Structural Elements of an Antioxidative Pectic Arabinogalactan from Solanum virginianum

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Abstract

The water-soluble polysaccharide-containing fraction from Solanum virginianum leaves upon anion exchange chromatography yielded a highly branched pectic arabinogalactan-containing fraction (F2). Herein, direct evidences for the (i) presence of a chain having 1,6-linked Gal units substituted at O-3, (ii) occurrence of 1,3-linked Ara residues substituted at C-6, and (iv) occurrence of 1,5-linked Araf residues substituted at O-3 were presented. This polysaccharide that showed a dose-dependent antioxidative property formed a water-soluble complex with bovine serum albumin. Thus, F2 could be used as a natural ingredient in functional food and pharmaceutical products to mollify oxidative stress.

Key words
Solanum virginianum · Solanaceae · polysaccharide structure · antioxidative effect · BSA-polysaccharide complex

Supporting information available online at http://www.thieme-connect.de/products

The importance of free radicals as an exacerbating factor in cellular injury and the aging process has attracted increasing attention over the years [1,2]. Recently, reactive oxygen species are evidenced to be closely related to degenerative diseases such as Alzheimer’s, neuronal death including ischemic stroke, and acute and chronic degenerative cardiac myocyte death [1,3,4]. Solanum virginianum L. (Solanaceae) is being utilized for the management of a number of diseases throughout the Indian subcontinent since ancient times [5,6]. Indeed, a range of pharmacological activities, such as antiurolithiatic [7], antihyperglycemic, and antioxidative [8], was observed from extracts and pure compounds from this herb. So far, only secondary metabolites, namely, glycosides [9] and steroid alkaloids [10], have been chemically characterized. Incidentally, polysaccharides from many medicinal plants stimulate a range of biological activities [11–14], but a report on the antioxidative activity of polysaccharides from S. virginianum leaves has not yet been explored. Herein, we report the isolation and structural analysis of a water-extracted polysaccharide from S. virginianum leaves. Moreover, we have evaluated the antioxidative property of this polysaccharide using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. Additionally, the interaction of this polysaccharide with bovine serum albumin has been investigated.

Results and Discussion

Extraction of the leaves of S. virginianum with water yielded a crude polysaccharide-containing fraction having antitussive activity [15]. This crude extract upon anion exchange chromatography (AEC) yielded two fractions: F1 and F2 eluted with 0.05 and 0.5 M NaOAc (pH 5.5), respectively. The major fraction F2, which consisted of 60% of the total material loaded onto the column, contained Ara, Gal, Rha, and Glc residues in the molar ratio of 44 : 40 : 2 : trace. F2 contained 71% (w/w) polysaccharide including 8% uronic acid. TLC analysis of the acid generated monosaccharides indicates the presence of 

<table>
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<tr>
<th>Methylation productsa</th>
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<th>Peak areab</th>
<th>PRF2</th>
<th>A1O</th>
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<tbody>
<tr>
<td>2,3,5-Me3-Ara</td>
<td>Terminal-</td>
<td>15</td>
<td>5</td>
<td>46</td>
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<tr>
<td>2,3-Me2-Ara</td>
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<td>24</td>
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<tr>
<td>2-Me-Ara</td>
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<td>9</td>
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<tr>
<td>3,4,6-Me3-Rha</td>
<td>1,2-</td>
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<td>2</td>
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<tr>
<td>2,3,6-Me2-Glc</td>
<td>1,4-</td>
<td>2</td>
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</tr>
<tr>
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<td>6</td>
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<tr>
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<tr>
<td>2,4-Me2-Gal</td>
<td>1,3,6-</td>
<td>14</td>
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a 2,3,5-Me3-Ara denotes 1,4-di-O-acetyl-2,3,5-tri-O-methylarabinitol, etc.; b Percentage of total area of the identified peaks

Table 1 Methylation analysis data of the water-extracted polysaccharide (F2) isolated from S. virginianum leaves and the ethanol-precipitated (PRF2) and soluble (A1O) fractions derived from F2 by Smith degradation.

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between 3.27 and 4.01 ppm, characteristic of ring protons (H2–H5). The other distinctive feature of F2 was the presence of esterified phenolic acid residues. Indeed, the total phenolic content of F2 was 10 mg GAE/g of sample and it contained p-coumaric acid, sinapic acid, ferulic acid, and 8,5-diferulic acid (diFA) in a molar ratio of 82:2:6:10.

