(20) Matsuzaki, T.; Iitaka, Y. *Acta Crystallogr., Sect. B* **1971**, *27*, 507.
(21) Galitsky, N. M.; Deigin, V. I.; Saenger, W.; Pletnev, V. Z. *Bioorg. Khim.* **1977**, *3*, 1445.
(22) Kartha, G.; Ashida, T.; Kakudo, M. *Acta Crystallogr., Sect. B* **1974**, *30*, 1861.
(23) Matsuzaki, T. *Acta Crystallogr., Sect. B* **1974**, *30*, 1029.
(24) Zimmermann, S. S.; Pottle, M. S.; Némethy, G.; Scheraga, H. A. *Macromolecules* **1977**, *10*, 1.
(25) IUPAC-IUB Commission on Biochemical Nomenclature *Biochemistry* **1970**, *9*, 3471.
(26) Balasubramanian, R.; Lakshminarayanan, A. V.; Sabesan, M. N.; Tegoni, G.; Venkatesan, K.; Ramachandran, G. N. *Int. J. Pept. Protein Res.* **1971**, *3*, 25.
(27) Tonelli, A. E. *J. Am. Chem. Soc.* **1970**, *92*, 6187.
(28) Tanaka, S.; Scheraga, H. A. *Macromolecules* **1974**, *7*, 698.
(29) Zimmermann, S. S.; Shipman, L. L.; Scheraga, H. A. *J. Phys. Chem.* **1977**, *81*, 614.
(30) Madison, V. *Biopolymers* **1977**, *16*, 2671.
(31) Rifkind, J. M.; Applequist, J. *J. Am. Chem. Soc.* **1968**, *90*, 3650.
(32) Deber, C. M. *Macromolecules* **1974**, *7*, 47.
(33) Benedetti, E.; Pavone, V.; Toniolo, C.; Bonora, G. M.; Palumbo, M. *Macromolecules* **1977**, *10*, 1350.
(34) Bonora, G. M.; Toniolo, C. *Gazz. Chim. Ital.* **1977**, *107*, 381.
(35) Pettit, G. R.; Gupta, S. K. *Can. J. Chem.* **1967**, *45*, 1600.
(36) Toniolo, C.; Palumbo, M.; Benedetti, E. *Macromolecules* **1976**, *9*, 420.
(37) Hamer, D.; Greenstein, J. P. *J. Biol. Chem.* **1951**, *193*, 81.
(38) Deber, C. M.; Bovey, F. A.; Carver, J. P.; Blout, E. R. *J. Am. Chem. Soc.* **1970**, *92*, 6191.
(39) Rapaka, R. S.; Bhatnagar, R. S. *Int. J. Pept. Protein Res.* **1975**, *7*, 475.
(40) Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286.
(41) Anderson, G. W.; Zimmermann, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.
(42) Rothe, M.; Theysohn, R.; Steffen, K. D. *Tetrahedron Lett.* **1970**, 4063.
(43) Jackson, M. B.; Becker, J. M.; Steinfeld, A. S.; Naider, F. *J. Biol. Chem.* **1976**, *251*, 5300.
(44) Šavrda, J. *J. Org. Chem.* **1977**, *42*, 3199.
(45) Helbecque, N.; Loucheux-Lefebvre, M. H. *Int. J. Pept. Protein Res.* **1978**, *11*, 353.
(46) Bruckner, P.; Rutschmann, B.; Engel, J.; Rothe, M. *Helv. Chim. Acta* **1975**, *58*, 1276.
(47) Rogozhin, S. V.; Davidovich, Yu. A.; Andreev, S. M.; Yurtanov, A. I. *Dokl. Chem. (Engl. Transl.)* **1973**, *212*, 108.
(48) Wieland, Th.; Lapatsanis, L.; Faesel, J.; Konz, W. *Justus Liebigs Ann. Chem.* **1971**, *747*, 194.
(49) Mukaiyama, T.; Soai, K.; Sato, T.; Shimizu, H.; Suzuki, K. *J. Am. Chem. Soc.* **1979**, *101*, 1455.
(50) Cromer, D. T.; Waber, J. T. In "International Tables for X-Ray Crystallography"; Kynoch Press: Birmingham, England, 1974; Vol. IV, Table 2.2B.
(51) Benedetti, E.; Ciajolo, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. *Macromolecules* **1979**, *12*, 438.
(52) Benedetti, E.; Palumbo, M.; Bonora, G. M.; Toniolo, C. *Macromolecules* **1976**, *9*, 417.
(53) Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. *Int. J. Biol. Macromol.* **1980**, *2*, 217.
(54) Ashida, T.; Kakudo, M. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1129.
(55) De Tar, D. L. F.; Luthra, N. P. *J. Am. Chem. Soc.* **1977**, *99*, 1232.
(56) Benedetti, E. In "Peptides"; Goodman, M., Meienhofer, J., Eds.; Wiley: New York, 1977; pp 257-73.
(57) Benedetti, E. In "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins"; Weinstein, B., Ed.; Dekker: New York, in press.
(58) Benedetti, E.; Pedone, C.; Toniolo, C.; Némethy, G.; Pottle, M. S.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1980**, *16*, 156.
(59) Dunitz, J. D.; Strickler, P. In "Structural Chemistry and Molecular Biology"; Rich, A., Davidson, N., Eds.; W. H. Freeman: San Francisco, Calif., 1968; pp 595-602.
(60) Mitra, J.; Ramakhrishnan, C. *Int. J. Pept. Protein Res.* **1977**, *9*, 27.
(61) Wolfenden, R. *Biochemistry* **1978**, *17*, 201.
(62) Bulmer, J. T.; Shurvell, H. F. *J. Phys. Chem.* **1973**, *77*, 256.
(63) MacPhee, J. A.; Dubois, J. E. *Tetrahedron Lett.* **1978**, 2225.
(64) Boussard, G.; Marraud, M.; Néel, J.; Maigret, B.; Aubry, A. *Biopolymers* **1977**, *16*, 1033.
(65) Toniolo, C. In "Bioorganic Chemistry"; van Tamelen, E. E., Ed.; Academic Press: New York, 1977; Vol. III, pp 265-91.
(66) Toniolo, C. *Crit. Rev. Biochem.*, in press.

