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MELTING BEHAVIOR OF *Schizophyllum commune* POLYSACCHARIDES IN MIXTURES OF WATER AND DIMETHYL SULFOXIDE*

TAKAHIRO SATO, TAKASHI NORISUYE, AND HIROSHI FUJITA
Department of Macromolecular Science, Osaka University, Toyonaka, Osaka 560 (Japan)

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ABSTRACT

The triple helix of a polysaccharide *Schizophyllum commune* (schizophyllan) of viscosity-average molecular weight 4.8×10^5 g.mol^{-1} (in water) was melted at denaturation temperatures T_d between 5 and 60° in water–dimethyl sulfoxide (Me_2SO) mixtures. The solutions for different T_d values were studied by viscometry and ultracentrifugation. As T_d was increased, the intrinsic viscosity of the mixture containing 12.76% (by weight) of water decreased sharply and, at about $T_d = 50°$, it approached the value expected for the single chain of schizophyllan in pure Me_2SO. Schlieren patterns of the sample "denatured" at T_d between 25 and 45° in the same mixed solvent showed the presence of two solute species. The fast-sedimenting species dominating at $T_d = 25°$ almost disappeared at $T_d = 45°$. From sedimentation-coefficient data for the mixture, pure water, and Me_2SO, the fast- and slow-sedimenting species could be identified with the triple helix and the single chain of schizophyllan, respectively. These findings allow us to conclude that, in water–Me_2SO mixtures containing ~13% of water, the schizophyllan triple-helix melts into single chains in all-or-none fashion with increasing temperature.

INTRODUCTION

Schizophyllan is an extracellular polysaccharide produced by *Schizophyllum commune*[1]. It consists of linearly linked, (1→3)-β-D-glucose residues with (1→6)-β-D-glucose residues as the side chains, one for every three consecutive repeating units[2]. In a recent paper[3], we reported that this polysaccharide is dispersed as a rigid, triple helix in water at 25°, whereas it is dissolved as a single, randomly coiled chain in dimethyl sulfoxide (Me_2SO) at the same temperature. We also found that, in mixtures of water and Me_2SO at 25°, the triple helix of schizophyllan is almost discontinuously decomposed into single chains when the water content is decreased to ~13 wt%. These results led us to the question of what would happen to the schizophyllan triple-helix if the solution of this polysaccharide in a water–Me_2SO mixed solvent near 13% (by weight) water were heated. With a sonicated sample of molecular weight

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*Triple Helix of *Schizophyllum commune* Polysaccharide in Dilute Solution, II. For Part I, see ref. 1.

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4.8×10^5 g.mol^{-1} (in water), we performed viscosity and sedimentation velocity experiments to answer this question.

RESULTS AND DISCUSSION

Viscosity measurement. — Fig. 1 illustrates the changes in $(\ln \eta_r)/c$ with time that occurred when a solution of the sample equilibrated at 20 or 25° was transferred into a thermostated bath at a higher temperature. Here, η_r is the relative viscosity, c is the polymer-mass concentration, and w_H is the weight fraction of water in the water–Me_2SO mixture used as the solvent. It may be seen that $(\ln \eta_r)/c$ at first decreases rather sharply and reaches an equilibrium value after ~20 h. This fact indicates that, if the schizophyllan solution having a w_H near the critical value (~13 wt%) is heated, some change in the solute occurs which brings about a decrease in solution viscosity, and that this change follows a very slow process until equilibrium is reached. The

