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ISOTOPE EFFECT ON THE ORDER–DISORDER TRANSITION IN AQUEOUS SCHIZOPHYLLAN*

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ABSTRACT

Optical rotation, heat capacity, and viscosity measurements were made on D_2O solutions of schizophyllan, a triple-helical polysaccharide. Solutions of schizophyllan in D_2O showed, at ~290 K, an order–disorder transition which depended remarkably on the molecular weight of the sample. The transition closely resembled the helix–coil transitions of polypeptides, and it was concluded that it was a cooperative transition between the ordered and disordered states of the triple helix. It was found that (*1*) the triple helix is kept intact throughout the transition region, (*2*) the transition is extremely cooperative, (*3*) the mobility of side chains is restricted in the ordered state, (*4*) the helix diameter is larger in the ordered state than in the disordered state, and (*5*) the transition undergoes a remarkable isotope effect, suggesting that water molecules, as well as side chains, play an important role in the transition. All these findings were taken into account in order to propose possible organized structure formed about the helix core in the ordered state.

INTRODUCTION

Schizophyllan, a (1→3)-β-D-glucan, produced by the fungus *Schizophyllum commune*, having the chemical structure shown in Fig. 1, dissolves in water as a triple-stranded helix of straight cylindrical shape[1–3]. Recently[4,5], we found from optical rotation and heat-capacity measurements that there exists a highly cooperative order–disorder transition in aqueous schizophyllan at ~280 K; a similar, but more strongly cooperative, transition is seen at ~291 K in heavy water[5]. With respect to the optical rotation and heat-capacity data, it closely resembles helix–coil transitions in polypeptides. Therefore, theories of the latter transitions[6–10] were used to analyze all the data, with the conclusion that there develops, among the side chains of the schizophyllan triple helix, some organized structure which persists

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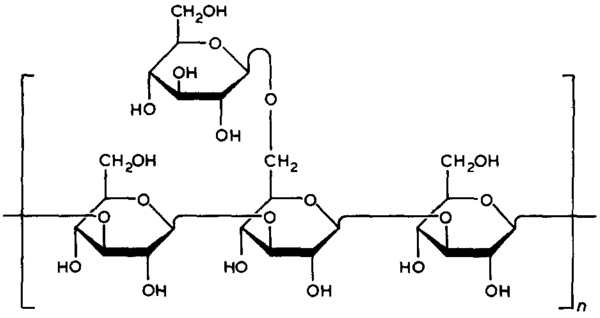

Fig 1 Repeat unit of schizophyllan

over a long distance along the helix axis, and gets disordered in a highly cooperative manner. It is suggested that this transition is associated with a change in side-chain orientation involving surrounding water molecules, with the conformation of the main chain kept intact. However, no side-chain orientation responsible for the transition, nor the role that water molecules play in the transition, has yet been elucidated.

To investigate the isotope effect on the transition in more detail, optical rotation, heat capacity, and viscosity measurements were made on solutions of schizophyllan samples having different molecular weights, in D_2O and mixtures of H_2O and D_2O Furthermore, ^{13}C-n m r. measurements were made on D_2O solutions, in order to examine dynamic aspects of the transition. We have combined now all of these data, in order to propose a possible molecular mechanism for the transition which may be consistent with the crystallographic structure of schizophyllan proposed by Takahashi and Kobatake[11]

EXPERIMENTAL

Schizophyllan samples and solutions. — For the present study, there were chosen from our stocks five schizophyllan samples which had been obtained by fractionation of sonicated schizophyllan provided by Taito Co., Ltd. Table I gives their intrinsic viscosities $[\eta]$ in water at 25° and viscosity-average molecular weights M_v; M_v was estimated from $[\eta]$ in H_2O, using the $[\eta]$ – M_w relationship established by Yanaki *et al.*[2], with M_w being the weight-average molecular weight. Solutions were prepared by mixing a weighed amount of a given sample with water in a stoppered flask under a nitrogen atmosphere. The weight fraction, w, of polysaccharide was determined gravimetrically and the mass concentration c (g.cm^{-3}) was calculated from w. Parts of the samples were lyophilized from D_2O solutions to prepare D_2O solutions with w >0.07. D_2O (Wako; deuteration level >99.75%) was used.

Physical measurements. — Measurements of optical rotation were made on a JASCO ORD/UV-5 spectropolarimeter, following the experimental procedure given before[5]

TABLE I

SCHIZOPHYLLAN SAMPLES USED

Sample	$[\eta]^a/10^2\ cm^3.g^{-1}$	$M_v{}^b/10^4 g.mol^{-1}$	P
J-4	0.320	8.4	130
J-22	0.458 (0.46)	10.8	167
R40	1.17	18.3	282
R-212	2.66 (2.68)	29.9	461
T-5	8.94 (9.31)	67	1033

[a]Intrinsic viscosities in H_2O at 25°, values in the parentheses refer to D_2O at 25° [b]Viscosity-average molecular weights determined from $[\eta]$ in H_2O at 25°, using the $[\eta]$ *vs* M_w relationship of Yanaki *et al* [2] $P = M_v/M_0$, where M_0 is the molar mass of the four-sugar repeat unit (648.6 g/mol), and M_w is the weight-average molecular weight

