

INFLUENCES OF MOLECULAR WEIGHT AND BRANCHING PARAMETER OF LACQUER POLYSACCHARIDE ON THE GROWTH OF LEUCOCYTES

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ABSTRACT

A method of determining branching parameter of lacquer polysaccharide was established by acid-base back-titration of terminal uronic acid of branches. The branching factors obtained are in agreement with the values determined by colorimetric method with carbazole and the results estimated by using Zimm-Stockmayer equation from viscosity data. Influences of molecular weights and branching factors of five fractions of lacquer polysaccharide on the bioactivities were studied. The results show that the polysaccharides have bioactivities in motivating the growth of leucocytes, and the effect increases with the decrease of molecular weight and branching factor in the range studied ($17 \times 10^4 > M_w > 4 \times 10^4$).

A great number of cultural treasures coated with Chinese lacquer have been preserved for more than a thousand years without having lost their original elegant beauty. Today fascinating objects of art made from lacquer are interesting to note that the lacquer is made up from novel plant polysaccharide. Studies on the lacquer polysaccharide showed that the macromolecule not only plays an important role in the durability of lacquer films^[1], but also has bioactivities in motivating the growth of leucocytes and anticoagulation activities^[2]. However, structural correlation to bioactivity

effects of the polysaccharide is not yet fully understood. This has prompted us to study the relationship of molecular weights and branching parameter of the polysaccharide on the growth of leucocytes in mice. Moreover it is an important project in today's fields of medicine to seek highly effective and non-toxic drug for the growth of leucocyte.

Oshima et al.^[3] reported that the lacquer polysaccharide from Chinese lac trees (*Rhus vernicifera*) consists of β -(1 $\rightarrow$ 3)-linked-D-galactose main chain with a number of branches having terminal uronic acid groups. In the previous work^[4], the molecular weights M_w and M_n of the polysaccharide from Jianshi lac trees in Hubei were determined by light scattering and membrane osmometry^[4]. However, the branching degrees of the polysaccharides are yet unknown. Usually the branching parameter (g) defined by Zimm and Stockmayer^[5] by Eq. (1) and (2) is taken as a measure of branching

$$g = \langle R \rangle_b^2 / \langle R \rangle_l^2 \quad (1)$$

$$g^\epsilon = [\eta]_b / [\eta]_l \quad (2)$$

where ϵ is the branching exponent dependent on the type of branching and the reported value of ϵ is 0.5 for starlike and random branching^[6], $\langle R \rangle_b$ and $\langle R \rangle_l$ are radius of gyration of the branched and linear polymer, respectively. The value of g decreases rapidly with the increase of the number of branches. To obtain the g -factor it requires the availability of the linear polymer having the same chemical composition as the branched polymer. But it is often not possible to have linear polysaccharide with the same chemical composition as the lacquer polysaccharide. In this work, a method for determining branching factor of lacquer polysaccharide by titration of terminal uronic acid of branches was presented.

EXPERIMENTAL

Potentiometric titration

The previously investigated Jianshi lacquer polysaccharide samples^[4] CJ-FR1, CJ-FR5, CJ-FR6, CJ-FR7, CJ-FR9, which gave the sodium salt of the polysaccharide (pH 7) were used in this work. Each sample was dissolved in distilled water, and the solution was passed through the cation-exchanger (No. 732 H⁺) to obtain an acid-form polysaccharide, followed by concentrating under reduced pressure below 50°C to give about 5 g/L

polysaccharide solution. The test solution (20 mL) was neutralized under a nitrogen atmosphere and stirred for 5 hr with 1.5 mL 0.1340 *N* sodium hydroxide (NaOH) aqueous solution. The excess base of the solution was titrated under the same condition with 0.1024 *N* hydrochloric acid (HCl). The concentration of uronic acid-group was determined according to data of NaOH in the test solution at the end point of the titration. For pH measurements, the pH S-10 B meter made by Xiaoshan Sci. Instrument Factory was used. The concentration of the polysaccharide solution was checked by ultraviolet spectroscopy (UV-240 Shimadzu, Japan) based on working curve of concentration dependence of UV-absorptivity (*A*) at 199nm. The glucuronic acid content (C_{GA}) of the polysaccharide was calculated according to the relation

$$C_{GA} = \frac{[N_b V_b - N_a (V_a - V_c)]}{V_p} / (C_p / 162) \quad (3)$$

where V_p -volume of test polysaccharide solution (mL); N_b and N_a -equivalent concentration of standard NaOH and HCl aqueous solution respectively; V_b -volume (mL) of standard NaOH solution added into test solution; V_a and V_c -volume of standard HCl consumed for test polysaccharide and blank assay (mL); C_p -concentration (g/L) of polysaccharide solution. For comparison, a colorimetric method of Dische's carbazole reaction for uronic acid in the presence of borate was used^[7].