Triple Helix of *Schizophyllum commune* Polysaccharide in Dilute Solution. 3. Hydrodynamic Properties in Water

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ABSTRACT: A native sample of a *Schizophyllum commune* polysaccharide (schizophyllan) and its sonicated fragments, ranging in weight-average molecular weight (M_w) from 10^5 to 6×10^6 g mol^{-1}, were studied by ultracentrifugation and viscometry in water at 25 °C, in which schizophyllan has been found to exist as a triple helix. Measurements of intrinsic viscosity $[\eta]$ and limiting sedimentation coefficient s_0 showed that the schizophyllan triple helix is almost perfectly rigid up to $M_w = 5 \times 10^5$ g mol^{-1} but acquires flexibility for higher M_w. The data in the lower molecular weight range were analyzed by Yamakawa's theory for $[\eta]$ and Yamakawa–Fujii's theory for s_0 of a long rigid cylinder, with the result that M_L (the molar mass per unit cylinder length) = 2150 ± 150 nm^{-1} and d (the diameter) = 2.6 ± 0.4 nm. This M_L gives the pitch of the triple helix per β-1,3-D-glucose residue a value of 0.30 ± 0.02 nm, which agrees closely with the reported pitches for lentinan and a β-1,3-D-xylan in the crystalline region. The d value 2.6 nm is consistent with the diameter of the model triple helix for schizophyllan. With $M_L = 2150 \pm 150$ nm^{-1} and $d = 2.6 \pm 0.4$ nm, the persistence length that allows the theoretical curves of s_0 and $[\eta]$ for wormlike cylinders to fit the present data was found to be 200 ± 30 nm. This value indicates that the schizophyllan triple helix is stiffer than that of native collagen, a known triple-helical biopolymer.

In part 1[2] of this series, we concluded that schizophyllan, $[\beta\text{-}1\rightarrow 3\text{-Glu-}\beta\text{-}1\rightarrow 3\text{-Glu-}(\beta\text{-}6\leftarrow 1\text{-Glu})\text{-}\beta\text{-}1\rightarrow 3\text{-Glu}]_n$, an extracellular polysaccharide produced by *Schizophyllum commune*,[3,4] is dispersed in 25 °C water as a triple helix similar to that proposed by Atkins et al.[5] for a crystalline β-1,3-D-xylan and that the triple helix is rigid for weight-average molecular weights M_w lower than 5×10^5 g mol^{-1} but has a certain flexibility for higher M_w. These conclusions were drawn from the following observations: (1) the M_w of the samples in water were approximately 3 times those in dimethyl sulfoxide, in which schizophyllan is dispersed as a single randomly coiled chain; (2) the exponents in the Mark–Houwink–Sakurada $[\eta]$ (intrinsic viscosity) vs. M_w relation in water were about 1.7 and 1.2 below and above $M_w = 5 \times 10^5$ g mol^{-1}, respectively; and (3) intrinsic viscosities in water for M_w lower than 5×10^5

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g mol^{-1} could be fitted by Yamakawa's theory[6] for long rigid cylinders if the length was estimated from the pitch of the triple helix proposed by Atkins et al.[5] and the diameter from the model helix of schizophyllan constructed according to Atkins et al.