Heat-capacity measurements were made on a renovated version[12] of the computerized, adiabatic calorimeter[13] according to the experimental and data-acquisition procedures given in detail before[5,12,13]. The measurements were made between 275 and 310 K on D_2O solutions of samples J-4, R40, and R-212 and one solution of R-212 in an equimolar mixture of H_2O and D_2O; their w values were ~0.08. The calorimeter gave as output the heat capacity C_p of a polysaccharide solution containing one mole of saccharide repeat unit. The apparent, molar heat-capacity C_s of the repeat unit was computed from C_p by the equation $C_s = C_p - C_w M_0(1 - w)/w$, where M_0 is the molar mass of the four-sugar repeat unit, and C_w the specific heat-capacity of water; M_0 is 648.6 g/mol for schizophyllan. The literature values[14,15] for C_w were used for the computation.

Relative viscosities, η_r, at zero shear rate[1,2] were measured on D_2O solutions of samples J-22, R-212, and T-5, with fixed concentrations, between 279 and 308 K. Additional measurements made at several temperatures gave the Huggins viscosity constant, k', ranging between 0.36 and 0.46, depending on sample and solvent conditions On the basis of this result, appropriate k' values were chosen to obtain $[\eta]$ from the measured η_r.

RESULTS

Figure 2 shows optical rotation data for D_2O solutions of three schizophyllan samples having different molecular weights. It can be seen that the specific rotation, $[\alpha]$, at 350 nm for R-212 undergoes, at ~291 K, a sharp change which manifests itself as a highly cooperative order–disorder transition. As the molecular weight of the sample is decreased, the transition is shifted to lower temperature and becomes more gradual. This trend is very similar to the transition[5] in H_2O, which appears at temperatures ~12 K lower than the former.

Fig 3 shows heat-capacity curves for the same solutions as in Fig. 2; the data for sample R-212 are reproduced from a previous paper[5]. The heat-capacity curve

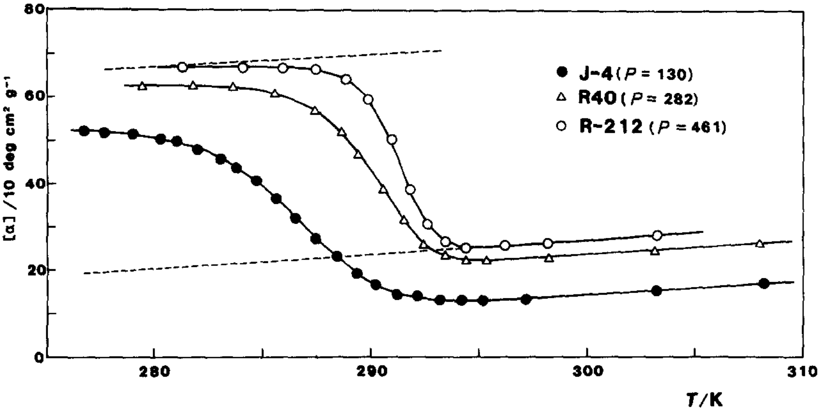

Fig 2 Temperature dependence of specific rotation [α] (light of wavelength 350 nm) for schizophyllan in D_2O [Key ●, J-4 (P = 130), △, R40 (P = 282), ○, R-212 (P = 461) Dashed lines, baselines (see text) $P = M_v/M_0$]

for R-212 shows a very sharp peak centered at ~291 K, the transition temperature already detected with [α], and the transition curve is displaced to lower temperature and gets more diffuse with decrease in molecular weight. Above 300 K, the data points for all the samples appear to follow a single straight line, within experimental error. This implies that, in this temperature range, the schizophyllan triple helix is

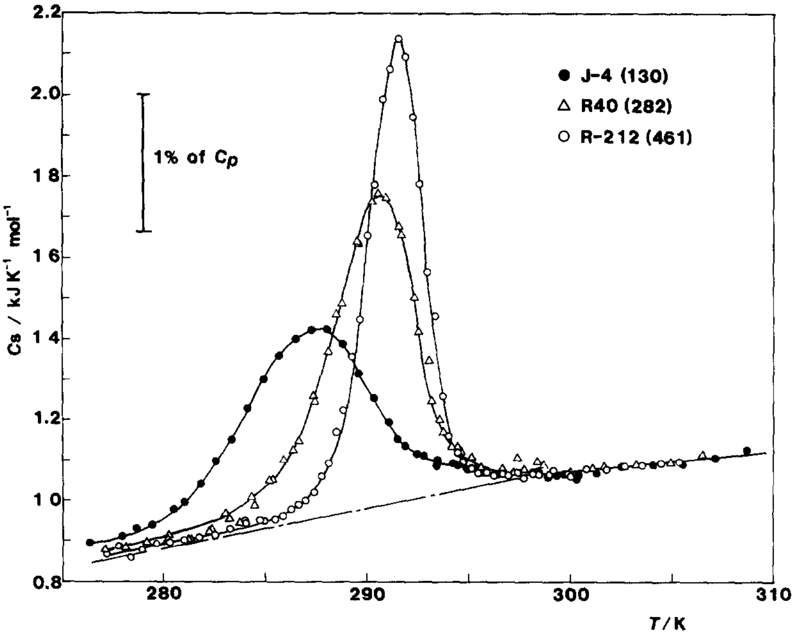

Fig 3 Apparent molar heat capacity (C_s) of the schizophyllan repeat unit in D_2O [Key ●, J-4, △, R40, ○, R-212 Dash-dot line, baseline]

in the disordered state, and has a specific heat-capacity independent of molecular weight; the helix is intact under these solvent conditions, as shown later. The transition for R-212 appears to end at ~282 K, and is well separated from the melting temperature of D_2O, 276.97 K, which is ~14 K lower than the transition temperature T_c defined as the temperature at which the transition curve displays a peak.

