Study on conformational structure of tamarind seed polysaccharide and its sulfated derivative by light scattering method

Bui Ngoc Tan¹,², Quach Thi Minh Thu², Thanh Thi Thu Thuy³*
¹Hai Phong University of Medicine and Pharmacy
²Institute of Chemistry Vietnam Academy of Science and Technology

Received 15 July 2016; Accepted for publication 11 April 2017

Abstract

The aim of this study was to study the conformational changing when sulfate groups were introduced to the molecular chain of native tamarind seed polysaccharide (TSP). Light Scattering (LS) method was used to determine conformation of TSP and its sulfated derivative TSPS. The results indicated that both TSP and TSPS have a highly branched and more sphere-like molecule; however, after sulfation, the TSPS became more branched structure than native polysaccharide.

Keywords. Tamarind seed polysaccharide, conformation, light scattering.

1. INTRODUCTION

Tamarind seed polysaccharide (TSP) belongs to xyloglucan family obtained from the seed of tamarind tree Tamarindus indica. TSP possesses properties like high viscosity, broad pH tolerance, noncarcinogenicity, mucoadhesive nature, and biocompatibility; therefore, it is used as stabilizer, suspending agent, thickener, gelling agent, and binder in food and pharmaceutical industries [1, 2].

Polysaccharides are known to reveal the biological functions by forming a specific conformation. For example, branched poly-β(1→3)-D-Glucan has a strong anti-tumor activity, which may be associated with its specific chain conformation, while curdlan, a linear poly-β(1→3)-D-Glucan, has no anti-tumor activity although it assumes a triple-stranded helical conformation, but by sulfation, curdlan sulfate has anti-HIV activity [3]. Therefore, the elucidations of the molecular structure for both chemical structure and conformation can expand the application of a particular polysaccharide.

With the development of high-resolution instrumental processes, such as scattering techniques (i.e. light scattering, x-ray and neutron scattering), it is possible to study the conformation of a polysaccharide at the molecular level. Light scattering (LS) is a powerful technique that can provide structural information of high-resolution structures, and determine the conformation of molecule in solution [4].

In our previous paper [5, 6], the chemical structure of TSP was determined and a sulfated derivative of TSP was produce, the result showed that the sulfation of TSP enhances the antitumor activity of the polysaccharide. In this work, we aim to know the conformational changing when sulfate groups were introduced to the molecular chain of native TSP, here, Light Scattering (LS) method including both static and dynamic techniques was used to determine conformation of TSP and TSPS.

2. MATERIALS AND METHODS

2.1. Materials

Tamarind seed polysaccharide TSP: Tamarind seed was collected at Thuy-nguyen (Hai-phong) in May, 2013. The extraction of TSP from tamarind seed kernel followed the method of R. Deveswaran et al. [7] and has been reported in our previous paper [5]. The yield of extraction was 38.7 % calculated based on dried tamarind seed kernel weight.

Sulfated derivative TSP: The sulfation was following the method of Lihong Fan et al. [8]. Determination of sulfate content was followed the gravimetric method [9]. The structure of sulfated derivative (TSPS) was confirmed by IR and NMR spectra. The results showed that TSP was sulfated to give a sulfate polysaccharide TSPS with a sulfate content of 26.2 %. The details of this study can be found in our previous work [6].
2.1. Gel Permeation Chromatography (GPC) measurement

GPC measurements were performed on an HPLC Agilent 1100 with a refractive index detector RID at 30 °C. The eluent was 0.1 N NaNO₃ with flow rate of 1.0 mL/min. The sample concentration was 1 mg/ml. Pullulan was used as a standard sample.

2.2. Light Scattering (LS) measurement

The LS measurements were performed at every 5° in the range 30°-150° on an SLS-6500 & EDLS-9000 spectrometer installed at the Osaka Electro-Communication, Japan. The light source is a He-Ne laser (λₑ₀ = 632.8 nm), and the spectrometer was calibrated using toluene as a primary standard. Cylindrical cells immersed in toluene at 25.0±0.2 °C were used for all the measurements. The alignment of the instrument was performed regularly by measuring of the light scattering of toluene in the range of angles described above. These solutions were filtered several times through 0.45 μm MF filters (Millipore) to remove dust from the solutions. The refractive index increments (dn/dc) of the solutions were measured at λₑ₀ = 632.8 nm using a Brice-Phoenix differential refractometer. The data were analyzed by the conventional Zimm’s method [8], which is a general procedure for the determination of the radius of gyration, Rₚ, the average molecular weight, Mₓw, and the hydrodynamic radius, Rₓ. The ρ value, defined as a ratio of Rₓ to Rₚ (ρ = Rₓ/Rₚ), was also determined in order to establish the shape of a solute molecule [4].