Assay of the growth of leucocyte

86 healthful mice of Kunming type (each weighing 18—22g) were divided into drug test and control group with 10—20 mice per group. The test polysaccharides, dissolved in distilled water, were injected intraperitoneally (i. p.) at dosages of 200 mg/Kg for 4 days. For control group, physiological salt solution was injected intraperitoneally at dosages of 0.2 mL/mouse for 4 days. At the 5th day, the mice were killed, and blood was taken to be investigated.

RESULTS AND DISCUSSION

The molecular weights M_w and M_n of the polysaccharide samples, which have been reported in our previous work, and glucuronic acid content C_{GA} calculated from Equation (3) are summarized in Table 1. The C_{GA} values are in good agreement with that determined by colorimetric method with carbazole reagent and the results (27.8 mol% for FR1, 26.2 mol% for

FR2) of the polysaccharide from Chinese lacquer reported by Oshima et al.^[3]. The polysaccharide is regarded as molecules with trifunctional branch units distributed at random. The branching frequency $\bar{\lambda}$ based on end-group analyses of branches are defined as

$$\bar{\lambda} = n_n / M_n \quad (4)$$

$$n_n = C_{GA} \times M_n / 162 \quad (5)$$

where n_n -the number average number of branch units per molecule; C_{GA} - glucuronic acid content. According to Zimm-Stockmayer^[5], the branching factor g can be similarly expressed in terms of n_n

$$g = \frac{6}{n_n} \left\{ \frac{1}{2} \left(\frac{2 + n_n}{n_n} \right)^{1/2} \ln \left[\frac{(2 + n_n)^{1/2} + n_n^{1/2}}{(2 + n_n)^{1/2} - n_n^{1/2}} \right] - 1 \right\} \quad (6)$$

The values of n_n and g of the lacquer polysaccharides were calculated from Equation (4), (5), (6), and are summarized in Table 1. These formulas are restricted in the application to trifunctional branch units distributed at heterogeneous systems.

Table 1 Molecular weights and branching parameters of the lacquer polysaccharides

Sample	$M_w \times 10^{-4}$	$M_n \times 10^{-4}$	C_{GA} (mol%)		n_n	$\bar{\lambda} \times 10^3$	g	
			Titration	Carbazole method			Estimated by Eq. (3)(5)(6)	Estimated by Eq. (7)(8)(2)
CJ-FR1	16.9	8.67	27.7	—	148	1.71	0.076	0.067
CJ-FR5	9.71	5.91	26.5	26.6	97	1.64	0.104	0.081
CJ-FR6	8.33	5.56	26.7	26.3	92	1.65	0.107	0.091
CJ-FR7	7.58	4.72	19.3	—	56	1.19	0.152	0.089
CJ-FR9	5.99	4.07	20.6	—	52	1.27	0.160	0.096

Branched molecules are more compact than linear molecules of the same molecular weight and chemical composition. Therefore intrinsic viscosity of the branched polymer $[\eta]_b$ is less than that of linear polymer $[\eta]_l$ in the same solvent. If pullulan (a linear polysaccharide with α -1,6-glucosidic linkage) is regarded as similar as linear lacquer polysaccharide, g -factor can be roughly estimated by Equation (2), (7), and (8)

$$[\eta] = 1.79 \times 10^{-2} M^{0.67} (\text{pullulan in } 0.1 \text{ M NaCl}^{[8]}) \quad (7)$$

$$[\eta] = 3.59 \times 10^{-2} M^{0.50} (\text{lacquer polysaccharide in } 0.1 \text{ M NaCl}^{[4]}) \quad (8)$$

The values are in good agreement with that obtained from end group analysis (see Table 1).

The bioactivities of the lacquer polysaccharide were tested on the

growth of leucocyte counts of mice. In Table 2, the leucocyte counts of mice are compared. The leucocyte counts of mice injected with the polysaccharides were significantly higher than that of control group. It suggests that the polysaccharides have bioactivities in motivating the growth of leucocytes. As shown in Table 2, the polysaccharide having low molecular weight and little branching gives high leucocyte counts.

Table 2 Comparison of bioactivities in motivating growth of leucocyte for fractions of lacquer polysaccharide

Group	Mice	Dose	Leucocyte count ($10^9/L$) $\bar{X} \pm SD$
		mg/kg $\times$ days	
CJ-FR1	11	200 $\times$ 4	5854 $\pm$ 2407
CJ-FR5	20	200 $\times$ 4	5590 $\pm$ 1309
CJ-FR6	10	200 $\times$ 4	7170 $\pm$ 1513
CJ-FR7	10	200 $\times$ 4	7076 $\pm$ 2052
CJ-FR9	10	200 $\times$ 4	7600 $\pm$ 1658
Control	16	0.2 mL	4900 $\pm$ 1263

We conclude that the molecular weight and branching factor of the polysaccharides obviously influence the growth of leucocytes, and the bioactivity increases with the decrease of both of them in the range studied ($17 \times 10^4 > M_w > 4 \times 10^4$). These results provide useful information on structure-activity relation of the polysaccharides.

Keywords:

Lacquer polysaccharide; Molecular weight; Branching parameter; Structure-activity relation; Bioactivity; Growth of leucocyte

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