The transition for sample R40 appears to end just above the melting temperature of D_2O although sample J-4 does not show a complete-transition curve. This trend differs from that for H_2O solutions[5], where T_c for R-212 is only 7 K above the melting temperature of ice, and the transition for R40 does not end before the solution freezes, thus making it impossible to obtain a full transition-curve for a sample having a lower molecular weight. Comparison of Figs. 2 and 3 clearly indicates that the same order–disorder transition is detected by the two different techniques. It is noted that, although the peak for R-212 appears very sharp and high, this thermal anomaly is only a small fraction (3%) of the total heat-capacity of the solution.

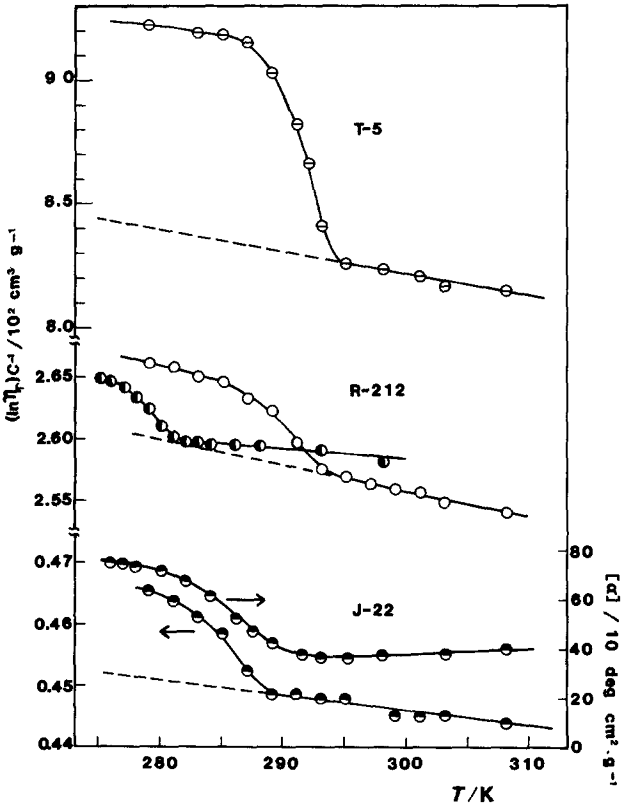

Fig 4 Temperature dependence of $(\ln \eta_r)/c$ and $[\alpha]$ [Key, ●, J-22, ○, R-212, ⊖, T-5 in D_2O, ◐, R-212 in H_2O]

It can be seen in Table I that, for samples J-22 and R-212, $[\eta]$ is practically the same in H_2O and in D_2O. This indicates that the triple helical conformation found in H_2O is preserved in D_2O as well. Fig. 4 shows plots of $(\ln \eta_r)/c$ in D_2O against temperature for three samples having different molecular weight; the specific rotation for J-22 is shown for comparison. For all samples, $(\ln \eta_r)/c$ increases slightly with lowering temperature, but the increase is, at most, 13% over the entire temperature range investigated. This implies that the triple helical conformation of the main chains is substantially intact throughout this temperature range. Examined in detail, however, $(\ln \eta_r)/c$ displays an anomalous change in the same temperature range, where the order–disorder transition is found from the specific rotation data. Thus, this anomalous change is ascribed to the order–disorder transition detected from the specific rotation and heat-capacity data already shown.

Bluhm *et al.*[16] noted similar sharp increases in $[\alpha]$, and reduced viscosity, with lowering temperature for a dilute aqueous solution of scleroglucan, which has the same chemical structure and triple helical conformation as schizophyllan. They attributed these changes to gelation of the solution. However, the aforementioned changes for aqueous schizophyllan are due neither to gelation nor to aggregation, for the reasons given later. First, they are reversible for any sample and in any solvent examined, which is a necessary condition for the absence of aggregation. Second, the Huggins viscosity constant, k', which ranges between 0.36 and 0.46,

