Structural Features and Anti-complementary Activity of Rehmannan SA, a Polysaccharide from the Root of *Rehmannia glutinosa*

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The structural features of rehmannan SA, a polysaccharide with remarkable reticuloendothelial system-potentiating activity obtained from the root of *Rehmannia glutinosa*, were investigated by methylation analysis and periodate oxidation. Rehmannan SA is mainly made up of arabino-3,6-galactan type structural units. Both rehmannan SA and rehmannan SB showed pronounced anti-complementary activity.

Keywords polysaccharide structure; anti-complementary activity; *Rehmannia glutinosa*; acidic arabinogalactan; rehmannan SA

Recently, we isolated and characterized two acidic polysaccharides, rehmannan SA and rehmannan SB, from the dried root of *Rehmannia glutinosa* LIBOSCHITZ, having remarkable activity on the recticuloendothelial system (RES).[1] The dried root of this plant is a well-known crude drug under the name of Kan-Jiou in Japan and Dihuang in China. Structural features of the major polysaccharide, rehmannan SB, have been elucidated by us.[1] The present paper describes methylation analysis and periodate oxidation studies of rehmannan SA, and presents structural features of this polysaccharide. Pronounced anti-complementary activity of the two polysaccharides is also described in this report.

Materials and Methods

Isolation of Polysaccharide This was performed as described in a previous report.[1] The material was imported from China. The yield of rehmannan SA was 20 mg from 1 kg of the material roots.

Reduction of Carboxyl Groups This was carried out with 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate and sodium borohydride as described previously.[2] The yield was 6.4 mg from 10 mg of rehmannan SA.

Methylation This was performed with powdered sodium hydroxide and methyl iodide in dimethyl sulfoxide as described previously.[3] The yields were 1.2 mg from 5.4 mg of rehmannan SA, and 0.8 mg from 6.4 mg of its carboxyl-reduced derivative.

Analysis of the Methylated Products The products were hydrolyzed with dilute sulfuric acid in acetic acid, then reduced and acetylated as described previously.[4] The partially methylated alditol acetates obtained were analyzed by gas chromatography–mass spectrometry (GC-MS) using a fused silica capillary column (0.32 mm i.d. × 30 m) of SP-2330 (Supelco Co.) with a programmed temperature increase of 4 °C per min from 160 to 220 °C at a helium flow of 1 ml per min. GC-MS was performed with a JEOL JMS-DX303 mass spectrometer. The relative retention times of the products with respect to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol in GC are listed in Table I.

Periodate Oxidation The polysaccharide (10 mg) was dissolved in water (2.5 ml), then 0.1 M sodium metaperiodate (2.5 ml) was added. The reaction mixture was kept at 4 °C in the dark, and the periodate consumption was measured by a spectrophotometric method.[5] The oxidation was completed after 4 d. The reaction mixture was successively treated with ethylene glycol (0.01 ml) at 4 °C for 1 h and sodium borohydride (30 mg) at 4 °C for 16 h, then adjusted to pH 5.0 by the addition of acetic acid. The solution was concentrated and applied to a column (2.6 × 84 cm) of Sephadex G-25. The column was eluted with water, and fraction of 10 ml were collected. The eluates obtained from tubes 20 to 22 were combined, concentrated and lyophilized. Yield, 5.2 mg.

Determination of Components Hydrolysis and cellulose thin-layer chromatography (TLC) of the component sugars were performed as described previously.[6] Neutral sugars were analyzed by GC after conversion of the hydrolyzate into alditol acetates as described previously.[7]

Anti-complementary Activity This was measured as described in a previous report.[8] Gelatin–veronal-buffered saline (pH 7.4) containing 500 μM Mg^{2+} and 150 μM Ca^{2+} (GVB^{2+}) was prepared, and normal human serum (NHS) was obtained from a healthy adult. Various dilutions of the samples in water were incubated and the residual total hemolytic complement (TCH_{50}) was determined using immunoglobulin M (Ig M)-hemolysis-sensitized sheep erythrocytes. NHS was incubated with water and GVB^{2+} to provide a control, and the activities of the samples were expressed as the percentage inhibition of the TCH_{50} of the control. Plantago-mucilage A from the seed of *Plantago asiatica* L.[9] was used as a positive control.

