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Studies on fractionation and molecular weights of Chinese lacquer polysaccharide

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An ionic branched polysaccharide, isolated from the sap of the Chinese lac tree (*Rhus vernicifera*), was separated into 10 fractions by isopropanol reprecipitation from an aqueous 0·1 M NaCl solution of 1 wt% polysaccharide. The molecular weights and solution properties were investigated by low angle laser light scattering, rapid static membrane osmometry and viscometry. The Mark-Houwink equations of the polysaccharide in aqueous 0·1 M NaCl at 30°C were established to be: $[\eta] = 3{\cdot}59 \times 10^{-2} M_w^{0{\cdot}50}$ and $[\eta] = 2{\cdot}15 \times 10^{-2} M_n^{0{\cdot}59}$. The second virial coefficients A_2, unperturbed dimensions $<h^2>_0/M$ and expansion factors α_η obtained suggested that the polysaccharide exists in aqueous solution as a dense random coil, in consequence of its highly branched structure. In addition, the differential molecular weight distribution curve of the polysaccharide obtained from the experimental data shows double peaks, which is in agreement with data in the literature.

INTRODUCTION

Polysaccharides have been utilized in a variety of ways (Kennedy, 1989) for a variety of reasons. However, little has been written on their use in any form of art. Lacquer has been used in Asian countries for thousands of years as a durable coating material. It has also been employed as a coating for objects in everyday use, works of art and industrial equipment. A great number of cultural treasures coated with Chinese lacquer have been preserved for more than 1000 years without having lost their original elegant beauty. Chinese lacquer was utilized in Europe hundreds of years ago when woodwork was sent to China for lacquering and returned. Today fascinating Chinese manufactured objects of art made from Chinese lacquer are being seen on the market again, and it is interesting to note that the lacquer is made up from novel plant polysaccharides. Chinese lacquer is composed of urushiol (60%), glycoprotein (2·1-1·8%), gummy substance (6-7%), which contains enzymes, stellacyanin and some of mono-, oligo- and polysaccharides, and water (30%).

In the orient, three kinds of lacquer trees are grown: *Rhus vernicifera* (China, Korea and Japan), *Rhus succedanea* (Vietnam and Taiwan) and *Melanorrhoea usitate* (Burma and Thailand). Chinese lacquers are named according to their place of production. Recent attention to the lacquer polysaccharide showed that the macromolecule plays an important role in the durability of lacquer films by protecting the polymerized urushiol from oxidative degradation (Kumanotani *et al.*, 1978). In addition, the polysaccharide was found to have bioactivities in motivating the growth of leucocytes and anticoagulation activities in mice (Kong, 1991). In this respect, the biological activities of the polysaccharide from Chinese Jianshi lacquer is more effective than others. Knowledge of the chemical structure, molecular weight and conformation of the

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polysaccharide is valuable for an understanding of these bioactivities, and low angle laser scattering has become an important technique (Jumel *et al.*, 1991). It has been reported that the lacquer polysaccharide from Chinese lac trees (*R. vernicifera*) consists of a (1→3)-linked β-D-galactose main chain with a number of branches, mostly having a terminal uronic acid group (Oshima & Kumanotani, 1984). In previous work (Zhang *et al.*, 1989), the molecular weights of the polysaccharide from the sap of the Vietnamese lac tree (*R. succedanea*) were determined by light scattering and membrane osmometry, but these values are much higher than those of Chinese lacquer polysaccharides estimated by aqueous-phase GPC (Oshima and Kumanotani, 1984). Therefore, the molecular weights and solution properties of Chinese lacquer polysaccharide have, until now, been inadequately researched.

This paper reports on some of the physicochemical aspects of lacquer polysaccharide. The solubility of the polysaccharide in water is very high, so it is not precipitated readily by organic solvent. The unusual solution behaviour of the polysaccharide in pure water is undoubtedly due to its ionic character. In the present study, a search was made for a suitable solvent for the fractional reprecipitation of Jianshi lacquer polysaccharide. Molecular weight data obtained experimentally are discussed in terms of the molecular size and conformation of the polysaccharide in aqueous solution.

