Notes

Ultrasonic Degradation of Schizophyllum Commune *Polysaccharide in Dilute Aqueous Solution*

INTRODUCTION

Schizophyllan, a water-soluble β-1,3-D-glucan produced by *Schizophyllum Commune* Fries,[1] is an extracellular polysaccharide consisting of a repeat unit[2] shown in Figure 1. This polysaccharide has specific biological activities[3,4] against cancer and infection. Recently, it was shown that ultrasonication of a native schizophyllan in dilute aqueous solution produced a lower molecular polysaccharide with its primary structure and biological activities retained[5] and that both native and sonicated schizophyllan were dissolved in water as a rodlike trimer with triple helical structure, the individual chain of which has approximately equal lengths.[6]

In this note, we investigate the ultrasonic degradation of the trimer of a native schizophyllan in dilute aqueous solution using a high performance gel permeation chromatograph (GPC) connected to a low-angle laser light scattering (LALLS) photometer.

Schizophyllan is a probably the first example of a rodlike polymer which has enabled to be determined the molecular weight distribution using this apparatus.

EXPERIMENTAL

Sonication

A native schizophyllan sample was prepared and purified in the same way as described elsewhere.[5,6] It was dissolved in water at different concentrations of 0.10×10^{-2}, 0.20×10^{-2}, and 0.40×10^{-2} g/cm^3, and 1 L each solution was sonicated in a Kaijo Denki ultrasonic oscillater, Model TA-6280N for 3–500 min at 500 W and 19.5 kHz (nominal values). The solution was kept below 20°C by circulating water through a jacket. The irradiated polymer was freeze-dried from an aqueous solution.

Viscometry

The decrease in molecular weight due to sonication was checked by measuring intrinsic viscosities $[\eta]$ in a four-bulb spiral capillary viscometer[7] at 25°C. Zero shear rate $[\eta]$ were determined by graphically extrapolating the measured $[\eta]$ to zero shear rate. No kinetic energy correction was necessary.

GPC and LALLS

GPC curves and scattering intensity curves for sonicated samples in water at 37°C were obtained in a Toyo Soda Type HLC-802UR high performance GPC connected in a series to a Toyo Soda LALLS photometer with a flow cell. Polymer concentrations were monitored by refractive index increment. The initial polymer mass concentration c_0 was so injected that the product of c_0 and M_v (the viscosity-average molecular weight) gave a value of 2.0×10^3 g/cm^3. The aqueous solution injected into the apparatus was recycled twice or more through a column (Type G-5000PW) and allowed to flow into the LALLS flow cell. Scattered intensities were measured at a fixed scattering angle of 5° with a laser beam of wavelength 633 nm. The pressure in a GPC column was less than 50 kg/cm^2 and the flow rate was 0.5 cm^3/min.

Since, as was reported elsewhere,[6,8] the second virial coefficients A_2 of aqueous schizophyllan solutions are about 10^{-4} cm^3·mol·g^{-2} in the range of molecular weight from 10^5–6×10^6, the dimensionless quantity $2A_2M_vc$ (c is the polymer mass concentration in the LALLS flow cell) for our native and sonicated samples should be sufficiently small compared with unity. Thus, the measured intensity may be regarded as the value at infinite dilution. We also found that the intensity can be approximated to that at zero-scattering angle with an error less than 4%, by calculating the contribution of the size of the schizophyllan chain to the intensity with the aid of the recent light scattering data of Kashiwagi et al.[9] for schizophyllan in dilute aqueous sodium hydroxide.
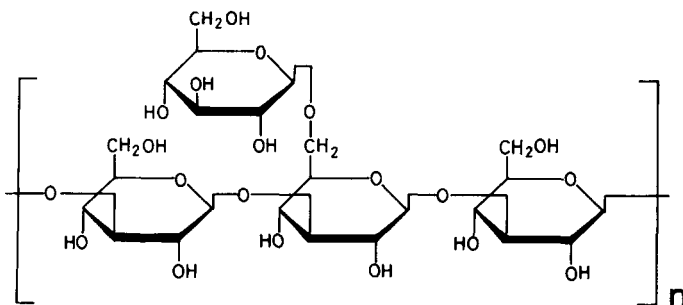
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Fig. 1. Repeat unit of schizophyllan.