In this study, we have accumulated more $[\eta]$ and M_w data as well as sedimentation coefficient data for a native sample of schizophyllan and its sonicated fragments in water, hoping to estimate the pitch, diameter, and stiffness of the schizophyllan triple helix on the basis of current hydrodynamic theories.

Experimental Section

Samples. Five sonicated samples of schizophyllan were added to the previously used six samples[2] for the present measurement. The new samples were prepared in the following way.

Two native schizophyllans supplied by Taito Co. were dissolved in water at concentrations between 0.2 and 1.1%. Each solution was exposed to a 19.5-kHz sonic wave for 25 min to 140 h for fragmenting the polymer to various lengths. From eight sonicated samples so obtained four samples, designated S-65, S-45, B, and E, were chosen. They were purified as previously described,[2] and each was separated into five to eleven parts by fractional precipitation with water as solvent and ethanol as precipitant. Appropriate middle fractions, designated below as S-65-2, S-45-4, B-4, and E-4, were selected, reprecipitated from aqueous solutions into acetone, and freeze-dried. To these four fractions was added one more fraction, H-3, which was prepared similarly for another purpose from a sonicated, purified schizophyllan. All of them and the previous samples were dried overnight in vacuo before use.

Ultracentrifugation. Sedimentation Equilibrium. Weight-average molecular weights of the five new samples in water at 25 °C were determined in a Beckman Spinco Model E ultracentrifuge equipped with an electronic speed-control system. A filled Epon 30-mm double-sector cell was used for samples S-65-2, S-45-4, and B-4; a Kel-F 12-mm double-sector cell was used for samples H-3 and E-4. The length of the liquid column was adjusted to 1.3–1.0 mm, and rotor speeds were chosen to be 2200 rpm for samples S-65-2 and S-45-4, 2600 rpm for sample B-4, 4800 rpm for sample H-3, and 7200 rpm for sample E-4. We used the previously determined values[2] 0.142 cm^3 g^{-1} for the specific refractive index increment at wavelength 546 nm and 0.619 cm^3 g^{-1} for the partial specific volume.

Sedimentation Velocity. Sedimentation velocities of the samples in water at 25 °C were measured in a Kel-F 30-mm single-sector cell. Rotor speeds were 40000 rpm for a native schizophyllan (sample N-1) and 48000 rpm for the rest. For any sample the observed schlieren patterns were single-peaked. Sedimentation coefficients s were determined by the usual peak method for a series of initial polymer mass concentration c_0 and analyzed by the equation $s^{-1} = s_0^{-1}(1 + k_s c_0)$ to obtain the limiting sedimentation coefficient s_0 and the constant k_s.

Viscometry. Viscosity measurements (in water at 25 °C) were made in a rotational viscometer[7] of the Zimm–Crothers type for samples S-65-2 and S-45-4, in a four-bulb capillary viscometer[8] for samples B-4 and H-3, and in a conventional capillary viscometer of the Ubbelohde type for sample E-4.

Results

Molecular Weight and Second Virial Coefficient. Figure 1 shows M_{app}^{-1} as a function of $\bar{c}$ for five samples of schizophyllan in water at 25 °C, where M_{app} is the apparent molecular weight and $\bar{c}$ is the mean concentration at sedimentation equilibrium. Table I summarizes the values of M_w and A_2 (the second virial coefficient) evaluated from the indicated straight lines and those of M_z/M_w (M_z is the z-average molecular weight) also estimated from the sedimentation equilibrium data (see eq 1–8 in ref 2), together with the reported values[2] for the previous samples. Except for the two lowest molecular weight samples, the values of A_2 scatter around 10^{-4} cm^3 mol g^{-2}. This feature is in qualitative agreement with the prediction from the Zimm theory[9] for long straight rods with a hard-sphere potential.

