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# Synthesis of sulfonated amino-polysaccharides having anti-HIV and blood anticoagulant activities

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**Abstract**

Sulfonated 3-amino-3-deoxy-(1→6)-α-D-allopyranans were synthesized to elucidate the relationship between structure and such specific biological activities as anti-HIV and blood anticoagulant activities. Ring-opening copolymerization of 1,6-anhydro-3-azido-2,4-di-*O*-benzyl-3-deoxy-β-D-allopyranose **1** with 1,6-anhydro- 2,3,4-tri-*O*-benzyl-β-D-allopyranose **2** with $PF_5$ catalyst gave copolymers having various proportions of the **1** unit. $^{13}C$ NMR spectroscopy showed the resulting copolymers to have α-(1→6)-stereoregularity, and the monomer reactivity ratios were calculated to be $r_1 = 0.92$ and $r_2 = 1.11$ by the Kelen–Tüdös method. Reduction of the azido groups and subsequent debenzylation of the copolymers afforded new amino-polysaccharides consisting of 3-amino-3-deoxy-allose and allose units. Sulfonation of 3-amino-3-deoxy-(1→6)-α-D-allopyranan and copolysaccharides consisting of 3-amino-allose and allose or glucose units was performed with piperidine-*N*-sulfonic acid to give polysaccharides containing sulfoamido and sulfonate groups in good yields. The sulfoamido-copolysaccharides containing of 3-amino-3-deoxy-allose and glucose units exhibited high anti-HIV activities manifested by the 50% protecting concentration ($EC_{50}$) of 0.2–0.5 mg/mL and low cytotoxicity shown by a 50% cytotoxic concentration ($CC_{50}$) of $> 1000\,\mu g/mL$. The sulfoamido-copolysaccharides containing 3-amino-allose and allose units had somewhat lower anti-HIV activities ($EC_{50} = 0.8$–$0.9\,\mu g/mL$) and slightly higher cytotoxicities ($CC_{50} = 740 \sim 797\,\mu g/mL$). In addition, it was found that the blood anticoagulant activity increased, with increasing proportion of the amino-allose unit, from 30 to 58 unit/mg (standard dextran sulfate, 22.7 unit/mg). These results suggest that the sulfonated 3-amino-3-deoxyallose unit plays important roles on both anti-HIV and blood anticoagulant activities. © 1998 Elsevier Science Ltd. All rights reserved

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## 1. Introduction

Recently, chitin, modified chitins, and chitosans containing acetamido and amino groups have attracted much attention because of their unique bioactivities and biocompatibilities, for example, wound healing activity [1], induction of basic fibloblast growth factor [2], wound dressings for soft tissues [3], regeneration of bone tissues [4], and induction of human tumor necrosis factor-$\alpha$ [5]. Heparin preparations having high blood anticoagulant activity contain residues of glucosamine and sulfonated glucuronic and iduronic acids in the repeating units [6]. In general, it is difficult to clarify the structures–activity relationships of naturally occurring poly- and oligosaccharides having amino sugar units, because of their complex structures. Therefore, the synthesis of amino-polysaccharides having well-defined structures is useful for elucidating these structures–activity relationships.

We have synthesized various sulfonated polysaccharides showing potent anti-HIV activities [7,8]. The molecular weight and the number of sulfonate groups per sugar unit are important for activity. In addition, we have synthesized amino polysaccharides by ring-opening polymerization of glucose- [9], allose- [10], xylose- [11], and ribose-type [12] anhydro sugar derivatives having an azido group at C-3.

In this paper, we report the synthesis of polysaccharides containing sulfoamido and sulfonate groups composed of 3-amino-allose and glucose or allose units and their anti-HIV and blood anticoagulant activities, and note that the sulfoamido group in the polysaccharide chain plays an important role in the biological activity.

## 2. Experimental

*General methods*.—The 400 MHz $^1$H and 100 MHz $^{13}$C NMR spectra were recorded on a Jeol JNM $\alpha$-400 spectrometer in $CDCl_3$ or $D_2O$ and with $Me_4Si$ or sodium 4,4-dimethyl-4-silapentanesulfonate (DSS) as the internal standard. 2D spectra for DQF-COSY (double quantum filtered correlation spectroscopy) and HMQC ($^1$H detected heteronuclear multiple quantum coherence) were obtained for the data size of 512 (t2) by 512 points (t1) after 2-fold zerofilling along the f1 axis. IR spectra were taken on a Jasco IR-700 spectrometer by the KBr pellet method. Specific rotations were measured on a Jasco DIP-140 digital polarimeter in $CHCl_3$ or water. All of the monomers were purified by HPLC (column; Tosoh TSK-gel silica-60, 21.5×300 mm) or by recrystallization. Molecular weights of polymers were estimated by gel permeation chromatography (GPC) (column; Tosoh TSK-gel G3000$H_{XL}$, G4000$H_{XL}$, and G5000$H_{XL}$, 7.6×300 mm×3; eluent, tetrahydrofuran) using polystyrene standards or by means of aqueous phase GPC (column; Tosoh TSK-gel G2500$PW_{XL}$, G3000$PW_{XL}$, and G4000$PW_{XL}$, 7.6×300 mm×3; eluent, 66.7 mmol/L of phosphate buffer, pH 6.86) using pullulan standards.