Determination of Molecular Weight Distribution

Figure 2 illustrates the GPC curve (weight fraction w vs. elution count) of a sonicated schizophyllan sample M-2 prepared for another purpose. The weight-average molecular weight M_w of this sample was 43.7×10^4 when determined by sedimentation equilibrium in water at 25° (see Refs. 6 and 8 for the experimental procedures). The upper abscissa scale of Figure 2 was converted to the molecular weight scale (the lower abscissa) using this M_w and the LALLS intensity curve corresponding to the GPC curve. In the actual procedure, we evaluated numerically the areas S_{GPC} and S_{LALLS} under the w vs. elution count curve and the corresponding intensity curve, and determined the apparatus constant K ($= wM/I$) of the photometer from the M_w value and the area ratio S_{LALLS}/S_{GPC}. Here I and M denote, respectively, the scattering intensity and the molecular weight at a given elution count. GPC curves for all the sonicated samples were converted to molecular weight distribution curves with the same K value and the intensities in the corresponding LALLS curves.

RESULTS AND DISCUSSION

Figure 3 shows the change in $[\eta]$ of schizophyllan that occurred when each of the aqueous solutions with the indicated concentrations was exposed to ultrasonic sound. We see that $[\eta]$ decreases first rapidly and then slowly with time and reaches a constant almost independent of concentration. This behavior implies that an average chain length of the schizophyllan trimer decreases with time and
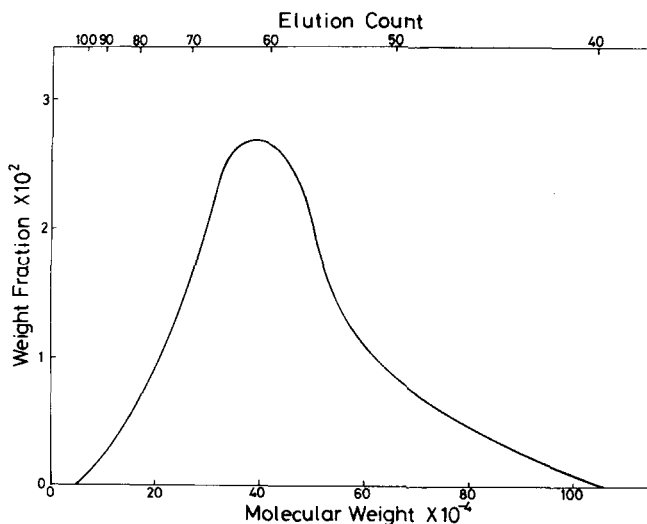

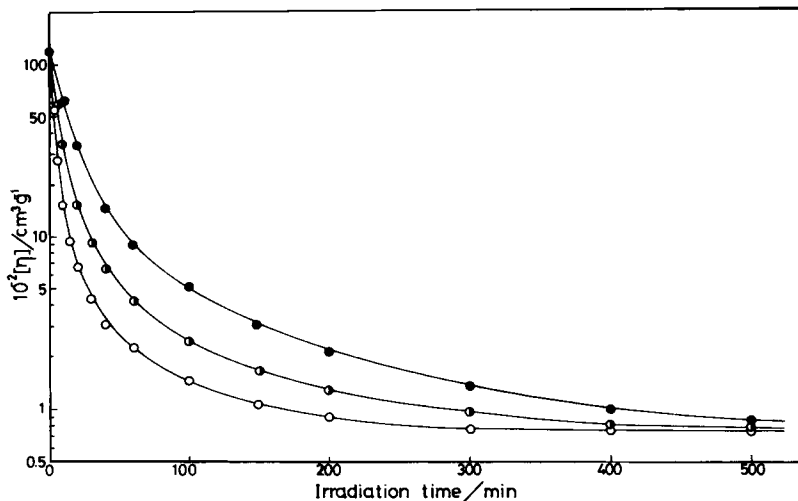Fig. 2. Molecular weight distribution of a schizophyllan sample M-2.