Results

The neutral sugar-rich polysaccharide fraction obtained from the root of *Rehmannia glutinosa* was applied to a column chromatography of diethylaminoethyl (DEAE)-Sephadex A-25. The eluate with 0.2 M ammonium carbonate was applied to a column chromatography of DEAE-Sephacel. The eluate with 0.1 M sodium chloride in a phosphate buffer was subjected to affinity chromatography on Con A-Sepharose. Rehmannan SA was isolated from the passed-through fraction.[1] The RES-potentiating activity of this polysaccharide is about two times that of rehmannan SB.[1]

Rehmannan SA was composed of α-L-arabinose, β-D-galactose, α-L-rhamnose and α-D-galacturonic acid in the molar ratio of 10 : 10 : 1 : 1.[1] The carboxyl groups of galacturonic acid in the polysaccharide were reduced to give the corresponding neutral sugar residues.[10] Both the original polysaccharide and the carboxyl-reduced derivative were methylated with solid sodium hydroxide and methyl iodide in dimethyl sulfoxide.[11] The methylated products thus obtained were hydrolyzed, then converted into partially methylated alditol acetates. The hexuronic acid methyl ether was removed from the hydrolysis products of the methylated original polysaccharide by treatment with an anion-exchange resin. Analysis by GC-MS gave the results shown in Table I. The result of methylation analysis of the carboxyl-reduced derivative was unsatisfactory in the molar ratio of products, probably because of its lower solubility in dimethyl sulfoxide.

Rehmannan SA was subjected to periodate oxidation followed by reduction. The component sugar analysis of

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TABLE I. Methylation Analysis of Rehmannan SA and Its Carboxyl-Reduced Product

Methylated sugars (as alditol acetates)	Relative retention times[a)]	Molar ratios	
		Original	Carboxyl-reduced
2,3,5-Me$_3$-L-arabinose	0.69	6.0	6.0
2,3,4-Me$_3$-L-arabinose	0.79	1.0	1.0
2,5-Me$_2$-L-arabinose	1.04	2.8	1.0
2,3-Me$_2$-L-arabinose	1.14	6.1	5.6
3-Me-L-arabinose	1.54	3.1	2.0
2,3,4,6-Me$_4$-D-galactose	1.10	3.0	2.7
2,4,6-Me$_3$-D-galactose	1.39	1.8	1.2
2,3,6-Me$_3$-D-galactose	1.47	4.2	5.7
2,3,4-Me$_3$-D-galactose	1.62	4.2	2.3
2,3-Me$_2$-D-galactose	1.98	0.8	0.6
2,4-Me$_2$-D-galactose	2.02	5.0	2.7
2,3,4-Me$_3$-L-rhamnose	0.64	0.1	0.1
3,4-Me$_2$-L-rhamnose	0.96	0.6	0.6
3-Me-L-rhamnose	1.31	1.2	1.2

a) Relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. Abbreviation: Me = methyl.

(sixty)[a)]	α-L-Ara*f* 1→	(thirty)[a)]	β-D-Gal*p* 1→
(ten)[a)]	α-L-Ara*p* 1→	(eighteen)[a)]	→3 β-D-Gal*p* 1→
(twenty-eight)[a)]	→3 α-L-Ara*f* 1→	(forty-two)[a)]	→4 β-D-Gal*p* 1→
(sixty-one)[a)]	→5 α-L-Ara*f* 1→	(forty-two)[a]	→6 β-D-Gal*p* 1→
(thirty-one)[a)]	→5 α-L-Ara*f* 1→ 2 ↑	(eight)[a)]	→6 β-D-Gal*p* 1→ 4 ↑
(one)[a)]	α-L-Rha*p* 1→	(fifty)[a)]	→6 β-D-Gal*p* 1→ 3 ↑
(six)[a)]	→2 α-L-Rha*p* 1→		
(twelve)[a)]	→4 α-L-Rha*p* 1→ 2 ↑	(nineteen)[a)]	→4 α-D-Gal*p*A 1→

Chart 1. Component Sugar Residues in the Minimal Unit in the Structure of Rehmannan SA

a) Number of residues. Ara*f*, arabinofuranose; Ara*p*, arabinopyranose; Gal*p*, galactopyranose; Rha*p* rhamnopyranose; Gal*p*A, galactopyranosyluronic acid.

the product showed that one-third of the arabinose units, three-eighths of the galactose units and two-thirds of the rhamnose units survived after periodate oxidation. All the galacturonic acid residues were completely destroyed by this treatment. In addition to the methylation analysis, this result showed that D-galacturonic acid units are present in a 1,4-linked form.

