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Melting Behaviour of a Triple Helical Polysaccharide Schizophyllan in Aqueous Solution

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SUMMARY

Two sonicated samples of schizophyllan in aqueous solution at temperatures from 20 to 160°C were investigated by viscometry. The temperature dependence of the viscosity coefficient η *showed that schizophyllan in water undergoes an irreversible thermal transition at about 135°C. The values of* $(\ln \eta_r)/c$ *(*η_r *is the relative viscosity and* c *is the polymer concentration (w/v)) at 25°C determined after preheating aqueous schizophyllan indicated that the major conformations of schizophyllan in water at 120 and 150°C are triple helix and single random coil, respectively. Thus, it was concluded that the change in* η *at about 135°C with an increase in temperature is due to the melting of triple helices to single chains. Schizophyllan denatured to single chains at about 150°C did not restore the intact triple helix, but formed aggregates, when the solution was cooled to 25°C. It was also found that the aggregates form a gel when* c *is higher than a certain value.*

INTRODUCTION

Schizophyllan is a water-soluble non-ionic polysaccharide produced extracellularly by the fungus *Schizophyllum commune* (Kikumoto *et al.*, 1971). It consists of linearly linked β-1,3-D-glucose residues with one β-1,6-D-glucose side chain for every three main chain residues. Recently, Norisuye *et al.* (Norisuye *et al.*, 1980; Kashiwagi *et al.*, 1981) found that this polysaccharide dissolves as a rigid triple helix in pure

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water or N/100 sodium hydroxide at 25°C and as a single randomly coiled chain in dimethyl sulphoxide (DMSO) at the same temperature. They (Norisuye *et al.*, 1980; Sato *et al.*, 1981, 1983) also found that, in mixtures of water and DMSO at 25°C, the triple helix of schizophyllan splits almost completely into single chains when the water composition in the binary mixture is decreased to about 13 wt%. This transition is temperature dependent and in this solvent the schizophyllan triple helix melts into single chains in an all-or-none fashion with increasing temperature. These results led us to question what would happen to the triple helix when an aqueous solution of this polysaccharide was heated. To answer this question, we made measurements of viscosity and viscoelastic properties.

EXPERIMENTS

Samples

The sonicated samples M-2 and U-1 studied in our previous work (Yanaki *et al.*, 1983*a*, *b*) were used. The weight-average molecular weights M_w, intrinsic viscosities $[\eta]$ and Huggins constants k' of these samples in water and DMSO at 25°C are presented in Table 1.

Viscometry

Samples M-2 and U-1 in pure water at temperatures from 20–160°C were investigated using a capillary viscometer of the Ubbelohde type or

TABLE 1
Molecular Characteristics (Yanaki *et al.*, 1983*a*, *b*) of the Schizophyllan Samples Used

Sample	*Solvent (25°C)*	$10^{-4}M_w$	$10^{-2}[\eta]/(cm^3 g^{-1})$	k'
U-1	Water	13·4	0·584	0·43
	DMSO	4·7	0·397	0·41
M-2	Water	43·7	4·79	0·42
	DMSO	13·6	0·820	0·31

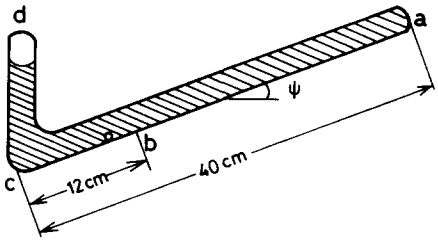

Fig. 1. Schematic diagram of the rolling-ball viscometer.

a rolling-ball viscometer (Höppler, 1933; Hubbard & Brown, 1943; Schmidt & Wolf, 1982). The former was used for determining the relative viscosity η_r and the latter the viscosity coefficient η.

A schematic diagram of the rolling-ball viscometer is shown in Fig. 1. The viscometer consists of a glass tube of inner diameter 7·60 ± 0·08 mm (Tokyo Garasu Kikai Co.) and a glass ball of 0·695 ± 0·003 mm in diameter, both made of SK soft glass with a density ρ_b of 2·50 g cm^{-3} (at 25°C) and a linear expansion coefficient α of 99×10^{-7} deg^{-1}.

The glass tube of the rolling ball viscometer was sealed after it was filled with a test solution; in fact, air (about 0·5 cm^3) was left near one end (d) of the tube to prevent the tube from breaking. The viscometer was placed in an oil thermostat in such a way that the tube (a–c) made an angle ψ (see Fig. 1) of 15–25°. The ball was allowed to roll down from points (b) to (c), and its velocity V was determined. The Reynolds number defined by Hubbard's equation (Hubbard & Brown, 1943) was smaller than 4 for any aqueous solutions studied.