EXPERIMENTAL

Isolation of polysaccharide

Sap from Chinese lac trees from Jianshi in Hubei province (total solids content 70–80%) was supplied by the Wuhan Chinese lacquer factory (Wuhan). The isolation process is as follows: the sap (1 kg of sap liquid) was mixed with five times its volume of acetone and stirred for 1 h. Then the mixture was filtered to yield urushiol acetone solution and residue (yellow acetone-dried powder), which was washed with 20 times its volume of acetone and dried in air (yield 95 g). The acetone-dried powder (50 g) was stirred in 1000 ml twice-distilled water for 16 h at 5–10°C, then filtered. The clear blue coloured filtrate was purified by adding CM-Sephadex C-50 (a carboxymethyl dextran gel) step by step in a chromatographic column (20 × 6 cm diameter). The blue band remained on the top of the column, and, at the end, could be eluted with 0·05 M phosphate buffer (pH 7) to give enzymes and stellacyanin, which were monitored according to Reinhammar's methods (1970) by Xiao and Du (1991). First, the yellow band of combined mono-, oligo- and polysaccharide was eluted from the column with distilled water. Then the carbohydrate elutent was injected into a column packed with cation-exchange resin (No. 732, H^+) to obtain an acid-form polysaccharide, followed by neutralization with 1·0 M NaOH to give the sodium salt of the polysaccharide (pH 7).

One tenth of the original polysaccharide solution was dialysed as an unfractionated sample against twice-distilled water for 3 days to remove mono- and oligosaccharide, and then rotary evaporated under reduced pressure below 50°C, dried *in vacuo* at 45°C to give a colourless powder of the polysaccharide (yield 1·43 g, coded as CJ-unFR). The polysaccharide content (based on this yield) in the Chinese lacquer was calculated to be 2·66%. The main impurities of the polysaccharide are the enzymes, stellacyanin and yellow pigments of different molecular weights, hence the purity was checked by ultraviolet spectroscopy (UV-300 Shimadsu, Japan) and high pressure liquid chromatographs (LC-6A, column: GPC DIOL-300, 50 cm × 7·9 mm diameter, Shimadzu, Japan). The results showed that in the UV absorption curve there was only a peak at approximately 220 nm containing polysaccharide, and absorptions at 250 nm, 280 nm and 600 nm of enzymes stellacyanin and pigments were zero. Only a bimodal molecular weight distribution represented the lacquer polysaccharide (Oshima and Kumanotoni, 1984) was obtained in the GPC graph. Kong (1991) demonstrated that the polysaccharide contains D-galactose (68 wt%), 4-O-methyl-D-glucuronic acid and D-glucuronic acid (24 wt%), L-arabinose (5·8 wt%) and L-rhamnose (1·9 wt%), and consists of (1→3)-D-galactopyran main chain.

Fractionation

The original polysaccharide solution mentioned above (900 ml) was diluted to 1000 ml, and NaCl (5·83 g) added to give 0·1 M NaCl aqueous solution. The polysaccharide solution was fractionated by addition of isopropanol as precipitant at 25°C according to the non-solvent addition method (Rabek, 1980). The first 800 ml of isopropanol was added until the solution turned milky. The turbid solution was then warmed through several degrees to make it transparent again. After standing for 16 h at 25°C, separation into liquid and gel phases (L_1 and G_1) occurred (Fig. 1). After the gel G_1 had been removed, the liquid L_1 was subjected to the next fractionation step. In order to decrease tail effect it is necessary to refractionate each fraction. Hence, the G_1 was dissolved in 0·1 M NaCl aqueous solution, and reprecipitated with isopropanol to give gel C_1 (the subscript to C means fraction number) and liquid phase (part of G_2). The C_1 was diluted with twice-distilled water and dialysed against twice-distilled water for 96 h at 25°C, then rotary evaporated at reduced pressure below 50°C to obtain fraction