3. RESULTS AND DISCUSSION

Chemical structure of TSP was determined [5]. TSP is a galactoxyloglucan composed of (1→4)-β-Glucan backbone substituted with side chains of α-Xylopyranose and β-Galactopyranosyl (1→2)-α-Xylopyranose linked (1→6) to glucose residues (figure 1).

The structure of native TSP and its sulfated derivative was confirmed by IR and NMR spectra and the results indicated that some hydroxyl groups of glucose, xylose and galactose of TSP were sulfated and sulfation enhances the antitumor activity of TSP [6].

\[ α-Xyl-(1→6) \]
\[ β-Glc-(1→4)β-Glc-(1→4)β-Glc-(1→4) \]
\[ β-Gal-(1→2)α-Xyl-(1→6) \]

Figure 1: Chemical structure of TSP

Molecular weight Mₓw and molecular weight distribution Mₓ/Mₓ were determined by GPC. Like other native sulfate polysaccharides, the molecular weight distribution of the TSP is highly polydisperse with Mₓ/Mₓ = 4.36 and Mₓ = 1.155×10⁶. The literature reports a very wide range of molecular weights of TSP samples from 1.15×10⁵ to 2.5×10⁶, mostly explainable as differences in the extraction processes, polydispersity of the samples [10]. The weight average molecular weight Mₓ of TSPS is 2.96×10⁵, the result indicated that under sulfation process, the native polysaccharide was hydrolyzed. The chromagrams of GPC measurements for TSP and TSPS are shown in figure 2a and b, respectively.

Conformational characteristic of TSP and TSPS are determined by SLS and DLD. The Zimm plots from light scattering measurement of TSP for static and dynamic light scattering measurements (SLS and DLS) are shown in figures 3a and 3b, respectively. The structural parameters estimated from GPC and LS measurement were summarized in table 1. The relationship between ρ value estimated from LS measurement and molecular architecture has been extensively summarized by Burchard [4]. Generally, Rh increases with branching due to a higher segment density, and thus branched chain will have a smaller ρ value than a linear chain. The ρ value of TSP and TSPS are 0.92 and 1.16, respectively, indicated that both TSP and TSPS have a highly branched and more sphere-like molecule, similar conformational characteristics with other plant polysaccharides from soybean [11] and flaxseed [12]. After sulfation, TSPS has a large ρ value indicating that TSPS have a more branched structure than that of TSP. The schematic conformation of TSP and TSPS are simulated in figures 4a and 4b, respectively.

Table 1: Results estimated from GPC and LS measurement

<table>
<thead>
<tr>
<th>Sample</th>
<th>dn/dc (ml/g)</th>
<th>Mₓw x 10⁶</th>
<th>Mₓ/Mₓ</th>
<th>Rₓ (nm)</th>
<th>Rₚ (nm)</th>
<th>ρ (=Rₓ/Rₚ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSP</td>
<td>0.137</td>
<td>11.55</td>
<td>4.36</td>
<td>211.7</td>
<td>229.4</td>
<td>0.92</td>
</tr>
<tr>
<td>TSPS</td>
<td>0.161</td>
<td>2.69</td>
<td>1.52</td>
<td>216.6</td>
<td>186.4</td>
<td>1.16</td>
</tr>
</tbody>
</table>
Figure 2: GPC chromatogram of TSP (a) and TSPS (b)

Figure 3: Zim plots for SLS (a) and DLS (b) of TSP

Figure 4: Schematic conformation of TSP (a) and TSPS (b)

4. CONCLUSION

Conformational structure of TSP and its sulfated derivative TSPS was determined by LS method. The results indicated that both TSP and TSPS have a highly branched and more sphere-like molecule, however, after sulfation, the TSPS became more branched structure than native one. Our results contributed to confirm that chemical modification affected not only biological activity, but also conformation of native polysaccharides.

Acknowledgment. We thank Dr. Yoshiaki Yuguchi of Osaka Electro-Communication University, Japan for LS measurements.

REFERENCES

1. Lang P., Masci G., Dentini M., Crescenzi V., Cooke.


7. Deveswaran R., Bharath S., Sharon Furtado, Sindhu Abraham, Basavaraj B. V., Madhavan V. Isolation...


Corresponding author: Thanh Thi Thu Thuy
Institute of Chemistry
Vietnam Academy of Science and Technology
No. 18, Hoang Quoc Viet, Cau Giay District, Hanoi
E-mail: thuyttt@ich.vast.vn; Telephone number: 0982262005 / 04-37563788.