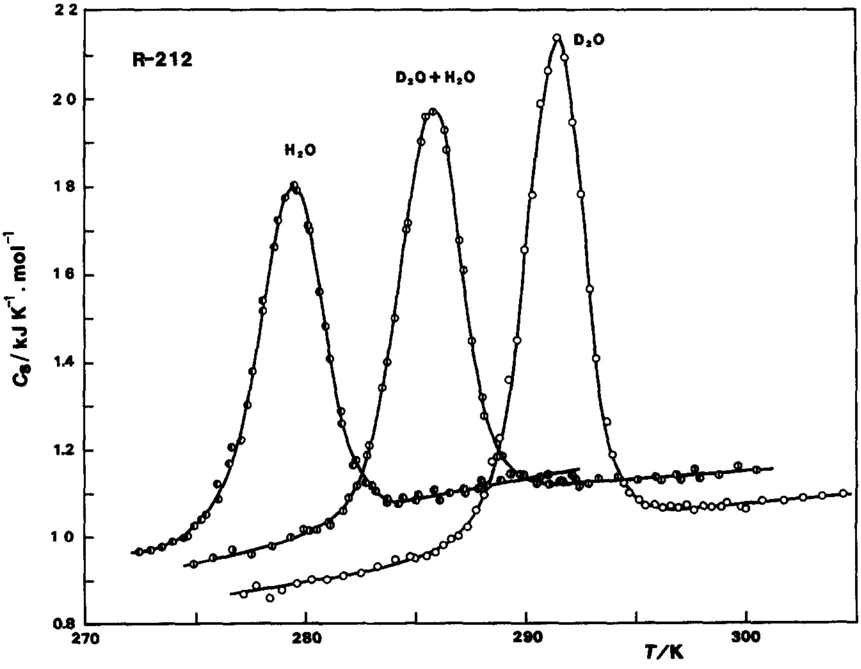

Fig 5 Isotope effect on C_s for R-212 [Solvent· ○, D_2O; ⦶, D_2O–H_2O equimolar mixture, ◐, H_2O]

depending on sample and solvent conditions is taken as evidence for negligible aggregation, because the helix dissolves in H_2O at 298 K without appreciable aggregation[1,2].

Fig. 5 illustrates that the transition for R-212 undergoes a remarkable isotope effect, indicating that the stability of the ordered structure increases on going from H_2O to D_2O. In conclusion, all these findings substantiate the previous suggestion[5] that this order–disorder transition is essentially associated with the side chains involving the surrounding water molecules, and not with the backbone.

DISCUSSION

Linear cooperative transition

As in the previous study[5], we assume that each saccharide unit of schizophyllan is either in the ordered state (1) or in the disordered state (2), and we represent the fraction of units in the ordered state at temperature T by f_N. Quantities associated with state $i (i = 1,2)$ are denoted by the subscript i. The specific rotation $[\alpha]$ at a given wavelength is related to f_N by

$$[\alpha] = f_N[\alpha]_1 + (1 - f_N)[\alpha]_2 \tag{1}$$

where $[\alpha]_1$ and $[\alpha]_2$ are the baseline values corresponding to the ordered and disordered states, respectively.

It has been shown[5] that C_s consists of the molar-transition heat-capacity ΔC_s and the baseline contribution, and ΔC_s is related to f_N by

$$\Delta C_s = -\Delta H_r(\partial f_N/\partial T), \tag{2}$$

where $\Delta H_r = H_2 - H_1$ is the molar transition enthalpy of the repeat unit. Assuming that ΔH_r is independent of T, we obtain from this equation

$$f_N = -(\Delta H_r)^{-1}\int_{T_r}^{T} \Delta C_s \, dT, \tag{3}$$

where T_r is a reference temperature chosen above the transition region. Note that $f_N = 0$ at T_r, and $f_N = 1$ at T well below T_c.

We follow a procedure similar to that used before[5] to determine the baselines $[\alpha]_1$ and $[\alpha]_2$. It is seen in Fig. 2 that, for all samples, $[\alpha]$ increases linearly with T above 295 K, *i.e.*, in the disordered state. The extension of this linear portion, shown by the dashed line, is taken as $[\alpha]_2$. The value of $[\alpha]$ for sample R-212 appears to reach a plateau at lower temperature. However, this plateau is not long enough to define $[\alpha]_1$ correctly. Here, we assume that $[\alpha]_1$ has the same temperature coefficient as $[\alpha]_2$, but differs by a constant K_2, and compute f_N by

$$f_N(T) = ([\alpha] - [\alpha]_2)/K_2, \tag{4}$$

with

$$[\alpha]_2 = K_1 + 3.08\,(T - 280), \tag{5}$$

where K_1 is determined for each sample so as to fit the data to the straight line at higher temperature. Computation was made with K_2 = 461 deg.cm^2.g^{-1}, which gives almost vanishing $1 - f_N$ for R-212 at 280 K.

As shown in Fig. 3, the C_s *vs* T curves in D_2O have a common straight portion at higher temperature, which represents the partial molar heat capacity of a repeat unit in the disordered state. The short straight portion at lower temperature for R-212 suggests that the transition is almost over for this sample in this temperature range. Thus, we take the dash-dot line connecting these two portions as the baseline, and regard the difference between the C_s curve and the baseline as ΔC_s The same baseline is assumed for the other samples.

Fig 6 shows plots of f_N and ΔC_s, thus computed, against temperature. Values of ΔH_r were obtained by numerical integration of the transition heat-capacity curves between 275 and 300 K on the basis of Eq. *3*. Table II summarizes the values of ΔH_r thus obtained, along with T_c and $\Delta S_r = \Delta H_r/T_c$, in comparison with those for H_2O solutions obtained previously[5]. It can be seen that ΔH_r is rather insensitive to molecular weight, but undergoes a remarkable isotope effect; the ordered state is more stable in D_2O than in H_2O.