Fig. 3. Change in $[\eta]$ of schizophyllan when aqueous solutions of concentrations 0.10 (O), 0.20 (◑), and 0.40 (●) $\times 10^{-2}$ g/cm^3 were sonicated.

approaches a minimum below which the chain no longer breaks. Nishihara and Doty[10] observed similar behavior for collagen a triple helical biopolymer.

Figure 4 shows the change in molecular weight distribution curve with sonication time for the solution of a concentration 0.10×10^{-2} g·cm^{-3}. Each curve is single-peaked, and the peak shifts from the higher to the lower molecular weight region with increasing sonication time t. The
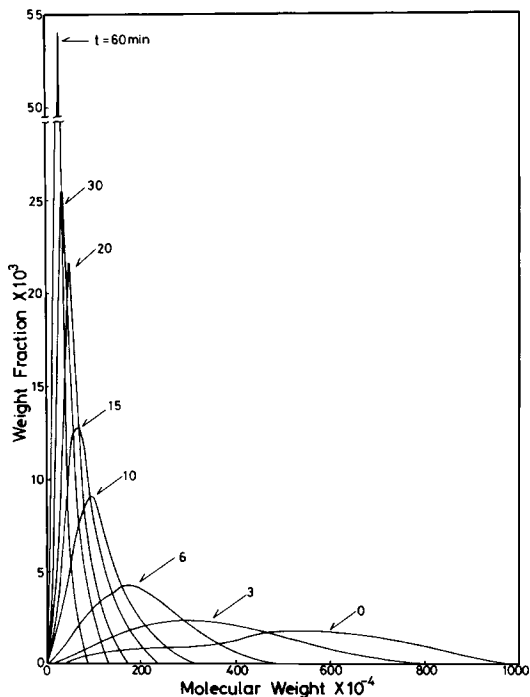

Fig. 4. Molecular weight distribution curves for schizophyllan irradiated in 0.10×10^{-2} g/cm^3 aqueous solution for indicated times.

TABLE I
Results from Gel Permeation Chromatography and Viscometry

Irradiation time (min)	$M_n \times 10^{-4}$	$M_w \times 10^{-4}$	M_w/M_n	$10^{-2} [\eta]$ (cm^3·g^{-1})	k'
0	385	526	1.37	120	0.44
3	250	347	1.39	56.3	0.45
6	147	198	1.35	28.0	0.42
10	77.8	102	1.31	15.4	0.43
15	58.9	75.8	1.29	9.37	0.41
20	46.9	57.9	1.23	6.64	0.43
30	33.4	43.7	1.31	4.36	0.42
40	27.4	34.8	1.27	3.13	0.42
60	23.0	27.8	1.21	2.28	0.42
100	19.5	24.1	1.24	1.49	0.40

weight-average molecular weights M_w and the number-average molecular weights M_n calculated from the curves are presented in Table I, together with the values of $[\eta]$ and the Huggins constant k'. A point to note in Table I is that the polydispersity index M_w/M_n gradually decreases with sonication time.

Figure 5 shows the relationship between $[\eta]$ and M_w shown in Table I, together with the reported values[6,8] (solid curve) in the same solvent. Though most of our data are lower than the solid line, they are close to it. This fact confirms Norisuye et al.'s conclusion[6] that schizophyllan exists as a rodlike polymer in water.