*Materials*.—1,6-Anhydro-3-azido-2,4-di-*O*-benzyl-3-deoxy-$\beta$-D-allopyranose **1** [5], 1,6-anhydro-2,3,4-tri-*O*-benzyl-$\beta$-D-glucopyranose **3** [13], and 1,6-anhydro-2,3,4-tri-*O*-benzyl-$\beta$-D-allopyranose **2** [14] were synthesized from 1,6-anhydro-$\beta$-D-glucopyranose according to literature methods. Piperidine-*N*-sulfonic acid was prepared as previously described [15].

*Copolymerization of* **1** *with* **2**.—Ring-opening polymerization and copolymerization were performed under high vacuum ($\sim 10^{-6}$ mm Hg) at low temperatures in $CH_2Cl_2$. The polymerization was terminated by the addition of MeOH. After the polymer had been extracted with $CHCl_3$, the $CHCl_3$ layer was neutralized with $NaHCO_3$, washed with water, dried ($Na_2SO_4$), and then concentrated. The residue was dissolved in a small amount of $CHCl_3$ and the product reprecipitated with MeOH several times, and freeze-dried from benzene to give a purified polymer as a white powder.

*Reduction of azido groups and debenzylation*.—Reduction of azido group in copoly(**1**–**2**) (148 mg, $M_n = 16.5\times10^3$, **1** unit = 47 mol%) was performed with $LiAlH_4$ (30 mg, 0.79 mmol) in dry THF (30 mL) for 1 h at 50 °C. A copolymer having free amino groups was obtained in 95% (136 mg) yield after freeze-drying from benzene. IR (KBr) $\nu = 3374\ cm^{-1}$ (-$NH_2$); $\bar{M}_n = 14.8\times10^3$. The ninhydrin test was positive.

A solution of the amino-copolymer (136 mg) in 1,2-dimethoxyethane (5 mL) was debenzylated with Na (0.6 g) in liquid $NH_3$ (50 mL) under $N_2$ for 1 h at −78 °C. After evaporation of $NH_3$, a small amount of 3 wt% HCl was added to the residue and the solution was immediately dialyzed with deionized water. After freeze-drying from water, the amino-polysaccharide (73 mg) was obtained as the HCl salt in 95% yield. The ninhydrin test was positive; $\bar{M}_n = 12.0\times10^3$; $[\alpha]_D^{25} = +119.1°$ (*c* 1%, $H_2O$).

*Sulfonation*.—3-Amino-3-deoxy-(1→6)-α-D-allopyranan (49 mg, $\bar{M}_n = 9.6\times10^3$) was treated with piperidine-*N*-sulfonic acid (390 mg) in anhydrous $Me_2SO$ (10 mL) at 85 °C under $N_2$ atmosphere. After stirring for 1.5 h, the mixture was cooled to room temperature, neutralized with saturated $NaHCO_3$ solution, and dialyzed for 24 h with deionized water. The dialyzate was concentrated to 20–30 mL and freeze-dried to give a sulfonated 3-amino-3-deoxy-(1→6)-α-D-allopyranan in 60 mg yield; $\bar{M}_n = 11.1\times10^3$; $[\alpha]_D^{25} = +86.5°$ (*c* 1%, $H_2O$). The ninhydrin test was negative, while the metachromasia test was positive. Elemental analysis data are shown in Table 2 (no. 2). Amino-polysaccharides consisting of 3-amino-3-deoxyallose and allose or glucose units were also sulfonated by the same procedure.

*Anti-HIV assay*.—Anti-HIV (human immunodeficiency virus) activity of sulfonated amino-polysaccharides was examined by the MTT method [16]. MT-4 cells were infected with HIV-$1_{HTLV\text{-}IIIB}$ at the multiplicity of infection (MOI) of 0.01. HIV- or mock-infected MT-4 cells ($1.5\times10^5$ cells/mL) were incubated in the presence of various concentrations of sulfonated amino-polysaccharides for 5 days at 37 °C. The cell viability was calculated by a spectrophotometric method via the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue-colored formazan product. The anti-HIV activity is represented as the $EC_{50}$, which denotes the concentration of the amino-polysaccharide to protect 50% infection of MT-4 cells from HIV. The cytotoxicity is designated as $CC_{50}$, that is, a 50% cytotoxic concentration of the amino-polysaccharide against on the MT-4 cell culture.