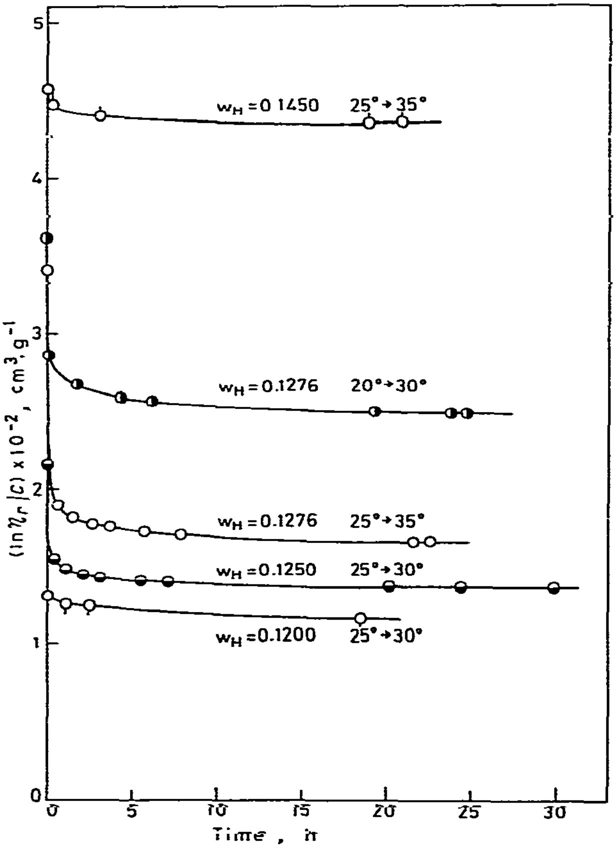

Fig. 1. Decreases in $(\ln \eta_r)/c$ with time, observed after the temperature of a solution of schizophyllan in water–Me_2SO mixtures of different w_H was changed as indicated by the arrows. The polymer concentrations are (from top to bottom) 0.0943×10^{-2}, 0.4979×10^{-2}, 0.2469×10^{-2}, 0.3122×10^{-2}, and 0.2157×10^{-2} g.cm^{-3}.

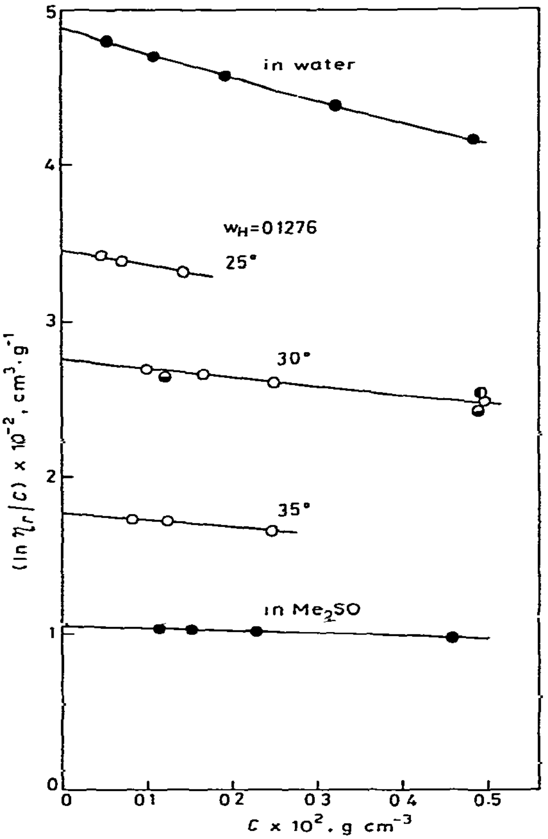

Fig. 2. Concentration dependence of ($\ln \eta_r$)/c for the schizophyllan sample in water and Me_2SO at 25° and that for the same sample denatured to equilibrium at 25, 30, and 35° in the water–Me_2SO mixture of w_H = 0.1276. The half-filled circles for w_H = 0.1276 at the denaturation temperature 30° refer to independent measurements.

solution equilibrated at a higher temperature was cooled to the original, lower temperature, but its ($\ln \eta_r$)/c value was not different from that before cooling and remained the same for at least a few days. Further cooling to as low a temperature as 5° caused no change in ($\ln \eta_r$)/c. Thus, we concluded that the change caused by heating in schizophyllan is irreversible. For descriptive convenience, this change is hereafter called thermal denaturation of schizophyllan.

Fig. 2 shows the variation of ($\ln \eta_r$)/c with c for the sample of schizophyllan denatured to equilibrium at 25, 30, and 35° in the water–Me_2SO mixture of w_H = 0.1276, together with that for the same sample in pure water and in Me_2SO at 25°. The plots are linear and give normal values ranging from 0.33 to 0.43 for the Huggins constant. Thus, we find that dilution does not give rise to any special effect on thermally denatured schizophyllan.

