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Chemical modification, characterization and bioactivity of Chinese lacquer polysaccharides from lac tree *Rhus vernicifera* against leukopenia induced by cyclophosphamide

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Abstract

Lacquer polysaccharide (LP) was isolated from the sap of lac tree (*Rhus vernicifera*). Its derivatives, carboxymethyl LP, sulfated LP and debranching LP were prepared. Their structure was analyzed by GPC, FT-IR and NMR spectroscopy. The sugar components of carboxymethyl and sulfated LPs hardly changed, but the molecular weight of the former decreased. The side chains of LPs were partially removed using sodium periodate in mild conditions and the pyranose ring β-configuration of products obtained was not changed. Bioactivity of natural and modified LPs against leukopenia induced by cyclophosphamide (CP) was investigated in mice. LP exhibited a significant bioactivity ($P < 0.05$) compared to positive control group (CP). The bioactivity could increase slightly with the increasing of the contents of carboxymethyl groups. However, with the removal of the side chains and the incorporation of sulfate groups, the bioactivity gradually decreased. These showed that the bioactivity of lacquer polysaccharides against leukopenia induced by CP was strongly dependent on the types of ionic groups of the polysaccharides and concerned with the side chains with 4-*O*-methyl-β-glucuronic acid in the terminal.

Keywords: Lacquer polysaccharide; Chemical modification; Leukopenia

1. Introduction

Varieties of polysaccharides from varieties of sources such as animal, plant cell walls, and fungal cells, possess marked immunological properties ranging from non-specific stimulation of host immune system, resulting in anti-tumor, anti-viral, and anti-infective effects, to anti-oxidant, anti-mutagenic or hematopoietic activity. The activity of polysaccharides is close related to their structure, i.e. molecular weight, degree of substitution, degree of branching, conformation in solution, sugar component and the structures of main chain and branches (Bohn & BeMiller, 1995; Kennedy & White, 1983; Kennedy, 1989). Every polysaccharide has its special biological activity because of its specific molecular structure. At present, the study on the structural correlation to bioactivity effects is a most important aspect in the fields of polysaccharides.

Chinese lacquer polysaccharide (LP), isolated from the sap of lac tree (*Rhus vernicifera*), is an acidic hetero-polysaccharide and has a 1,3-β-linked D-galactopyranosidic main chain having complex branches with 4-*O*-methyl-β-glucuronic acid in the terminal (Oshima and Kumanotani, 1984; Du, Kong, & Li, 1994). The lacquer polysaccharides contained in the gummy substanse of the lacquer coating play a significant role in making the characteristic architecture, and are concerned with the emulsion polymerization of urushiol with laccase and the highly durable properties of lacquer films (Kumanotani, 1998). The investigation of the molecular weights and solution properties by low angle laser light scattering, rapid static membrane osmometry and viscometry suggested that the polysaccharide exists in aqueous solution as a dense random coil, in consequence of its highly branched structure (Zhang et al., 1992. The lacquer polysaccharide having complex branches might have a swelled structure in the sap. The urushiol and laccase in the sap were dispersed as an emulsion and penetrated into the branches of the swelled polysaccharide and then polymerized enzymatically

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and oxidatively to afford the durable polymers with the polysaccharide in the network (Lu, Yoshida, & Uryu, 1999b). Recently, the polysaccharide was found to have bioactivity in motivating the growth of leucocytes (Du, Yang, Kong, & Xiao, 1999) and anti-tumor (Lu et al., 2000). In this work, we prepared several derivatives, such as carboxymethyl LP (CMLP), sulfated LP (SLP) and debranching LP (DLP). Their bioactivity against leukopenia induced by cyclophosphamide was first estimated and the structural correlation to bioactivity was primarily described.

2. Experimental

2.1. Materials

Lacquer polysaccharide was obtained from the sap of Chinese lac tree from Maoba in Hubei province. The isolation process is based on the process described in the literature (Du et al., 1994). D-glucuronic acid was purchased from Sigma Chemical Co. (St Louis, USA). All other chemicals used were of analytical grade.