*Anticoagulant activity*.—Anticoagulant activity of sulfonated amino-polysaccharides were measured according to a modified United States Pharmacopoeia procedure using bovine plasma [17]. Dextran sulfate (Meito Sangyo Co., Ltd., H-039, $\bar{M}_n = 8.5\times10^3$, S content; 18.4%, AA = 22.7 unit/mg) was used as a reference.

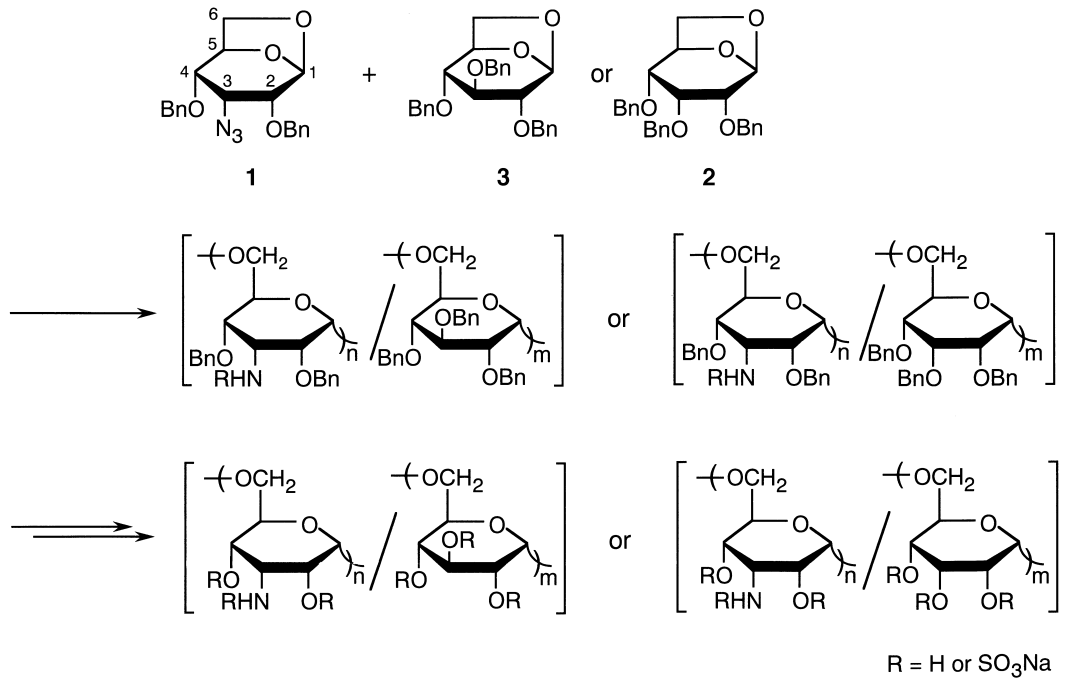
## 3. Results and discussion

*Copolymerization of* **1** *with* **2**.—Recently, we reported the ring-opening polymerization of 1,6-anhydro-3-azido-2,4-di-*O*-benzyl-3-deoxy-β-D-allopyranose **1** to give 3-amino-3-deoxy-(1→6)-α-D-allopyranan after reducing the azido groups and debenzylation [10]. Furthermore, copolysaccharides consisting of 3-amino-allose and glucose units were obtained by the ring-opening copolymerization of **1** with benzylated 1,6-anhydro-glucose **3** in various feeds and subsequent reduction of the azido and benzyloxy groups. In this study, allopyranan derivatives composed of both 3-amino-allopyranose and allopyranose units were prepared. Since these copolysaccharides contained amino groups and alternately sulfoamido groups at specific positions and in fixed quantities, these polysaccharides make it possible to elucidate relationships between the polymer structures and such biological activities as anti-HIV and anticoagulant activities. Ring-opening copolymerizations of **1** with **2** were performed with phosphorus pentafluoride as the catalyst to give copolymers, as shown in Scheme 1. The results are summarized in Table 1.

The proportion of **1** in the feed was decreased by intervals of 25 mol% from 100 to 0 mol%. The copolymer yields and number-average molecular weights increased with increasing proportion of **2** units in the feed, which suggested that **2** had higher polymerizability than **1**. The specific rotations also increased linearly with increasing proportion of **2** units in the copolymer. The copolymers had number-average molecular weights of $21.8\times10^3$ to $27.8\times10^3$, being thus almost half of those of the copolymers consisting of **2** with **3** units reported previously [10]. The monomer reactivity ratios of **1** ($r_1$) and **2** ($r_2$) were estimated by use of the Kelen–Tüdös method [18–20] in the polymerizations at low conversions less than 10% as follows:

$$r_1 = 0.92 \text{ and } r_2 = 1.11$$

In the copolymerization of **1** with 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (**3**), the $r_1$ and $r_3$ values were 0.66 and 1.67, respectively [10]. Accordingly, the present copolysaccharides having




Scheme 1. Synthetic route for sulfonated polysaccharides having 3-amino-3-deoxyallose residues.