The viscosity coefficient of a given test solution was evaluated from the equation (Höppler, 1933; Schmidt & Wolf, 1982):

$$\eta = \frac{K}{V}(\rho_b - \rho)$$

where ρ is the solution density and K is a constant dependent on ψ and the diameters of the tube and the ball. The viscometer was calibrated for different values of ψ with aqueous sucrose; K was independent of temperature T in the range 20–150°C and of the inner pressure generated in the glass tube. The values of ρ_b at different temperatures were calculated from ρ_b at 25°C and α, and those of ρ estimated from density

data for pure water and the partial specific volume of schizophyllan (Norisuye *et al.*, 1980) in water at 25°C. The values of V ranged from $6{\cdot}3 \times 10^{-4}$ to $1{\cdot}5$ cm s^{-1}, and were reproducible within ±3% in the entire range of T studied. No corrections for non-Newtonian effects were made.

Viscoelasticity

Dynamic shear moduli were measured on sample M-2 in water at 25°C using a rheometer of the coaxial cylinder type (Iwamoto Seisakusyo

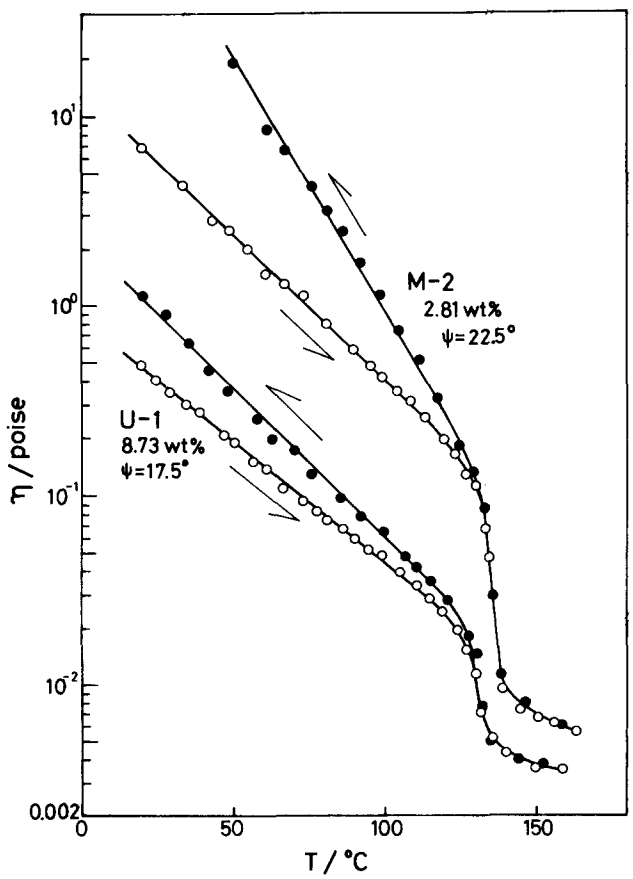

Fig. 2. Temperature dependence of η for schizophyllan samples M-2 and U-1 in water: unfilled circles, heating; filled circles, cooling. Rate of heating or cooling, $0{\cdot}50 \pm 0{\cdot}03$ deg min^{-1}.

Co., Kyoto). The diameters of the inner and outer cylinders of the viscometer were 1·6 (or 2·0) and 2·2 cm, respectively, and the length of the inner cylinder was 7·1 cm. Lissajous' figures were recorded and analysed by Markovitz's equation (Markovitz, 1952).

RESULTS AND DISCUSSION

Figure 2 shows the variations of η with T observed when aqueous solutions of samples M-2 and U-1 were heated from 20 to about 160°C and cooled to 20°C, at a rate of $0{\cdot}50 \pm 0{\cdot}03$ deg min^{-1}. It can be seen that, on heating, η for either sample decreases almost linearly up to 130°C, very sharply at about 135°C, and again linearly above 140°C. The linear decreases in η below 130°C and above 140°C may be described by an Arrhenius type temperature dependence, but the sharp decrease in η at $T \sim 135$°C cannot. On the other hand, on cooling, η follows the heating curve down to about 130°C, and deviates progressively upwards from it below 130°C. Thus, schizophyllan in water undergoes an irreversible thermal change at $T \sim 135$°C.

Similar measurements on the same samples at different polymer concentrations (0·8–12 wt%) gave substantially the same results as above. Hence, we can conclude that the thermal change in schizophyllan occurs virtually independently of the concentration and molecular weight of the polymer.