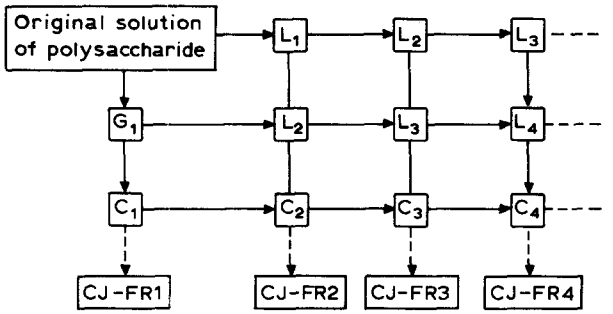

Fig. 1. Fractionated scheme for Chinese lacquer polysaccharide with 0·1 M NaCl as solvent and isopropanol as precipitant at 20°C.

CJ-FR1. The other fractions shown in Fig. 1 were obtained by the same process.

Light scattering measurements

The scattering light intensities were observed on a chromatic low angle laser light scattering photometer KMX-6 (LDC/Milton Roy, Sunnyvale, CA) at 30 ± 0·5°C. The light source was an unpolarized 2 mM He-Ne laser of 632·8 nm and scattering angle 4·96°. The polysaccharide solutions were prepared with aqueous 0·1 M NaCl and used within 8 h after preparation. Optical clarification of the solution was made by centrifugation at 10^4 rpm for 30 min, and then filtered through a 0·2 μm pore size (MILLEX-HV) filter directly into the scattering cell. The refractive index increment dn/dc was measured on a Chromatic KMX-16 at 632·8 nm at 30 ± 0·5°C. The value of dn/dc was 0·139 cm^3/g for the polysaccharide solution, which dialysed against aqueous 0·1 M NaCl for 48 h.

Osmometry

Osmotic pressure (π) of the polysaccharide in 0·1 M NaCl aqueous solution was measured with an improved Bruss membrane osmometer by rapid static equlibrium at 30 ± 0·5°C (Zhang *et al.*, 1991). A regenerated cellulose membrane coded ZNA67-2 with a pore size of 7 nm, prepared in our Chinese laboratory, was used. The values of number average molecular weight M_n and second virial coefficient A_2^{OS} were given by:

$$\pi/RTc = 1/M_n + A_2^{OS}c + \ldots \quad (1)$$

where R is the gas constant, T is the absolute temperature (K), and c is the polysaccharide concentration (g/cm^3).

Viscometry

Viscosities of the polysaccharide in aqueous 0·1 M NaCl were measured at 30 ± 0·5°C using a modified capillary viscometer supplied by the Institute for Industrial Science in Tokyo University. The kinetic energy correction was always negligible. Huggins' plot was used to estimate the intrinsic viscosity $[\eta]$ and Huggins' constant k'.

RESULTS AND DISCUSSION

The values of weight average molecular weight M_w and second virial coefficient A_2^{LS} of the samples evaluated from the scattering intensity by the conventional method are presented in Table 1, together with those of M_n, A_2^{OS}, $[\eta]$, k' and polydispersity indexes M_w/M_n. All the extrapolations of η_{sp}/c against c are based on good linear relationships, and the values of k' are within the range 0·22–0·34. This suggests that the Donnan effects of the polyelectrolyte solution have been inhibited, and the normal polymer solution behaviours are exhibited for the lacquer polysaccharide in aqueous 0·1 M NaCl. The values of M_w and M_n for fractions and unfractioned samples are close to those of Vietnam

Table 1. Molecular size measurements for Chinese lacquer polysaccharide in 0·1 M NaCl at 30°C