2.2. Carboxymethylation of lacquer polysaccharides

CMLP1 was prepared as follows (Misaki, Kakuta, Sasaki, Tanaka, & Miyaji, 1981): a suspension of 2.5 g of the lacquer polysaccharide in 50 ml of 2-propanol was stirred at room temperature for 60 min. Then, 7.3 ml of a 30% solution of sodium hydroxide was slowly added with stirring over a period of about 20 min. Vigorous stir was continued at room temperature for about 60 min. Then, 1.25 g of monochloroacetic acid were added and the mixture was stirred at 60 °C for 5 h. The supernate was poured out and the precipitate was thoroughly washed with a mixture of methanol and acetic acid (v/v, 7:3), then, dissolved in 50 ml distilled water, followed by neutralization with 1.0 mol/l NaOH (pH 7). The solution was dialyzed against distilled water for 72 h. The dialysate was concentrated under reduced pressure below 45 °C, then, precipitated with anhydrous alcohol. The precipitate was collected after drying over phosphorus pentoxide under reduced pressure.

CMLP2 was prepared according to a similar procedure, except that the mass of chloroacetic acid and the volume of a 30% solution of NaOH used were 1.87 g and 9.0 ml, respectively.

2.3. Sulfation of lacquer polysaccharides

SLP1 was prepared as followed (Inoue et al., 1983): the LP powder (2 g) was dissolved in dry formamide (100 ml), and the mixture was stirred at room temperatures for 30 min, the sulfating reagent (1 ml $ClSO_3H + 4.5$ ml pyridine) was added. The stirring was continued at room temperature for 8 h, then, 750 ml of methanol was added. The precipitate was collected by centrifugation, washed successively with methanol, then dissolved in 110 ml distilled water, followed by neutralization with 1.0 mol/l NaOH (pH 7.0), dialyzed against distilled water for 72 h. The dialysate was concentrated under reduced pressure below 45 °C, then, precipitated with anhydrous alcohol. The precipitate was collected after drying over phosphorus pentoxide under reduced pressure.

SLP2 was prepared as followed: the LP powder (2 g) was dissolved in dry formamide (100 ml), and the mixture was stirred at room temperature for 30 min, the sulfating reagent (4.1 ml $ClSO_3H + 2$ ml pyridine) was added. The stirring was continued at room temperature for 2.5 h, then at 45 °C for 4 h. The mixture was treated in accordance with the operating steps mentioned above.

2.4. $NaIO_4$ oxidation of lacquer polysaccharides

DLP was prepared as follows (Chihara, Hamuro, Maeda, Arai, & Fukuoka, 1970): the LP powder (3.0 g) was dissolved in 200 ml distilled water, then 400 ml of a 0.075 mol/l solution of $NaIO_4$ was added. The reaction was maintained under 5 °C in dark place. In the reaction process, the solution was monitored with UV spectrophotometer until absorbance did not change, then 5 ml of ethylene glycol was added, and stirred for 30 min. The mixture was dialyzed against distilled water for 24 h, the dialysate was concentrated to 300 ml under reduced pressure bellow 45 °C, then 3.0 g of $NaBH_4$ in distilled water was added, kept dark and stirred at room temperature for 24 h, 1.2 g of $NaBH_4$ was added again. The reaction was continued at room temperature for 24 h, then adjusted PH to 5.5–6.0 with acetic acid to make the reaction over, stirred and hydrolyzed under 0.05 mol/l H_2SO_4 for 24 h. The mixture was dialyzed against distilled water for 72 h. The dialysate was concentrated under reduced pressure below 45 °C, then, precipitated with anhydrous alcohol. The precipitate was collected after having dried over phosphorus pentoxide under reduced pressure.

2.5. Characterization

The number average molecular weight (M_n) and weight average molecular weight (M_w) of sample were measured by a gel permeation chromatography (GPC). GPC system incorporated a TSP P100 instrument. Two columns in series (TSK G5000-pw and TSK G3000-pw) were used.The eluent was 0.01 mol/l phosphate buffer solution containing 0.2 mol/l $NaSO_4$. The flow rate was maintained at 1.0 ml /min. The temperature of the column was maintained at 30 °C. The eluent was monitored with RI 150 refractive index detector. The sample concentration was 0.4 mg/ml. The standards used to calibrate the column were TOSOH pullulan of defined Mw ranging from 2.7 to 788 kDa. All data provided by the GPC system were collected and analyzed using the Jiangshen Workstation software package.