The solid curves in panel (b) of Fig 6 represent the values of f_N computed by Eq *3* from the ΔC_s data given in panel (a). The curve for each sample follows the data points obtained from $[\alpha]$ data for the same sample, given in Fig 2. Thus, we see that optical rotation and heat capacity-changes both correspond accurately to each other, and originate from some common, molecular mechanism.

From the close resemblance of the transition in H_2O to helix-coil transitions in polypeptides, we concluded it to be a linear cooperative transition between the ordered and disordered states[5]. The optical rotation and heat-capacity data for D_2O solutions (in Fig. 6) are also reminiscent of the helix–coil transitions in polypeptides; indeed, transition curves remarkably dependent on molecular weight are characteristic of the latter transitions[9,10] Therefore, as before, we analyze these data by helix–coil-transition theories, with f_N being compared to the helical fraction. It is implicit from the foregoing discussion that the terms "ordered" and "disordered" refer to the state of the side chains, and not that of the backbone

In the Zimm–Bragg–Nagai formalism of the helix–coil transition[6–10], f_N for a polypeptide with N peptide units is expressed by a function of N, s, and σ, where s is regarded as the equilibrium constant between the ordered and disordered states, and σ is the cooperativity parameter. Because the triple helix of schizophyllan consists of three identical D-glucan chains with the repeat units shown in Fig 1, we combine three such units belonging to the three chains at the same level on the

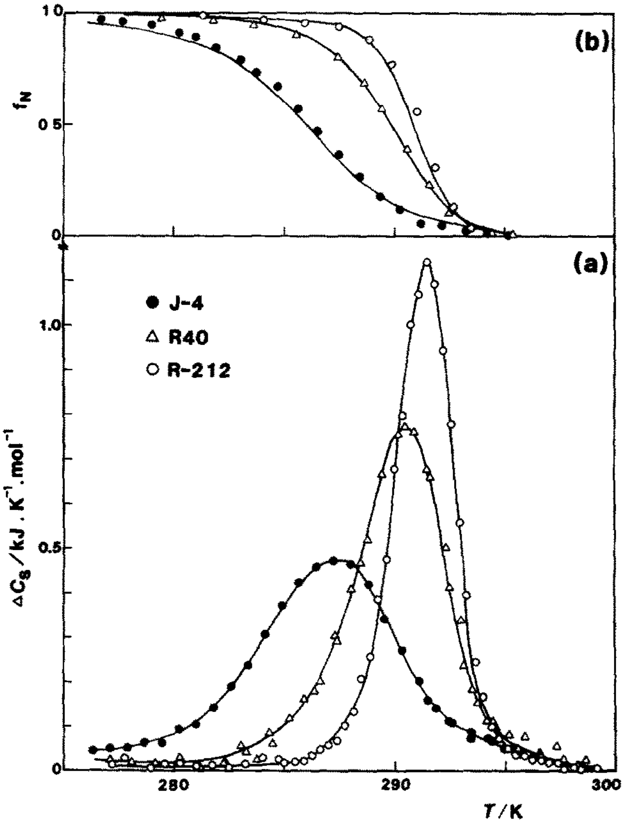

Fig 6. (a) Transition heat capacity, ΔC_s for J-4 (●), R40 (△), and R-212 (○) in D_2O; data points are connected by solid curves (b) Temperature dependence of fraction of the saccharide units in the ordered state {Data points, the values estimated from [α] in Fig 2; solid lines, the values calculated from the ΔC_s vs T curves in panel (a).}

TABLE II

THERMODYNAMIC QUANTITIES ASSOCIATED WITH THE ORDER-DISORDER TRANSITION IN AQUEOUS SCHIZOPHYLLAN

Sample	*Solvent*	*w*	T_c/K	*Observed*		*Calculated*
				ΔH_r *kJ/mol*	ΔS_r *J/K mol*	$(\Delta H_r)_{cal}$ *kJ/mol*
J-4	D_2O	0.08084	287.3	4.09	14.2	4.23
R40	D_2O	0.07540	290.5	4.34	14.9	4.42
	H_2O^a	0.07825	278.7	2.81	10.1	
R-212	D_2O^a	0.07189	291.5	4.17	14.3	4.48
	H_2O–D_2O	0.008022	285.7	3.56	12.5	
	H_2O^a	0.007722	279.5	2.95	10.6	
N	H_2O^b		280.2	3.7		
	D_2O^b		292.3	4.6		

[a] Taken from ref 5 [b] The values used for theoretical calculations. $(\Delta H_r)_{cal}$, values of the molar-transition enthalpy, calculated from the theoretical ΔC_s vs. T relationships.