Figure 3 shows the changes in $(\ln \eta_r)/c$ (c is the polymer concentration (w/v)) at 25°C with time, that occurred after an aqueous solution of sample M-2 with $c = 0{\cdot}106 \times 10^{-2}$ g cm^{-3} was preheated at 150°C for different periods Δt_p (1–10 min) and cooled to 25°C. The curves for $\Delta t_p \leqslant 4$ min depend strongly on Δt_p, whereas those for $\Delta t_p \geqslant 6$ min almost superimpose. The initial $(\ln \eta_r)/c$ values (about 1×10^2 cm^3 g^{-1}) for the latter group of curves are comparable to $[\eta]$ of the same sample in DMSO at 25°C. This indicates that schizophyllan is dispersed in pure water at 150°C as single random coils.

Similar measurements on aqueous solutions of sample M-2 preheated at 120°C showed that $(\ln \eta_r)/c$ are virtually independent of Δt_p, ranging from $4{\cdot}2 \times 10^2$ to $4{\cdot}4 \times 10^2$ cm^3 g^{-1}. The close agreement of these $(\ln \eta_r)/c$ values with $[\eta]$ for the intact triple helix of the same sample in water at 25°C (see Table 1) indicates that the majority of the schizophyllan chains in water maintained the triple helical structure up to as high a temperature as 120°C.

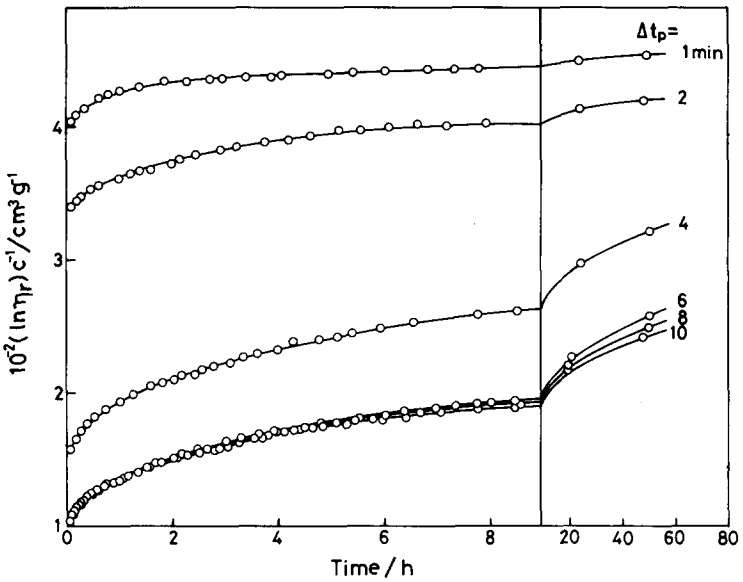

Fig. 3. Increases in $(\ln \eta_r)/c$ at 25°C with time that occurred after aqueous schizophyllan solutions preheated at 150°C for periods Δt_p of 1-10 min were cooled to 25°C. Samples, M-2; c, $0{\cdot}106 \times 10^{-2}$ g cm^{-3}.

Now that the major conformations of schizophyllan in water at 120 and 150°C have been found to be triple helix and single random coil, respectively, the sharp drop in η at about 135°C in Fig. 2 may be interpreted as due to the melting of triple helices to single chains.

Figure 4 shows the time dependence of $(\ln \eta_r)/c$ at 25°C for aqueous solutions of sample M-2 preheated at 150°C for 10 min. The horizontal lines in this figure represent the values of $(\ln \eta_r)/c$ for the intact triple helix at the indicated c. The curves resemble those observed previously for water–DMSO mixtures (Norisuye *et al.*, 1980): the curve for the lowest c levels off at a value far below that expected for the intact triple helix at this c value, while that for $c = 0{\cdot}493 \times 10^{-2}$ g cm^{-3} exceeds the level of the triple helix value after 4 h and appears to rise indefinitely. These features indicate that schizophyllan single chains in water at 150°C no longer restore the intact triple helix but aggregate when the solution is cooled to 25°C.

The curve for the highest c in Fig. 4 rises very sharply. The solution at this c formed a gel after 30 min. Similar behaviour was observed for

