Studies on Interaction Mechanism of Sulfated Polysaccharides as an AIDS Drug by NMR

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ABSTRACT: Various sulfated polysaccharides exhibit antiviral activity *in vitro* against certain viruses, including HIV, via an unknown mechanism. It has been known that the polysaccharides appear to bind strongly to the HIV virions and inhibit their association with the CD4 receptor of T lymphocytes. Ionic interactions are assumed to play a major role, as has been demonstrated in the interaction between a negatively charged polysaccharide portion of heparin and three lysine residues in antithrombin-III. A model system for interaction was constructed using sodium curdlan sulfate, a sulfated polysaccharide with extremely potent *in vitro* inhibition against HIV (abbreviated "CS"), and poly-L-lysine·HBr (abbreviated "PL") to simulate the α-6 helix of HIV gp120. The oligomeric nature of both species allowed characterization by ^{1}H and ^{13}C NMR. Upon mixing certain ratios of the two compounds in aqueous solution, a gel formed in the sample tube. The gel isolated was found to be composed primarily of poly-L-lysine and curdlan sulfate, having excluded most of the sodium and bromide ions. NMR analyses revealed a single species that is quite different from either of the two original species. The extent of gelling was affected by pH, average $\bar{M}_w$ of the PL, temperature, and absolute concentration. Maximal gelling was attained at pH 4.0 ∼ 8.4, 10000 Da, and 37 °C, respectively. It was concluded that CS can bind strongly with PL via ionic interactions, in such a way as to produce a polyion complex with a conformation distinct from either of the two original compounds. It was suggested that this interaction may help to explain the observed inhibition of HIV and other viruses by CS, in that CS may bind to highly basic helical segments of viral proteins and in so doing induce a conformational change that affects their functions.

Introduction

Since the antiviral activity of sulfated polysaccharide was first reported by Gerber et al.,[1] various sulfated polysaccharides such as dextran sulfate,[2] heparin,[3] and sulfated glycosaminoglycan[4] were found to inhibit viral replications of herpes simplex, yellow fever, and polio viruses. Recently, it was revealed that fucoidan,[5] lentinan sulfate,[6] pentosan sulfate,[7] and curdlan sulfate[8] showed anti-HIV activity *in vitro*. In particular, we have reported that curdlan sulfate has potent inhibitory activity against HIV-1 and HIV-2 without serious toxicity and stability problems.[9,10]

It is assumed that ionic interactions of sulfated polysaccharides with proteins caused various biological activities. Interactions between bio-molecules have been well analyzed in the case of anticoagulant heparin with antithrombin-III.[11] In this case, a negatively charged pentasaccharide portion of heparin is supposed to interact with a positively charged portion of antithrombin-III containing three positively charged lysine residues in appropriate spatial positions for the interaction.[12]

According to the secondary structure of HIV envelope glycoprotein gp120, it contains six α-helices in which four helices, i.e., α1, α4, α5, and α6, were found in the conserved C1, C4, C5 regions and two helices, i.e., α2 and α3, were predicted in the V2 and pseudo-conserved C3 regions.[13,14] In particular, a helical portion containing several basic amino acids such as lysine and arginine were found in residues 506 through 518 (Thr−Lys$^+$−Ala−Lys$^+$−Arg$^+$−Arg$^+$−Val−Val−Gln−Arg$^+$−Glu−Lys$^+$−Arg$^+$) in the α6 helical region.

Taking into account the interaction mechanism of heparin with antithrombin-III, negatively charged portions of sulfated polysaccharides may have interactions with positively charged helical regions in the HIV envelope glycoprotein gp120.[15,16] By these interactions probably causing conformational change in the gp120, the sulfated polysaccharide seems to inhibit binding of the virus protein to the CD4 receptor of T lymphocytes.[17] Examining in detail the inhibitory effects of curdlan sulfate against HIV infection, Aoki found out that the curdlan sulfate interacts strongly with HIV virions and weakly with T lymphocytes.[18]

We examined interactions of curdlan sulfate with polylysine as model compounds for the sulfated polysaccharide and the positively charged protein portion, respectively. In this study, we wish to report detection of the gellike material formed by interactions between curdlan sulfate and polylysine by ^{1}H and ^{13}C NMR spectroscopy. In addition, effects of various factors on the gel formation are examined.

