Triple Helix and Random Coil of Scleroglucan in Dilute Solution

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ABSTRACT: Eleven sonicated samples of a polysaccharide scleroglucan (chemically identical with but different in biological origin from schizophyllan) in 0.01*N* sodium hydroxide (NaOH) and dimethylsulfoxide (DMSO) at 25°C were studied by light scattering, viscometry, and ultracentrifugation. Data for the radius of gyration $\langle S^2 \rangle^{1/2}$, the intrinsic viscosity $[\eta]$, and the sedimentation coefficient s_0 as functions of weight-average molecular weight M_w , combined with those for the ratio M_w (0.01*N* NaOH)/ M_w (DMSO), showed that scleroglucan dissolves in DMSO as a single randomly coiled chain very similarly to that of schizophyllan in the same solvent, while it dissolves in 0.01*N* NaOH as a rodlike trimer or higher aggregates, depending on whether M_w (DMSO) is lower or higher than 2×10^5 . From $\langle S^2 \rangle$, $[\eta]$, and s_0 for samples with M_w (DMSO) $\leq 1 \times 10^5$ in 0.01*N* NaOH, the contour length per main chain residue and the diameter of the scleroglucan trimer rod were found to be 0.30 ± 0.03 and 2.6 ± 0.5 nm, respectively, which agree with the reported pitch (per residue) and diameter of the schizophyllan triple helix. Thus, it was concluded that the scleroglucan trimer has essentially the same triple helical structure as that of the schizophyllan trimer. Evidence was obtained showing that the higher aggregates in 0.01*N* NaOH consist of trimers as building units.

KEY WORDS Polysaccharide / Scleroglucan / Schizophyllan / Triple Helix
/ Radius of Gyration / Intrinsic Viscosity / Sedimentation Coefficient /

Scleroglucan is the general name for capsular polysaccharides elaborated by species of the genus *Sclerotium*. Recently, we¹ showed that one of these polysaccharides, commercially available under the trade name of Polytran (Ceca S. A., France), is a β -1,3-D-glucan consisting of the same repeating units as those of schizophyllan,^{2,3} an extracellular polysaccharide produced by a fungus *Schizophyllum commune*. We also found that Polytran is dissolved as a rodlike trimer in 0.01*N* aqueous sodium hydroxide (NaOH) and as a single randomly coiled chain in dimethylsulfoxide (DMSO), as is schizophyllan^{4,5} in these solvents. In fact, these two glucans in the former solvent showed no discernible difference with respect to dimensional and viscosity behavior. However, their random coils in the latter solvent were distinctly different in the degree of swelling by excluded-volume effect. Actually, Polytran's coil appeared to be in the unperturbed state. It is not easy to reconcile this difference with the finding that Polytran and

schizophyllan are chemically indistinguishable.

The point is that our experiment was done only on two Polytran samples. It seemed mandatory for a definitive elucidation of Polytran's random coil in DMSO to repeat a similar experiment with more samples covering a wide range of molecular weight. We therefore prepared 11 such Polytran samples and carried out light scattering, viscosity, and sedimentation velocity measurements both in 0.01*N* NaOH and in DMSO. This paper presents the experimental data obtained and their theoretical interpretation.

EXPERIMENTAL

Samples

Eight scleroglucan samples different in molecular weight were prepared by sonicating a purified sample¹ of Polytran R. Each was separated into four to seven parts by fractional precipitation with water as the solvent and acetone as the precipitant.

Appropriate middle fractions, designated below as H-1, F-1, D-3, I-1, I-3, C-2, C-5, B-3, J-1, J-3, and E-4, were selected, reprecipitated from aqueous solutions into acetone, and freeze-dried from aqueous solutions. These fractions were dried overnight *in vacuo* before use.

Light Scattering

Intensities of light scattered from 0.01 *N* NaOH and DMSO solutions of scleroglucan at 25°C were measured on a Fica 50 automatic light scattering photometer in an angular range from 22.5 to 150°. Vertically polarized incident light of 436 or 546 nm wavelength was used. The instrument was calibrated with benzene at 25°C as the reference liquid. The Rayleigh ratios of 25°C benzene at 436 and 546 nm were taken to be 46.5×10^{-6} and 16.1×10^{-6} cm, respectively.⁶ The depolarization ratio of benzene was estimated to be 0.41₀ for 436 nm and 0.40₀ for 546 nm by the method of Rubingh and Yu.⁷ For the specific refractive index increments of scleroglucan in 0.01 *N* NaOH and DMSO, the previously determined values¹ were used.

