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Synthesis and Anti-HIV Activity of Sulfonated Amino Ribofuranans

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New sulfonated amino ribofuranans were synthesized to elucidate the relationship between structure and specific biological activities such as anti-HIV and blood anticoagulant activities. The synthesis was performed by sulfonation of copolymers having various proportion of (1→5)-α-D-ribofuranosidic unit. The sulfonation with piperidine *N*-sulfonic acid produced the sulfonated amino ribofuranans in high yield. The anti-HIV activity of sulfonated 3-amino-3-deoxy-(1→5)-α-D-ribofuranan showed more potent by increasing the degree of sulfonation and the average molecular weights. This activity was almost equal to the activities of sulfonated ribofuranans and ribopyranans reported before in spite of low molecular weight. The blood anticoagulant activities was observed at 36-48 mg/units, more potent than standard dextran sulfonate, 22.7 mg/units. In addition, the blood anticoagulant activities of sulfamide-copolysaccharide consisting various proportion of (1→5)-α-D-ribofuranan units were potentiated by increasing sulfonated amino-ribofuranan units from 13 to 21 mg/units.

Key words: Sulfonated amino ribofuranans, Anti-HIV activity, Anticoagulant activity

INTRODUCTION

Amino sugars are found as components of poly- and oligosaccharides in various glycoproteins, glycopeptides, and proteoglycans on the surface of various cells and are deeply related to the biological interactions (Garegg and Lindberg 1993; Spiro, 1965). Heparin is a natural sulfonated mucopolysaccharide having repeated units of glucuronic acid or iduronic acid and glucosamine, and is well known to have a strong blood anticoagulant activity (Van Boeckel and petitou, 1993).

Natural and synthetic sulfonated polysaccharides showed strong anti-AIDS virus activity (Uryu, 1993). The disease of AIDS (acquired immunodeficiency syndrome) was firstly reported in 1981 and the causative virus was discovered to be a retrovirus termed Human Immunodeficiency Virus (HIV) (Broder, 1987; Dalgeish and Weiss, 1990). Sulfonated polysaccharides inhibit the binding of HIV to the outside of T4 cells (Mitsuya *et al.*, 1990). Therefore, the inhibitory mechanism of sulfonated polysaccharides for HIV is completely different from those of nucleic analogues and protease inhibitors. Hattori's group synthesized sulfonated 3-amino-3-deoxy-(1→6)-α-D-allopyranan and found strong anti-HIV activities (Hattori *et al.*, 1998).

Additionally, the blood anticoagulant activity was raised by increasing amino-allose unit from 30 to 58 mg/unit (for example; standard dextran sulfonate, 22.7 mg/unit). But, these sulfamide polysaccharides have hexose type in amino sugar, while pentose type in amino sugar is rare. To examine the structure and biological activity relationships on amino polysaccharides, it is required to synthesize amino polysaccharide having defined structure. To know the structural information of amino polysaccharides, the synthesis of pentose type polysaccharides having defined structures is important. Recently, we reported the anti-HIV activity of sulfonated arabinofuranan and xylofuranan type polysaccharides (Yoshida *et al.*, 2001).

In this paper, 3-amino-3-deoxy-(1→5)-α-D-ribofuranan having various ratios of amino group, (Kang *et al.*, 1997) were sulfonated and then their biological activities such as anti-HIV and anticoagulant activities were examined to clarify the relationship between their structures and biological activities.

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MATERIALS AND METHODS

Measurements

^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained in $CDCl_3$ or D_2O by a JEOL α-400 spectrometer. Specific rotations were measured in chloroform or water at 25°C by Perkin-Elmer 241 and JASCO DIP-140 polarimeter. Molecular weights were measured at 40°C on a GPC apparatus with THF, DMSO, or water as elution solvents and standard polystyrene or curdlan were used as reference (columns: Toso TSK-gel, G3000HXL, G4000HXL, and G5000HxL for THF, GMHHR-M(S) for DMSO, and G2500SW and G3000SW for water).