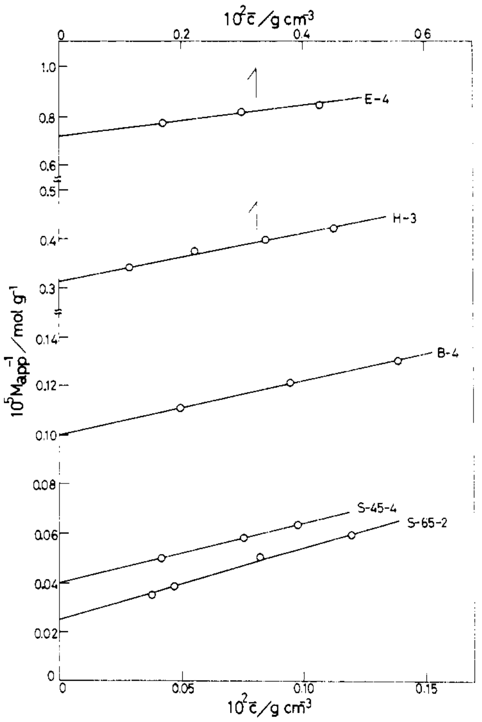

Figure 1. Plots of M_{app}^{-1} vs. $\bar{c}$ for schizophyllan samples in water at 25 °C.

Table I
Results from Sedimentation Equilibrium Measurements on Schizophyllan Samples in Water at 25 °C

sample	$10^{-4}M_w$/g mol^{-1}	$10^4 A_2$/cm^3 mol g^{-2}	M_z/M_w
N-1[a]	570	1.0	
S-65-2	400	1.4	1.5
S-45-4	248	1.2	1.5
S-1-2[a]	161	1.1	1.4
B-4	100	1.1	
S-148-2[a]	72.4	0.6	1.8
S-144-2[a]	42.9	1.0	1.4
H-3	32.0	1.2	1.5
S-166-4[a]	19.6	1.4	1.2
E-4	13.9	1.6	1.2
S-164-3[a]	9.60	2.2	1.3

[a] Taken from ref 2.

Intrinsic Viscosity and Sedimentation Coefficient. Numerical results for $[\eta]$ and k' (Huggins' constant) from the previous and present studies are given in Table II. Figure 2 displays the double-logarithmic plot of $[\eta]$ vs. M_w. The data points for M_w lower than 5×10^5 g mol^{-1} are fitted accurately by a straight line with slope 1.7 (K in the Mark–Houwink–Sakurada equation $[\eta] = KM_w^{1.7}$ is 1.3×10^{-7} cm^3 g^{-1}), whereas those for higher M_w follow a slightly bent curve with an asymptotic slope of about 1.2. These slope values agree with those estimated previously from a limited number of data points and confirm the conclusion that the schizophyllan triple helix is rigid below $M_w = 5 \times 10^5$ g mol^{-1} and flexible at higher molecular weights.

Figure 3 shows the concentration dependence of s^{-1} for all samples studied. The plot for the highest molecular weight sample, N-1, is markedly bent downward, but, since it is almost linear below $c_0 = 2 \times 10^{-4}$ g cm^{-3}, the intercept can be determined with no great ambiguity. The values of s_0 and k_s evaluated from the intercepts and slopes of the indicated straight lines are presented in the fourth and

Table II
Results from Viscosity and Sedimentation Velocity Measurements on Schizophyllan Samples in Water at 25 °C

sample	$10^{-2}[\eta]$/ cm³ g⁻¹	k'	$10^{13}s_0$/s	$10^{-2}k_s$/ cm³ g⁻¹
N-1	120[a]	0.44[a]	15.0	26.1
S-65-2	71.9	0.46	13.0	16.4
S-45-4	43.7	0.42	11.2	7.66
S-1-2	25.0[a]	0.43[a]	10.3	4.26
B-4	15.4	0.40	9.12	2.55
S-148-2	9.62[a]	0.42[a]	8.74	2.12
S-144-2	4.78[a]	0.44[a]	8.01	1.29
H-3	3.01	0.42	7.36	1.17
S-166-4	1.32[a]	0.42[a]	6.57	0.72
E-4	0.736	0.43	6.09	0.52
S-164-3	0.401[a]	0.43[a]	5.25	0.34

[a] Taken from ref 2.

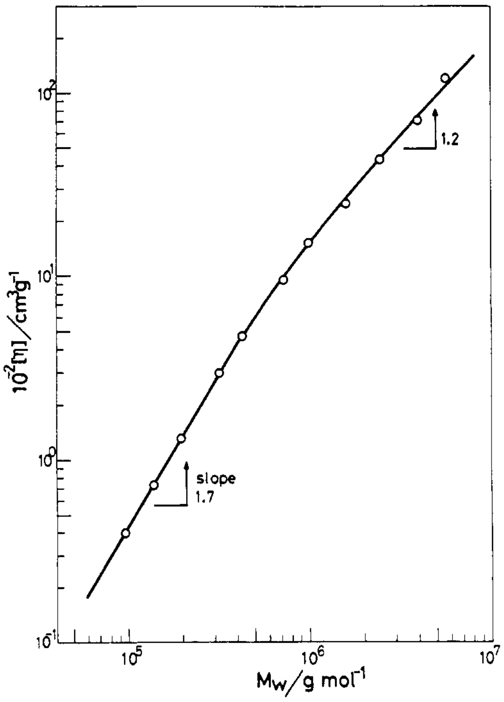

Figure 2. Double-logarithmic plot of $[\eta]$ vs. M_w for schizophyllan in water at 25 °C.

