulfuric acid [13] method for neutral glycoses, the carbazole reagent assay [14] for hexuronic acids, the method of Chen *et al.* for phosphate [15], the method of Lowry *et al.* for protein [16], the method of Hestrin for *O*-acetyl [17] determination. The sulfate [18], silica [19], and ash [20] contents were determined by using the procedures as previously reported. Potassium [21] was estimated in a digital flame photometer using a calibration graph prepared using standard potassium solution.

The electron micrography [22] of the gold coated finely ground polysaccharide was taken with a scanning electron

microscope S450 Hitachi, Japan. The diffraction [23] patterns were recorded using a JEOL (JD × 8p) X-ray diffractometer. The Iodine binding [24] capacity of the polysaccharides was determined by potentiometric titration. The blue values [25], of the polysaccharide in sodium hydroxide solution (10%, 5 ml) were determined using a Beckmann DB spectrophotometer at 680 mm. Reducing values [26] of the polysaccharide samples was determined by the ferricyanide method.

Analytical and preparative p.c. was performed by the descending mode on Whatman Nos. 1 and 3 MM papers, respectively, using (A), 1-butanol : benzene : pyridine : water (4 : 1 : 3 : 3, upper layer); (B) ethyl acetate : pyridine : water (8 : 2 : 5); (C) 1-butanol : pyridine : water (6 : 4 : 3); (D) 1-butanol : ethanol : water (4 : 1 : 5, upper layer); (E) 1-butanol : acetic acid : water (4 : 1 : 5, upper layer); (F) ethyl acetate : pyridine : acetic acid : water (5 : 5 : 1 : 3); (G) 1-butanol : acetic acid : pyridine : water (4 : 1 : 3 : 3). Solvents A–D were used for neutral sugars, and solvents E–G for acidic sugars. Sugars were detected with p-anisidine hydrochloride [27] or alkaline silver nitrate [28]. Gas-liquid chromatography (GLC) was performed as described previously [3]. GLC-MS analyses were carried out on a VG Micromass 16F instrument with electron impact ionization at 70 eV, equipped with a Pye Unicam chromatograph series 204, and using capillary columns as reported [3]. Compositional analysis of the polysacchrides was carried out as described previously [3].



Scheme 1. Flow diagram of isolation of polysaccharides from the scape of *M. paradisiaca.*

Preparation of acetone powder

Fresh samples of the scape of *M. paradisiaca* (rasabale, local exotic variety) was collected after the harvest of bananas. The scape (1480 g) was cut into small pieces, blended with acetone (500 ml) in a blender to pulp and then suspended in excess of acetone (4000 ml). The mixture was allowed to stand for 4 days. The light brown acetone extract was decanted and the insoluble residue was extracted twice with acetone (2 × 500 ml). The combined acetone extract was evaporated to a syrupy mass which was dried over phosphorus pentoxide under vacuum (19.98 g). The insoluble residue was washed with acetone, dry ether and air dried at 50 °C. The resulting pale brown fibrous powder (152 g) was milled to a fine powder and sieved with a 60 mesh sieve.

Delignification [29] of acetone powder

A solution of acetone powder (22 g) in water (1000 ml) was treated with glacial acetic acid (1.2 ml) and sodium chlorite (15 g) for 1 h at 60 °C. Delignification was carried out for an additional 1 h with two more additions of same amount of reagents at 30 min intervals. The solution was cooled to room temperature, filtered through a linen cloth and the filtrate was centrifuged to remove the residual insoluble particles. The insoluble residue was washed with water followed by ethanol and ether, and dried (14 g). The filtrate was neutralized with aqueous sodium hydroxide (5%, 25 ml) and the polysaccharide (water soluble polysaccharide, WSP, Scheme 1) was collected (8 g) by ethanol precipitation.

The insoluble residue (14 g) was repeatedly extracted with an aqueous solution of EDTA (2%, w/v, 3 × 50 ml) at 90 °C. Polysaccharide fraction (EDTA soluble, EDTA-SP) presented in the extracts was recovered by ethanol precipitation (5.0 g). The insoluble residue after extraction with EDTA was further extracted with aqueous sodium hydroxide (0.1 N, 3 × 100 ml). The alkaline extracts were pooled, neutralized with aqueous acetic acid (5 M, 60 ml), and the polysaccharide (alkali-soluble, ASP) was collected by ethanol precipitation (4 g). The final insoluble residue (alkali-insoluble, AISP) was washed with water, followed by ethanol and ether, and then dried (5 g).