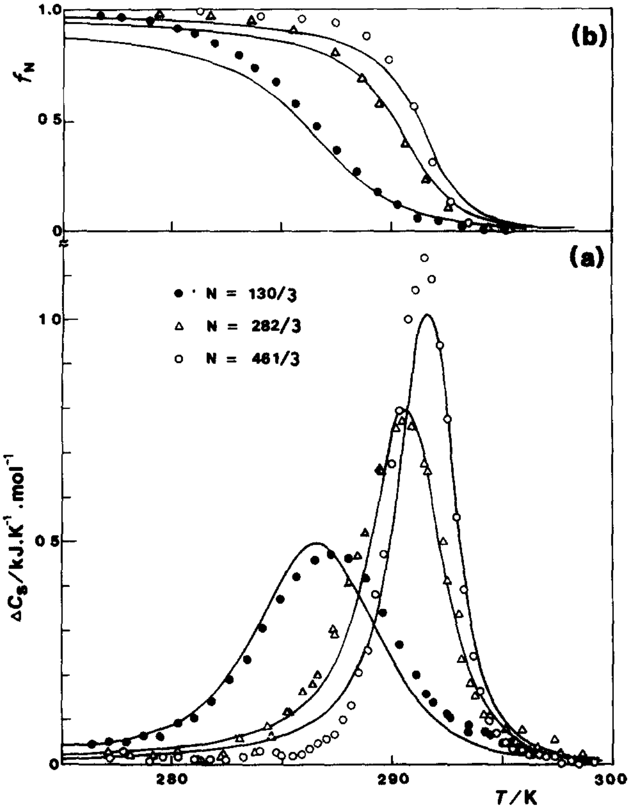

Fig. 7 Comparison of the theoretical and experimental values for (a) ΔC_s and (b) f_N. [Theoretical values calculated for $T_c^\infty = 292.3$ K, $\Delta H_r^\infty = 4.6$ kJ/mol, and $\sigma^{1/2} = 0.0135$ The symbols are the same as in Fig. 2 $N = M_v/3M_0$]

helix axis as a theoretical repeat unit, with the assumption that the ordered state be stabilized by nearest-neighbor interactions between such theoretical units. Thus, s is expressed as a function of T as

$$\ln s = (3\Delta H_r^\infty/RT)(1 - T/T_c^\infty), \qquad (6)$$

where ΔH_r^∞ and T_c^∞ are ΔH_r and T_c for infinite N. When ΔH_r^∞ is assumed to be independent of T, f_N is a known function of N and T, with ΔH_r^∞, T_c^∞, and σ as unknown, temperature-independent parameters.

We sought for these parameters, by trial and error, values which best fitted the data for f_N and ΔC_s shown in Fig. 6, with an emphasis being placed on how accurately the positions and heights of the ΔC_s *vs.* T curves could be predicted.

Here, the samples were assumed to be monodisperse*, and N was equated to $M_v/3M_0$, with M_0 being the molar mass of the four-sugar repeat unit, namely, 648.6 g/mol. The parameter $\sigma^{1/2}$ was assumed to be independent of temperature, and much smaller than unity. The best values for the parameters are ΔH_r^∞ = 4.6 kJ/mol, T_c^∞ = 292.3 K, and $\sigma^{1/2}$ = 0.0135. In Fig. 7, the theoretical ΔC_s *vs.* T relationships calculated with these parameter values (solid curves) are in good agreement with the experimental data. Table II contains values of the molar-transition enthalpy, $(\Delta H_r)_{cal}$, obtained by numerical integration of the calculated ΔC_s *vs.* T curves over the temperature range between 275 and 300 K. Their good agreement with the measured values, ΔH_r, confirms the consistency of this analysis.

In Table II, ΔH_r and ΔS_r are seen to increase linearly with D_2O content. Although the present $\sigma^{1/2}$ is close to 0.012 obtained for H_2O solutions**, stabilization of the ordered state in D_2O as measured by ΔH_r and T_c is remarkable if their increases are compared with the corresponding increases in heat of fusion and melting temperature of ice[14,15].

When compared with $\sigma^{1/2}$ values reported for polypeptides[9,10], the $\sigma^{1/2}$ values obtained for schizophyllan indicate that the transition is highly cooperative. Theoretically, $\sigma^{-1/2}$ is the average length of ordered (and disordered) sequences on a triple helix for infinite N at T_c^∞, which contains 222 side chains in the present case. Thus, it is expected that, once initiated, an ordered sequence should grow over a long distance on the helix, and will not be fully stabilized on such a short helix as sample J-4 (carrying only 130 side chains). Therefore, near the middlepoint of a transition, a long, schizophyllan triple-helix tends to consist of relatively long, ordered and disordered sequences appearing alternately, but a shorter helix can accommodate, at most, one ordered sequence, or none.

Side-chain mobility

Schizophyllan dissolves[1,2] as disordered, single chains in dimethyl sulfoxide (Me_2SO). However, as already shown, in D_2O at 313 K, schizophyllan exists as an intact, triple helix, but is in the disordered state, presumably with respect to the side-chain conformation. In order to get information about the conformation of the side-chain D-glucose residues of schizophyllan, ^{13}C-n.m.r. spectra of one schizophyllan sample, of molecular weight 150,000, in Me_2SO-d_6 were recorded at 313 K, and in D_2O, between 278 and 313 K. Use was made of a Bruker WM 360 WP spectrometer at the Institute for Protein Research, Osaka University. The

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*The samples used were obtained by careful fractionation, but were not monodisperse They are thought to be characterized by a polydispersity index M_z/M_w ranging between 1.1 and 1.4[1,2,17] Effects of polydispersity on the transition were estimated by using a molecular-weight-distribution function of the logarithmic, normal type It was found that the ΔC_s and $[\alpha]$ data could be fitted to the calculated curves for M_z/M_w values in the range, using ΔH_r^∞ and σ not very much different from those used in Fig. 7 Thus, our conclusion with respect to the cooperativity of transition need not be altered by the polydispersity of the sample