In a given water–Me_2SO mixture, the sample was denatured to equilibrium at a series of increasing temperatures, and the intrinsic viscosity [η] at each denatura-

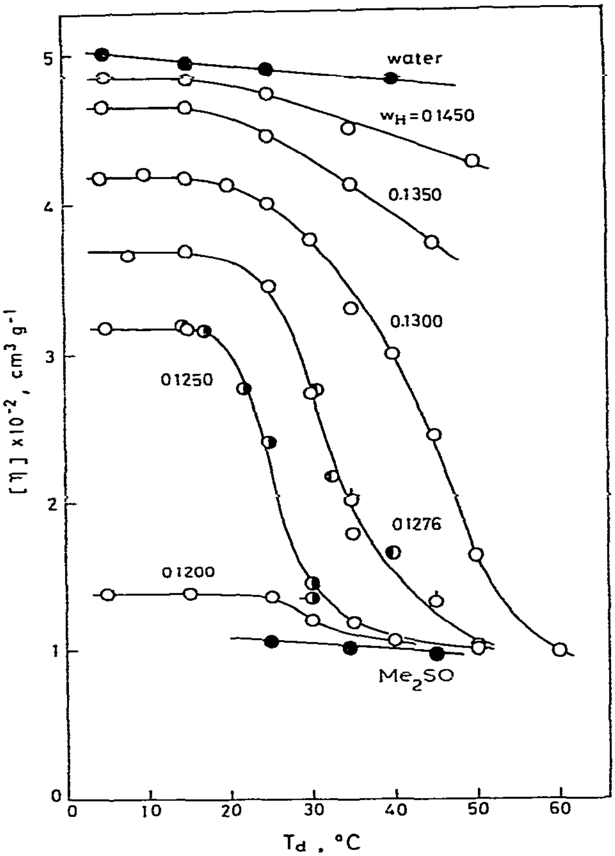

Fig. 3. Curves of [η] vs. denaturation temperature T_d in water–Me_2SO mixtures of different w_H. The different symbols for w_H = 0.1276 and 0.1250 refer to different series of measurements.

tion temperature T_d was determined. The results from these experiments at different w_H are shown in Fig. 3. It is seen that [η] in pure water is almost independent of T_d, which implies that the schizophyllan triple-helix is thermally stable in pure water. Below T_d = 18°, [η] does not change with T_d for any w_H examined, although it is larger for higher w_H values. On the other hand, above T_d = 20°, [η] for w_H = 0.1300, 0.1276, and 0.1250 decreases rather sharply with increasing T_d and, at about 50°, approaches the line for pure Me_2SO, in which schizophyllan exists as a single, randomly coiled chain[3]. From these viscosity data, it may be considered that the irreversible change in schizophyllan caused by heating is a decomposition of the triple helix and that all helices in the solution are eventually broken into single chains at $T_d > 50°$.

Sedimentation-velocity measurement. — The sample dissolved in the water–Me_2SO mixture of w_H = 0.1276 was denatured to equilibrium at temperatures between 25 and 45°. The solutions for different T_d were cooled to 20° and then subjected to sedimentation-velocity experiments. The expectation was that sedimentation patterns of these solutions would reveal the polymer components in equilibrium at the respective denaturation temperatures since, as already noted, cooling does not restore schizophyllan denatured at higher temperature.

Fig. 4 shows the schlieren-boundary curves taken after solutions of initial concentration $c_0 = 0.4976 \times 10^{-2}$ g.cm^{-3} had been centrifuged for a time t of ~150 min. The boundary curves for the solutions in water ($c_0 = 0.3463 \times 10^{-2}$ g.cm^{-3}; $t = 38$ min) and in Me$_2$SO ($c_0 = 0.4937 \times 10^{-2}$ g.cm^{-3}; $t = 147$ min) are also included for comparison. The curve for the solution at $T_d = 25°$ [panel (b)] is single-peaked and as sharp as that observed in pure water [panel (a)], but it unmistakably displays a tail on the left side of the peak. In the solution for $T_d = 30°$, this tail develops to a second peak, indicating the presence of a slower-sedimenting species [panel (d)]. At the higher denaturation-temperatures, the sharp, faster-sedimenting peak almost disappears, and the boundary curves resemble that for the Me$_2$SO solution in panel (g). From these results, we find that when schizophyllan is thermally denatured in a water–Me$_2$SO mixture, there appear two predominant polymer species, but no more, and that one sediments faster than the other in the centrifugal field.