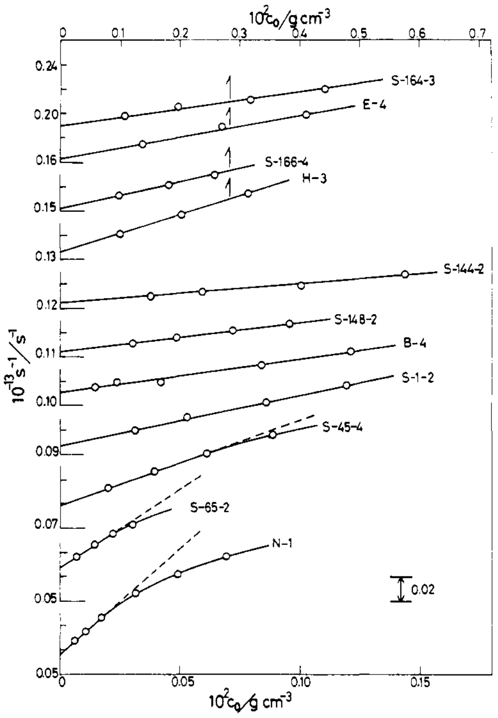

Figure 3. Concentration dependence of s^{-1} for schizophyllan samples in water at 25 °C.

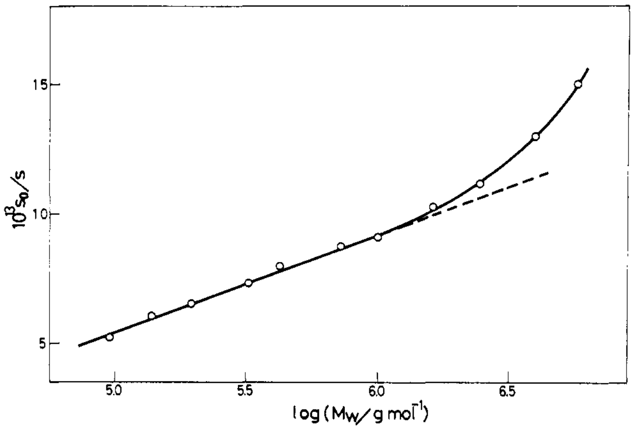

Figure 4. Molecular weight dependence of s_0 for schizophyllan in water at 25 °C.

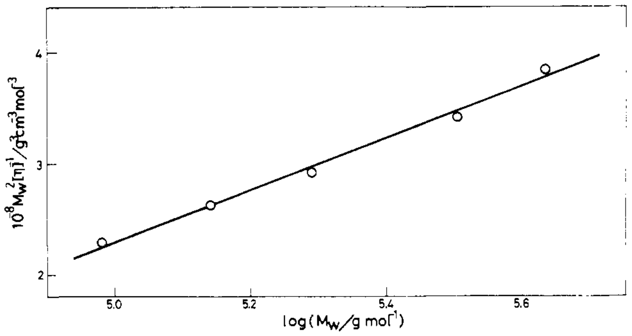

Figure 5. Plot of $M_w^2/[\eta]$ vs. log M_w for schizophyllan in water at 25 °C.

fifth columns of Table II. These s_0 values, plotted against log M_w in Figure 4, follow a straight line for M_w lower than 10^6 g mol⁻¹. This linearity between s_0 and log M_w is further evidence that the schizophyllan triple helix is rigid and is in conformity with the theoretical prediction[10] for long rigid rods (see below). Thus the upswing of s_0 for M_w higher than 10^6 g mol⁻¹ must be ascribed to the fact that the helix becomes flexible at such high molecular weight.

Discussion

Pitch of the Triple Helix. Yamakawa's expression[6] for $[\eta]$ of a long straight cylinder may be written

$$M^2/[\eta] = (45M_L^3/2\pi N_A)[\ln M - 0.6970 - \ln (dM_L)] \tag{1}$$

where M is the molecular weight of the polymer, M_L is the molar mass per unit cylinder length, d is the diameter of the cylinder, and N_A is Avogadro's constant. The values of $M_w^2/[\eta]$ for the five lowest molecular weight samples are plotted against log M_w in Figure 5 according to eq 1. The plotted points can be fitted by a straight line, with a maximum deviation of less than 2.5%. If analyzed in terms of eq 1, the slope and intercept (at log M_w = 0) of the straight line yield 2100 ± 100 nm⁻¹ for M_L and 2.6 ± 0.6 nm for d. The large uncertainty in d reflects the theoretical fact that $[\eta]$ is not sensitive to d.