Materials

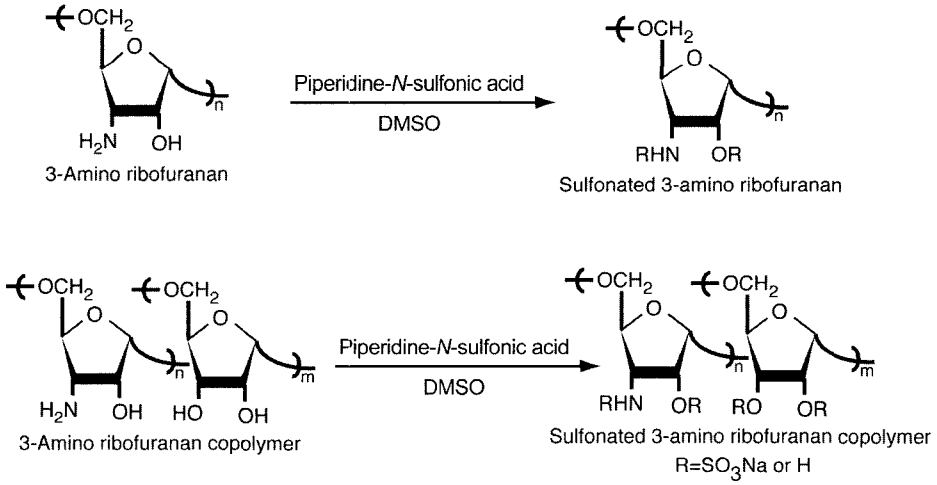
Piperidine *N*-sulfonic acid was prepared from piperidine and chlorosulfonic acid according to the protocol in a previous report (Nagasawa and Yoshidome, 1969). 3-amino-3-deoxy-(1→5)-α-D-ribofuranan having various ratios of amino group was synthesized by the previous method (Kang *et al.*, 1997). All solvents were distilled before use. Other commercial reagents were used without further purification.

Sulfonation

The amino ribofuranan (0.2 g, 1.39 mmol) was sulfonated at 85°C with piperidine *N*-sulfonic acid (2.3 g, 13.9 mmol) in anhydrous DMSO under nitrogen atmosphere for 1.5 h. After cooling, the reaction mixture was neutralized with saturated $NaHCO_3$ solution, and then acetone (200 mL) was poured into the reaction mixture to form white precipitate. The precipitate was centrifuged and washed three times with acetone, then was dissolved in water and finally dialyzed for a day. The dialysate was freeze-dried completely to afford powder state of sulfonated amino polysaccharides. The degree of sulfonation was calculated from the results of the elementary analysis. The synthetic route was shown in Scheme 1.

Measurement of anti-HIV activity

Anti-HIV activities of sulfonated amino-polysaccharides were carried out by measuring the inhibitory activities of HIV-induced cytopathic effects and expression of virus antigen in HIV-sensitive MT-4 cells (Nakashima *et al.*, 1987). Curdlan and dextran sulfonates were used as reference polysaccharides. The anti-HIV activity was calculated by viable cells counting and percentage of virus antigen positive cells on 3- and/or 5-day incubations after HIV infection. The highest value of complete inhibition (EC_{100}) for sulfonated polysaccharides against HIV infection, was determined as 3.3 mg/mL, which is nearly equal to the EC_{50} of 0.13 mg/mL for curdlan sulfonate. The anti-HIV activity was also measured by the MTT method using $HIV_{HTLV\text{-}IIIB}$ virus and MT-4 cells (Pauwels *et al.*, 1988), which is a more convenient method in the preliminary screening of anti-HIV assay for test compounds. Practically, MT-4 cells were infected with HIV at 0.01 multiplicity and then MT-4 cells (1.5×10^5 cells/mL) and HIV-infected MT-4 cells were co-incubated in the presence of sulfonated amino polysaccharides for five days in a CO_2 incubator at 37°C. The viable cells number of both HIV infected and uninfected MT-4 cells was measured by the changing color of the test solutions from yellow to blue according to the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT). The 50% inhibitory concentration of test compounds against infection of HIV to MT-4 cells was represented as EC_{50}. For measuring the cytotoxicity of the sulfonated amino polysaccharides, uninfected MT-4 cells were cultured with sulfonated amino polysaccharides in various concentrations. The cytotoxicity was defined as CC_{50} which means a 50% preventive concentration for MT-4 cells growth.