Now we consider a polymer sample with the most probable distribution which is well known as a special case of the Schulz–Zimm distribution. In an initial stage of a random degradation process
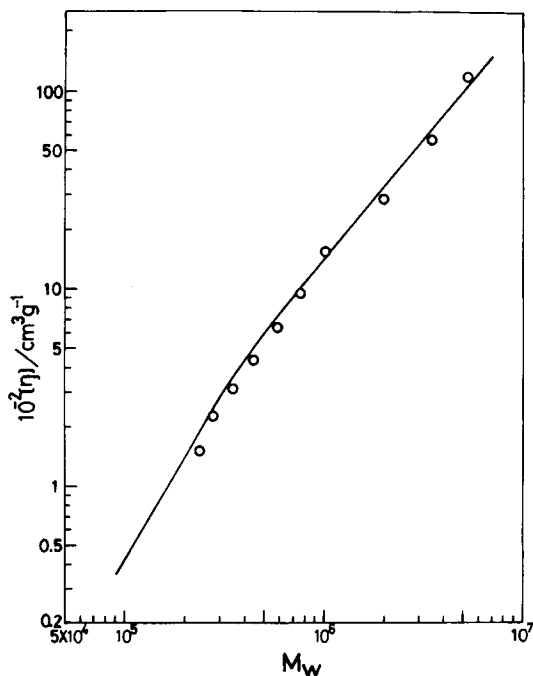

Fig. 5. Relationship between $[\eta]$ and M_w for schizophyllan in aqueous solution. The solid curve represents the reported values[6,8] in the same solvent.
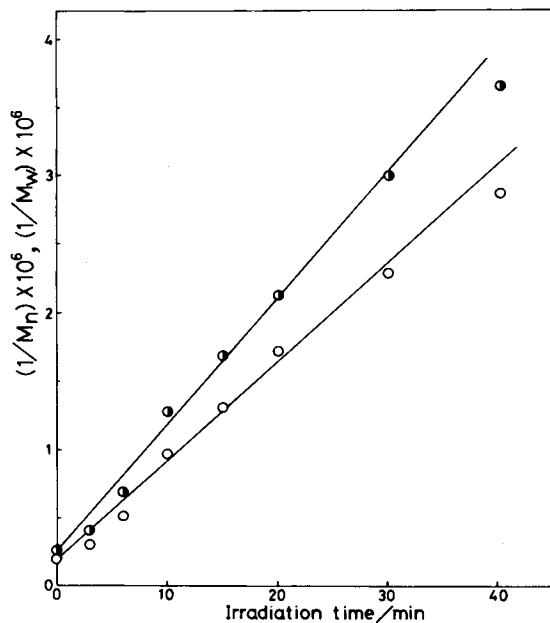
Fig. 6. Plots of M_n^{-1} (●) and M_w^{-1} (○) vs. t for sonicated schizophyllan.

for this sample, M_n and M_w are given by[11]

$$1/M_n = 1/(M_n)_0 + kt \qquad (1)$$

$$1/M_w = 1(M_w)_0 + \tfrac{1}{2} kt \qquad (2)$$

where $(M_n)_0$ and $(M_w)_0$ are the initial values of M_n and M_w, respectively, k is the constant, and t is degradation time.

On the other hand, Charlesby[12] has theoretically shown that whatever the initial molecular distribution, the decrease of M_n with degradation is identical with that of the most probable destribution, while M_w only decreases in this manner after an adequate amount of main chain fracture. Therefore, if our polymer experienced random degradation, time dependence should obey the above equations. Equations (1) and (2) predict that $1/M_n$ and $1/M_w$ should vary linearly with time and that the slope of the straight line for the former should be twice as great as that for the latter.

Figure 6 shows the plots of $1/M_n$ and $1/M_w$ vs. t constructed from our data for times shorter than 40 min. As expected from eqs. (1) and (2), the plots are linear. However, the ratio of the slope for $1/M_n$ to that for $1/M_w$ is 1.31, being significantly smaller than 2. Since, if each molecule is broken only at the center of the chain, the ratio must be closer to unity, our value 1.31 suggests that the schizophyllan chain tends to break at middle portions when sonicated. This suggestion is consistent with the fact that the polydispersity index M_w/M_n decreases gradually with time (see Table I).

In conclusion, we interpret the results from our sonication experiments on schizophyllan as follows: (i) There is a minimum molecular length below which no further breaking occurs, and (ii) the probability of breaking seems to be higher at middle portions than portions near the ends of the schizophyllan chain.

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