In Fig. 5, the boundary curves for the sample denatured at 30° are shown as a function of the time of centrifugation t. All of the curves exhibit two peaks. The distance between the peaks increases with increasing t. The $\ln r_m$ vs. t plots derived

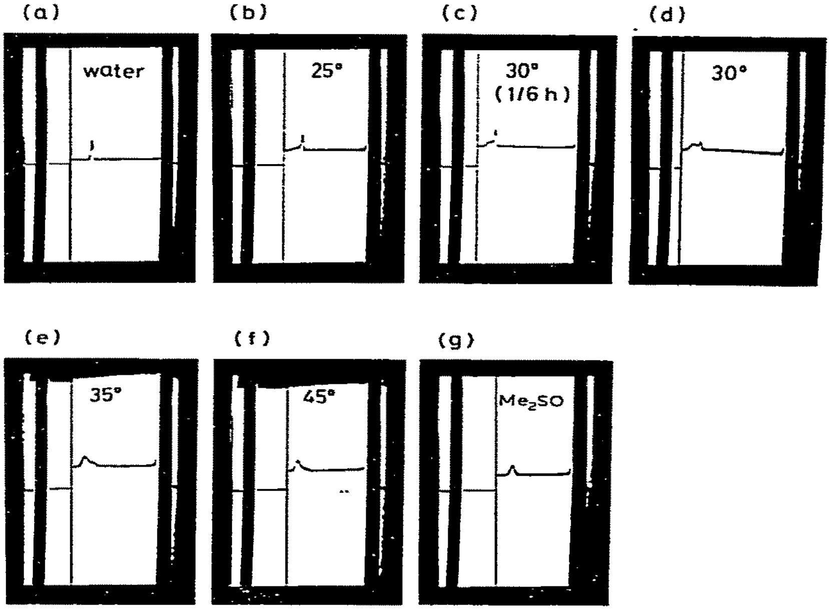

Fig. 4. Schlieren-boundary curves at 20° for the schizophyllan sample in water and Me$_2$SO and those at 20° for the same sample denatured for 10 min [panel (c)] and 24 h [panels (b), (d), (e), and (f)] at the indicated temperatures in the water–Me$_2$SO mixture of $w_H = 0.1276$. Bar angles are 80° for panel (a), 70° for panels (b) and (g), 65° for panels (c), (d), and (f), and 60° for panel (e). See the text for other experimental conditions.

from these data are presented in Fig. 6, where r_m is the distance from the center of rotation to each peak. Similar, linear plots were obtained for the solutions denatured at other temperatures. The sedimentation coefficients s evaluated from these plots are summarized in Table I, together with those in water and in Me_2SO reduced to the water–Me_2SO mixture of w_H = 0.1276, using the familiar relation[4] (see the Experimental section). It is seen that the s values of the fast-sedimenting species in the mixed solvent at different T_d agree with that in water, whereas those of the slow species in the same mixture are approximately equal to that in Me_2SO. These results lead us to conclude that the fast- and slow-sedimenting peaks appearing in the boundary curves correspond to the triple helix and the single chain, respectively.

CONCLUSIONS

Our sedimentation experiment made it clear that no predominant solute species other than the triple helix and the single chain appear when schizophyllan is thermally denatured in water–Me_2SO mixed solvents. This finding implies that the melting of the schizophyllan triple-helix to single chains proceeds not through intermediate steps such as partial breaking of helical portions or formation of double helices, but in all-or-none fashion. Thus, only the fraction of triple helices in the solution decreases continuously with increasing T_d. This effect should be responsible for the decrease in $[\eta]$ for w_H = 0.1276 (Fig. 3), as well as for the increase in the amount of the slow species (Fig. 4) with increasing T_d.

