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Chemical analysis of a polysaccharide of unripe (green) tomato (*Lycopersicon esculentum*)

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ABSTRACT

A polysaccharide (PS-I) isolated from the aqueous extract of the unripe (green) tomatoes (*Lycopersicon esculentum*) consists of D-galactose, D-methyl galacturonate, D-arabinose, L-arabinose, and L-rhamnose. Structural investigation of the polysaccharide was carried out using total acid hydrolysis, methylation analysis, periodate oxidation study, and NMR studies (^{1}H, ^{13}C, DQF-COSY, TOCSY, NOESY, ROESY, HMQC, and HMBC). On the basis of above-mentioned experiments the structure of the repeating unit of the polysaccharide (PS-I) was established as:

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                          G                B               F                   C
              →4)-β-D-Galp-(1→4)-β-L-Arap(1→4)-α-D-GalpA6Me-(1→2)-α-D-GalpA6Me-(1→
                                           3
                                           ↑
                                           1
α-L-Rhap-(1→4)-α-D-Galp-(1→5)-α-D-Araf
     D              A              E
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1. Introduction

Plant extracts are very attractive source of additive for food and drug industries because of their use in complementary medicinal supplement.[1] It has been observed that bio-active plant polysaccharides exhibit antioxidant,[2] immunomodulatory as well as antitumor activity[3,4] and have a large range of applications in industrial field.[5] Plant polysaccharides such as neutral xyloglucan, glucuronic acid containing arabinogalactan, and 4-*O*-methyl glucuronoxylans are involved in macrophage stimulation.[6] Several plant polysaccharides isolated from *Aloe barbadensis*,[7] fruit juice of *Morinda citrifolia*[8] (noni), *Morus alba*, *Chlamydomonas mexicana*, and *Poria cocos*[9] show immunomodulatory and antitumor activity. Structural characterization of some plant[10,11] and mushroom[12–19] polysaccharides has been carried out in our laboratory and reported in this journal.

Lycopersicon esculentum (Tomato) is a popular, versatile, easily grown plant with great tasting fruit. Tomato belongs to the solanaceae family. It has medicinal uses for the treatment of rheumatism and severe headache,[20] its root is useful in the treatment of toothache.[21] The fruits are used for first aid treatment for burns, scalds, and sunburn.[22] Some pectic polysaccharides[23] were isolated from the ripe and unripe tomatoes using different extraction procedures, and the side chain of the pectic polysaccharides were reported to be composed of mainly β-(1→4)-linked galactopyranosyl and α-(1→5)-linked arabinofuranosyl residues. A polysaccharide[24] isolated from green tomatoes consisted of D-galactose (58%), L-arabinose (14.5%), L-rhamnose (3.5%), D-galacturonic acid (22%), and some minor components (2%). It has been reported that an enzyme galacturonase[25] isolated from the ripe tomato degraded the polysaccharide of green tomato to low molecular weight galactourans and (1→4)-linked β-galactose. In the present study we have isolated a polysaccharide from green tomatoes consisting of almost similar sugar residues from hot water extract and detailed structural studies are reported here in.

2. Result and discussion

Fresh green (unripe) tomatoes (1 kg) were collected from local market of Midnapore town and were washed with water. The unripe tomatoes (*L. esculentum*) were boiled with distilled water for 5 h and then filtered through linen cloth. The filtrate was centrifuged at 4 °C and the supernatant was precipitated with 1:5 water/EtOH. The precipitated material was collected through centrifugation and then dialyzed through cellulose tubing bag to remove low molecular weight material. The whole solution was

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centrifuged at 4 °C, filtrates were taken and freeze-dried, yielding 1.3 g of crude polysaccharide. The water-soluble crude polysaccharide was fractionated through Sepharose S-6B column into two fractions (PS-I and PS-II). Detailed structural investigation was carried out with PS-I, which was identified as a heteropolysaccharide composed of galactose, galacturonic acid, rhamnose, and arabinose. The molecular weight[26] of PS-I was estimated from a calibration curve prepared with standard dextran as $\sim 2.0 \times 10^5$ Da. It showed a specific rotation of $[\alpha]_D^{25}$ +131.3 (*c* 0.8, water). The analysis for neutral sugars gave rhamnose, arabinose, and galactose in the molar ratio of nearly 1:2:2, as shown by GLC of their alditol acetate. But the GLC analysis of the alditol acetates of carboxy-reduced[27] polysaccharide showed the presence of rhamnose, arabinose, and galactose in the molar ratio of nearly 1:2:4. This result indicates that two galacturonic acid unit may be present in PS-I. Absolute configuration of monosaccharide residues in PS-I was determined through the method of Gerwig et al.[28]

PS-I was methylated using the method of Ciucanu and Kerak,[29] followed by hydrolysis and conversion into alditol acetates. The alditol acetates of the methylated product were analyzed by GLC and GLC–MS using HP-5 fused silica capillary column. The polysaccharide showed the presence of 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-galactitol; 1,3,4,5-tetra-*O*-acetyl-2-*O*-methyl-arabinitol; 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-arabinitol; and 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-rhamnitol in the molar ratio of nearly 2:1:1:1. These results indicate that (1→4)-galactpyranosyl or (1→5)-galactfuranosyl, (1→3,4)-arabinopyranosyl or (1→3,5)-arabinofuranosyl, (1→5)-arabinofuranosyl or (1→4)-arabinopyranosyl, and terminal L-rhamnopyranosyl moieties may be present in PS-I. GLC analysis of the alditol acetates of methylated-reduced[30] polysaccharide showed above peaks along with two new peaks of 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-galactitol and 1,2,5,6-tetra-*O*-acetyl-3,4-di-*O*-methyl-galactitol. This GLC analysis indicates (1→2) and (1→4)-linked galacturonic acid is also present in PS-I. The periodate oxidized, reduced material upon hydrolysis with trifluoroacetic acid followed by GLC and paper chromatographic[31] studies' analysis showed the presence of only arabinose. These results signify only (1→3,4) or (1→3,5)-linked arabinose is retained during periodate oxidation study; the exact linkage of which was confirmed through NMR experiments.

