Vietnam Journal of Chemistry, International Edition, **55**(2): 236-239, 2017
DOI: 10.15625/2525-2321.2017-00451

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

Bui Ngoc Tan[1,2], Quach Thi Minh Thu[2], Thanh Thi Thu Thuy[2*]

[1]*Hai Phong University of Medicine and Pharmacy*

[2]*Institute 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 sulfated 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 TSPS: 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 ^oC. The eluent was 0.1 N $NaNO_3$ 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 (λ_o= 632.8 nm), and the spectrometer was calibrated using toluene as a primary standard. Cylindrical cells immersed in toluene at 25.0±0.2 ºC 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 λ = 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, R_g, the average molecular weight, M_w, and the hydrodynamic radius, R_h. The ρ value, defined as a ratio of R_g to R_h ($\rho = R_g/R_h$), 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].

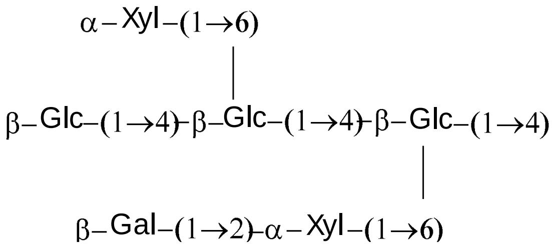

Figure 1: Chemical structure of TSP

Molecular weight M_w and molecular weight distribution M_w/M_n were determined by GPC. Like other native sulfate polysaccharides, the molecular weight distribution of the TSP is highly polydisperse with M_w/M_n = 4.36 and $M_w = 1.155\times10^6$. The literature reports a very wide range of molecular weights of TSP samples from 1.15×10^5 to 2.5×10^6, mostly explainable as differences in the extraction processes, polydispersity of the samples [10]. The weight average molecular weight M_w of TSPS is 2.96×10^5, the result indicated that under sulfation process, the native polysaccharide was hydrolyzed. The chromatograms 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

Sample	dn/dc (ml/g)	M_w x 10^{-5}	Mw/Mn	R_g (nm)	R_h (nm)	ρ (=R_g/R_h)
TSP	0.137	11.55	4.36	211.7	229.4	0.92
TSPS	0.161	2.69	1.52	216.6	186.4	1.16

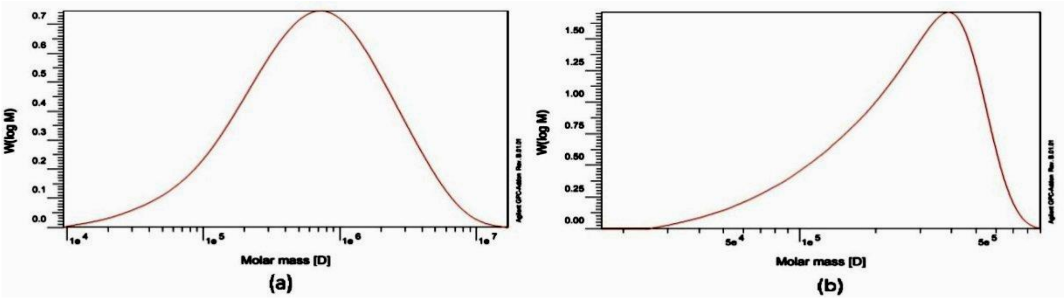

Figure 2: GPC chromatogram of TSP (a) and TSPS (b)