Results and Discussion

NMR Spectra of Polyion Complexes Composed of Curdlan Sulfate and Polylysine. When curdlan sulfate (CS) was mixed with low molecular weight lysine compounds such as lysine, lysine dimer, and lysine trimer, clear solutions were obtained. On the other hand, it was found that gellike complexes were formed by mixing curdlan sulfate with lysine compounds larger than a tetramer. It is assumed that several sulfate anions in the curdlan sulfate interacted with several ammonium cations in the lysine compound to form an insoluble gellike complex which must have very high molecular weight by cross-linking.

In order to elucidate structures of the gellike complex, interactions between curdlan sulfate with $\bar{M}_w$ of 79 000

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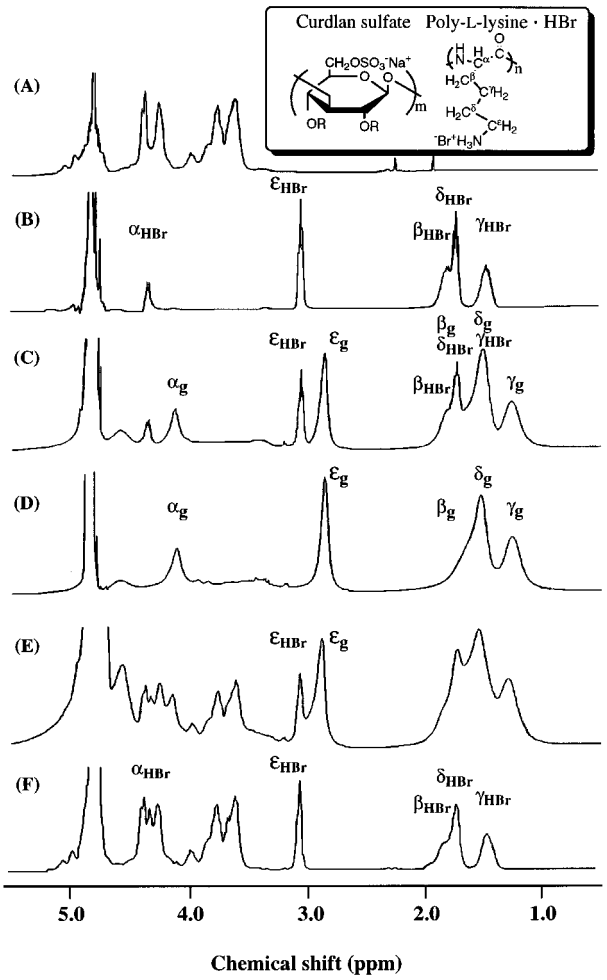

Figure 1. 400 MHz ^{1}H NMR spectra of curdlan sulfate (CS), poly-L-lysine·HBr (PL), and polyion complexes (PIC) between CS and PL at different molar ratios (CS/PL): (A) curdlan sulfate; (B) poly-L-lysine·HBr; (C) PIC at a molar ratio of 0.5; (D) PIC at a molar ratio of 0.8; (E) PIC at a molar ratio of 1.0; (F) PIC at a molar ratio of 2.0. The concentration of PL is 2.8% (w/v).

and polylysine hydrobromide (PL) with $\bar{M}_w$ of 10 700 were examined by means of NMR spectroscopy.

Figure 1 shows the NMR spectra of mixtures of curdlan sulfate and polylysine in different molar ratios. Absorptions due to curdlan sulfate and polylysine in D_2O solution were assigned according to the references.[10,19] As seen in spectra C−F, ^{1}H NMR absorptions due to polylysine component to a large extent changed with the molar ratio of curdlan sulfate to polylysine. The variation in the molar ratio seemed to cause a difference in the quantities of the gellike complex and free polylysine.

For a molar ratio of repeating units (CS/PL) of 0.5 (spectrum C), absorptions due to polylysine appeared as two peaks. One peak appearing at 3.03 ppm is assignable to the ϵ proton (designated as ϵ_{HBr}) of the side chain in the starting polylysine hydrobromide, and the other (ϵ_g at 2.84 ppm) is due to a new species formed by interactions between curdlan sulfate and polylysine. In this case, a gellike material attaching to the NMR tube was observed. In addition, absorptions due to the curdlan sulfate disappeared probably as broad baseline peaks. This phenomenon has been often seen in NMR spectra of locally immobile gelled polymers. Similarly, other protons due to the gellike material for the polylysine side chain appeared 0.2 ppm upfield from the corresponding proton peaks. Accordingly, it was revealed that both the starting polylysine and the gellike complex formed by the interaction of CS with PL were existing in this system.