Uronic acid was determined colorimetrically using the method described in the literature (Bitter & Muir, 1962) with a Simadz 1601 UV spectrophotometer. A calibration curve was constructed with D-glucuronic acid as standard. Sulfated content was determined colorimetrically by Antonopoulos' method (Antonopoulos, 1962). A calibration curve was constructed with sodium sulfate as standard. The content of carboxymethyl group was determined using acid–base back-titration described in the literature (Zhang & Ding, 1995).

FT-IR spectra were recorded with KBr pellets on a Nicolet FT-IR 360 and Nicolet 170SX FT-IR spectrophotometers. 16 scans at a resolution of 4 cm^{-1} were averaged and referenced against air.

^{13}C NMR spectra (125.7 MHz) were recorded on a Bruker AMX-500 NMR spectrometer at ambient temperature. The samples were dissolved in D_2O. Tetramethylsilane (TMS) was used as internal standard.

2.6. Bioactivity assay

In the bioactivity experiment, Kunming mice used were 10–12weeks-old, and weighed 18–20 g. The number of female and male mice was equal. The animals were randomly divided into eight groups with 10 animals each group. The control group was treated with 0.2 ml saline each animal. The dose of LP and its derivatives was 50 mg/kg. The dose of CP was 90 mg/kg, and the positive group was treated with only CP on seventh day. Six groups of mice were injected with LP and its derivatives and continued for six days, then injected with CP and polysaccharide solution on the seventh day. All mice were sacrificed on the ninth day. The number of leucocytes was counted before experiment and after experiment, respectively.

3. Results and discussion

3.1. Carboxymethylation of lacquer polysaccharides and characterization

Carboxymethylation is usually performed on suspensions in water-miscible solvent such as 2-propanol. In order to form active nucleophilic reagent, 30% NaOH is usually used. Depending on different molar ratio of $ClCH_2COOH$ to NaOH, the carboxymethyl polysaccharides with different degree of substitution can be obtained. The products obtained from lacquer polysaccharide by reaction with $ClCH_2COOH$ and NaOH were listed in Table 1. The degree of substitutions of CMLP1 and CMLP2 were 0.24 and 0.74, respectively.

Fig. 1 showed the FT-IR spectra of LP and CMLP2. In FT-IR spectrum of LP, the absorb band at 1612 cm^{-1} is due to asymmetrical COO^- stretching vibration, the absorb band at 1420 cm^{-1} is due to symmetrical COO^- stretching vibration. In the spectrum of CMLP2, the two bands shifted to 1607 and 1422 cm^{-1}, respectively. The increase of their absorbance showed that the carboxymethxyl groups were incorporated into the polysaccharides. However, in the reaction process, degradation was companied (Table 1). It was due to the β-elimination in an alkaline medium (Whistler & BeMiller, 1958). Although the molecular weights decreased, the content of uronic acid did not change basically (Table 1). The ^{13}C NMR spectra of LP and CMLP2 were shown in Fig. 2. In the ^{13}C NMR spectrum of LP, the signals at 178.3 and 177.8 ppm attribute to carbonyl carbon of 4-*O*-methyl-D-glucuronic acid, and D-glucuronic acid, respectively. The signals at 111.8, 107.1, 106.3, 105.2, 103.3, 102.8 ppm attribute to the anomeric carbons of α-L-AraF, β-D-GluA, β-D-Galp, 4-*O*-Me-GluA, α-L-Rha and α-D-Galp, respectively. The assignments of these signals are based on data found in the literatures (Oshima, 1984; Lu et al., 1999a). In the ^{13}C NMR spectrum of CMLP2, these signals mentioned above were still kept. These indicated that the structure of the original polysaccharides still remained except the molecular weights. The ^{13}C NMR spectrum also showed a new broad peak at 180 ppm. It was contributed to the signal of carbonyl group of the carboxymethyl group. Concerning the positions of substitution, due to the overlap of the signals at 70–78 ppm, their arrangement was difficult.