Sample number	Fraction weight	Light scattering		Osmometry		Viscometry		M_w/M_n
	w_i (g)	$M_w \times 10^{-4}$	$A_2 \times 10^4$ (cm^3mol/g^2)	$M_n \times 10^{-4}$	$A_2 \times 10^4$ (cm^3mol/g^2)	$[\eta]$ (cm^3/g)	k'	
CJ-FR1	0·449	16·9	3·86	8·67	10·05	14·7	0·22	1·95
CJ-FR2	0·794	14·7	3·86	7·69	9·95	12·9	0·22	1·91
CJ-FR3	0·902	13·5	3·90	6·77	5·30	12·2	0·27	1·99
CJ-FR4	1·232	11·3	4·01	6·34	6·20	11·9	0·25	1·79
CJ-FR5	1·616	9·71	2·90	5·91	6·40	11·2	0·26	1·64
CJ-FR6	1·467	8·33	2·80	5·56	6·95	10·7	0·28	1·50
CJ-FR7	1·356	7·58	4·03	4·72	6·25	9·94	0·30	1·61
CJ-FR8	1·303	7·14	3·74	4·53	6·35	9·71	0·31	1·58
CJ-FR9	1·954	5·99	3·86	4·07	7·10	8·80	0·34	1·47
CJ-FR10	0·946	5·32	3·52	3·73	10·6	8·36	0·34	1·43
CJ-unFR	1·430	9·86	0·22	5·71	9·61	10·2		1·73
CJ-unFR[a]		9·31		5·44		10·6		1·71

[a]Values calculated from data of 10 fractions by definition.

lacquer polysaccharide (Zhang *et al.*, 1989). The experimental values of M_w, M_n and $[\eta]$ of CJ-unFR are the same as those estimated from 10 fractions according to the definition. The satisfactory results indicate that the fractionation method for the polysaccharide in aqueous 0·1 M NaCl using isopropanol precipitation was successful.

The integral molecular weight distribution of Chinese lacquer polysaccharide was obtained from M_w and weights of fraction w_i according to the Tung method (1956) as follows:

$$I_j = \left(\sum_{i=1}^{j-1} w_i + \frac{1}{2} w_j \right) / \sum_{i=1}^{10} w_i \qquad (2)$$

where I_j is the fraction of cumulative weights of the fractions. Thus the differential molecular weight distribution curve can be drawn with the slope values of integral curve (Fig. 2). The differential distribution curve consists of two peaks, which is similar to the GPC chromatogram reported by Oshima and Kumanotani (1984). The values of M_w for two peaks were estimated to be $13{\cdot}4 \times 10^4$ and $7{\cdot}45 \times 10^4$, respectively, which are much higher than M_w ($8{\cdot}40 \times 10^4$ and $2{\cdot}77 \times 10^4$) obtained by Oshima and Kumanotani (1984) using GPC. The error of their molecular weights may be attributed to the GPC calibration being achieved by dextrans which are largely unbranched. As the degree of branching increases (amylose 0% branched; amylopectin 4–5% branched; glycogen 10% branched) for a common elution volume the molecular weight increases, as evidenced by Yu and Rollings (1987).

Estimation of the dependence of A_2^{LS} on M_w for the fractionated samples in aqueous 0·1 M NaCl at 30°C (Fig. 3) showed that A_2^{LA} is almost independent of molecular weight. Similar results have been reported by Takahashi *et al.* (1967) who showed that the A_2 of

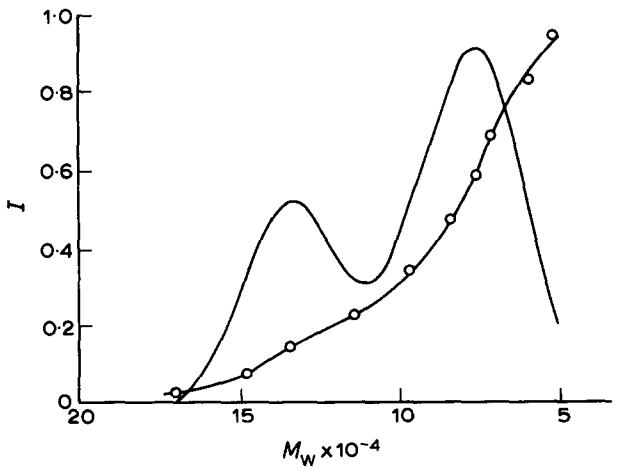

Fig. 2. Plot of integral molecular weight (M_w) distribution curve (—O—) and differential molecular weight distribution curve (———) of Chinese lacquer polysaccharide.