On the other hand, in the molar ratio of 0.8 (spectrum D), the absorptions due to the gellike material exclusively appeared. The absolute amount of the gellike material is recorded as a maximum in this molar ratio. Since the curdlan sulfate had 1.5 sulfate groups per glucose unit, the molar ratio of CS to PL of 0.8 indicates that the ratio of $-OSO_3^-$ anions to $-NH_3^+$ cations is about 1.2. From the ionic interactions between anionic and cationic polymers, the hydrophobicity of polyion complexes is assumed to reach a maximum around this molar ratio, affording the insoluble gel.

For a molar ratio of 1 (spectrum E), absorptions due to the ϵ protons of polylysine appeared as two peaks, indicating the existence of two species. One appeared at the same chemical shift of the molar ratio of 0.8, while the other appeared at 0.2 ppm downfield from that of the gellike complex. The chemical shift of the latter species was equivalent to that of free polylysine. Absorptions due to excess curdlan sulfate appeared in chemical shifts between 3.5 and 4.5 ppm. Accordingly, when excess curdlan sulfate existed in the mixture, there were three species: that is, the complex, curdlan sulfate, and polylysine. It is not clear whether the curdlan sulfate and the polylysine existed free or in a form of soluble complex. For a molar ratio of 2, a sample solution became clear without forming the gellike material. Absorptions due to curdlan sulfate and polylysine appeared separately (spectrum F).

The observation that, for a molar ratio of 0.5−0.8, the absorption of curdlan sulfate disappeared into the baseline might imply that the polysaccharide structure is so rigid as to inhibit local motions of the polysaccharide included in the complex. Thus, absorptions due to curdlan sulfate were not detected in the presence of the gellike complex. pH values of the mixtures used for spectra C−F ranged from 6.4 to 6.8.

In accordance with ^{1}H NMR spectra, ^{13}C NMR spectra also varied by the presence of the gellike complex. Typical spectra are presented in Figure 2. All carbons were observed separately, and absorptions due to curdlan sulfate and polylysine were assigned according to the references.[10,21]

The characteristics of the ^{13}C NMR spectra are as follows. By formation of the gellike complex, absorptions due to curdlan sulfate disappeared as background for a molar ratio smaller than 0.8. Concerning absorptions of polylysine, α and β carbon peaks of polylysine side chains were broadened by the formation of the complex. All absorptions due to γ to ϵ carbons of polylysine were split individually into two peaks due to the coexistence of unreacted free polylysine and the gellike complex. New peaks appeared upfield by approximately 0.07−0.25 ppm composed with those of the polylysine. For a molar ratio of 0.8 (spectrum C), peak broadening of the α and β carbons is the most prominent. In addition, all absorptions of polylysine shifted upfield, with individual carbon peaks appearing as a single peak. Disappearance of the absorption due to curdlan sulfate and broadening of α and β carbon peaks clearly demonstrated the lack of local motion of the polymer backbone due to the formation of high-molecular-weight polyion complexes.