Yamakawa-Fujii's expression[10] for s_0 of a long straight cylinder is given by

$$s_0 = \frac{(1 - \bar{v}\rho_0)M_L}{3\pi\eta_0 N_A}[\ln M + 0.3863 - \ln (dM_L)] \tag{2}$$

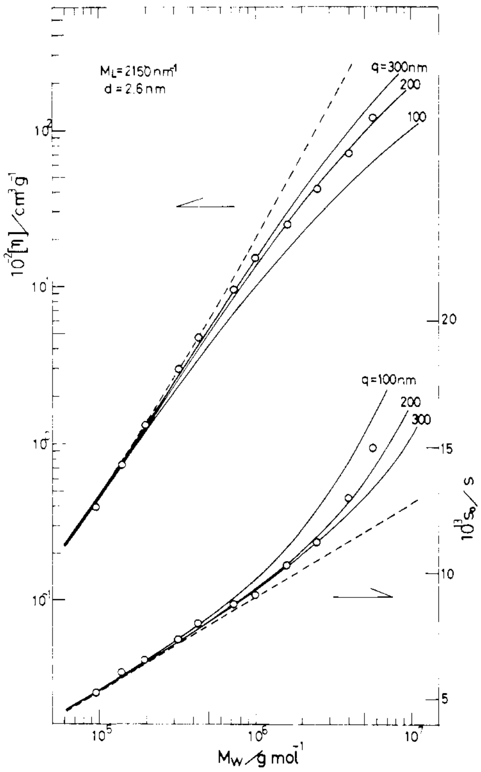

Figure 6. Comparison of the experimental s_0 and $[\eta]$ for schizophyllan with the theoretical values for wormlike cylinders[10,13] calculated for q = 100, 200, 300, and ∞ (the dashed lines) with M_L and d fixed at 2150 nm^{-1} and 2.6 nm, respectively.

where $\bar{v}$ is the partial specific volume of the polymer and ρ_0 and η_0 are the solvent density and viscosity, respectively. From this equation and the linear relation between s_0 and log M_w shown in Figure 4 we find that $M_L = 2200 \pm 100$ nm^{-1} and $d = 2.6 \pm 0.4$ nm. These values are in substantial agreement with those estimated above from the $[\eta]$ data. Further, the d value 2.6 nm is consistent with the previous estimation[2] (2.5–3.0 nm) from a molecular model for the triple helix of schizophyllan.

Each repeat unit of the main chain of schizophyllan contains three β-1,3-D-glucose residues (see Figure 1 of ref 2). Hence, the pitch h of the triple helix per β-1,3-D-glucose residue is related to M_L by

$$h = (M_0/3)/(M_L/3) \qquad (3)$$

with M_0 the molar mass of the schizophyllan repeat unit (=648 g mol^{-1}). Substituting 2150 ± 150 nm^{-1} for M_L (which is the average of the estimates from s_0 and $[\eta]$) into eq 3, we find $h = 0.30 \pm 0.02$ nm, which may be compared with 0.306 nm for the right-handed triple helix of a β-1,3-D-xylan[5] and with 0.29 (right-handed) or 0.33 nm (left-handed) for the triple helix of lentinan[11] (a β-1,3-D-glucan), both in the crystalline region. This agreement confirms our previous conclusion that the triple-helical structure of schizophyllan in dilute aqueous solution should be very similar to that of these other polysaccharides in the crystalline state.

Flexibility of the Triple Helix. The viscosity and sedimentation velocity data described above suggest that the schizophyllan triple helix with an M_w higher than 10^6 g mol^{-1} can be better represented by a wormlike cylinder than by a straight cylinder. In the hydrodynamic theories of Yamakawa and co-workers,[10,12,13] both s_0 and $[\eta]$ for wormlike cylinders are expressed as functions of M_L, d, and persistence length q. We sought q that allows the Yamakawa–Fujii theory[10] for s_0 and the Yamakawa–Yoshizaki theory[13] (a modification of the Yamakawa–Fujii theory[12]) for $[\eta]$ to fit our experimental data, with M_L and d fixed at 2150 nm^{-1} and 2.6 nm, respectively. The curves in Figure 6 show theoretical values of $[\eta]$ (on a logarithmic scale) and s_0 (on a linear scale) calculated for q = 100, 200, 300, and ∞. Similar analyses were made by allowing M_L and d to vary within the above-mentioned ranges of uncertainty, and it was found that the persistence length for the schizophyllan triple helix is in the range 170–230 nm. These q values are larger than 130 nm for triple-helical collagen[14] and 60 nm for double-stranded DNA,[15] indicating a very high stiffness of the schizophyllan triple helix.