These results indicated that the minimal unit of rehmannan SA is composed of fifteen kinds of component sugar units, as shown in Chart 1.

The anti-complementary activities of rehmannan SA and rehmannan SB are shown in Fig. 1. The activities of these polysaccharides were higher than that of the positive control, Plantago-mucilage A. Further, the activity of rehmannan SA was a little higher than that of rehmannan SB.

Discussion

Two RES-potentiating polysaccharides, rehmannan SA and rehmannan SB, have been obtained from the dried root of *Rehmannia glutinosa*.[1)] Rehmannan SA, having higher activity on the RES than rehmannan SB, belongs to an acidic arabino-3,6-galactan. To date, we have identified 34 polysaccharides as RES-activating substances in crude drugs obtained from various plant sources. The majority of these are acidic arabino-3,6-galactan type polysaccharides. In addition to rehmannans SA and SB, those are saposhnikovan A from the root and rhizome of *Saposhnikovia divaricata*,[7)] MVS-IIIA, -IVA and -VI from the seed of *Malva verticillata*,[12-14)] ukonans A, B and C from the rhizome of *Curcuma longa*,[15-17)] glycyrrhizans UA, UB and GA from the root of *Glycyrrhiza uralensis* and the stolon of *G. glabra* var. *glandulifera*,[18,19)] eucomman A from the bark of *Eucommia ulmoides*,[20)] AMon-S from the root of *Astragalus mongholicus*,[21)] cnidirhan AG from the rhizome of *Cnidium officinale*,[22)] ginsenans PA, PB, S-IA and S-IIA from the root of *Panax ginseng*,[23,24)] peonan SB from the root of *Paeonia lactiflora*,[25)] and alisman PII from the tuber of *Alisma orientale*.[26)]

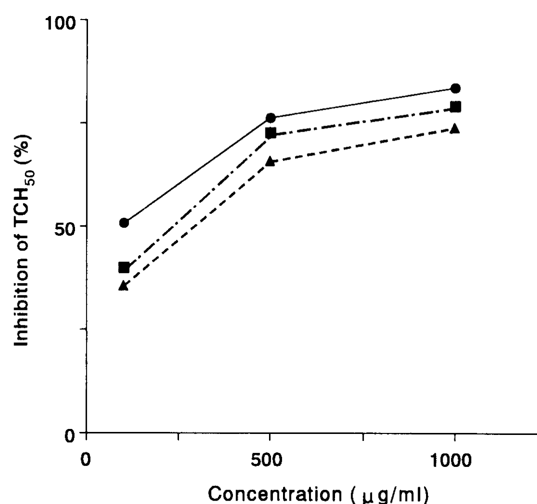

Fig. 1. Anti-complementary Activity of Rehmannan SA and Rehmannan SB

Rehmannan SA, —●—; rehmannan SB, ---■---; plantago-mucilage A, ---▲---. Each point represents the mean ($n=3$).

The RES activity of rehmannan SA is superior to most of the other acidic arabino-3,6-galactan type polysaccharides. Its value in the phagocytic index is about two times that of rehmannan SB. Both polysaccharides, rehmannans SA and SB, possess basically common structural units; those are, terminal, 1,5-linked and 2,5-branched L-arabinose units, terminal, 1,3-, 1,4- and 1,6-linked, 3,6- and 4,6-branched D-galactose units, terminal, 1,2-linked and 2,4-branched L-rhamnose units, and 1,4-linked D-galacturonic acid residues. In addition to these component sugar residues, rehmannan SA has a considerable number of α-1,3-linked L-arabinofuranosyl units. Further, the ratio of β-1,4- and 1,6-linked and 3,6-branched D-galactopyranosyl units in rehmannan SA is much higher than in rehmannan SB. It is conceivable that these factors may contribute to the high RES-potentiating activity of rehmannan SA.

The anti-complementary activity of rehmannan SA is also superior to rehmannan SB, though the difference between the two is not as apparent at high doses. Further investigation of the relationship between the biological activities and structural features of rehmannan SA is in progress.

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