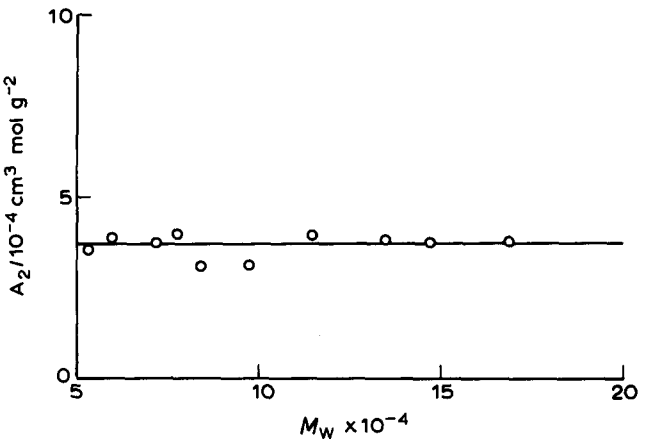

Fig. 3. Plot of A_2^{LS} versus M_w for fractionated samples of Chinese lacquer polysaccharide in 0·1 M NaCl at 30°C.

sodium polystyrene sulphonate in aqueous NaCl exhibits no molecular weight dependence at ionic strength $c_s < 0{\cdot}05$ M, and a slight dependence at higher ionic strength. In addition, the data for A_2^{LS} and A_2^{OS} suggest that aqueous 0·1 M NaCl has a much better solvent power than θ solvent. The reduced viscosity η_{sp}/c of sample CJ-unFR in aqueous 0·1 M NaCl remained unchanged for 2 weeks at room temperature. This implies that neither gelation nor degradation had occurred and that the polysaccharide solution is perfectly stable. It can be concluded that aqueous 0·1 M NaCl is a good solvent for the lacquer polysaccharide.

From double-logarithmic plots of $[\eta]$ against M_w and M_n, respectively, for 10 fractions in aqueous 0·1 M NaCl at 30°C, the Mark-Houwink equations for Chinese lacquer polysaccharide were established as follows:

$$[\eta] = 3{\cdot}59 \times 10^{-2} M_w^{0{\cdot}50} \qquad (3)$$

$$[\eta] = 2{\cdot}15 \times 10^{-2} M_n^{0{\cdot}59} \qquad (4)$$

The two exponents (α) approximate to 0·5, and are close to 0·52 and 0·56 the values of Vietnam lacquer polysaccharide in aqueous 0·08 M KCl/0·1 M NaAc and in aqueous 0·5 M KCl/10% cadoxen at 30°C (Zhang *et al.*, 1989; Zhang & Zhang, 1989). The values of α close to 0·5 suggest that the molecules of the lacquer polysaccharide in aqueous solution exist as random coils with compact chain conformation. As mentioned above, aqueous 0·1 M NaCl is a good solvent, therefore the 'compact aspect' should represent dense chains, and results from highly branched structure. This is in agreement with Granath (1958), who found that α decreases with increasing degree of branching of dextran.

It is worth noting that the plot of $[\eta]$-M_w relationship shows a slight downward curvature at larger fractions of CJ-FR2 - CJ-FR4 (Fig. 4). Possibly, the samples of CJ-FR2 - CJ-FR4 contain not only high molecular

Table 2. Comparison of the unperturbed dimensions and expansion factors for Chinese lacquer polysaccharide, pullulan and dextran

Sample	Solvent	$M_w \times 10^{-4}$	α_η	$K_\theta \times 10^2$ (cm^3/g)	$<h^2>_0/M$ (cm^2mol/g)	Source
CJ-FR4	0·1 M NaCl	11·3	1·21	3·55	$2·69 \times 10^{-17}$	This work and
CJ-FR5		9·71	1·19			Qiu *et al.* (1991)
CJ-FR6		8·33	1·18			
CJ-FR7		7·58	1·16			
CJ-FR8		7·14	1·16			
Pullulan PF806	0·02% NaN_3	34·7	1·17[a]	11·5	$5·59 \times 10^{-17}$	Kato *et al.* (1982)
Pullulan PF305		29·1	1·16[a]		$6·06 \times 10^{-17}$	
Dextran	Water	3·2		9·1	$5·03 \times 10^{-17}$	Gekko (1981)

[a]Value of α_s.