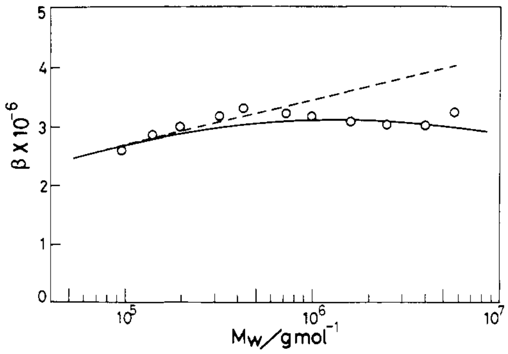

Figure 7. Flory–Scheraga–Mandelkern parameter β (in conventional units) for schizophyllan in water at 25 °C. The solid line refers to the wormlike cylinder with q = 200 nm, M_L = 2150 nm^{-1}, and d = 2.6 nm and the dashed line to the rigid cylinder with the same M_L and d values.

Figure 7 shows the molecular weight dependence of the Flory–Scheraga–Mandelkern parameter β defined by

$$\beta = N_A \eta_0 s_0 [\eta]^{1/3} / [(1 - \bar{v}\rho_0) M^{2/3} 100^{1/3}] \qquad (4)$$

The solid curve refers to the wormlike cylinder with q = 200 nm, M_L = 2150 nm^{-1}, and d = 2.6 nm and the dashed line to the rigid cylinder with the same M_L and d values. The former and the experimental values first increase and then gradually decrease as M_w is increased, whereas the dashed curve shows a monotonic increase. The rise in experimental β in the lower molecular weight range is ascribed to the increasing axial ratio of the rigid triple helix, while the decline in the higher molecular weight range is due to an increasing bending of the helix.

Summarizing our hydrodynamic study, we conclude that the triple helix of schizophyllan in water has a pitch (per residue) of 0.30 ± 0.02 nm and a diameter of 2.6 ± 0.4 nm. The pitch is approximately the same as the reported values for similar polysaccharides[5,11] in the crystalline state, while the diameter is consistent with the model triple helix for schizophyllan. Although the hydrodynamic theories used for data analysis in this work are considered to be accurate at present, it is apparent that these values of the pitch and diameter depend on the hydrodynamic model assumed for the schizophyllan triple helix. Light-scattering measurements of the radius of gyration are now in progress in order to estimate the pitch more directly.

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References and Notes

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(2) Norisuye, T.; Yanaki, T.; Fujita, H. *J. Polym. Sci., Polym. Phys. Ed.* **1980**, *18*, 547.
(3) Kikumoto, S.; Miyajima, T.; Yoshizumi, S.; Fujimoto, S.; Kimura, K. *J. Agric. Chem. Soc. Jpn.* **1970**, *44*, 337.
(4) Kikumoto, S.; Miyajima, T.; Kimura, K.; Okubo, S.; Komatsu, N. *J. Agric. Chem. Soc. Jpn.* **1971**, *45*, 162.

(5) Atkins, E. D. T.; Parker, K. D.; Preston, R. D. *Proc. R. Soc. London, Sect. B* **1969,** *173,* 209. Atkins, E. D. T.; Parker, K. D. *J. Polym. Sci., Part C* **1969,** *28,* 69.
(6) Yamakawa, H. *Macromolecules* **1975,** *8,* 339.
(7) Einaga, Y.; Miyaki, Y.; Fujita, H. *J. Soc. Rheol. Jpn.* **1977,** *5,* 188.
(8) Einaga, Y.; Miyaki, Y.; Fujita, H. *J. Polym. Sci., Polym. Phys. Ed.* **1979,** *17,* 2103.
(9) Zimm, B. H. *J. Chem. Phys.* **1946,** *14,* 164.
(10) Yamakawa, H.; Fujii, M. *Macromolecules* **1973,** *6,* 407.
(11) Bluhm, T. L.; Sarko, A. *Can. J. Chem.* **1977,** *55,* 293.
(12) Yamakawa, H.; Fujii, M. *Macromolecules* **1974,** *7,* 128.
(13) Yamakawa, H.; Yoshizaki, T. *Macromolecules* **1980,** *13,* 633.
(14) Utiyama, H.; Sakato, K.; Ikehara, K.; Setsuiye, T.; Kurata, M. *Biopolymers* **1974,** *12,* 53.
(15) Godfrey, J. E.; Eisenberg, H. *Biophys. Chem.* **1976,** *5,* 301.