DMSO solutions were optically clarified by centrifugation at about 4×10^4 gravities for 2 h, after being allowed to stand at 25–50°C for 16–52 h. For 0.01 *N* NaOH solutions, optical clarification was made as described by Kashiwagi *et al.*⁵

Analysis of the data for samples I-1, C-2, and E-4 by the method described elsewhere⁸ indicated that the optical anisotropy of scleroglucan in 0.01 *N* NaOH is negligible, as found by Kashiwagi *et al.*⁵ and Sato *et al.*⁹ for schizophyllan in the same solvent.

Ultracentrifugation

Samples B-3 and E-4 in water at 25°C were investigated by sedimentation equilibrium, using a Spinco Model E ultracentrifuge equipped with an electronic speed-control system. A Kel-F 12 mm double-sector cell was used. The liquid column was adjusted to 1.4–1.6 mm, and rotor speeds were chosen as 5200 and 7200 rpm for samples B-3 and E-4, respectively. The partial specific volume of scleroglucan in water at 25°C was determined to be $0.620 \text{ cm}^3 \text{ g}^{-1}$.

Sedimentation velocities of samples C-2, C-5, B-3, J-1, J-3, and E-4 in 0.01 *N* NaOH at 25°C were measured at a rotor speed of 48000 rpm, using a

Kel-F 30 mm single-sector cell. For any of these samples, the observed schlieren patterns were single-peaked, so that sedimentation coefficients *s* for a series of initial polymer mass concentrations *c*₀ were determined by the usual peak method.

Viscometry

Zero shear-rate viscosities of samples H-1, F-1, D-3, I-1, and I-3 in 0.01 *N* NaOH at 25°C were determined using four-bulb capillary viscometers. For other samples in 0.01 *N* NaOH and all the samples in DMSO at 25°C, conventional capillary viscometers of the Ubbelohde type were used.

RESULTS

Molecular Weight

Figure 1 illustrates the concentration dependence of Kc/R_0 for samples I-3, C-5, and J-3 in 0.01 *N* NaOH and DMSO. Here, *K* is the optical constant, *c*, the polymer mass concentration, and *R*₀, the reduced scattering intensity at zero scattering angle. It can be seen that the data points for each sample in 0.01 *N* NaOH follow a straight line, whereas those in DMSO follow a curve bending upward. Since similar curvatures were observed for the concentration dependence of Kc/R_0 for all other samples in DMSO, the Kc/R_0 data in this solvent were anal-

Figure 1. Plots of Kc/R_0 vs. *c* for scleroglucan samples in 0.01 *N* NaOH (the lower *c* scale) and DMSO (the upper *c* scale) at 25°C.

alyzed using the Berry square-root plot.¹⁰

The values of M_w (the weight-average molecular weight) and A_2 (the second virial coefficient) for all the scleroglucan samples in 0.01 *N* NaOH and DMSO are summarized in Table I, along with those of M_w and M_z/M_w (M_z is the *z*-average molecular weight) in pure water determined by sedimentation equilibrium. The M_z/M_w values for samples B-3 and E-4 indicate that these two samples are narrow in molecular weight dispersion.

The values of M_w (0.01 *N* NaOH)/ M_w (DMSO) presented in Table I indicate that the predominant species of scleroglucan in 0.01 *N* NaOH is a trimer for M_w (DMSO) below 2×10^5 and an aggregate higher than a trimer for M_w (DMSO) above 2×10^5 and that the number of chains in the aggregate increases with an increase in M_w (DMSO). This was an unexpected finding, since our previous determination⁵ of M_w (0.01 *N* NaOH)/ M_w (DMSO) for schizophyllan exhibited no significant deviation from 3 throughout the entire range of M_w (DMSO) treated, *i.e.*, 3.7×10^4 – 1.6×10^6 .