Gel-permeation chromatography of WSP

WSP (~25 mg) was allowed to swell in glass distilled water (1 ml) and sodium hydroxide was added (0.4 N, 0.25 ml). Any insoluble material was removed by centrifugation and the centrifugate was chromatographed on a Sephadex G-25 column (2.6 cm × 75 cm). The column was eluted with a solution of 1 M NaCl containing 0.1 M NaOH pH 10.5. Fractions of 5 ml were collected and assayed by the phenol-sulfuric acid method [13] for carbohydrates.

Fractionation of WSP with iso-amylalcohol

WSP (1 g) was resuspended in phosphate buffer, pH 8.0 (0.3 ml) and stirred for 1 h at room temperature. To this iso-amylalcohol (10 ml) was added and the mixture was heated in a boiling water bath for 3 h. The solution was cooled and kept at 4 °C for 24 h. The precipitate formed was collected by centrifugation (30 mg). The soluble fraction was recovered from the centrifugate by ethanol (3 vol) precipitation (870 mg).

Enzyme digestions

WSP (500 mg) was treated with α-amylase (500 U, barley malt, Sigma) in an acetate buffer, pH 4.7 (25 ml) at 37 °C for 24 h. The reaction was stopped by heating the mixture in a boiling water bath for 10 min, cooled to room temperature, centrifuged and then dialysed. The dialysate was analysed by p.c. for released sugars. The undialysed material was recovered by ethanol precipitation and analysed for monosaccharide composition. WSP (500 mg) was treated with glucoamylase (500 U, *Aspergillus niger*, Sigma) in a phosphate buffer, pH 7.5, at 37 °C for 24 h. The reaction was stopped by heating the solution on a boiling water bath for 10 min. A portion of the solution was dialysed against distilled water and the dialysate was analysed by p.c. for released monosaccharides. To the remaining portion, alkaline ferricyanide solution (25 ml) and water (25 ml) were added and heated in a boiling water bath for 15 min. The solution was cooled and a zinc sulfate-acetic acid reagent (1 ml) and potassium iodide (5 g) were added. The liberated iodine was titrated against a sodium thiosulfate solution. A control containing all the reagents except the glucoamylase digestion products was also performed.

Compositional analysis

Polysaccharide samples (500–600 μg) were initially solubilized in aqueous sulfuric acid (72%, 0 °C, 100 μl). After 1 h at room temperature, the acid strength was adjusted to 8% and hydrolysed for 6 h at 100 °C. The acid was neutralized with solid barium carbonate and filtered. The filtrate was deionized by passing through successive columns of Amberlite IRA-120H^+ and IRA-400 CO_3^{2-} resins. The eluant and washings were combined, concentrated and then divided into two portions. One portion was analysed by p.c. and the other by GLC.

Butanolysis

Absolute configuration of monosaccharides were established by conversion into 2-(*S*)- and 2-(*R*)-butyl glycosides [30] and GLC, and GLC-MS analysis as their acetate derivatives. Briefly, the polysaccharides (100–150 μg) were solvolysed with butanolic 2 M hydrochloric acid for 5 h at 100 °C, treated with silver carbonate (~5 mg) in the presence of a drop of acetic anhydride at ambient temperature overnight, centrifuged and the centrifugate was evaporated to dryness. The residue was acetylated with pyridine-acetic anhydride (1 : 1, v/v, 0.3 ml) for 1 h at 100 °C, evaporated to dryness, and then analysed by GLC, and GLC-MS. Further, the polysaccharides (50–100 mg) were hydrolysed with 2 M trifluoroacetic acid for 6 h at 100 °C, the acid was removed and the resulting monosaccharides were separated by paper chromatography on a Whatmann 3 MM paper using solvent C. Specific rotation of the isolated monosaccharides was determined. Only Ara had the L-configuration, all other sugars had the D-configuration.