Triple-Helical Structure of $(1\rightarrow3)$-β-D-Glucan[†]

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ABSTRACT: The molecular and crystal structure of the anhydrous form of curdlan, a $(1\rightarrow3)$-β-D-glucan of bacterial origin, has been determined by combined X-ray diffraction analysis and stereochemical model refinement. The anhydrous polymorph crystallizes as a triple-stranded helix in a hexagonal unit cell with parameters $a = b = 14.41 \pm 0.05$ Å and c (fiber repeat) $= 5.87 \pm 0.05$ Å. The space group is $P6_3$ and there is one helix per unit cell. As in the triple-helical $(1\rightarrow3)$-β-D-xylan, the three strands of the glucan helix are parallel, right-handed, 6/1 helices repeating in 3c, and they are in phase along the helix axis. The hydroxymethyl group of each glucose residue is in the tg position, and the crystal structure is extensively hydrogen-bonded. The reliability of the structure analysis is indicated by the residual index $R = 0.233$.

Introduction

Polysaccharides are important constituents of plants and microorganisms, where they perform a variety of functions. Among the latter, the structural function of cellulose in the plant cell wall and the food-reserve role of granular starch are well-known. In many cases, the function of a polysaccharide appears to be determined by its structure in the solid state, i.e., its molecular conformation and crystal structure. A variety of conformational and crystalline forms of polysaccharides have been observed, including the less common multiple-helical forms. For example, double-helix crystal structures exist in native starch[1] and in carrageenan[2,3] and a triple-helix structure appears in the $(1\rightarrow3)$-β-D-xylan of some green algae.[4]

A triple-helical structure has been proposed for $(1\rightarrow3)$-β-D-glucan,[5,6] a polysaccharide which occurs in many fungi, bacteria, plants, and algae and is known variously as curdlan, pachyman, laminaran, laricinan, callose, and paramylon. Its functions appear to range from the structural in plants and fungi to food reserve in the granules of paramylon, where it is also more than 90% crystalline.[7,8] This polysaccharide exhibits interesting and useful gel properties,[9] and of additional interest is that curdlan has been reported to possess antitumor activity.[10]

In common with many other polysaccharides, $(1\rightarrow3)$-β-D-glucan crystallizes in different hydrates, not all of which are thought to be triple-helical or triplexes of the same type. For example, curdlan[9] is poorly crystalline in its native state, but upon annealing it yields two polymorphs: the "dry" form obtained in vacuo and the "hydrate" form obtained at 75% or higher relative humidity.[5] Sixfold, triple-helical crystalline forms have been proposed for these two polymorphs,[5,6] although various other structures, ranging from a single helix[11] to a sevenfold triplex,[12] have been put forth. In this study, a detailed X-ray crystallographic refinement of the "dry" form of curdlan has shown it to be a sixfold, triple-helical structure, as suggested earlier.

Table I
Comparison of Observed and Predicted d Spacings (Å) for the "Dry" Unit Cell of Curdlan

hkl	obsd	pred	*hkl*	obsd	pred
100	12.6	12.48	201	4.26	4.27
110	7.25	7.21	121	3.67	3.68
200	6.26	6.24	301	3.38	3.36
120	4.73	4.72	131	2.98	2.98
300	3.60	3.60	401	2.75	2.76
220	3.60	3.60	002	2.90	2.935
130	3.46	3.46	102	2.84	2.86
400	3.12	3.12	112	2.69	2.72
230	2.86	2.86	202		2.66
140	2.72	2.72	122	2.52	2.49
101	5.32	5.31	302	2.40	2.40
111	4.53	4.51			

Experimental Section

Commercially available curdlan powder (Takeda Chemical Co., Japan) was the source of $(1\rightarrow3)$-β-D-glucan. Oriented fibers were prepared from a 10% solution in dimethyl sulfoxide by extruding with a syringe into a methanol bath at room temperature. Fibers were washed with water, allowed to dry at constant length, and annealed under tension in a sealed bomb at 145 °C in the presence of water and this was followed by evacuation for 12 h at room temperature. The crystallinity of the resulting "dry" polymorph, as shown in Figure 1, was improved dramatically. The density of the fibers was measured by flotation in a p-xylene–chloroform mixture.

The unit cell parameters of the crystal structure were refined by least-squares procedures, using 23 measured reflections. Relative intensities of the reflections were obtained from radial tracings of the X-ray films, recorded with a Joyce-Loebl microdensitometer. The areas under the tracings were resolved into

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[†] Part 13 in the series "Packing Analysis of Carbohydrates and Polysaccharides".

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