Table 1
Ring-opening copolymerization of 1,6-anhydro-3-azido-2,4-di-*O*-benzyl-3-deoxy-β-D-allopyranose (**1**) with 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-allopyranose (**2**) [a]

| No. | Mole fraction [b] mol% | | Time h | Yield % | $\bar{M}_n$ [c] $\times 10^3$ | $\bar{M}_w/\bar{M}_n$ | $[\alpha]_D^{25}$ [d] deg | Mole fraction of **1** unit [e] mol% |
|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | | | | | | |
| 1 | 100 | 0 | 24 | 65 | 20.1 | 1.8 | +79.4 | 100 |
| 2 | 75 | 25 | 24 | 66 | 21.8 | 1.8 | +92.0 | 73 |
| 3 | 50 | 50 | 20 | 74 | 26.5 | 1.8 | +112.5 | 47 |
| 4 | 25 | 75 | 12 | 82 | 27.8 | 1.7 | +130.7 | 22 |
| 5 | 0 | 100 | 12 | 80 | 34.1 | 1.7 | +141.0 | 0 |

[a] Temp; −60 °C, Solvent: $CH_2Cl_2$; 0.4 ml, $PF_5$: 5 mol%.
[b] Total monomers; 0.20 g.
[c] Measured in $CHCl_3$ (c 1%).
[d] Determined by GPC using polystyrene as a standard eluted with THF.
[e] Calculated from $^{13}C$ NMR spectra.

both types of allose residues must have a more-random distribution of both monomeric units in the backbone than the copolysaccharides composed of allose and glucose residues.

Fig. 1 shows the $^{13}C$ NMR spectra of copoly-(**1**–**2**)s in the proportions of (B) 73 : 27, (C) 47 : 53, and (D) 22 : 78 mol%, in addition to (A) 3-azido-2,4-di-*O*-benzyl-3-deoxy-(1→6)-α-D-allopyranan and (E) 2,3,4-tri-*O*-benzyl-(1→6)-α-D-allopyranan. The anomeric C-1 carbon resonances in the copolymers appeared as two peaks at 96.2 and 98.1 ppm assigned to **1** and **2** units in spectra B, C, and D, respectively, indicating that the copolymers had the α-(1→6)-pyranosidic structure. The mole fraction of **1** units in the copolymers was almost the same as that in the feeds, because the polymerizations of the two monomers with somewhat different monomer reactivity ratios were performed in high conversions.

It was reported that, in the polymerization of **2**, the stereoregularity of the polymers is influenced by the monomer concentration in the feed and the poly(**2**)s obtained with monomer concentrations less than 0.5 mol/L had mixed structures consisting of α- and β-(1→6)-pyranosidic units [21]. However, as shown in Table 1, probably because the monomer concentrations were in the range of 0.65∼1.2 mol/L, all copolymers in this experiment possessed only the α-(1→6)-structure.

Table 2
Sulfonation of 3-amino-3-deoxy-(1→6)-α-D-allopyranans [a]

| | Free amino-polysaccharide | | | | | Sulfated amino-polysaccharide | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Mole fraction of sugar unit [c] mol% | | | Yield | $[\alpha]_D^{25}$ [d] | $\bar{M}_n$ [b] | Elemental analysis % | | | | |
| No | mg | $\bar{M}_n$ [b] $\times 10^3$ | 3-amino-allose | allose | glucose | mg | deg | $\times 10^3$ | C | H | N | S | DS [e] |
| 1 | 53 | 7.3 | 100 | 0 | 0 | 58 | +84.9 | 8.8 | 26.95 | 5.10 | 3.90 | 15.28 | 2.2 |
| 2 | 49 | 9.6 | 100 | 0 | 0 | 60 | +86.5 | 11.1 | 27.63 | 5.02 | 3.85 | 15.64 | 2.2 |
| 3 | 52 | 12.0 | 78 | 22 | 0 | 78 | +65.3 | 13.0 | 22.29 | 3.68 | 3.26 | 14.07 | 2.1 |
| 4 | 73 | 12.0 | 51 | 49 | 0 | 90 | +61.7 | 12.1 | 24.49 | 4.02 | 2.99 | 14.07 | 2.1 |
| 5 | 44 | 11.1 | 24 | 76 | 0 | 63 | +62.8 | 13.0 | 20.29 | 3.21 | 2.00 | 15.20 | 2.2 |
| 6 | 44 | 7.8 | 57 | 0 | 43 | 68 | +92.7 | 8.6 | 24.48 | 4.39 | 3.17 | 14.97 | 2.2 |
| 7 | 68 | 9.5 | 37 | 0 | 63 | 91 | +95.4 | 9.0 | 20.44 | 3.89 | 2.27 | 14.12 | 2.1 |
| 8 | 41 | 8.3 | 16 | 0 | 84 | 50 | +98.3 | 9.1 | 18.78 | 3.25 | 1.17 | 14.44 | 2.1 |
| 9 | 50 | 15.0 | 0 | 0 | 100 | 80 | +104.3 | 14.6 | 17.36 | 3.10 | 0 | 14.32 | 2.1 |