Radius of Gyration

Figure 2 depicts the particle scattering function $P(\theta)$ for scleroglucan samples in 0.01 *N* NaOH. The values of $\langle S^2 \rangle^{1/2}$ (the radius of gyration) evaluated from the slopes of the indicated dashed lines are presented in Table I, along with those in DMSO. In Figure 3, these and previously reported $\langle S^2 \rangle^{1/2}$ values¹ in 0.01 *N* NaOH and DMSO are compared with the data (the solid lines) of Kashiwagi *et al.*⁵ for schizophyllan in the same solvents.

All the data points for scleroglucan in 0.01 *N* NaOH fall close to the solid line for schizophyllan in the same solvent. The slope of this line equals 1.0 for M_w (0.01 *N* NaOH) below 3×10^5 and decreases gradually with increasing M_w (0.01 *N* NaOH). Thus, it may be concluded that the scleroglucan trimer [M_w (0.01 *N* NaOH) $< 8 \times 10^5$] is almost rigidly rod-like below and semiflexible above M_w (0.01 *N* NaOH) $\sim 3 \times 10^5$. However, the finding that $\langle S^2 \rangle^{1/2}$ of both scleroglucan and schizophyllan in 0.01 *N* NaOH follow the same line throughout the entire range of M_w (0.01 *N* NaOH) must be considered accidental, since, as has been mentioned above, the predominant species of these glucans in 0.01 *N* NaOH are different aggregates when M_w (DMSO) is higher than about 2×10^5 .

In Figure 3, except for the two highest-molecular

Figure 2. Particle scattering function for scleroglucan samples in 0.01 *N* NaOH at 25°C.

Figure 3. Comparison of $\langle S^2 \rangle^{1/2}$ data for scleroglucan in 0.01 *N* NaOH (○) and DMSO (●) with those (the solid lines) for schizophyllan⁵ in the same solvents. The filled circles represent the data for scleroglucan samples H-1 and F-1 in 0.05 *N* NaOH.

weight samples, $\langle S^2 \rangle^{1/2}$ of scleroglucan in DMSO falls on a straight line of slope 0.58, fitting schizophyllan $\langle S^2 \rangle^{1/2}$ in the same solvent. This indicates

that, in DMSO, the scleroglucan random coil is perturbed by the excluded-volume effect as much as the schizophyllan random coil, and thus contradicts

our previous conclusion¹ that Polytran's coil in DMSO is virtually in the unperturbed state. The present A_2 values for DMSO solutions (Table I)

Table I. Results from light scattering measurements on scleroglucan samples in 0.01*N* NaOH and DMSO at 25°C

Sample	0.01 <i>N</i> NaOH				DMSO			M_w (0.01 <i>N</i> NaOH)
	$10^{-4}M_w$	10^4A_2	$\langle S^2 \rangle^{1/2}$	M_z	$10^{-4}M_w$	10^4A_2	$\langle S^2 \rangle^{1/2}$	
		cm ³ mol g ⁻²	nm	M_w		cm ³ mol g ⁻²	nm	M_w (DMSO)
H-1	370	2.8	264		51.8	3.9	46.0	7.1
	163 ^a	2.4 ^a	151 ^a					
F-1	239	5.4	198		37.2	5.3	33.1	6.4
	129 ^a	3.2 ^a	134 ^a					
D-3	141	4.4	152		29.5	4.3	27.1	4.8
I-1	110	1.7	127		25.8	4.9	26.4	4.3
I-3	81.1	4.1	92.7		22.5	4.7	23.2	3.6
C-2	37.6	5.7	51.4		12.8	6.8	17.8	2.9
C-5	31.0	4.3	42.1		11.0	6.3	16.3	2.8
B-3	22.0	6.2	31.1		7.89	6.5	13.8	2.8
	21.2 ^b	1.5 ^b		1.1 ₂ ^b				
J-1	16.7	8.0	23.3		6.57	5.5	12.6	2.5
J-3	12.0	5.8	17.4		4.68	8.3		2.6
E-4	9.62	7.8	13.4		3.56	6.7		2.7
	9.01 ^b	3.2 ^b		1.1 ₉ ^b				

^a In 0.05*N* NaOH.

^b Sedimentation equilibrium in water.