3.2. Sulfation of lacquer polysaccharides and characterization

Lacquer polysaccharides were sulfated using $ClSO_3H$ as sulfated reagent in the presence of pyridine. The rate

Table 1
Molecular structure parameters of lacquer polysaccharide and its derivatives

Polysaccharide	Molecular weight			Uronic acid (mol%)	DS (CH_2COO^-)	S(wt%)
	$M_w \times 10^{-4}$	$M_n \times 10^{-4}$	PD[a]			
LP	8.36	5.19	1.61	25.89	–	–
CMLP1	4.53	2.55	1.78	26.20	0.24	–
CMLP2	4.56	2.77	1.65	25.56	0.74	–
SLP1	9.25	5.66	1.63	25.10	–	0.43
SLP2	9.14	5.53	1.65	25.62	–	5.14
DLP	4.12	2.25	1.83	7.45	–	–

[a] Polydispersity.

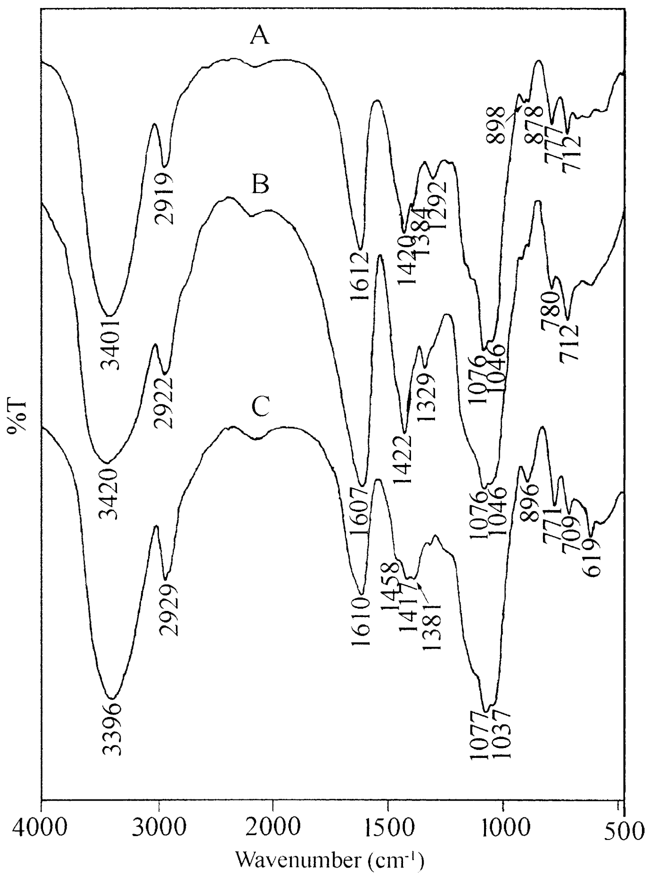

Fig. 1. FT-IR spectra of natural and modified lacquer polysaccharides (A) LP; (B) CMLP2; (C) DLP.