**The previous $\sigma^{1/2}$ refers to one four-sugar repeat unit[5], and should be multiplied by three to compare with the present one

Me_2SO-d_6 spectrum agreed with that reported by Tabata *et al.*[18]. The spectrum in D_2O at 313 K appeared well resolved, but showed fewer peaks than the Me_2SO-d_6 spectrum. On the basis of the peak assignments for the Me_2SO-d_6 spectrum due to Tabata *et al.*, we found that all the peaks in the D_2O spectrum were assigned to the side-chain carbon atoms of schizophyllan; the signals from the main chain carbon atoms were hardly visible. As the temperature was lowered into the transition region, the D_2O spectrum became increasingly blurred. We conclude from these findings that the side-chain D-glucose residues, which are not completely free to move, even in the high-temperature, disordered state, may become less mobile in the low-temperature, ordered state

Helix diameter

Because of the intact, cylindrical shape, $[\eta]$ of the helix of the schizophyllan triple-helix, is well represented by the theory of Yoshizaki and Yamakawa[19] for spheroid cylinders when its molecular weight is lower[1,2] than 300,000. Above this molecular weight, the triple helix exhibits increasing flexibility; more correctly, it is represented by a worm-like chain (Kratky and Porod) with a persistence length[2] of 200 nm. The viscosity equation of Yoshizaki and Yamakawa, in terms of the axial ratio, $p = L/d$, is written

$$[\eta] = (2\pi N_A d^3/45M)P^3F_\eta(p,L) \qquad (7)$$

where M, L, and d are respectively the molecular weight, length, and diameter of the cylinder, and $F_\eta(p,L)$ is a known function of p given in their paper; N_A is Avogadro's number. Alternatively, $[\eta]$ may be expressed as a function of M, d, and M_L, with M_L being defined by M/L Yamakawa's asymptotic expression of $[\eta]$, valid for large p, reads[20]

$$[\eta] = (2\pi N_A M^2/45M_L^3)[\ln(M/M_L\mathrm{d}) + 2\ln 2 - 25/12]^{-1}. \qquad (8)$$

Both viscosity equation show that any change in $[\eta]$ for a given sample (at a fixed M) may be due to the change either in d or M_L, or in both of them. With one of either d or M_L held constant, the change in $[\eta]$ with the other parameter was calculated as a function of M by Eq. 7; appropriate values were chosen for d and M_L, according to Yanaki *et al.*[2]. Fig. 8 shows $[\eta]$ in a particular state relative to that in some reference state s. Panel (a) shows the molecular-weight dependence of $[\eta]/[\eta]_s$ at a fixed value of M_L. For a fixed increase in diameter, d/d_s, the relative $[\eta]$ decreases sharply with increasing molecular weight, and tends to vanish logarithmically at large M

It is indicated in panel (b) that, for a fixed $(M_L)_s/M_L$, $[\eta]$ shows a slight increase with M. These calculations show that the ratio of $[\eta]$ for two solvent conditions should decrease, or slightly increase, with increasing M, depending on whether it is due to the change in d or due to the change in M_L.

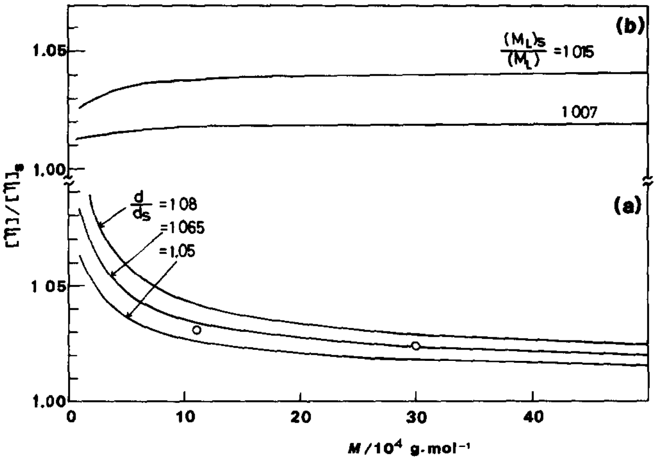

Fig 8 Dependence of $[\eta]/[\eta]_s$ on molecular weight, $[\eta]_s$, $[\eta]$ in the reference state {Data points for J-22 and R40 (open circles), obtained from the data in Fig 4, solid lines, calculated according to the Yoshizaki–Yamakawa theory for spheroid cylinders with d_s = 2.6 nm and $(M_L)_s$ = 2150 nm^{-1} (a) $[\eta]/[\eta]_s$ as a function of d/d_s (b) $[\eta]/[\eta]_s$ as a function of $(M_L)_s/M_L$ }

It is seen in Fig. 4 that the data points at temperatures higher than 295 K follow approximately the straight line indicated, which accounts for the temperature dependence of $[\eta]$ in the disordered state; the extended, dashed line is taken as the baseline $[\eta]_2$ in this state. The viscosity data given in Fig. 4 are reproduced as plots of $[\eta]/[\eta]_2$ *vs.* T in Fig. 9. For either of samples R-212 and J-22 belonging to the rigid-rod regime, $[\eta]/[\eta]_2$ undergoes a transition quite similar to those found from other measurements, and appears to approach another straight line at lower temperature, which may be taken as the baseline $[\eta]_1$ for the ordered state. Relative