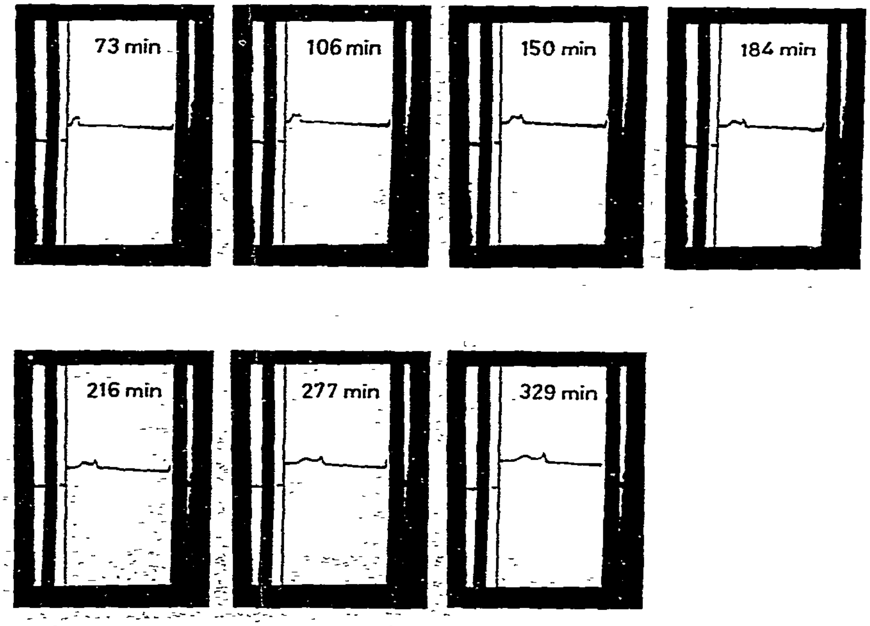

Fig. 5. Schlieren-boundary curves at 20° as a function of the time of centrifugation for the sample of schizophyllan denatured to equilibrium at 30° in the water–Me_2SO mixture of w_H = 0.1276.

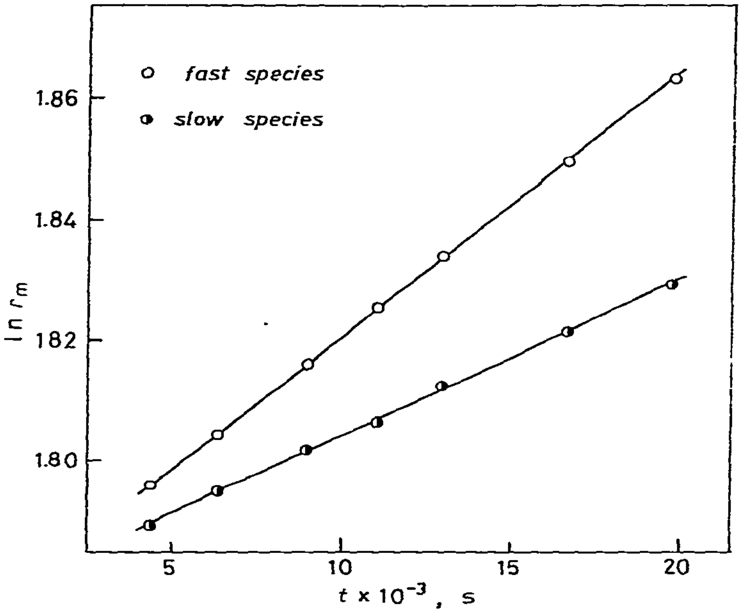

Fig. 6. Plots of $\ln r_m$ vs. t for the fast- and slow-sedimenting species appearing in the boundary curves in Fig. 5. r_m is expressed in cm.

TABLE I

SEDIMENTATION COEFFICIENTS FOR TWO SPECIES PRESENT IN SOLUTIONS OF SCHIZOPHYLLAN DENATURED TO EQUILIBRIUM AT DIFFERENT TEMPERATURES IN A WATER–Me_2SO MIXTURE CONTAINING 12.76% (BY WEIGHT) OF WATER

Denaturation temperature, T_d	$c_0 \times 10^2$ *($g.cm^{-3}$)*	*Fast-sedimenting species* $s \times 10^{13}$ *(sec) (at 20°)*	*Slow-sedimenting species* $s \times 10^{13}$ *(sec) (at 20°)*
(Pure water)	0.4836	1.11[a]	
25°	0.4976	1.11	
30° [b]	0.4976	1.09	
30°	0.4976	1.12	0.66
35°	0.4976		0.66
45°	0.4976		0.62
(Pure Me_2SO)	0.4937		0.62[a]

[a] Reduced to the water–Me_2SO mixture of $w_H = 0.1276$. [b] Denatured for 10 min.