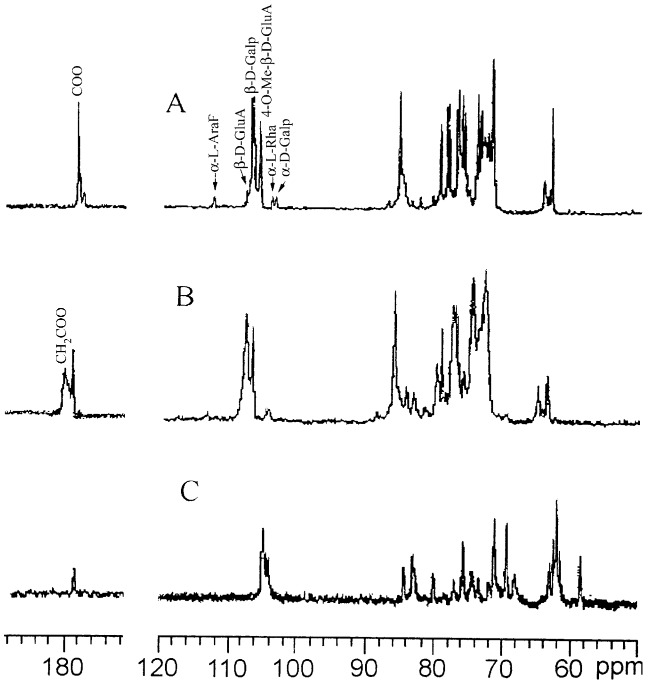

Fig. 2. ^{13}C NMR spectra of natural and modified lacquer polysaccharides (A) LP; (B) CMLP2; (C) DLP.

of degradation at high temperature was rather high, thus, the temperature of the reaction was selected for room temperature and 45 °C. Under this mild reaction condition, two products, SLP1 and SLP2 were prepared. FT-IR spectrum of SLP2 was shown in Fig. 3. The spectra showed two characteristic absorption bands, one at 1259 cm^{-1} describing an asymmetrical S=O stretching vibration and the other at 817 cm^{-1} indicating a symmetrical C–O–S vibration associated with a C–O–SO$_3$ group (Falshaw & Furneaux, 1998; Liao et al., 1996). These indicated incorporation of the sulfating group.

Molecular structure parameters of SLP1 and SLP2 were listed in Table 1. Under the reaction condition used, their degrees of sulfation were low, and their molecular weights were higher than LP's, due to incorporation of the sulfating group. Compared with SLP1, there was the very slight degradation in the process of SLP2's preparation. At the same time, the content of uronic acids of SLP1 and SLP2 were 25.10 and 25.62 mol%, and seldom changed compared with the content of LP during the sulfation reaction. In the spectrum of SLP2, two bands at 1623 and 1423 cm^{-1} were due to COO$^-$ stretching vibrations. The shoulder peak at 1734 cm^{-1} indicated the presence of COOH. Based on their integration, the same conclusion could be drawn about the content of uronic acids. Because of the structure of complex branches with 4-*O*-methyl-β-D-glucuronic acid in the terminal, it could be assumed that two sulfated polysaccharides kept the structure of LP.

3.3. Sodium periodate oxidation of lacquer polysaccharides and characterization

The carbon–carbon linkage between vicinal hydroxyl groups can be broken and the hydroxyl groups can be oxidized by periodic acid oxidation. After the newly formed aldehyde groups are reduced using NaBH$_4$, during the following hydrolysis step the acetal bonds in the opening ring sugar residue are hydrolyzed. Glycosidic linkages are not hydrolyzed except for sugars in furanose

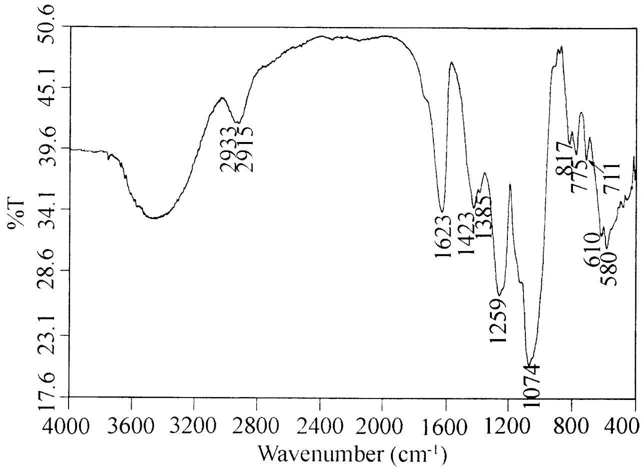

Fig. 3. FT-IR spectrum of SLP2.

form that are hydrolyzed under mild conditions. Lacquer polysaccharides are acidic polysaccharides having a 1,3-β-linked D-galactopyranosidic main chain. Thus 1,6-linked side chains of LP may be cut off by periodic acid oxidation and the main chain isn't influenced. Lacquer polysaccharides were debranched by oxidative degradation using sodium periodate in mild conditions. In the reaction process, the solution was monitored with UV spectrophotometer, until absorbance didn't change. The pH of the final solution was 3.17 at 6 °C.