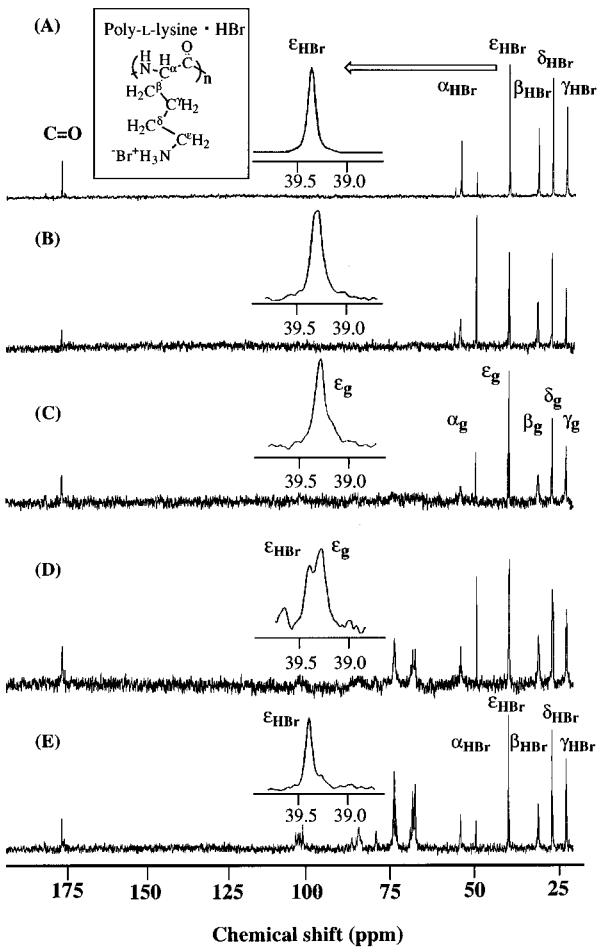

Figure 2. 100 MHz ^{13}C NMR spectra of poly-L-lysine·HBr (PL) and polyion complexes (PIC) between CS and PL at different molar ratios: (A) poly-L-lysine·HBr; (B) PIC at a molar ratio of 0.5; (C) PIC at a molar ratio of 0.8; (D) PIC at a molar ratio of 1.0; (E) PIC at a molar ratio of 2.0. The concentration of PL is 2.8% (w/v).

For a molar ratio of 1 (spectrum D), small absorptions due to curdlan sulfate appeared between 60 and 110 ppm. The α and β peaks of polylysine in the complex became sharp, and the γ to ϵ absorptions split into two peaks due to the coexistence of free polylysine and the gellike complex. In the molar ratio of 2 (spectrum E), absorptions due to both curdlan sulfate and polylysine appeared separately.

CD spectra of mixtures of CS with PL were also measured, revealing that the PL existed in the random coil conformation in neutral to basic pH ranges.

Taking into account results on both NMR and CD measurements, the curdlan sulfate forms complexes with the polylysine in certain molar ratios. The NMR spectrum also demonstrated that in the complex the CS took a rigid immobile conformation, while the flexible PL took a random conformation which afforded rather sharp absorptions for individual protons or carbons.

Similarly to an interaction between heparin and antithrombin-III,[12] the ionic interaction between CS and PL is assumed to be based on segmental interactions. Since both polymeric CS and PL had such several functional segments, multiple interactions took place among two kinds of polymers to cause cross-linkings, providing gels.

Counterion Analysis of Curdlan Sulfate and Polylysine. To examine the complex state, the quantitative analysis of counterions of sodium curdlan sulfate (=curdlan sulfate) and polylysine hydrobromide (PL·HBr), i.e., Na^+ and Br^-, was carried out by atomic absorption spectroscopy and ion chromatography, respectively.

Table 1. Results of Elemental Analysis for Polyion Complex

sample	^1H NMR spectrum in Figure 1		anal. % C	% H	% Na^+ [a]	% Br^- [b]
CS[c]	A	calcd	22.9	2.6	10.9	
		found	20.7	4.3	10.6	
PL[d]	B	calcd	34.5	6.3		38.2
		found	31.5	6.9		34.0
PIC[e]	D					
before dialysis		calcd	28.1	4.2	6.0	17.3
		found	31.3	6.1	1.0	3.3
after dialysis[f]		found	35.9	6.9	0	0

[a] Analyzed by atomic absorption spectroscopy (AAS). [b] Analyzed by ion chromatography (IC). [c] Sodium curdlan sulfate (abbreviated as curdlan sulfate). [d] Polylysine hydrobromide. [e] PIC means a polyion complex prepared by mixing curdlan sulfate and polylysine in a molar ratio of 0.8. [f] It was done at room temperature for 48 h.

The gellike complex was formed by mixing sodium curdlan sulfate and polylysine hydrobromide in the molar ratio of 0.8 at 0.4 M concentration of polylysine. Table 1 shows results on the counterion analysis for the gellike complex which was separated from the mixture.

One percent of sodium and 3.3% bromide were contained in the as formed gellike material before dialysis. The calculated contents of sodium and bromide were 6.0% and 17.3%, respectively. It was suggested that reaction of sodium curdlan sulfate with polylysine hydrobromide took place by causing precipitation or exclusion of sodium bromide out of the gel. Since both Na^+ and Br^- ions were scarcely detected in the mixture, the dialysis removed the remaining small amount of both counterions out of the gel. Accordingly, it was concluded that the gellike material was composed of a polyion complex between curdlan sulfate having $-OSO_3^-$ anions and polylysine having $-NH_3^+$ cations and that since the complex lost Na^+ and Br^- ions to form an ultrahigh-molecular-weight cross-linked polymeric material, it became insoluble.