EXPERIMENTAL

Schizophyllan sample. — An extensively purified sample of schizophyllan was supplied by Taito Co. for the present study. It had been prepared by sonicating a native schizophyllan to fragments, followed by fractionation of the fragments. The details of these procedures have been described previously[3]. The viscosity-average molecular weight (M_v) of the sample in water at 25° was estimated to be 4.8×10^5 g.mol^{-1} from $[\eta]$ at zero shear-rate and the previously established $[\eta]$ *vs.* M_w (weight-average molecular weight) relation[3]. The M_v value in Me_2SO at 25° was 1.8×10^5 g.mol^{-1}. The ratio of 4.8×10^5 to 1.8×10^5 is ~ 3, which confirms our previous finding[3] that schizophyllan exists as a trimer in water.

Dimethyl sulfoxide, dehydrated with calcium hydride, was fractionally distilled at 3 torr (b.p. 65°).

Viscometry. — A weighed amount of schizophyllan was dissolved in the water–Me_2SO mixture of desired composition, at $\sim 3°$ in a cold room, at $\sim 7°$ in a refrigerator, or at room temperature. The resulting solution was subjected to viscosity measurement successively in order from low to high denaturation temperature, with viscometers of the Ubbelohde, suspended-level type; no correction for the effect of shear rate was made. The data were treated with the Mead–Fuoss plot[5] as well as the Huggins plot[6] to determine $[\eta]$ from the common intercept. In some instances, $[\eta]$ was estimated from $(\ln \eta_r)/c$ at a single concentration, with an appropriate value assumed for the Huggins constant.

Densities of water–Me_2SO mixtures at different temperatures were estimated by interpolating or extrapolating the reported values[7] and our previously measured values[3].

Ultracentrifugation. — A Hitachi 65P-7, automatic, preparative ultracentrifuge was used, with a 12-mm aluminum single-sector cell. The initial concentration of polymer (c_0) was adjusted to $\sim 0.5 \times 10^{-2}$ g.cm^{-3} in most experiments, and the rotor was revolved at 60000 r.p.m. Schlieren curves were photographed on Polaroid Type 107 Land films, and the peak positions were read with a travelling microscope. The sedimentation coefficients in water and Me_2SO at 20° were reduced to those in the water–Me_2SO mixture of $w_H = 0.1276$ by using the relation[4]:

$$s = \left(\frac{1 - \bar{v}\rho_0}{\eta_0}\right)\left(\frac{\eta_0}{1 - \bar{v}\rho_0}\right)_i s_i,$$

where $\bar{v}$ is the partial specific volume of the polymer, ρ_0 and η_0 are the solvent density and viscosity, respectively, and the subscript i refers to the value in either water or Me_2SO. The following values were assumed for $\bar{v}$ of schizophyllan: 0.619 cm^3.g^{-1} in water, and 0.623 cm^3.g^{-1} in Me_2SO and in the mixture of $w_H = 0.1276$. These are the experimental values[3] previously determined at 25°.

The concentration dependence of sedimentation coefficient for the sample in water at 20° was examined from measurements at three c_0 values between 0.2×10^{-2} and 0.5×10^{-2} g.cm^{-3}. The results yielded 7.4×10^{-13} s for the limiting sedimentation-coefficient s_0 and 1.3×10^2 cm^3.g^{-1} for k_s defined by the equation $s^{-1} = s_0^{-1}(1 + k_s c_0)$. The former gives 8.3×10^{-13} s for s_0 at 25°. These reduced s_0 and $M_v = 4.8 \times 10^5$ g.mol^{-1} values are consistent with the s_0 *vs.* M_w relation (in water at 25°), reported shortly recently by Yanaki *et al.*[8].

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