The spectrum of DLP was shown in Fig. 1. In the spectrum of DLP, the band of asymmetrical COO^- stretching vibration was at 1607 cm^{-1}, and its absorbance was weak. The band of symmetrical COO^- stretching vibration at 1420 cm^{-1} disappeared. Quantitative analysis showed that the content of D-glucuronic acid was only 7.45 mol% (Table 1). These indicated that the side chains of polysaccharides were partially cut off. In the spectrum of LP, the band at 898 cm^{-1} attributes to β-anomer (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000). In the spectrum of DLP, the band still remained, but shifted to 896 cm^{-1}. This indicated that LP's pyranose ring β-configuration was not changed during the oxidative degradation using sodium periodate.

The NMR analysis showed in the spectra of debranching polysaccharides, DLP, the anomeric carbons signals of α-L-ArabF, α-L-Rha, -D-glucuronic acid and α-D-Galp disappeared. This suggested that these sugar units had been cut off (Fig. 2). The polysaccharides only consisted of β-D-galactose and 4-*O*-methyl-D-glucuronic acid. Based on the DEPT-135 and 90^{13}C NMR of DLP (data not shown), the signals at 61–64, 68 and about 71 ppm attributed to the primary hydroxyl groups. The integration of the signal of unlinked C-6 at 61–64 ppm increased, and that of linked C-6 at about 71 ppm decreased. These further indicated that the side chains of the polysaccharides were partially cut off. Furthermore, a new peak at 68 ppm appeared, corresponding to linked C-6 based on α-effect (Srivastava & Kulshreshtha, 1989). This also suggested that the sugar residues in side chains were partially cut off. At the same time, the signals of the LP and DLP spectra at high fields, ie, δ60.9 and 58.5 ppm, belonged to the methoxy carbons. The integration of OCH_3 in the DLP spectrum became less, and this also indicated that the branches were partially cut off.

3.4. Bioactivity against leukopenia induced by cyclophophamide

Table 2 showed the bioactivity of natural and modified LPs against leukopenia induced by cyclophophamide. CP is frequently used as cytostatic agent that exerts adverse immunosuppressive effects (Wilmer, Colvin, & Bloom, 1992). CP administration induced statistical decrease of the number of leucocytes in all CP-treated groups compared to saline control group. Among the groups of polysaccharide treatment prior to CP injection, LP, CMLP1, CMLP2, SLP1 had statistically significant ($P < 0.05$) bioactivity compared to cyclophosphamide control group. For CMLP1 and CMLP2, their M_ws were 4.53×10^4 and 4.56×10^4, respectively, and were lower than that of LP. The contents of carboxyl groups were 42.40 and 74.56 mol%, respectively, and were higher than that of LP (Table 1). Rate of decrease of leucocyte counts were less than that of LP. Although charge is a factor influencing immunological regulation of polysaccharides (Weiner, Langille, & Quintero, 1995), charge density was not apparently proportional to the bioactivity. In addition, this could also be related to their M_ws, because LP had the bioactivity in motivating the growth of leucocytes, and the effect increased with the decrease of M_ws in the range of 17×10^4–4×10^4 (Zhang & Ding, 1995). However, DLP had low M_w (4.12×10^4), less carboxyl groups (7.45 mol%) and side chains. Its rate of decrease of leucocyte counts was 33.42%, more than that of LP, 17.34%. Thus, the bioactivity of LP should be related to its complex branching structures and the content of carboxyl groups. But the effect of carboxymethyl groups was minor. Since the activity of polysaccharide is also close related to their solubility and conformation in solution (Bohn & BeMiller, 1995), this could be because

Table 2
The changes of leucocyte counts in mice before experiment and after experiment