Factors Controlling Formation of the Polyion Complex Gel. Since it was assumed that the gel formation would be affected by other factors than the CS-to-PL ratio, the gel formation was examined in detail by changing several reaction conditions.

Effects of pH. To this section, the gel formation was examined in nearly neutral pH ranges. However, it is clear that the equilibrium between amino groups and ammonium ions in the polylysine in addition to the dissociation of sodium curdlan sulfate depends on the pH of the mixture solution. Thus, effects of the pH of the mixture solution on the complex formation were investigated. As shown in Figure 3, spectra of mixtures consisting of curdlan sulfate and polylysine hydrobromide varied to a large extent with pH of the mixture. At nearly neutral pH 6.6 (spectrum D), ϵ proton of the polylysine side chain shows two peaks which are due to free polylysine hydrobromide and polyion complex at 3.05 and 2.89 ppm, respectively. Below pH 2.0, these two peaks are incorporated into a broad single peak at 3.04 ppm. The broad single peak might indicate that since in strong acidic conditions the amino group neutralized by HBr was changed into alkyl ammonium group and free HBr, a rapid exchange between the

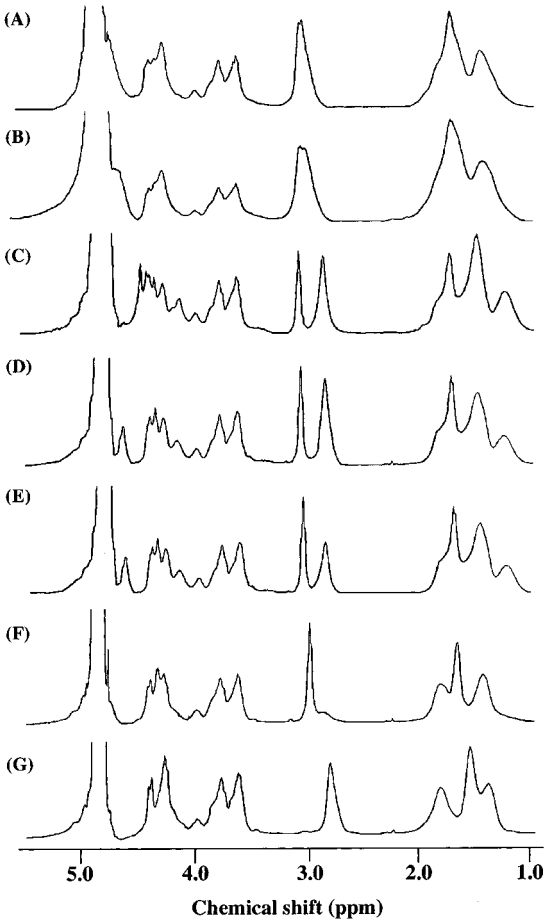

Figure 3. 400 MHz ^{1}H NMR spectra of polyion complexes between CS and PL in different pH's in the molar ratio of 1.0. The concentration of PL is 1.0% (w/v): (A) pH 0.4; (B) pH 2.0; (C) pH 5.0; (D) pH 6.6; (E) pH 8.4; (F) pH 9.8; (G) pH 11.5.

formed alkyl ammonium group and the alkyl ammonium contained in the polyion complex occurred.

On the other hand, since, in an alkaline condition at pH 9.8, the almost NH_3^+ groups were changed into the NH_2 groups, the formation of the polyion complex was to a large extent suppressed. At a stronger alkaline condition of pH 11.5, the ϵ proton neighboring to the NH_2 shifted upfield to 2.79 ppm by the influence of solvent.

Proportions of the gel were determined by measuring intensities of ϵ_g and ϵ_{HBr} proton absorptions corresponding to the gel and the free polylysine, respectively, by means of ^{1}H NMR spectroscopy. As shown in Figure 4, the percentage of the gel was in the range of approximately 50−67% at pH 4 to pH 8.4, and it to a great extent decreased to 11% at pH 9.8. It is assumed that since the dissociation of sulfate groups of CS into sulfate anions was not affected by pH, a nearly constant amount of the gellike complex was produced in acidic to neutral pH ranges.