Table II. Results from viscosity and sedimentation velocity measurements on scleroglucan samples in 0.01*N* NaOH and DMSO at 25°C

Sample	0.01 <i>N</i> NaOH				DMSO	
	$10^{-2}[\eta]$	k'	$10^{13}s_0$	k_s	$10^{-2}[\eta]$	k'
	cm ³ g ⁻¹		s	cm ³ g ⁻¹	cm ³ g ⁻¹	
H-1	43.7	0.45			2.17	0.32
	25.3 ^a	0.44 ^a				
F-1	26.1	0.43			1.80	0.34
	17.0 ^a	0.44 ^a				
D-3	14.2	0.42			1.39	0.34
I-1	10.5	0.43			1.26	0.36
I-3	6.59	0.41			1.09	0.34
C-2	2.65	0.41	7.42	75	0.781	0.35
C-5	2.03	0.42	7.17	64	0.740	0.33
B-3	1.34	0.42	6.80	62	0.562	0.38
J-1	0.809	0.42	6.29	53	0.462	0.35
J-3	0.497	0.43	5.73	42	0.376	0.40
E-4	0.345	0.44	5.34	34	0.305	0.42

^a In 0.05*N* NaOH.

Figure 4. Comparison of $[\eta]$ data for scleroglucan in 0.01N NaOH ($\circ$) and DMSO ($\bullet$) with those (the solid lines) for schizophyllan^{4,5} in the same solvents. The filled circles represent the data for scleroglucan samples H-1 and F-1 in 0.05N NaOH.

support this finding, being much larger than those previously obtained and consistent with random coils perturbed by excluded-volume effect. Thus, it appears convincing that our previous conclusion was incorrect. But what is responsible for this?

We suspect that the previous test solutions might have contained a small amount of aggregates since they were prepared at room temperature. We therefore heated DMSO solutions of our previous Polytran samples at 50°C for 48 h and then measured their light scattering intensities. However, the results were substantially the same as before, giving $A_2 \sim 0$. At present, it is not clear as to why the two scleroglucan samples chosen in our previous study behave in DMSO so differently from those investigated in the present work.

Intrinsic Viscosity and Sedimentation Coefficient

Numerical data for $[\eta]$ (the intrinsic viscosity) and k' (Huggins' constant) in 0.01 N NaOH and DMSO are summarized in Table II. These and previous data¹ of $[\eta]$ are compared with the corresponding data (the solid lines) for schizophyllan^{4,5} in Figure 4. The present data for M_w below 3×10^5 in 0.01 N NaOH can be fitted by a straight line of slope 1.7 (the dashed line), which almost merges with the solid line for this solvent and is consistent with the finding from $\langle S^2 \rangle$ that scleroglucan trimers with M_w (0.01 N NaOH) lower than 3×10^5 are rigidly

Figure 5. Concentration dependence of s^{-1} for scleroglucan samples in 0.01N NaOH at 25°C.

Figure 6. Plot of s_0 vs. $\log M_w$ for scleroglucan in 0.01N NaOH.

rodlike. On the other hand, except for the highest-molecular weight sample, the DMSO data fall on the schizophyllan line of slope 0.69, indicating that both scleroglucan and schizophyllan single chains in this solvent are in the random coil conformation perturbed by volume effect.

Figure 5 shows the concentration dependence of s^{-1} in 0.01 N NaOH. The values of s_0 (the limiting sedimentation coefficient) and k_s (the constant defined by the equation $s^{-1} = s_0^{-1} + k_s s_0^{-1} c_0$) determined from the indicated straight lines are presented in the fourth and fifth columns of Table II. Figure 6 shows that these s_0 vary linearly with $\log M_w$. This linear relation is additional evidence for the fact that the scleroglucan trimer is rigidly rodlike.

DISCUSSION

Data Analysis

Our dimensional and hydrodynamic data along with those of the molecular weight ratio M_w (0.01 N NaOH)/ M_w (DMSO) show that scleroglucan dissolves in 0.01 N NaOH as a rigid trimer for M_w (DMSO) lower than 1×10^5 . The well-known expression for $\langle S^2 \rangle$ of a rigid rod is

$$\langle S^2 \rangle = M / (12M_L) \quad (1)$$

where M is the molecular weight of the polymer and M_L , the molar mass per unit length of the rod. If our $\langle S^2 \rangle$ data for M_w (DMSO) below 1×10^5 in 0.01 N NaOH are substituted into this equation, M_L is found to be $2050 \pm 50 \text{ nm}^{-1}$.