Polysaccharide	Dose (mg/kg) and days	Mice	Source	LCb[a] ($\bar{X} \pm SD \times 10^{12}$/L)	LCa[a] ($\bar{X} \pm SD \times 10^{12}$/L)	Rate[b] (%)
LP	50 × 8	10	i.p.	8.20 ± 1.44	6.77 ± 1.65[c]	17.43
CMLP1	50 × 8	10	i.p.	7.70 ± 0.29	6.74 ± 1.47[c]	12.47
CMLP2	50 × 8	10	i.p.	8.23 ± 0.17	7.23 ± 0.23[c]	12.15
SLP1	50 × 8	10	i.p.	7.33 ± 2.77	5.70 ± 2.36[c]	22.24
SLP2	50 × 8	10	i.p.	11.23 ± 1.05	5.14 ± 1.55	54.23
DLP	50 × 8	10	i.p.	7.60 ± 1.95	5.06 ± 0.63	33.42
CP		10	i.p.	8.00 ± 2.78	4.48 ± 2.15	44.00
Saline		10	i.p.	7.13 ± 1.40	7.57 ± 2.37	

[a] LCb and LCa are average leucocyte counts in mice before experiment and after experiment, respectively.

[b] Rate of decrease of leucocyte counts = [(LCb − LCa)/LCb] × 100.

[c] Significant bioactivity against leukopenia ($P < 0.05$) compared to CP control group.

the incorporation of carboxymethyl groups into LP caused little effect on its solubility and conformation in water.

For SLP1 and SLP2, their M_ws and the content of uronic acid were almost the same as those of LP. However, incorporation of the sulfating group resulted in the decrease of LP's activity and this activity greatly decreased with the increase of sulfating group (Table 2). This indicated that the sulfating group was an adverse factor on the bioactivity of LP preventing leukopenia induced by CP, and the bioactivity of LP was strongly dependent on the types of ionic groups of the polysaccharide. That polysaccharides protected the immune system against toxins, antibiotics and poisons was because they could directly interact with some leucocytes to form a physical protective barrier (Weiner et al., 1995). The affinities between polysaccharides and cell surfaces took an important role in the protective function of polysaccharides. Thus incorporation of the sulfating group into LP could disturb the affinities between LP and leucocyte surfaces. Similarly, removal of side chains resulted in the decreasing of charge density, thus the affinities could be reduced. For the carboxymethyl LPs, the incorporation of carboxymethyl groups only caused slight effects on the bioactivities of LP. This could also be because the recognition sites on the cell surfaces had been saturated.

In conclusion, LP had the significant bioactivity against leukopenia induced by cyclophosphamide in mice. The increase of the content of carboxyl groups could result in the increase of the bioactivity. However, incorporation of the sulfating groups made the bioactivity lower. The loss of side chains also weakened the activity of lacquer polysaccharide. Further detailed works on the biological activity and mechanism of action are now in progress.

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References

Antonopoulos, C. A. (1962). A modification for the determination of sulfate in mucopolysaccharides by the benzidine method. *Acta Chimica Scandinavica*, *16*, 1521–1522.

Bitter, T., & Muir, H. M. (1962). A modified uronic acid carbozole reaction. *Analytical Biochemistry*, *4*, 330–334.

Bohn, J. A., & BeMiller, J. N. (1995). (1 → 3)-β-D-Glucan as biological response modifiers: A review of structure–functional activity relationships. *Carbohydrate Polymers*, *28*, 3–14.

Chihara, G., Hmuro, J., Maeda, Y., Arai, Y., & Fukuoka, F. (1970). Antitumor polysaccharide derived chemically from natural glucan (pachyman). *Nature*, *225*, 943–944.

Du, Y., Kong, Z., & Li, H. (1994). Studies on saparation and structure of lacquer polysaccharides. *Acta Polymerica Sinica*, *3*, 301–306.

Du, Y., Yang, J., Kong, Z., & Xiao, L. (1999). Structure and bioactivity of lacquer polysaccharides from Chinese lac tree of wild species and cultispecies. *Chemical Journal of Chinese Universities*, *20*(3), 399–402.

Falshaw, R., & Furneaux, R. H. (1998). Structural analysis of carrageenans from the tetrasporic stages of the red algae, *Gigartina lanceata* and *Gigartina chapmanii* (gigartinaceae, Rhodophyta). *Carbohydrate Research*, *307*, 325–331.