Effects of Concentration and Temperature. The proportion of the gellike complex also depended on concentration of the starting polylysine hydrobromide. The result is depicted in Figure 5. When the molar ratio of CS to PL·HBr was 0.8, the percentage of the gel was almost constant at 93−100% independent of the PL concentration. On the other hand, for molar ratios of 0.67 and 1.0, the percentage of the gel increased with increasing concentration of PL to reach 100 and 75%, respectively, at the PL concentration of 2.8%. For a molar ratio of 2.0, the gel was not produced at any PL

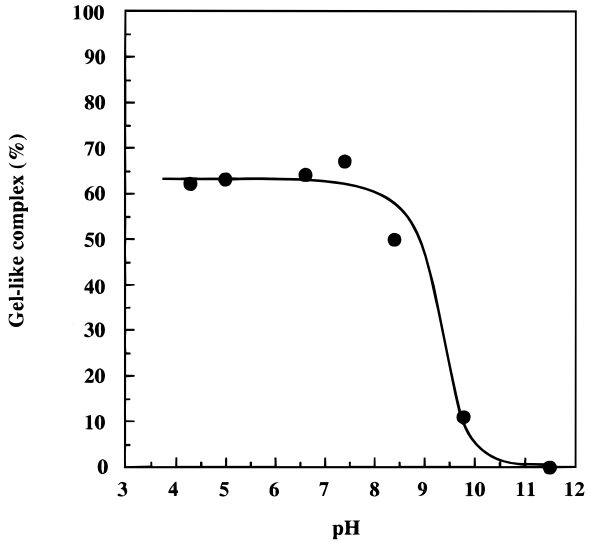

Figure 4. Dependence of the amount of gellike complex on pH in the molar ratio of 1.0. The concentration of PL is 1.0% (w/v).

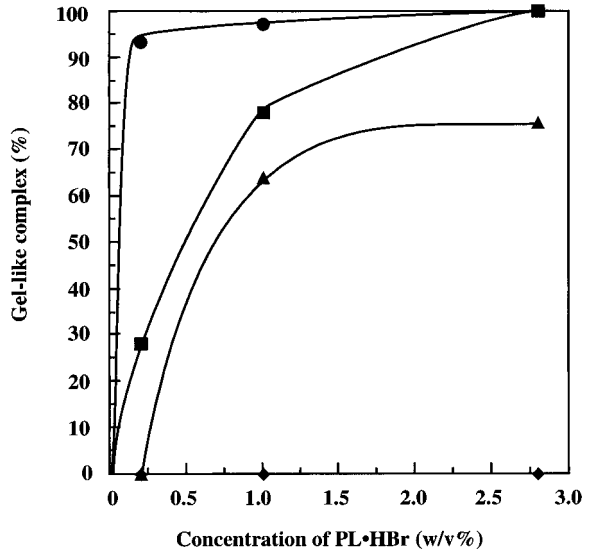

Figure 5. Dependence of the amount of gellike complex on molar ratio and concentration: (■) molar ratio of 0.67; (●) molar ratio of 0.8; (▲) molar ratio of 1.0; (◆) molar ratio of 2.0.

concentration. When the concentration of PL was low, the gel to be formed might have not a high enough molecular weight as to precipitate. In addition, a large excess amount of CS seemed not to cause the gel formation, probably because an appropriate ratio of anionic CS to cationic PL is necessary for ionic interactions to form cross-linkings.

When the reaction of CS with PL was compared at 25 and 37 °C, the latter temperature was more advantageous than the former, because the higher temperature completed the gel formation in a shorter time. Thus, NMR measurements were performed at 37 °C.

Effects of the Molecular Weight of Polylysine. As shown in Figure 6, no gel was observed by using oligolysines smaller than tetramer. The proportion of gels increased with increasing molecular weight from 530 to 10 700 which showed the maximum proportion. Using polylysines with molecular weights greater than 25×10^3, the proportion of gels exhibited a tendency to decrease with the molecular weight. Since the cross-linking was assumed to be caused by multiple interac-