Yamakawa's expressions^{11,12} for $[\eta]$ and s_0 of a long straight cylinder are

$$\begin{aligned} M^2/[\eta] &= (45M_L^3/2\pi N_A) \\ &[\ln M - 0.6970 - \ln(dM_L)] \end{aligned} \quad (2)$$

$$\begin{aligned} s_0 &= [(1 - \bar{v}\rho_0)M_L/3\pi\eta_0 N_A] \\ &[\ln M + 0.3863 - \ln(dM_L)] \end{aligned} \quad (3)$$

where d is the diameter of the cylinder, N_A Avogadro's constant, $\bar{v}$ the partial specific volume of the polymer, and ρ_0 and η_0 the solvent density and viscosity, respectively. The values of $M_w^2/[\eta]$ calculated from our data for the four lowest-molecular weight samples in 0.01 N NaOH are plotted against $\ln M_w$ in Figure 7. If a straight line is drawn to fit the plotted points as indicated and the values of its slope and intercept at $\ln M_w = 0$ are substituted into eq 2, M_L and d are found to be $2200 \pm 60 \text{ nm}^{-1}$ and $2.6 \pm 0.4 \text{ nm}$, respectively. Further, when compared with eq 3, the linear re-

lation between s_0 and $\log M_w$ in Figure 6 yields $2140 \pm 70 \text{ nm}^{-1}$ for M_L and $2.5 \pm 0.4 \text{ nm}$ for d .

Triple Helical Structure

The repeating unit of scleroglucan contains three main chain glucose residues (see Figure 1 of ref 1). Hence, the length h per main chain residue of the scleroglucan trimer along the rod axis can be written

$$h = (M_0/3)/(M_L/3) \quad (4)$$

with M_0 the molar mass of the scleroglucan repeating unit ($= 648 \text{ g mol}^{-1}$). The values of h calculated from our M_L and those of d estimated above are summarized in Table III, along with the pitches and diameters for the schizophyllan triple helix in water¹³ and 0.01 N NaOH⁵ and the model triple helix⁴ of schizophyllan. Our h values estimated by different methods agree with one another and also with the schizophyllan helix pitches. Our d values are also in substantial agreement with

Figure 7. Plot of $M_w^2/[\eta]$ vs. $\ln M_w$ for scleroglucan in 0.01 N NaOH.

Table III. Pitches and diameters of the triple helices of scleroglucan and schizophyllan

Polysaccharide	Solvent	Pitch ^a	or	h	d	Method
		nm		nm	nm	
Scleroglucan	0.01 N NaOH	0.32 ± 0.01				Light scattering
		0.29 ± 0.01			2.6 ± 0.4	Viscosity
		0.30 ± 0.01			2.5 ± 0.4	Sedimentation
Schizophyllan	Crystalline state	0.30^a				X-Ray ^{14,15}
	Water	0.30 ± 0.02^a			2.6 ± 0.4	Viscosity and sedimentation ¹³
	0.01 N NaOH	0.30 ± 0.01^a			2.2 ± 0.6	Viscosity and light scattering ⁵
		0.30^a			$2.5-3.0$	Molecular model ⁴

^a Per residue.

those for schizophyllan and its model helix. Thus, it may be concluded that the scleroglucan trimer has a triple helical structure very similar to that of schizophyllan.

Very recently, Marchessault *et al.*^{14,15} concluded from X-ray and conformational studies that scleroglucan in the crystalline state has a triple helical structure with a pitch (per residue) of 0.30 nm, which can be favorably compared with the values from our solution study. Thus, it may be concluded that the triple helical structure of scleroglucan in the crystalline state is maintained in dilute aqueous NaOH.

Aggregates of Triple Helices

We have mentioned that our scleroglucan samples with M_w (DMSO) higher than 2×10^5 are dispersed in 0.01 N NaOH as aggregates higher than a trimer. In the following, we present some convincing data that these higher aggregates are built up of two or more trimers.