The ^1H NMR (500 MHz) spectrum (Fig. 1, Table 1) of PS-I at 27 °C showed seven anomeric proton signal at δ 5.37, 5.20, 5.07, 5.06, 4.96, 4.95, and 4.61 ppm which were labeled as **A–G** according to their decreasing anomeric proton signal (Table 1). The ^{13}C NMR (125 MHz) spectrum (Fig. 2, Table 1) at 27 °C showed five anomeric carbon signals appeared at 100.2, 100.8, 103.7, 104.8, and 108.5 ppm. Further δ 53.3 was assigned for carbomethoxy carbon. All ^1H and ^{13}C signals were assigned from DQF-COSY, TOCSY, HMQC, and HMBC NMR experiments.

Residue **A** has an anomeric proton chemical shift at δ 5.37 ppm, and $J_{H-1,H-2} \sim 3.6$ Hz, $J_{H-1,C-1} \sim 171$ Hz indicating that it is an α-linked sugar residue. The $J_{H-2,H-3}$ value ($\sim$10 Hz) and $J_{H-3,H-4}$ value ($\sim$4 Hz) of residue **A** suggest that it is an α-D-galactosyl residue. The downfield shift of C-4 (δ 76.2 ppm) and a little upfield shift of C-3 (δ 68.2 ppm) and C-5 (δ 69.1 ppm) with respect to standard methyl glycosides[32,33] indicate that residue **A** is (1→4)-linked α-D-galactose.

Residue **B** was assigned as Ara*p* as it showed a large coupling constant $J_{H-2,H-3}$ ($\sim$8 Hz), $J_{H-3,H-4}$ ($\sim$4.8 Hz) and relatively small coupling constant for $J_{H-1,H-2}$. It also showed two H-5 signals at δ 3.81 and δ 3.94 ppm. The anomeric proton and carbon chemical shifts at δ 5.20 ppm and δ 103.7 ppm ($J_{H-1,C-1} \sim 170$ Hz) indicate that residue **B** is β-L-arabinose.[11] The downfield shift of C-4 (δ 79.4 ppm) and C-3 (δ 81.0 ppm) with respect to standard methyl glycosides indicates that residue **B** is (1→3,4)-linked.

The spin system of residue **C**, which consisted of only five protons with a relatively high chemical shift of the H-5 signal (δ 4.43 ppm) and weak coupling between H-3, H-4, and H-5, indicates that residue **C** is a D-galacturanosyl residue. The anomeric proton at δ 5.07 ppm ($J_{H-1,H-2} \sim 3.0$ Hz) and $J_{H-1,C-1} \sim 170$ Hz of residue **C** indicates that it is α-linked sugar residue.[10,11] The C-2 (δ 78.1 ppm) signal of residue **C** showed a downfield shift compared to the standard methyl glycosides. The carbon signals of residue **C** were observed at δ 69.1, 70.0, 73.2, and 171.1 ppm corresponding to C-3, C-4, C-5, and C-6 (ester carbonyl), respectively. The appearance of intra-residual coupling between carbonyl carbon (δ 171.1 ppm) and carboxymethyl proton (δ 3.78 ppm) in the HMBC experiment clearly indicates that carboxyl group of galacturonic acid is present as methyl ester. These data confirmed that residue **C** is (1→2)-linked.

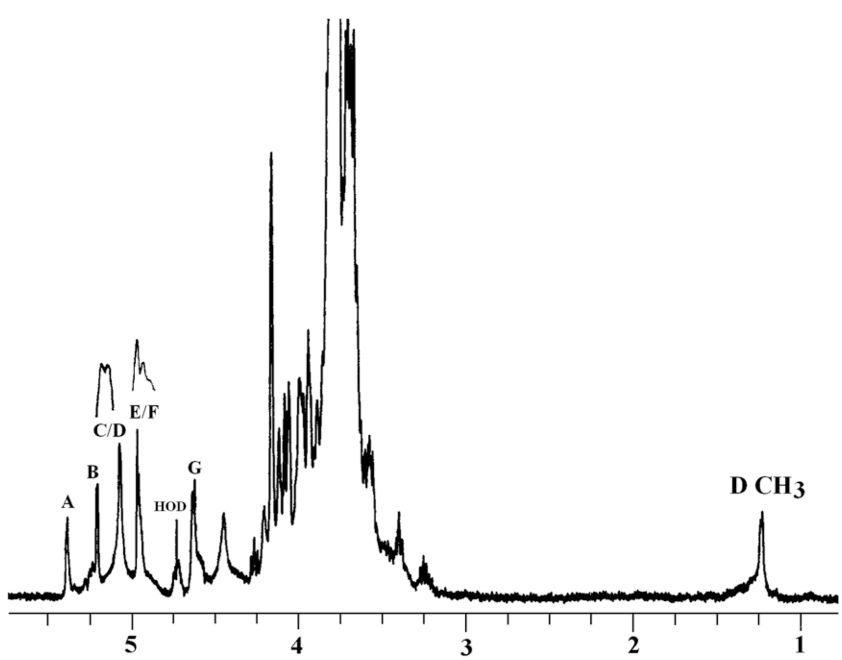

Figure 1. ^1H NMR spectrum (500 MHz, D_2O, 27 °C) of the polysaccharide (PS-I), isolated from green tomato (*Lycopersicon esculentum*).

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