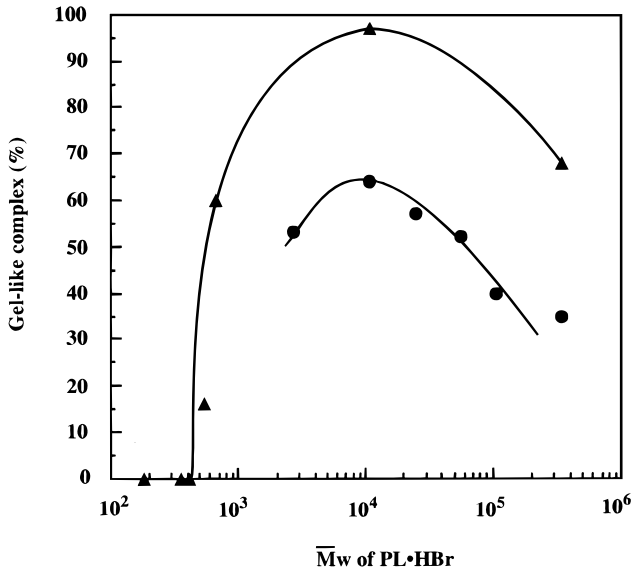

Figure 6. Dependence of the proportion of gellike complex on the molecular weight of PL. The reaction was carried out for 1 h by use of 1.0% (w/v) of polylysine concentration: (▲) Molar ratio of 0.8; (●) molar ratio of 1.0.

tions of both anionic and cationic segments, this fact indicates that oligolysines up to trimer did not contain multiple segments. On the other hand, it is assumed that as the molecular weight of PL was higher than a few ten thousands, CS and PL chains could not interpenetrate for CS to interact with every segment of the PL chain. Such molecular weight dependence of ionic interactions was reported on a mixture of polymethacrylic acid with quarterized oligo(ethyleneimine).[20]

In addition, when salts with strong ionic strengths such as $NaClO_4$ and KSCN were added to the aqueous solution of sodium curdlan sulfate and PL·HBr, formation of the gellike complex was decreased to a large extent. These salts are known not only to have high solubilities in water but also to affect conformations of polylysine and other polyamino acids.[21−23] Thus, it is also suggested that the gellike complex between CS and PL was formed with an accompaning precipitation of NaBr out of the gel.

In this study, we presented by use of NMR spectroscopy evidence that curdlan sulfate has a strong interaction with polylysine, the amino group of which is known to act as a cation in biological systems. Since it is presumed that an anti-HIV activity of the curdlan sulfate originates from its ionic interactions with a virus envelope glycoprotein,[16] NMR studies on the interaction of CS with the virus protein segments might afford a clear evidence on the biological activity.

Experimental Section

Materials. Sodium curdlan sulfate (abbreviated as curdlan sulfate) containing 14.4% of sulfur with a weight average molecular weight of 79 000 was provided from Ajinomoto Co., Ltd. Lysine hydrochloride was purchased from Junsei Chemical Co., Tokyo. Lysine dimer hydrochloride, oligolysine acetates (from trimer to pentamer) and poly-L-lysine hydrobromide ($\bar{M}_w$ 2700−346500, which was measured by LALLS) were purchased from Sigma and used without further purification.

Preparations of Polyion Complexes. Samples for the NMR measurements were prepared as follows. A certain concentration of lysine compounds of 2.8% (w/v) were added to a curdlan sulfate (CS) solution of different concentration in an NMR tube and then were mixed for 2 min. The solution was incubated at 37 °C for 1 h, unless otherwise mentioned. To measure the amount of gellike complexes, 1.0% (w/v) of polylysine solution was used. The pD of the samples were adjusted using NaOD or DCl. pD was measured with a TOA HM-30V pH meter with a GS-5016 electrode by reading the pH of solutions in D_2O without correction.

Measurements. 400 MHz ^{1}H and 100 MHz ^{13}C NMR spectra were recorded on a JEOL Lambda-400 spectrometer. 4,4-Dimethyl-4-silapentane-1-sulfonate (DSS) and methanol solution were used as the internal standards for ^{1}H and ^{13}C NMR measurements, respectively. TMS was used as reference zero.