Methylation linkage analysis

Methylation analyses were performed on 0.3–1.0 mg polysaccharide samples using the method of Ciucanu and Kerek [9]. Briefly, the polysaccharide was suspended in pure dimethyl sulfoxide (0.3–0.5 ml), stirred at ambient temperature for 15 min and ultrasonicated at ~40 °C for 10 min. This step was repeated until a clear solution of polysaccharide was obtained. The solution was treated with pulverized sodium hydroxide (~5 mg) and methyl iodide (0.3–0.5 ml) for 30 min at room temperature. The permethylated product was isolated by partition with dichloromethane and used for linkage analysis, after conversion into partially methylated alditol acetates by hydrolysis, reduction, and acetylation as described above, by GLC, and GLC-MS. Haworth and Purdie methylation was carried out as described [7, 8].

Sedimentation analysis [31]

A solution (1%) of WSP in 0.1 M sodium chloride was analysed in a Beckman analytical ultracentrifuge Model E at 25 °C at 59 780 r.p.m. The movement of the boundary was followed using Schlieren optics.

Results

Extraction of fresh samples of scape of *M. paradisiaca* afforded acetone soluble (~1.5%) and acetone insoluble (~10%) fractions. The acetone soluble fraction was examined by IR which showed strong absorption at 3500–3300 cm^{-1} due to –OH, 3100 cm^{-1}, and 1600–1400 cm^{-1} absorptions due to aromatic hydrocarbons. Further, examination of the acetone soluble fraction by UV showed a strong absorption band at λmax 270 nm. These results indicated that the acetone soluble fraction contained a mixture of polyphenols.

The pale brown fibrous acetone insoluble fraction contained ~13.37% ash, 1.6% potassium, ~6.5% silica and was free from lipids. The presence of silica and a high ash content is an interesting feature of this plant. Sugar composition of the acetone insoluble fraction indicated that it contained L-Ara, D-Glc and D-Xyl in ~0.5 : 1.0 : 1.0 proportions respectively. Uronic acids were found to be absent.

Delignification of the acetone insoluble fraction gave an insoluble residue and a soluble extract which upon neutralization followed by ethanol precipitation afforded a water soluble polysaccharide fraction (WSP) in ~36% yield. Sugar composition of WSP indicated that it is a homopolymer of D-Glc. WSP was highly hygroscopic, gave opalescent solution in water and the aqueous solution of polysaccharide underwent slow retrogradation, a unique characteristic of amyloid polysaccharides [4].

The insoluble residue of acetone powder obtained upon delignification was extracted repeatedly with aqueous EDTA which gave an EDTA-soluble polysaccharide fraction (EDTA-SP) in ~35% yield and an EDTA-insoluble fraction (EDTA-ISP) in 65% yield. The sugar composition of EDTA-SP showed that the polysaccharide contained only D-Glc indicating that it is a D-glucan type homopolymer. The EDTA-ISP was extracted with 0.1 M NaOH which gave an alkali-soluble polysaccharide fraction (ASP) and an alkali-insoluble polysaccharide fraction (AISP) in 30% and 35% yield respectively. The sugar composition of ASP gave D-Glc, L-Ara and D-Xyl in ~1:1:10 proportions respectively, indicating that the polysaccharide is mainly a xylan type polymer. The AISP contained D-Glc, L-Ara and D-Xyl in ~10:1:2 proportions respectively.

The above results indicates that the acetone powder of the stalk of rhizome of *M. paradisiaca* contains at least four different polysaccharide fractions (WSP, EDTA-SP, ASP and AISP). Further, the fractionation also indicates that the scape is essentially a solid state gel containing ~1.5% polyphenols and ~10% of polysaccharides with the rest being water (~88.5%) held by a matrix of at least four different polysaccharides in association with minerals, potassium, silica, lignin and polyphenolic pigments.

Gel-permeation chromatography of WSP on a Sephadex G-200 column gave a major peak at the column void volume indicating that the polysaccharide fraction is homogeneous. However quantitative purification of WSP on a Sephadex G-200 column was not possible since the aqueous solution undergoes retrogradation.