[a] Solvent; $Me_2SO$ 20 mL, Temp; 85 °C, Time; 1.5 h, Piperidine-*N*-sulfonic acid; 300–500 mg.
[b] Determined by GPC using pullulan as a standard.
[c] Calculated from $^{13}C$ NMR spectra.
[d] Measured in $H_2O$ (c 1%).
[e] Degree of sulfation (maximum, 3.0).

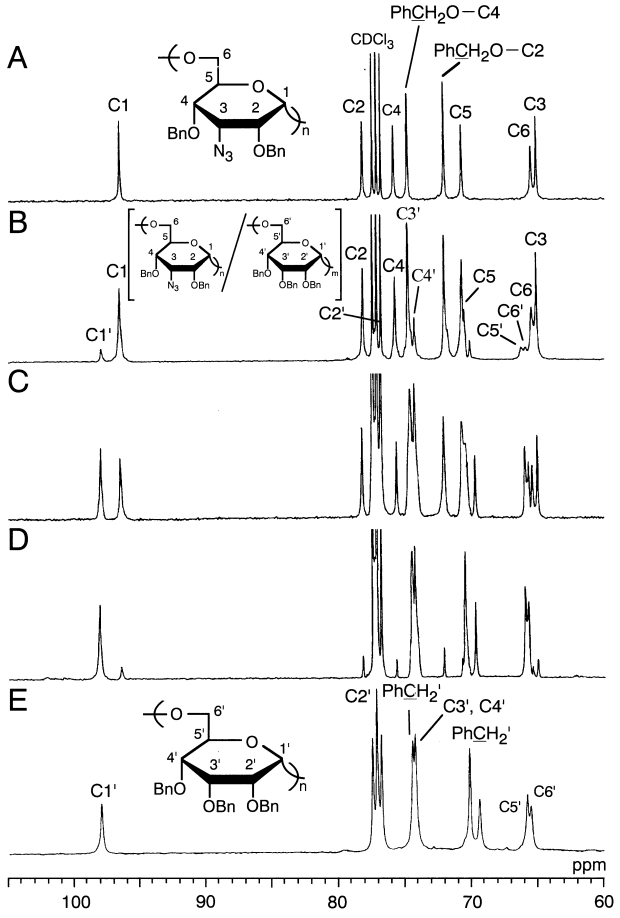



Fig. 1. 100 MHz $^{13}C$ NMR spectra of (A) 3-azido-2,4-di-*O*-benzyl-3-deoxy-(1→6)-α-D-allopyranan, (B)–(D) copoly(**1**–**2**)s, and (E) 2,3,4-tri-*O*-benzyl-(1→6)-α-D-allopyranan (in $CDCl_3$ as solvent). Monomer feed, (B) **1**:**2** = 75:25, (C) = 50:50, (D) = 75:25 mol%.

*Amino-polysaccharides*.—For the synthesis of amino-polysaccharides having free amino and hydroxyl groups, reduction of azido groups to amino groups by lithium aluminum hydride in the copolymers was first carried out. The IR absorption at 2102 $cm^{-1}$ for azide disappeared completely and a broad absorption (N–H stretching vibration) appeared around 3500 $cm^{-1}$.

Next the reduced copolymers were debenzylated with Na in liquid $NH_3$ at −78 °C to give new amino-polysaccharides having various ratios of 3-amino-3-deoxyallose units without irregular structures. Since these amino-polysaccharides were soluble in 3 wt% HCl solution and in $Me_2SO$, the amino-polysaccharides were isolated as their HCl salts after freeze-drying from water. Fig. 2 shows the $^{13}C$ NMR spectra of the HCl salt of amino-polysaccharides consisting of 3-amino-3-deoxyallose and allose units in the ratio of 76:24, 49:51, and 22:78 mol% in (B)–(D). All signals were assigned by their DQF-COSY and HMQC spectra. The C-1 signals at 99.7 and 100.5 ppm were assigned to C-1 of the 3-amino-3-deoxyallose and allose units, respectively, suggesting that the amino-polysaccharides had a complete α-(1→6) stereoregularity.