References and Notes

(1) Gerber, P.; Dutcher, J. D.; Adams, E. V.; Sherman, J. H. *Proc. Soc. Exp. Biol. Med.* **1958**, *99*, 590.
(2) Nahmias, A. J.; Kibrick, S.; Bernfeld, P. *Proc. Soc. Exp. Biol. Med.* **1964**, *115*, 993.
(3) Vaheri, A.; Cantell, K. *Virology* **1963**, *21*, 661.
(4) Voss, H.; Sensch, K. H.; Panse, P. *Zentralbl. Bacterior. Orig. A* **1974**, *229*, 1.
(5) Baba, M.; Snoeck, R.; Pauwels, R.; De Clercq, E. *Antimicrob. Agents Chemother.* **1988**, *32*, 1742.
(6) Yoshida, O.; Nakashima, H.; Yoshida, T.; Kaneko, Y.; Yamamoto, I.; Uryu, T.; Matsuzaki, K.; Yamamoto, N. *Biochem. Pharmacol.* **1988**, *37*, 2887.
(7) Baba, M.; Nakajima, M.; Schlos, D.; Pauwels, R.; Balzarini, J.; De Clercq, E. *Antiviral Res.* **1988**, *9*, 335.
(8) Kaneko, Y.; Yoshida, O.; Nakagawa, R.; Yoshida, T.; Date, M.; Ogihara, S.; Shiyoya, T.; Matsuzawa, Y.; Shinkai, H.; Yasuda, N.; Matsuzaki, K.; Uryu, T.; Yamamoto, N. *Biochem. Pharmacol.* **1990**, *39*, 793.
(9) Yoshida, T.; Hatanaka, K.; Uryu, T.; Kaneko, Y.; Suzuki, E.; Miyano, H.; Mimura, T.; Yoshida, O.; Yamamoto, N. *Macromolecules* **1990**, *23*, 3717.
(10) Yoshida, T.; Yasuda, Y.; Mimura, T.; Kaneko, Y.; Nakashima, H.; Yamamoto, N.; Uryu, T. *Carbohydr. Res.* **1995**, *276*, 425.
(11) Lindahl, U.; Backstrom, G.; Thunberg, L. *J. Biol. Chem.* **1983**, 258, 9826.
(12) Villanueva, G. B. *J. Biol. Chem.* **1984**, *259*, 2531.
(13) Ratner, L.; Haseltine, W.; Patarca, R.; Livak, K. J.; Starcich, B.; Josephs, S. F.; Doran, E. R.; Rafalski, J. A.; Whitehorn, E. A.; Baumeister, K.; Ivanoff, L.; Petteway, S. R. Jr.; Pearson, M. L.; Lautenberger, J. A.; Papas, T. S.; Ghrayeb, J.; Chang, N. T.; Gallo, R. C.; Wong-staal, F. *Nature* **1985**, *313*, 277.
(14) Hansen, J. E.; Lund, O.; Nielsen, J. O.; Brunak, S.; Hansen, J. E. S. *Proteins* **1996**, 25, 1.
(15) Uryu, T.; Ikushima, N.; Katsuraya, K.; Shoji, T.; Takahashi, N.; Yoshida, T.; Kanno, K.; Murakami, T.; Nakashima, H.; Yamamoto, N. *Biochem. Pharmacol.* **1992**, *43*, 2385.
(16) Jagodzinski, P. P.; Wiaderkiewicz, R.; Kurzawski, G.; Kloczewiak, M.; Nakashima, H.; Hyjek, E.; Yamamoto, N.; Uryu, T.; Kaneko, Y.; Posner, M. R.; Kozbor, D. *Virology* **1994**, *202*, 735.
(17) Lederman, S.; Gulick, R.; Chess, L. *J. Immunol.* **1989**, *143*, 1149.
(18) Aoki, T.; Kaneko, Y.; Stefanski, M. S.; Nguyen, T.; Ting, R. C. Y. *AIDS Res. Human Retroviruses* **1991**, *7*, 409.
(19) Bovey, F. A. *High Resolution NMR of Macromolecules*; Academic Press: New York, 1972; p 320.
(20) Wilson, A. D.; Prosser, H. J., Eds. *Developments in Ionic Polymers-2*; Elsevier Applied Science: London, 1986; p 212.
(21) Saito, H.; Ohki, T.; Kodama, M.; Nagata, C. *Biopolymers* **1978**, *17*, 2587.
(22) Joseph, M. R. *Biopolymers* **1969**, 8, 685.
(23) Dubin, P.; Bock, J.; Davis, R.; Schulz, D. N.; Thies, C. Ed. *Macromolecular Complexes in Chemistry and Biology*; Springer-Verlag: Berlin, 1994; p 260.

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