Lansky *et al.* [4], have been able to fractionate starches from corn, wheat, tapioca, potato, sago, eastern lily and lily into linear (amylose type) and branched (amylopectin type) starch components by complexation with Pentasol, a commercially available mixture of amylalcohols. Since the aqueous solution of WSP underwent retrogradation and contained only D-Glc, the polysaccharide fraction was subjected to complexation with iso-amylalcohol which afforded a minor insoluble amylose type fraction in 3% yield and a major soluble, amylopectin type fraction in 87% yield. The major amylopectin type fraction was found to disperse in water. Repeated complexation of the major polysaccharide with iso-amylalcohol did not yield any more amylose-type polysaccharide A. Thus WSP with only 3% of amylose type polysaccharide is essentially a amylopectin type polysaccharide. For further studies of WSP, the purified polysaccharide was used.

An aqueous solution of WSP showed a specific rotation of +109.53 indicating the presence of α-glucosidic bonds in the polymer. The presence of α-glucosidic bonds was confirmed by hydrolysis with amylases [5]. Treatment of purified WSP with salivary α-amylase gave maltose and D-Glc in ~2:1 ratio respectively along with the α-limit dextrin in 6.8% yield.

WSP contained the iodine affinity value of 5.09% indicating that the polysaccharide has very limited iodine binding capacity and hence is highly branched. The blue value, determined for WSP (0.023) was found to be very low when compared to those values of potato starch (0.4), standard amylose (1.212) and standard amylopectin (0.285), suggesting that the polysaccharide is more branched than the standard amylose and amylopectin and potato starch. The reducing value of WSP was 21.09 as determined by the ferricyanide reducing method. The reported reducing value for standard amylose and amylopectin was 5.17 and 0.72 respectively. These data suggest that although WSP is highly branched, the polymer has low molecular weight compared to the standard amylose and amylopectin. Hydrolysis of WSP and standard amylose with glucoamylase followed by ferricyanide number determination of the hydrolysates gave 52.76 and 133.18 reducing values, respectively. Glucoamylase [6] hydrolyses α-D-(1 → 4)-glucosidic bonds with a relatively high rate compared to α-D-(1 → 3) and α-D-(1 → 6)-glucosidic bonds. The results suggest that the WSP has a high degree of branching with either α-D-(1 → 3) or α-D-(1 → 6) linked Glc residues in the side chains. Electron micrography of WSP indicated a highly irregular amorphous cotton-like appearance. The X-ray powder diffractograms of WSP also indicated the amorphous nature of the polysaccharide.

Attempts to permethylate WSP using Haworth [7] and Purdie [8] methods were unsuccessful. However the permethylated polysaccharide was obtained by Ciucanu method [9] using solid sodium hydroxide in DMSO and methyl iodide. The GLC-MS of the partially methylated alditol acetates afforded 2,3,4,6-tetra-*O*-methylglucose, 2,3,6-tri-*O*-methylglucose, 2,6-di-*O*-methylglucose and 2,3-di-*O*-methylglucose in ~3.0:45:1.0:2.0 respectively. The presence of large proportions of 2,3,6-tri-*O*-methylglucose indicated that the polysaccharide contained a (1 → 4) linked Glc*p* chain as backbone. The presence of 2,6-di-*O*-methylglucose and 2,3-di-*O*-methylglucose indicated that the main chain was substituted at *O*-3 and *O*-6 with one or two non-reducing D-Glc*p* residues. From the foregoing results the structure shown in Figure 1 is proposed for WSP isolated from the scape of *M. paradisiaca*.

Discussion

Acetone extraction of the scape of *M. paradisiaca* afforded a soluble fraction containing polyphenols in ~1.5% and an