Scheme 1. Synthesis of sulfonated 3-amino ribofuranan and 3-amino ribofuranan copolymer

Measurement of blood anticoagulant activity

Anticoagulant activity of sulfonated amino polysaccharides was determined by using whole bovine plasma according to a modified method of US Pharmacopoeia National Formulary (1985). The coagulation time of bovine plasma with different concentrations of sulfonated amino polysaccharides was calculated and compared with that of standard dextran sulfonate (H-039, 22.7 mg/unit).

RESULTS AND DISCUSSION

Sulfonation

3-amino-3-deoxy-(1→5)-α-D-ribofuranan and its copolymers were sulfonated with piperidine *N*-sulfonic acid in DMSO to give the corresponding sulfonated amino polysaccharides. Commercially available amino ribofuranan was sulfonated having a sulfonation degree between 0.3 and 1.3 by the results of the elemental analysis. The results are summarized in Table I.

The specific rotation was changed to +7.21°~+10.67° after sulfonation. The average molecular weights were 6.5×10^3~9.3×10^3. Fig. 1 showed the ^{13}C-NMR spectra of 3-amino-3-deoxy-(1→5)-α-D-ribofuranan (A) before and (B) after sulfonation. The degree of sulfonation was 1.2 and sulfonate group should be introduced in C-3 position. The sulfonation shifted each signal to lower or upper magnetic field and was broadened compared with the spectrums before sulfonation.

Anti-HIV activity and blood anticoagulant activity

In order to investigate the relationship between structures and biological activities, the anti-HIV and blood anticoagulant activities of the sulfonated amino ribofuranan having various ratios amino group were tested. The results on anti-HIV activity and blood anticoagulant activity of sulfonated amino ribofuranans are summarized in Table II. In No. 1~4, sulfonated amino ribofuranans exhibited strong

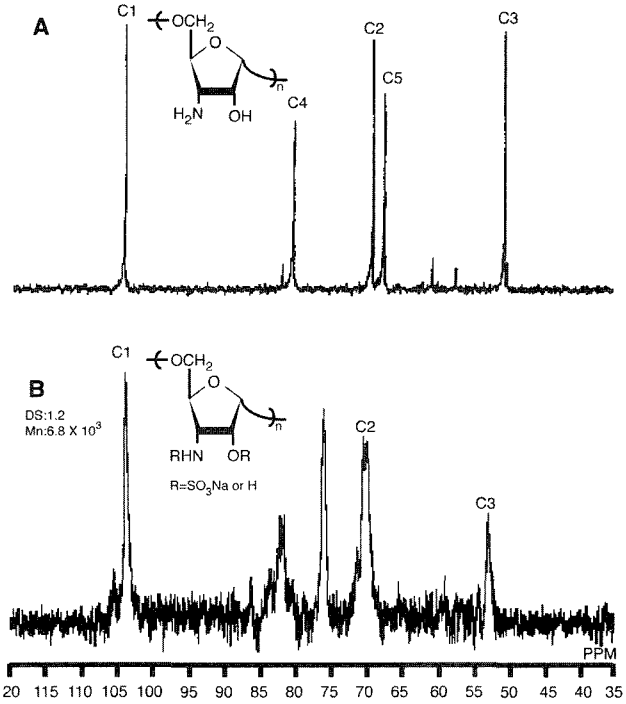


Fig. 1. 100 MHz ^{13}C-NMR spectra of (A) 3-amino-3-deoxy-(1→5)-α-D-ribofuranan and (B) Sulfonated 3-amino-3-deoxy-(1→5)-α-D-ribofuranan (D_2O as solvent).s

Table . Sulfonation of 3-amino-3-deoxy-(1→5)-α-D-ribofuranans by piperidine-*N*-sulfonic acid[a]