The polysaccharide F2, on Smith degradation using the standard protocol, yielded a periodate resistant polymeric fraction (PRF2) together with an aqueous 80% ethanol soluble fraction (PRF2S) containing oligomeric fragments. As shown in Fig. 1A “A1O”, the acetylated derivative of PRF2S comprises three series of oligomeric fragments, many of which contained a glycerol label (O-Gly). The O-Gly tag at the reducing end of oligomeric fragments such as Ara2Gly1Ac7–[(CH2)OAc], Ara2Gly1Ac7–[CH3CHOAc + CO] (molecular rearrangement products), Ara2Gly1Ac9, and others were generated from the oxidative cleavage of 1,6-linked Gal units by periodate. These oligomeric fragments together with the presence of Ara2Gal1Gly1Ac7 and Ara3Gal1Gly1Ac9, impared evidences for the existence of Ara and Gal residues in the same molecule, indicating that the polymer was an arabinogalactan and not a mixture of arabinan and galactan. Moreover, the presence of a segment containing at least three consecutive 1,3,5-linked Ara in accord with Ara3Gly1Ac9 was revealed. Incidentally, the presence of a segment containing at least three consecutive 1,3,5-linked Gal units (Table 1). Fragments such as Gal3Ac8, Ara3Ac7−[CO], and Ara1Ac7−[CH3OAc], probably arisen by the removal of O-Gly tag with CF3CO2H during Smith degradation, were also present. In addition, electrospray mass spectrometry (ESMS) analysis coupled with glycosidic linkage analysis confirmed the existence of two consecutive 1,6-linked Gal units each substituted at O-3 in the parent F2. The periodate-resistant polymeric product (PRF2) contained Gal and Ara in a molar ratio of 74:26. Indeed, large quantities of Ara residues were destroyed during Smith degradation. Certainly F2 possesses a large amount of terminal- and 1,5-linked Araf residues (Table 1), both of which were destroyed by periodate. Remarkably, the proportion of 1,6-linked residues increased significantly after Smith degradation (Table 1). Hence, the presence of a chain containing 1,6-linked Galp residues substituted at O-3 was ascertained. Moreover, the existence of a chain containing 1,3-linked Gal residues substituted at C-6 was also established. Notably, a major part of the Ara residues was either degraded or present in the ethanol-soluble part (PRF2S) as a monomer or oligomer. Afterwards, oligosaccharide subunits generated from F2 using a commercial enzyme preparation possessing endogalactanase activity [12] were then acetylated. ESMS spectrum (Fig. 1B) of acetylated oligomers (A2O) revealed the presence of numerous fragments. On the basis of their molecular weight, ions at m/z 413, 557, 701, 845, 989, 1205, and 1277 could be assigned to [M + Na]+ of Hex1Ac5 (Hex1 = one hexose residue, Ac5 = five acetyl substituent), Pent2Ac6 (Pent2 = two pentose residues), Hex2Ac8, Hex1Pent2Ac9, Hex2Pent1Ac11, Hex3Pent1Ac13, and Hex4Ac14, respectively. Fragment ions at m/z 437 and 455 could have arisen from Pent2Ac6−[2AcOH] and Pent2Ac6−[CH3OAc + CO] units. Sugar compositional analysis indicated that A2O contained galactose and arabinose residues as neutral sugars. Taken together, Hex1Ac5, Pent2Ac6, Hex2Ac8, Hex1Pent2Ac9, Hex2Pent1Ac11, Hex3Pent1Ac13, and Hex4Ac14 could be assigned to Gal1Ac5, Ara2Ac6, Gal2Ac8, Gal1 Ara3Ac9, Gal1Ac11, Gal1Ara3Ac13, and Gal4Ac14, respectively. Remarkably, A2O showed UV absorption bands at 230 and 277 nm, characteristic of phenolic acids. The presence of two phenolic acids containing oligosaccharides in A2O was also indicated by the presence of fluorescent spots on TLC. Especially p-coumaric acid was the predominant phenolic acid of F2. Taken together, ions at m/z 989 and 1205 may also be due to [M + Na]+ of Gal1Ara2CA1Ac9 (CA = coumaric acid) and Gal1Ara3CA1Ac11, respectively. Notably, the presence of Gal1Ara2CA1Ac9, Gal1Ara3CA1Ac11, Gal1Ara2Ac9, and Gal3AraAc13 suggested that galactose, arabinose, and phenolic acid residues were an integral part of the same polysaccharide. Therefore, it may be concluded that the studied biomacromolecule is a pectic arabinogalactan esterified with phenolic acid.
F2 showed a dose-dependent DPPH-radical scavenging activity up to 200 µg/mL (Fig. 2S, Supporting Information). Interestingly, at the 200 µg/mL concentration, F2 scavenged 88 ± 0.3% DPPH radicals, whereas for butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT), the values were 92.8 ± 0.3% and 92.3 ± 0.3%, respectively (Fig. 2).

This biopolymer interacts with BSA. Indeed, with the gradual addition of F2 to the BSA solution (pH 7.4), the intensity of the peak at 215 nm in the UV spectrum of BSA decreased (Fig. 3A). Moreover, the $\lambda_{\text{max}}$ (maximum wavelength) for the particles in the solution shifted toward a longer wavelength. The spectral changes around $\lambda_{\text{max}}$ (215 nm) might occur from the disturbance of the microenvironment around the polypeptide caused by the binding of F2 with BSA.

Moreover, the maximum fluorescence emission wavelength ($\lambda_{\text{em}}$) of BSA also showed a red shift (24 nm at pH 7.4; Fig. 3B). This phenomenon was likely due to the complexation of F2 with BSA, resulting in the latter’s conformational changes. According to a modified Stern-Volmer equation, the binding constant ($K$) for the F2-BSA complexation at pH 7.4 was 2.68 × 10$^5$/M. This value was analogous to other strong ligand-protein complexes with binding constants ranging from 10$^6$ to 10$^8$/M [18].

Thus, a polysaccharide containing esterified phenolic acids in monomeric and dimeric types was isolated from S. virginianum. ESMS analysis of a spectrum of per acetylated oligomeric fragments generated by Smith degradation and enzyme hydrolysis imparts finer structural details of this polymer. Remarkably, F2 formed a water-soluble complex with BSA and, hence, was biocompatible with the transport protein. Furthermore, antioxidative activity of the studied biomacromolecule provides a scientific basis for the use of this herb in traditional medicine.

Materials and Methods

This material is available as Supporting Information.

Supporting Information

The general experimental procedures, extraction, and purification of the pectic arabinogalactan followed by analytical methods as well as copies of the $^1$H NMR spectrum and scavenging effect on DPPH radicals of this polysaccharide are available as Supporting Information.

Acknowledgements

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Conflict of Interest

The authors declare no competing financial interest.

References

3 Beckman KB, Ames BN. The free radical theory of aging matures. Physiol Rev 1998; 78: 547–581
5 Rita P, Animesh DK. An updated overview of Solanum xanthocarpum Schrad and Wendl. JFRAP 2011; 2: 730–735