*Sulfonation*.—Sulfonation of the amino-polysaccharides was carried out with piperidine-*N*-sulfonic acid in $Me_2SO$ to give the sulfonated amino polysaccharides. Table 2 summarized the results of sulfonation of 3-amino-3-deoxy-(1→6)-α-D-allopyranan (nos. 1 and 2), amino-copolysaccharides

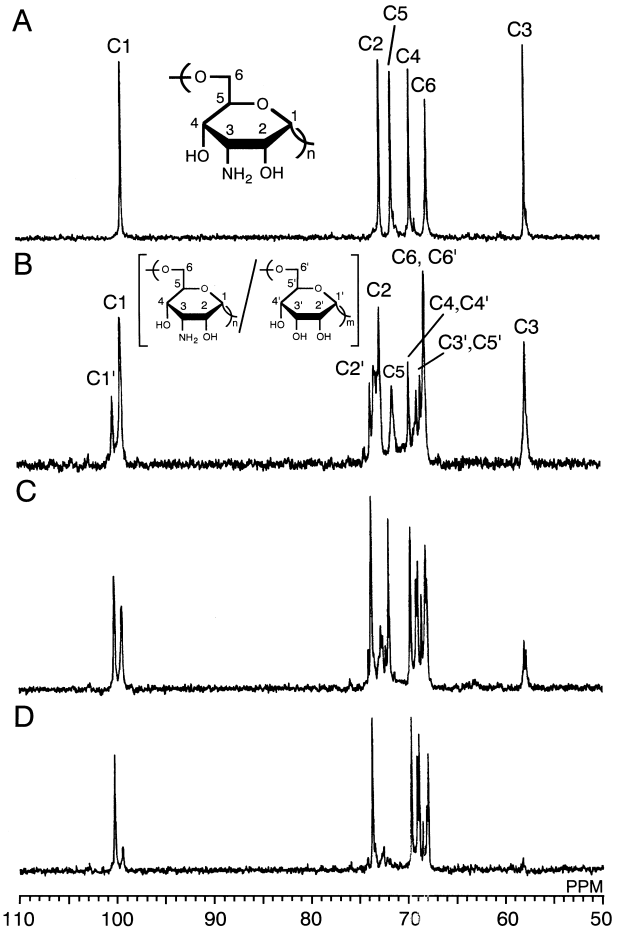



Fig. 2. 100 MHz $^{13}C$ NMR spectra of (A) 3-amino-3-deoxy-(1→6)-α-D-allopyranan, (B)–(D) amino-copolysaccharides consisting of 3-amino- 3-deoxy-allose and allose units in the ratio of (B) 73:27, (C) 47:53, and (D) 22:78 mol% (in $D_2O$ as solvent).

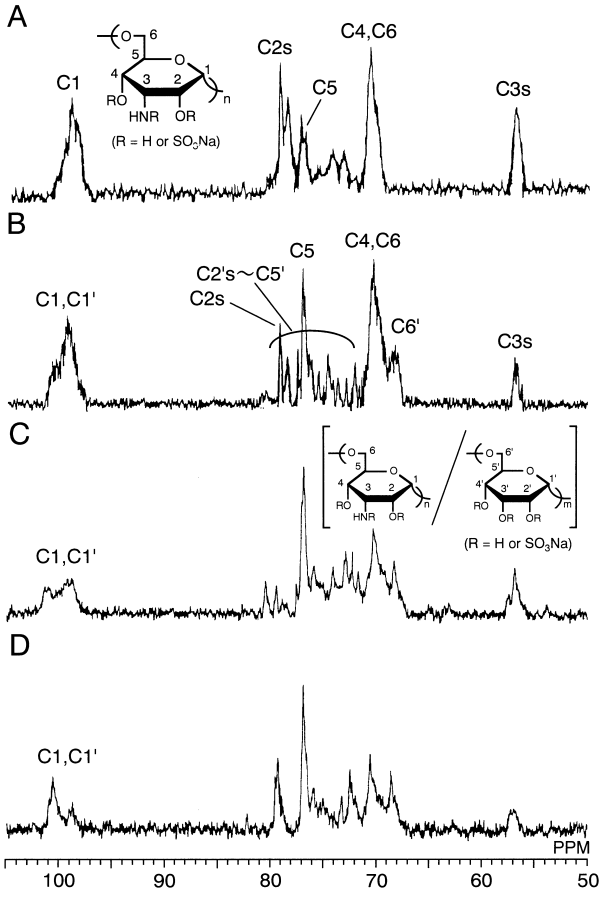



Fig. 3. 100 MHz $^{13}C$ NMR spectra of (A) sulfonated 3-amino-3-deoxy-(1→6)-α-D-allopyranan, (B)–(D) sulfonated amino-copolysaccharides consisting of 3-amino-3-deoxy-allose and allose units in the ratio of (B) 73:27, (C) 47:53, and (D) 22:78 mol% (in $D_2O$ as solvent). (R = H or $SO_3Na$, s: sulfonate substituted carbon).