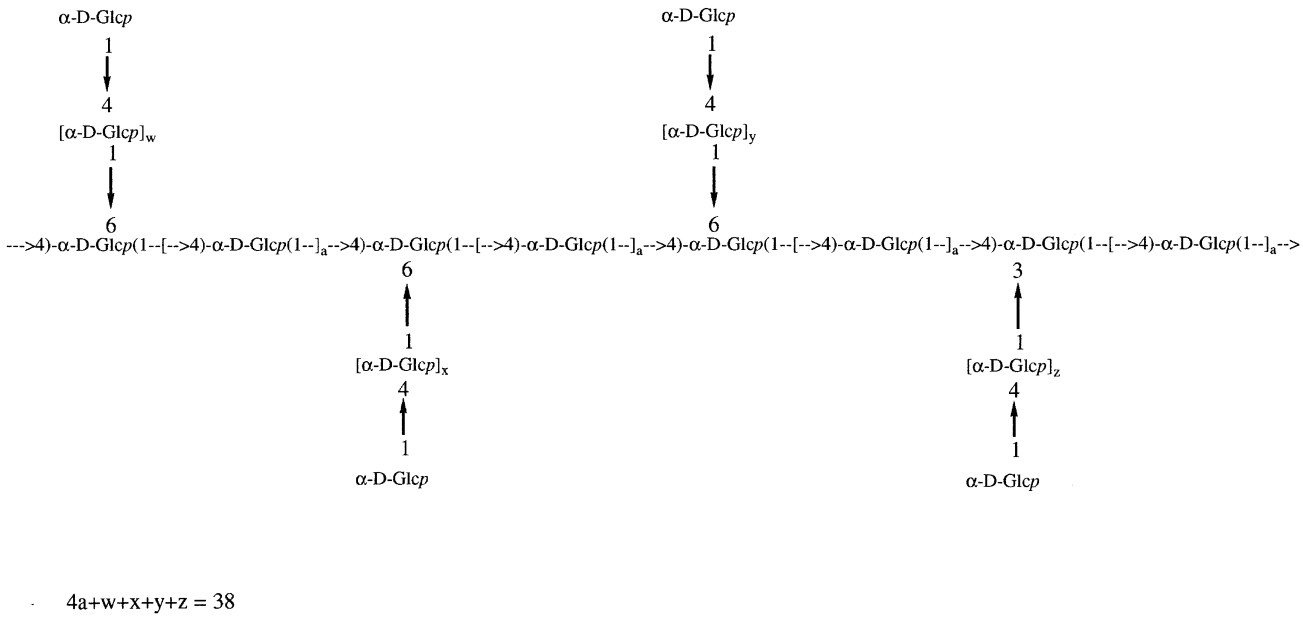


Figure 1. Proposed average structure for WSP isolated from *M. paradisiaca.*

insoluble fraction containing a mixture of polysaccharides in ~10% yield suggesting that the scape contained ~88.5% water presumably held by a matrix of different polysaccharide components in the swollen state. This was evident from the fractionation of the acetone insoluble fraction with aqueous solvents which gave at least four different polysaccharide components. Further, analysis of the acetone insoluble fraction showed the presence of silica (~6.5%) and potassium (~1.6%) suggesting that the minerals are also involved in the scape matrix.

Compositional analysis of the polysaccharide fractions indicated that they are mainly glucan and xylan type polysaccharides. WSP and EDTA-SP underwent retrogradation in aqueous solutions, indicating that these are amyloid type glucans. WSP was further purified by iso-amylalcohol fractionation and characterized by specific rotation, IR spectroscopy, iodine affinity, ferricyanide reducing number, blue value, enzymatic hydrolysis with α-amylase and glucoamylase, methylation linkage analysis and by electron microscopy and X-ray diffraction studies. The combined data indicate that WSP is a highly branched amylopectin type polysaccharide and the average structure for WSP is shown in Figure 1.

Conflicting reports have been made on the scape of *M. paradisiaca* [2, 10]. It has been showed that the *M. paradisiaca* scape starch and the starch from cereals and tubers are similar [2, 10]. The drawback of these studies is the lack of methylation analysis and other chemical investigations to determine the branching pattern. Our investigation showed that WSP is a highly branched amylopectin type α-D-glucan and is different from the starch from cereals and tubers. This might be the reason the scape of *M. paradisiaca* is used as medicine as well as food.

Structure and functional properties of the main starches of commerce such as wheat, corn, potato and rice have resulted in their extensive utilization in industrial or food products [11]. Further, food uses and functional properties of legume flours, protein isolate and concentrates have also been studied extensively [12]. However the chemical nature of starch from the plant sources which have medicinal properties has not been studied extensively. Our investigation on the scape of *M. paradisiaca* polysaccharides indicated the presence of highly branched low mol wt amyloid polysaccharides which are unique.

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