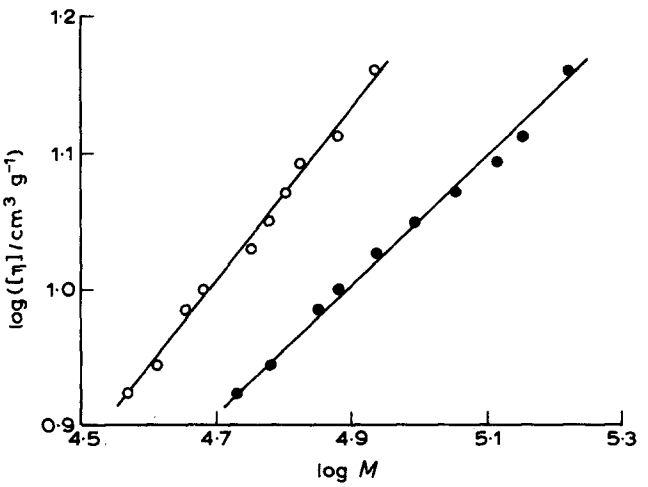

Fig. 4. Double-logarithmic plots of $[\eta]$ versus M_w (●) and $[\eta]$ versus M_n (○) for fractionated samples of Chinese lacquer polysaccharide in 0·1 M NaCl at 30°C.

weight components but also more highly branched components, which result from fractional precipitation. However, the slight difference in the branching of the polysaccharide has not markedly influenced the $[\eta]$-M_w and $[\eta]$-M_n equations in the range studied.

In our recent work (Qiu *et al.*, 1991), the intrinsic viscosities $[\eta]_\theta$ in θ solvent for samples of CJ-FR4 - CJ-FR8 were measured, and expansion factors of $\alpha_\eta = ([\eta]/[\eta]_\theta)^{1/3}$ were estimated. According to the Stockmayer-Fixman theory (1963) the two curves of $[\eta]/M_w^{1/2}$ against $M_w^{1/2}$ and $[\eta]_\theta/M_w^{1/2}$ against $M_w^{1/2}$ for the polysaccharide solution crossed to achieve the common intercept with $K_\theta = 3·55 \times 10^{-2}$. The K_θ relates to unperturbed dimensions $<h^2>_0/M$ of polymer by:

$$K_\theta = \phi_0(<h^2>_0/M)^{3/2} \quad (5)$$

where the value of ϕ_0 is assumed to be $2·55 \times 10^{23}$; $<h^2>_0$, the mean-square end distance under θ condition (Scholtan and Ying, 1967). The values of α_η and $<h^2>_0/M$ of the lacquer polysaccharide are summarized in Table 2. The expansion factors α_s and $<h^2>_0/M$ of pullulan and dextran, which are linear and 5% branched polysaccharide, respectively, were collected here (Gekko, 1981; Kato *et al.*, 1982). The values of α_s or α_η (1·1-1·2) of these polysaccharides are very low, and the $<h^2>_0/M$ value of the lacquer polysaccharide is lower than that of the pullulan and dextran. It implied that the lacquer polysaccharide molecules in aqueous solution behave as significantly flexible chains with limited expansion, as a consequence of highly branched structures.

From the above results it is concluded that Chinese lacquer polysaccharide has the following properties: the molecular weights, $M_w = 9·86 \times 10^4$ and $M_n = 5·71 \times 10^4$; conformation, the molecules exist as a dense random coil in aqueous solution; it exhibits regular polymer solution behaviour in aqueous 0·1 M NaCl. The $[\eta]$-M_w and $[\eta]$-M_n relationships established can be conveniently used for molecular weight determination by viscometry or GPC. However, as for the connection of the molecular weight and solution properties with bioactivities further investigation will be necessary.

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