No	Free amino copolysaccharide				Yield (%)	Sulfonated amino copolysaccharide						DS[c]
	$\overline{M}_n$ ($\times10^3$)[d]	$[\alpha]_D^{25}$ (deg)[e]	Molfraction of sugar unit[b] (mol%)			$\overline{M}_n$ ($\times10^3$)[d]	$[\alpha]_D^{25}$ (deg)[e]	Elemental analysis				
			3-amino-ribose	ribose				C (%)	H (%)	S (%)	N (%)	
1	1.44	+142.1	100	0	66	8.2	+10.42	25.47	4.39	15.46	4.58	1.3
2	1.44	+142.1	100	0	84	6.8	+8.04	25.64				1.2
3	1.44	+142.1	100	0	79	6.5	+7.21	28.50				0.9
4	1.44	+142.1	100	0	69	9.3	+8.93	28.73				0.8
5	1.44	+142.1	100	0	71	7.2	+10.67	29.48				0.5
6	1.44	+142.1	100	0	65	7.1	+9.31	40.38				0.3
7	1.44	+142.1	77	23	72	7.8	+15.6	29.12				0.8
8	1.44	+142.1	58	42	78	8.3	+13.8	28.49				0.5
9	1.44	+142.1	49	51	71	8.5	+10.1	28.58				0.6
10	1.44	+142.1	41	59	84	7.4	+14.9	33.27				0.5
11	1.44	+142.1	18	82	60	7.9	+8.5	27.68				0.4

a) Solvent DMSO-d_6 40 mL, Temp; 85°C, Time; 1.5 h. b) Calculated from ^{13}C-NMR spectra. c) Degree of sulfonation. d) Determined by GPC using pullulan as a standard. e) Measured in water at 25°C (c 1%).

Table II. Anti-HIV and Anticoagulant Activities of Sulfonated 3-amino-3-deoxy-(1→5)-α-D-ribofuranans

No	Sulfonated 3-amino polymers						EC_{50} (μg/mL)[d]	Anticoagulant activity (mg/unit)[e]
	Molfraction of sugar unit[b] (mol%)		$\overline{M}_n$ ($\times10^3$)[a]	S content (%)	$[\alpha]_D^{25}$ (deg)[b]	DS (%)[c]		
	3-animo-ribose	ribose						
1	100	0	8.2	15.46	+10.42	1.3	0.1	48
2	100	0	6.8	15.12	8.04	1.2	0.1	42
3	100	0	6.5	13.84	7.21	0.9	0.2	43
4	100	0	9.3	13.30	8.93	0.8	0.1	36
5	77	23	7.8	11.08	15.6	0.8	0.8	21
6	58	42	8.3	10.21	13.8	0.5	>1000	18
7	49	51	8.5	12.67	10.1	0.6	>1000	15
8	41	59	7.4	8.64	14.9	0.5	>1000	16
9	18	82	7.9	10.23	8.5	0.4	>1000	13
	RPS[f]		12.0	17.9	8.9	1.8	0.1	36
	RFS[g]		17.0	17.6	83.0	1.9	3.3[h]	56
	DS[i]		8.5	18.4	92.1	1.8	0.86	22.7
	CS[j]		79.0	14.1	3.0	1.6	0.13	<10

a) Determined by GPC. b) Measured in water at 25°C (c, 1%). c) Degree of sulfonation per sugar unit. d) 50% Effective concentration. e) Dextran sulfonate H-039, 22.7 mg/unit. f) Sulfonated ribopyranan. g) Sulfonated ribofuranan. h) Minimum effective concentration for 100% inhibition of HIV infection. i) Standard dextran sulfonate, H-039. j) Standard curdlan sulfonate.

anti-HIV activity (EC_{50}; 0.1~0.2 μg/mL) and potentiated by increasing degree of sulfonation.

By comparison with previous results of sulfonated ribofuranan (Yoshida *et al.*, 1992) and ribopyranan (Yoshida *et al.*, 1994), the anti-HIV activity of the sulfonated amino ribofuranans represented almost equal in spite of lower degree of sulfonation and average molecular weights. However, sulfonated copolysaccharides (No. 6~9) exhibited weak anti-HIV activity by low degree of sulfonation.

The blood anticoagulant activity of sulfonated amino polysaccharides potentiated by increasing the degree of sulfonation as shown in No. 1~4 on Table II. Although the sulfonated 3-amino-ribofuranans (No. 1~4) showed strong anticoagulant activity of 36~48 mg/unit, the sulfonated copolysaccharide containing ribose units had lower anticoagulant activity of 13~21 mg/unit. In addition, it was found that the anticoagulant activity of the sulfonated copolysaccharides containing ribose units gradually was decreased by lowering the proportion of 3-amino-ribofuranan unit. These results suggest that the sulfamide group plays an important role on the interactions of sulfamide or sulfonate groups-containing copolysaccharides with blood coagulation factor.

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