According to Kashiwagi *et al.*,⁵ the addition of NaOH to an aqueous solution of schizophyllan enhances the solubility of the triple helix, but leads to both dissociation (to single chains) and gradual degradation of the helix when the NaOH concentration x exceeds 0.1 N. We suspected that this was probably the case with scleroglucan, and tried to check it by measuring the viscosity of higher-molecular weight samples H-1 and F-1 in aqueous NaOH of different x . For comparison, we also investigated lower-molecular weight samples B-3 and J-1, which dissolve as trimers in 0.01 N NaOH.

The results are illustrated in Figure 8. It can be seen that $[\eta]$ for samples B-3 and J-1 are constant up to $x \sim 0.05$ N, decrease sharply at $x \sim 0.1$ N, and level off at constant values above $x \sim 0.2$ N. The abrupt change in $[\eta]$ at $x \sim 0.1$ N is probably a reflection of the dissociation of the triple helix to single chains, since the leveling-off values of $[\eta]$ for x above 0.2 N are comparable to $[\eta]$ of the respective samples in DMSO. The curves for samples H-1 and F-1 comprise two distinct steps, each showing a shoulder around $x = 0.05$ N. Probably, the decline to the shoulder represents the dissociation of a higher aggregate to trimers, and the subsequent sharp decline, the breaking of trimers into single chains.

In order to confirm this expectation, light scattering and the viscosity of samples H-1 and F-1 in 0.05 N NaOH were measured. The specific refrac-

Figure 8. Dependence of $[\eta]$ on the concentration of NaOH for scleroglucan samples in aqueous NaOH at 25°C. The dashed line indicates the data of Bluhm *et al.*¹⁵

tive index increment in this solvent at either 436 or 546 nm wavelength was not different from that in 0.01 N NaOH. The data obtained are given in Tables I and II and shown by filled circles in Figures 3 and 4. It can be seen that these circles lie close to the solid lines for schizophyllan in 0.01 N NaOH and also give M_w values approximately 3 times as large as those in DMSO. This demonstrates that the aggregates of samples H-1 and F-1 are dissociated in 0.05 N NaOH to trimers which have the same structure as the schizophyllan triple helix.

Recently, Bluhm *et al.*¹⁵ investigated the pH dependence of the reduced viscosity η_{sp}/c ($c = 4.9 \times 10^{-4}$ g cm⁻³) of a Polytran sample in 8×10^{-2} M aqueous sodium chloride containing NaOH (for pH > 7) or hydrochloric acid (for pH < 7). Their experimental data are shown by a dashed line in Figure 8 after pH has been converted to x assuming the relation $\log x = \text{pH} - 14$. Bluhm *et al.* remarked that the values of η_{sp}/c for x above 0.03 N are comparable to the reported $[\eta]$ for schizophyllan in DMSO, and attributed a fairly sharp decrease in η_{sp}/c at $x \sim 0.015$ N to the dissociation of triple helices to random coils. However, their remark on η_{sp}/c for x above 0.03 N is incorrect, since such η_{sp}/c values far exceed the high-

est of $[\eta]$ ($4.65 \times 10^2 \text{ cm}^3 \text{ g}^{-1}$) ever obtained for schizophyllan in DMSO.⁴ More reasonably, the sharp decrease in η_{sp}/c at $x \sim 0.015 N$ may be explained as due to the dissociation of higher aggregates to triple helices.

CONCLUDING REMARKS

The present study has shown that the trimer of Polytran scleroglucan in dilute aqueous NaOH and its monomer in DMSO assume essentially the same conformations as those of schizophyllan in these solvents. This finding is compatible with the results from a chemical analysis¹ and C^{13} -NMR measurements¹⁶ that the two glucans have the same chemical structure. However, these glucans in 0.01 N NaOH differed in solubility behavior when M_w (DMSO) was higher than 2×10^5 . In this region, triple helices of schizophyllan remained intact, while those of scleroglucan tended to associate further with one another to higher aggregates. This difference is striking, but we do not know the reason for it. Native schizophyllan produced by *Schizophyllum commune* in a culture medium separates spontaneously from the mycelium and migrates freely into the liquid phase.¹⁷ On the other hand, scleroglucan produced by *Sclerotium rolfsii* adheres to the mycelium as a gellike aggregate and cannot be dispersed in the liquid phase unless the culture medium is heated and homogenized.¹⁸ Such a difference in the native states of these two glucans may bear some relation to the difference in their solubility in 0.01 N NaOH.

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