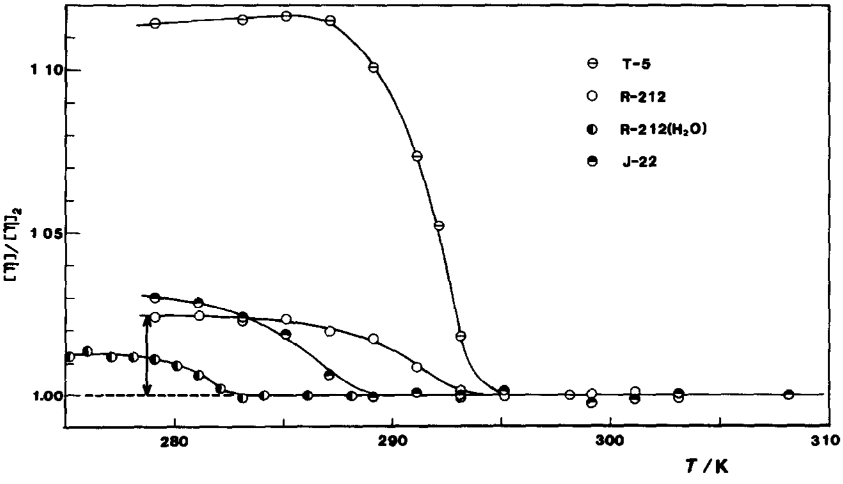

Fig 9 Plots of $[\eta]/[\eta]_2$ *vs* temperature for schizophyllan in water [The symbols are the same as in Fig. 4]

changes in $[\eta]$ across the transition region, $[\eta]_1/[\eta]_2$, may be estimated from the distance between the two baselines. The estimated values for R-212 and J-22 (open circles) are plotted in Fig. 8, which shows that the aforementioned trend is consistent with the prediction in panel (a). Thus, we see that the change in $[\eta]$ between the two states is due to the change in hydrodynamic diameter of the helix, amounting to ~6.5%. A similar analysis of the data for R-212 in H_2O gives a somewhat smaller diameter-increase of ~3.5%. For sample T-5, which is semi-flexible in the disordered state[2], the change in $[\eta]$ is much larger than those for the other samples. This is because it contains two contributions: one is due to the increase in diameter, and the other to the increased stiffness of the helix resulting from the formation of the ordered structure surrounding the helix core.

Proposed structure for the ordered state

Takahashi and Kobatake[11] elucidated, by X-ray analysis, a structure for the schizophyllan helix, where three equivalent D-glucan chains are wound together with their side-chain D-glucose residues at the same height along the helix axis. Fig. 10 shows schematically their structure, drawn roughly to scale, where the side chain D-glucose residues belonging to the same chains are placed ~0.9 nm apart along the helix axis. The nearest-neighbor side-chains, either belonging to the same chain or not, are too far apart to form direct hydrogen bonds between them.

On the basis of this structure, we sought, for the ordered state, a possible structure which is consistent with the findings from the foregoing analysis: (*1*) the remarkable cooperativity of the transition, (*2*) the reduced side-chain mobility in

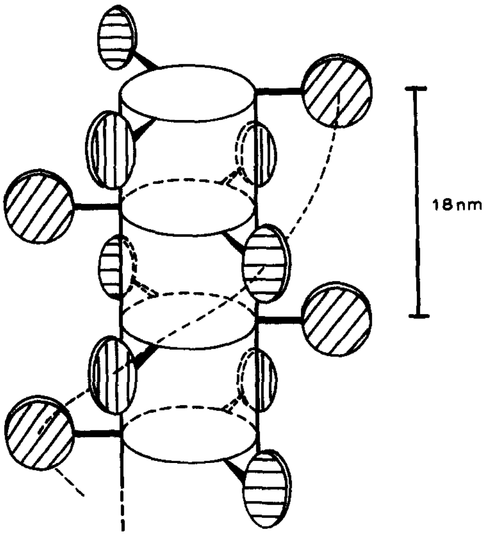

Fig 10 Schematic representation of the schizophyllan triple helix. [Hatched disks, side-chain D-glucose residues, the central cylinder, the helix core The disks, along with intervening water molecules, form a helical chain indicated by the dashed line]

the ordered state, (*3*) the increased helix diameter in the ordered state, and (*4*) the remarkable isotope effect on the transition.

Finding (*2*) indicates that fixation of side chains at a specific conformation may be a primary process toward the ordered state. However, it is apparent from finding (*4*) that water molecules are also associated with it. Thus, it is suggested that the ordered state may be characterized by an intermediate layer surrounding the helix core, where the side chains and associated water molecules are oriented into some organized structure to yield the increased diameter. Finally, finding (*1*) requires that such a structure be formed among neighboring side-chains, continuously along the helix axis; theoretically, no ordered conformation of non-interacting side-chains can lead to a cooperative transition. Thus, we propose a structural model in which side chains are linked together, with water molecules sandwiched between them, into a helical chain, as indicated by the dashed line in Fig. 10; three such chains surrounding the helix core form the intermediate layer, and give rise to the enhanced stiffness of the triple helix.

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