consisting of 3-amino-3-deoxyallose and allose (nos. 3–5), of 3-amino-3-deoxyallose and glucose units (nos. 6–8), and synthetic dextran (no. 9). The sulfonated amino-copolysaccharides possessed fairly high molecular weights, ranging from $8.6\times10^3$ to $13.0\times10^3$, and considerably high degrees of sulfonation of 2.1–2.2 per sugar residue. For sulfonated 3-amino-3-deoxy-(1→6)-α-D-allopyranan and sulfonated amino-polysaccharides consisting of 3-amino-3-deoxyallose and allose residues, $^{13}C$ NMR spectra assigned by DQF-COSY and HMQC techniques are shown in Fig. 3. The degree of sulfonation (DS) was calculated from the elemental analysis. It was noted that the C-2 and C-3 absorptions were shifted downfield and upfield, respectively, but the chemical shift of the C-4 absorption did not change (Fig. 3A). This suggests that sulfonation of 3-amino-3-deoxy-(1→6)-α-D-allopyranan having DS = 2.2 occurred mainly at the C-2 hydroxyl and C3 amino groups, and only little at C-4 because of steric hindrance. For the sulfonated copolysaccharides in Fig. 3B–D, taking into account the DS and the NMR spectra, the sulfonation evidently occurred mainly at 2-OH and 3-$NH_2$ groups. This phenomenon was also observed in the sulfonation of 3-amino-3-deoxy-(1→6)-α-D-glucopyranan [22].

Fig. 4 shows spectra of the sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and glucose units. Sulfonation again appears to occur at the C-2 and C-3 functional groups, because shifts of the C-2 and C-3 resonances are seen.

*Anti-HIV and blood anticoagulant activities of sulfonated amino-polysaccharides.*—Anti-HIV and blood anticoagulant activities of sulfonated amino-polysaccharides are shown in Table 3. Sulfonated polysaccharides having almost the same molecular

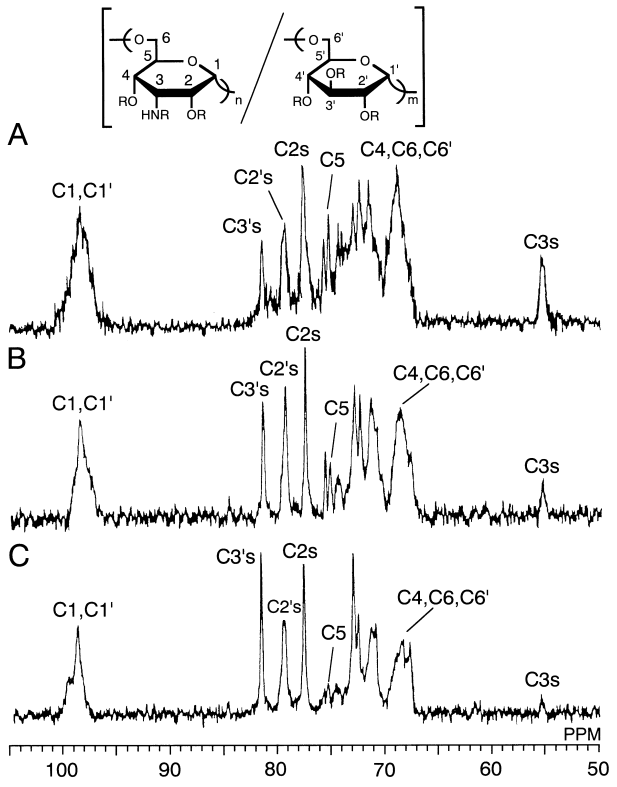



Fig. 4. 100 MHz $^{13}C$ NMR spectra of sulfonated amino-copolysaccharides consisting of 3-amino-3-deoxy-allose and glucose units in the ratio of (A) 71:29, (B) 43:57, and (C) 21:79 mol% (in $D_2O$ as solvent). (R = H or $SO_3Na$, s: sulfonate substituted carbon).

weight and the degree of sulfonation were used for the biological assays. The anti-HIV activity and the blood anticoagulant activity (A.A.) were examined by the MTT method using MT-4 cells [16] and by the United States Pharmacopoeia method [17] using bovine plasma, respectively. The anti-HIV activity and cytotoxicity of sulfonated amino-polysaccharides were evaluated by their $EC_{50}$ values, namely the 50% inhibitory concentration for infection of HIV into MT-4 cells, and by the $CC_{50}$, that is, the 50% cytotoxic concentration for MT-4 cells, respectively. In nos. 1 and 2, the sulfonated 3-amino-allopyranans showed potent anti-HIV activities, as manifested by the $EC_{50}$ of 0.1 and 0.2 $\mu$g/mL, which is almost equivalent to that of curdlan sulfate ($EC_{50} = 0.13\ \mu$g/mL), which has potent anti-HIV activity [23]. In addition, these sulfonated amino-allopyranans (nos. 1 and 2) exhibited low cytotoxicities ($CC_{50} > 1000\ \mu$g/mL).

The sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and glucose units (no. 6–8) also had high anti-HIV activities ($EC_{50} = 0.2$–$0.5\ \mu$g/mL), and low cytotoxicities of $CC_{50} > 1000\ \mu$g/mL. In contrast, the sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and allose units (no. 3–5) exhibited somewhat lower activities ($EC_{50} = 0.8$–$0.9\ \mu$g/mL) as well as rather higher cytotoxicities ($CC_{50} = 740$–$797\ \mu$g/mL). The reason for this slight increase in cytotoxicity might be attributable to the presence of allose in a structure also containing sulfoamido substituted allose. Such a phenomenon was reported for sulfonated alkyl oligosaccharides containing octadecyl groups, which showed both high anti-HIV activity and increased cytotoxicity [24,25].

The blood anticoagulant activity (AA) of sulfonated amino-polysaccharides, as determined by use of whole bovine plasma, depended on the number of sulfoamido groups and the kinds of sugar units in the polysaccharide main chain. The anticoagulant activity increased with increasing proportion of sulfoamido groups in the polysaccharide chains, as shown from no. 5 to no. 3 and from no. 9 to no. 6 in Table 3. Although the sulfonated 3-amino-3-deoxyallopyranans (nos. 1 and 2) had high anticoagulant activities of 44 and 58 units/mg, the sulfonated copolysaccharide consisting of 3-amino-3-deoxyallose and allose units in the mole ratio of 78:22 had also high anticoagulant activity of 56 unit/mg (no. 3). In addition, the blood anticoagulant activity of the sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and allose units decreased with decreasing proportion of the 3-amino-3-deoxyallose unit from 56 to 42 unit/mg. In contrast, the sulfonated copolysaccharides consisting of 3-amino-3-deoxyallose and glucose units showed only moderate anticoagulant activity, 30–35 units/mg (nos. 6–8).

Based on the results of the anticoagulant assay, three conclusions may be drawn: (1) the sulfoamido group plays an important role in interactions of the sulfoamido and sulfonate group-containing copolysaccharides with blood coagulation factors, (2) the anticoagulant activity increases which a random distribution of 3-amino-3-deoxyallose units in the amino-copolysaccharide chain, and (3) the configuration of the C-3 sulfonate group is important for high anticoagulant activity, that is, the *allo* configuration is more effective than the *gluco* one. In addition, it has been demonstrated that the sulfoamido group in the sulfoamido and sulfonate group-containing polysaccharides not only has nearly the same effect as that of the sulfonate group for anti-HIV activity, but also plays an important role in increasing anticoagulant activity.

Table 3
Anti-HIV and anticoagulant activities of sulfated 3-amino-3-deoxy-(1→6)-α-D-allopyranans

| No | Mole fraction of sugar unit mol% | | | $\bar{M}_n \times 10^3$ | S content % | DS | $EC_{50}$[a] μg/mL | $CC_{50}$[b] μg/mL | Anticoagulant activity[c] unit/mg |
|---|---|---|---|---|---|---|---|---|---|
| | 3-amino-allose | allose | glucose | | | | | | |
| 1 | 100 | 0 | 0 | 8.8 | 15.28 | 2.2 | 0.1 | > 1000 | 44 |
| 2 | 100 | 0 | 0 | 11.1 | 15.64 | 2.2 | 0.2 | > 1000 | 58 |
| 3 | 78 | 22 | 0 | 13.0 | 14.07 | 2.1 | 0.8 | 740 | 56 |
| 4 | 51 | 49 | 0 | 11.0 | 14.63 | 2.1 | 0.9 | 743 | 50 |
| 5 | 24 | 76 | 0 | 13.0 | 15.20 | 2.2 | 0.9 | 797 | 42 |
| 6 | 57 | 0 | 43 | 8.6 | 14.97 | 2.2 | 0.2 | > 1000 | 35 |
| 7 | 37 | 0 | 63 | 9.0 | 14.12 | 2.1 | 0.3 | > 1000 | 31 |
| 8 | 16 | 0 | 84 | 9.1 | 14.44 | 2.1 | 0.4 | > 1000 | 30 |
| 9 | 0 | 0 | 100 | 14.6 | 14.32 | 2.1 | 0.5 | > 1000 | 27 |
| Dextran sulfate (H-039) | | | | 8.5 | 18.4 | 2.7 | 0.84 | > 1000 | 22.7 |
| Curdlan sulfate | | | | 79.0 | 14.1 | 2.1 | 0.13 | > 1000 | < 10 |

[a] 50% Effective concentration.
[b] 50% Cytotoxic concentration.
[c] Standard dextran sulfate H-039, 22.7 unit/mg.

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