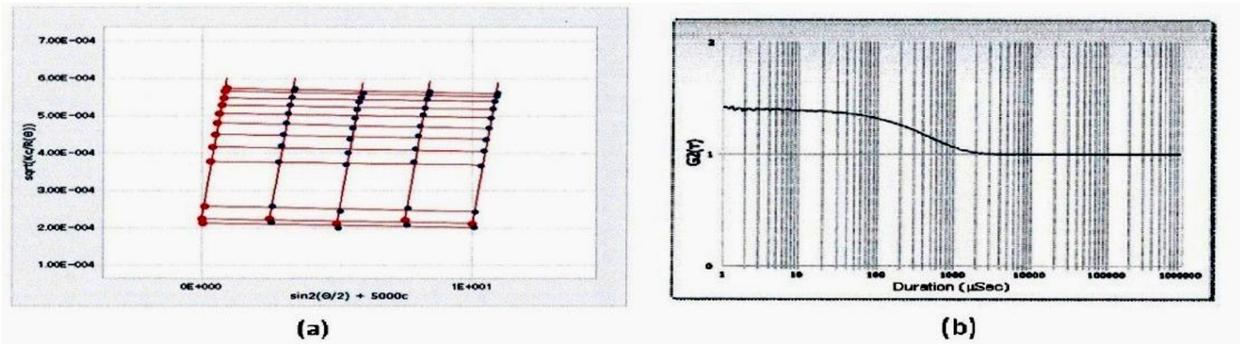

Figure 3: Zim plots for SLS (a) and DLS (b) of TSP

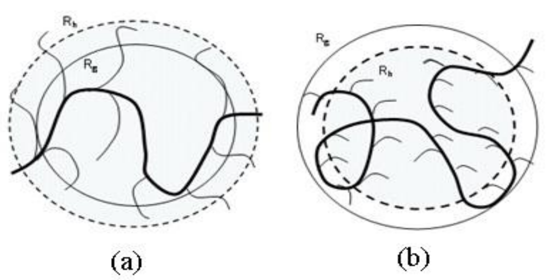

Figure 4: Schematic conformation of TSP (a) and TSPS (b)

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. D., Gidley M. J., Fanutti C. & Reid J. S. G. *Tamarind seed polysaccharide: preparation, characterisation and solution properties of carboxylated, sulphated and alkylaminated derivatives*, Carbohydrate Polymers, **17**, 185-198 (1992).
2. Shailaja T., Latha K.,Sasibhushan P., Alkabab A. M., Uhumwangho M. U. *A novel bioadhesive polymer: grafting of tamarind seed polysaccharide and evaluation of its use in buccal delivery of metoprolol succinate*, Der Pharmacia Lettre, **4(2)**, 487-508 (2012).
3. Gao Y., Fukuda A., Katsuraya K., Kaneko Y., Mimura T., Nakashima H. and Uryu T. *Synthesis of regioselective substituted curdlan sulfates with medium molecular weights and their specific anti-HIV-1 activities*, Macromolecules, **30**, 3224-3228 (1997)
4. Burchard W. *Physical Techniques for the Study of Food Biopolymers*, Blackie Academic and Professional, Glasgow 151-214 (1994).
5. Bui Ngoc Tan, Thanh Thi Thu Thuy, Nguyen Thi Nu, Dang Vu Luong, Nguyen Tuan Anh. *Structural Determination of Tamarind Seed Polysaccharide (TSP) Extracted from Tamarind Seed*, Journal of Chemistry, **53(6e3)**, 62-64 (2015).
6. Bui Ngoc Tan, Quach Thi Minh Thu, Dang Vu Luong, Nguyen Tuan Anh and Thanh Thi Thu Thuy. *Structure and antitumor activity of tamarind seed polysaccharide and its sulfated derivative*, Journal of Science and Technology, **54(2B)**, 170-176 (2016).
7. Deveswaran R., Bharath S., Sharon Furtado, Sindhu Abraham, Basavaraj B. V., Madhavan V. *Isolation*

and Evaluation of Tamarind Seed Polysaccharide as a Natural Suspending Agent, International Journal of Pharmaceutical & Biological Archives, **1(4)**, 360-363 (2010).

8. Lihong Fan, Lan Jiang, Yongmei Xu, Yue Zhou, Yuan Shen, Weiguo Xie, Zhongheng Long, Jinping Zhou. *Synthesis and anticoagulant activity of sodium alginate sulfates*, Carbohydrate Polymers, **83(4)**, 1797-1803 (2011).
9. Camila F. Becker, Jorge A. Guimarães, Paulo A.S. Mourão, Hugo Verli. *Conformation of sulfated galactan and sulfated fucan in aqueous solutions Implications to their anticoagulant activities*, Journal of Molecular Graphics and Modelling, **6**, 391-399 (2007).
10. Trushar R. Patel, Gordon A. Morris, Anna Ebringerová, Melita Vodenicarová, Vladimír Velebny´, Alvaro Ortega, Jose Garcia de la Torre, Stephen E. Harding. *Global conformation analysis of irradiated xyloglucans*, Carbohydrate Polymers, **74**, 845-851 (2008).
11. Wang Q., Huang X., Nakamura A., Burchard W., & Hallett F. R. *Molecular characterization of soybean polysaccharides: an approach by size exclusion chromatography, dynamic and static light scattering methods,* Carbohydrate Research, **340(17)**, 2637-2644 (2005).
12. Huihuang Ding. Doctoral thesis *Dietary Fibres from Flaxseed Kernel: Structural and Conformational Characterization, and Structure-Function Relationship*, The University of Guelph, Ontario, Canada (2015).

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.