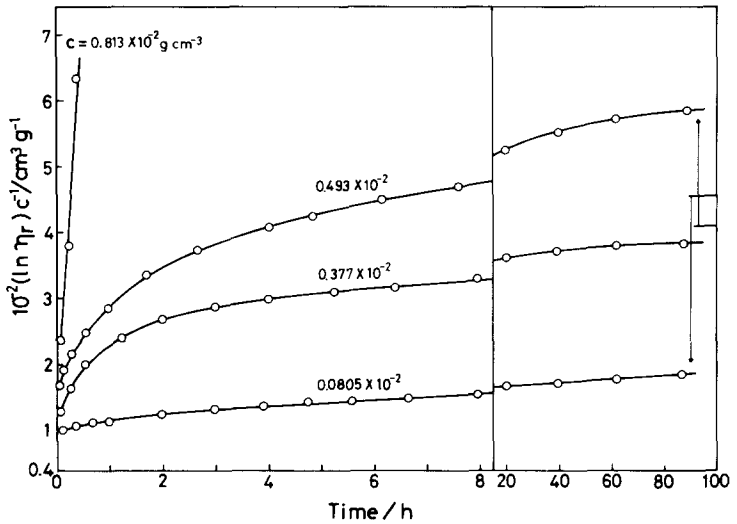

Fig. 4. Time dependence of $(\ln \eta_r)/c$ at 25°C for aqueous solutions of sample M-2 preheated at 150°C for 10 min.

aqueous M-2 solutions after the thermal curves of η in Fig. 2 had been determined. Figure 5 illustrates the frequency dependence of dynamic strage modulus G' and dynamic loss modulus G'' at 25°C for a 3·23 wt% aqueous M-2 solution. The filled circles represent the data obtained 5 h after a test solution was preheated at 150°C for 10 min, while the unfilled ones refer to a non-preheated solution. The curves of G' and G'' for the two solutions are distinctly different. For the non-preheated solution, G'' is larger than G' throughout the entire frequency range studied, and both exhibit behaviour typical of a molecularly dispersed system. On the other hand, the two moduli for the preheated solution show behaviour characteristic of gels; G' is larger than G'', and both moduli depend little on frequency. From these data it may be concluded that schizophyllan forms a gel when its aqueous solution with a concentration higher than a certain value is heated to about 150°C and cooled to room temperature.

The triple helical structure of schizophyllan is stabilized by interchain hydrogen bonds (Norisuye *et al.*, 1980; Takahashi *et al.*, 1985). Thus, when the temperature is lowered from 150°C to room temperature, the hydroxyl groups of single, randomly coiled schizophyllan chains should have chances to form interchain hydrogen bonds. How-

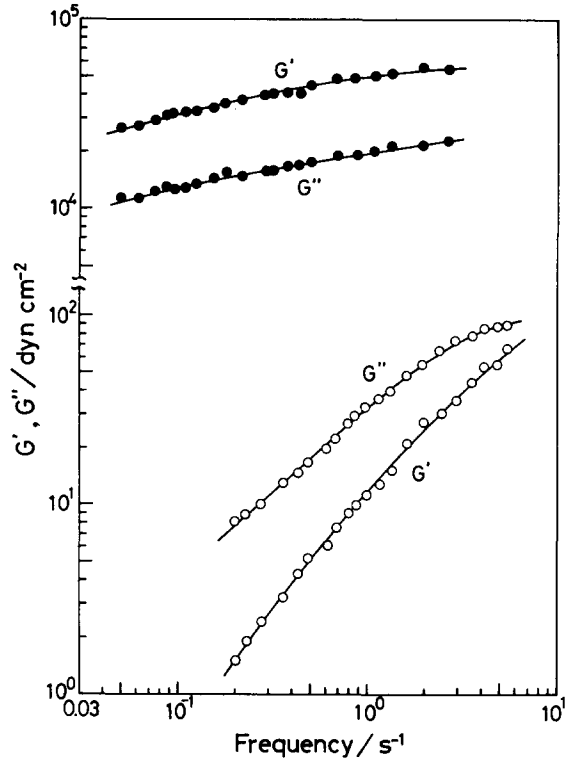

Fig. 5. Frequency dependence of G' and G'' at 25°C for an aqueous M-2 solution of 3·23 wt% polymer. (○) and (●), before and after preheating at 150°C for 10 min, respectively.

ever, it must be extremely unlikely that three non-specific chains in dilute solution are hydrogen bonded to reform a triple helix. It is far more likely that two, three, or more chains are randomly hydrogen bonded to form dimers, trimers and higher aggregates. As c is increased, there are more chances for schizophyllan chains to meet one another, and hence larger aggregates are formed, leading to a viscosity higher than that expected for a rod-like triple helix. When c exceeds a certain value, the aggregates may extend throughout the aqueous solution to form a gel.

These considerations are consistent with the viscosity behaviour observed in Fig. 4. In this connection, we remark that collagen, heat denatured at high concentrations, does not restore the intact triple

helical structure but forms large aggregates when the aqueous solution is cooled (Boedtker & Doty, 1956; Engel, 1962; Beier & Engel, 1966). The kinetic curves obtained by Engel (Engel, 1962) in a renaturation experiment on collagen are similar to those found for schizophyllan in Fig. 4.

In conclusion, the schizophyllan triple helix in water melts to single randomly coiled chains at about 135°C, but is not recoverable once denatured to single chains at about 150°C.

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