Inoue, K., Kawamoto, K., Nakajima, H., Kohno, M., Kadoya, S., & Mizuno, D. (1983). Chemical modification and antitumor activity of a D-Manno-D-Glucan from *Microellobosporia grisea*. *Carbohydrate Research*, *115*, 199–208.

Kačuráková, M., Capek, P., Sasinková, V., Wellner, N., & Ebringerová, A. (2000). FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydrate Polymers*, *43*, 195–203.

Kennedy, J. F. (1989). *Carbohydrates*. Oxford: Oxford University Press.

Kennedy, J. F., & White, C. A. (1983). *Bio-active carbohydrates*. Chichester: Ellis Horwood, pp. 116–180.

Kumanotani, J. (1998). Enzyme catalyzed durable and authentic oriental lacquer: A natural microgel-printable coating by polysaccharide-glycoprotein-phenolic lipid complexes. *Progress in Organic Coatings*, *34*, 135–146.

Liao, M.-L., Chiovitti, A., Munro, S. L. A., Craik, D. J., Kraft, G. T., & Bacic, A. (1996). Sulfated galactans from Australian specimens of the red alga *phacelocarpus peperocarpos* (Gigartinales, Rhodophyta). *Carbohydrate Research*, *296*, 237–247.

Lu, R., Hattori, K., Xia, Z., Yoshida, T., Yang, J., Zhang, L., Du, Y., Miyakoshi, T., & Uryu, T. (1999a). Structural analysis of polysaccharides in Chinese lacquer by NMR spectroscopy. *Sen'i Gakkaishi*, *55*(2), 47–56.

Lu, R., Yoshida, T., Nakashima, H., Premanathan, M., Aragaki, R., Mimura, T., Kaneko, Y., Yamamoto, N., Miyakoshi, T., & Uryu, T. (2000). Specific biological activity of Chinese lacquer polysaccharides. *Carbohydrate Polymers*, *43*, 47–54.

Lu, R., Yoshida, T., & Uryu, T. (1999b). Viscosity behaviors of Chinese lacquer polysaccharide in aqueous solutions. *Sen'i Gakkaishi*, *55*(9), 402–407.

Misaki, A., Kakuta, M., Sasaki, T., Tanaka, M., & Miyaji, H. (1981). Studies on interrelation of structure and antitumor effects of polysaccharides: Antitumor action of periodate-modified, branched (1 → 3)-β-D-glucan of Auricularia auricula-judae, and other polysaccharides containing (1 → 3)-glycosidic linkages. *Carbohydrate Research*, *92*, 115–129.

Oshima, R., & Kumanotani, J. (1984). Structural studies of plant gum from sap of the Lac tree, Rhus vernicifera. *Carbohydrate Research*, *127*, 43–57.

Srivastava, R., & Kulshreshtha, D. K. (1989). Bioactive polysaccharides from plants. *Phytochemistry*, *28*(11), 2877–2883.

Weiner, R., Langille, S., & Quintero, E. (1995). Structure, function and immunochemistry of bacterial exopolysaccharides. *Journal of Industrial Microbiology*, *15*, 339–346.

Whistler, R. L., & BeMiller, J. N. (1958). Alkaline degradation of polysaccharides. In M. L. Wolfrom, & R. S. Tipson (Eds.), (*Vol. 13*) (pp. 289–329). *Advances in carbohydrate chemistry*, New York: Academic Press.

Wilmer, J. L., Colvin, O. M., & Bloom, S. E. (1992). Cytogenetic mechanisms in the selective toxicity of cyclophophamide analogs and metabolites towards avian embryonic B lymphocytes in vivo. *Mutation Research*, *268*(1), 115–130.

Zhang, L., & Ding, Q. (1995). Influences of molecular weight and branching parameter of lacquer polysaccharide on the growth of leucocytes. *Chinese Journal of Polymer Science*, *13*, 107–111.

Zhang, L., Qiu, X., Xu, J., Du, Y., Qian, B., & Kennedy, J. F. (1992). Studies on fractionation and molecular weights of Chinese lacquer polysaccharide. *Carbohydrate polymers*, *19*, 161–166.