Triple Helix of the Polysaccharide Cinerean in Aqueous Solution

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Received March 28, 1997
Revised Manuscript Received July 25, 1997

Introduction

Cinerean is a microbial β-(1,3)(1,6)-D-glucan[1] produced by the fungus *Botrytis cinerea*.[2,3] Electronmicrographs show that the native cinerean consists of wormlike molecules.[4] By ultrasonic degradation rodlike fragments can be formed.[4] The mass per length (M/L = (2300 ± 400) Da nm^{-1}) of the cinerean rods in aqueous solution measured with SAXS[4,5] and also the comparison with scleroglucan,[7] schizophyllan,[8,9] and xanthan[10] suggests a multihelical conformation for cinerean. In NaOH the multihelix disentangles, and the single strands take the conformation of random coils.[5] In this work this phenomenon is used to determine the helicity of cinerean in aqueous solution in a direct fashion: namely, the weight-average molecular weight M_w was measured by light scattering for samples in H_2O, 0.01 N NaOH, and 1 N NaOH. The ratio of M_w in 0.01 N NaOH (multihelix) and that in 1 N NaOH (single strand) gives the helicity. The kinetics of the disentanglement was followed also by light scattering.

Experimental Section

The cultivation of *Botrytis cinerea*, the cinerean production, and the treatment with ultrasound are described elsewhere.[5,6] The filtration of the samples for the light scattering experiments was done with Nacalai filters (Millipore, cosmonice *W* 0.45 μm).

Gel Permeation Chromatography (GPC). The GPC column (Tosoh TSK4000PWxl and TSK6000PWxl, Tokyo, Japan, flow rate 0.6 mL/min) was calibrated with poly(ethylene oxide) (Tosoh TSK-standards with $M_w = 2.6 \times 10^4 - 9.96 \times 10^6$) and polyethylene glycols. As eluent 0.3 N CH_3COONa with 0.01 N NaOH was used. The polysaccharide in the eluent was detected by a differential refractometer.

Light Scattering. The light-scattering measurements were performed with a Otsuka Electronics dynamic light-scattering photometer DL-7000KC (Osaka, Japan; wavelength λ = 632.8 nm). For calibration benzene was used. The refractive index increments were measured with a Otsuka Electronics double beam differential refractometer DRM-1030. The results are dn/dc = (0.1365 ± 0.0020) mL/g in 0.01 N NaOH and dn/dc = (0.1367 ± 0.0020) mL/g) in 1 N NaOH at 25 °C. Effects of selective adsorption on scattered light intensities should be unimportant at these concentrations of NaOH.

Results and Discussion

By treatment with ultrasound, native cinerean was fragmented and then characterized by GPC. The results are shown in Table 1. The polydispersity is rather large, and the chromatograms can be described by Schulz−Zimm distributions.[11,12]

In the light-scattering study, we first used pure H_2O as solvent. For sample FC12-2-96, the observed scattering envelopes had an anormalous curvature. In addition the evaluated molecular weight M_w = (355 000 ± 14 000) was unreasonably high. Thus it has to be concluded that cinerean in pure H_2O agglomerates even in dilute solutions. To prevent this agglomeration, a little amount of NaOH (0.01 N) was added. Under these conditions, the Zimm plots show no anomaly (cf. Figure 1A), and the results from GPC and the light scattering are corresponding quite well as shown in Table 1. The negative value for the second virial coefficient A_2 indicates the tendency of cinerean to form agglomerates at higher concentrations. This seems to explain why the fractional precipitation of this polymer is so difficult.[11]

An interesting question that has been remaining open for a long time is how many cinerean single strands are wound around each other in a helical manner (helicity). With light scattering this problem could be solved. In 0.01 N NaOH the helix is still intact, and M_w of one multihelical rod is determined. For higher NaOH concentrations (for 25 °C at about 0.15 N NaOH) the multihelix undergoes a conformational change:[5] it disentangles into its single chains which take a coil conformation. Thus for comparison the same samples were resolved in 1 N NaOH (10 days, 25 °C, constant stirring) and measured. The values for M_w from these measurements are also shown in Table 1. The ratio M_w (0.01 N NaOH)/M_w (1 N NaOH) gives the helicity. The values in Table 1 clearly show that the helicity of cinerean is 3.0. The polydispersity does not affect the result here, because ratios of the moments of the chain length distribution should be unchanged before and after the disentanglement of a triple helix.

An evaluation in terms of $[Q\Delta R_\Theta/\pi Kc]_{c\to 0} = M/L$ for large Q (not shown here) yield a mass per length of $(M/L)^{0.01N} \approx 1950$ Da nm^{-1} for cinerean in 0.01 N NaOH and $(M/L)^{1N} \approx 350$ Da nm^{-1} in 1 N NaOH. $(M/L)^{0.01N}$ agrees with the results from SAXS[5] (M/L = (2400 ± 350) Da nm^{-1}) for the intact triple helix. In 1 N NaOH the value is smaller than that of a hypothetical single helix[4] ($M/L \approx 560$ Da nm^{-1}). This is in good agreement with the coillike structure.[5]

With the helicity known the molecular weight of the native cinerean could be determined in an indirect way. Solutions of native cinerean are difficult to handle because of the high viscosity. Even a few mg/mL make an aqueous solution highly viscous. In addition due to the high molecular weight and the large molecular size the solutions could not be filtrated for the light-scattering experiments. Thus the triple helix of the native cinerean was disentangled in 1 N NaOH (10 days, 25 °C, constant stirring). Now the viscosity was drastically reduced, and the samples could be filtrated directly into a light-scattering cell. These measurements yield M_w = (1 157 000 ± 171 000) for the single strands of the native cinerean. Thus it can be concluded that the weight average of the intact cinerean triplehelix is about 3.5×10^6. This is in agreement with the values extrapolated from the ultrasonic degradation experiments[11] and the values reported from viscometry[13] and ultracentrifugation.[14]

The kinetics of the disentanglement was followed with light scattering qualitatively. Figure 2 shows the excess Rayleigh ratio ΔR_Θ observed at a constant concentration (c = 5 mg/mL, Θ = 150°) as a function of time for sample FC12-2-96 in 0.5 N NaOH at 25 °C. For short times

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S0024-9297(97)00433-6 CCC: $14.00

Table 1. Results from GPC and Light Scattering[a]

	GPC			light scattering		
	M_w	M_n	M_w/M_n	M_w (0.01 N)	M_w (1 N)	helicity
FC16296	149 000	78 000	2.05	148 000 ± 2000	49 000 ± 2200	3.02 ± 0.14
FC12296	134 000	73 000	1.72	109 000 ± 1000	37 000 ± 5000	2.95 ± 0.40

[a] The values for M_w from GPC and from light scattering in 0.01 N NaOH are consistent. The ratio of M_w (0.01 N) and M_w (1 N) is the helicity h. For cinerean it is 3.

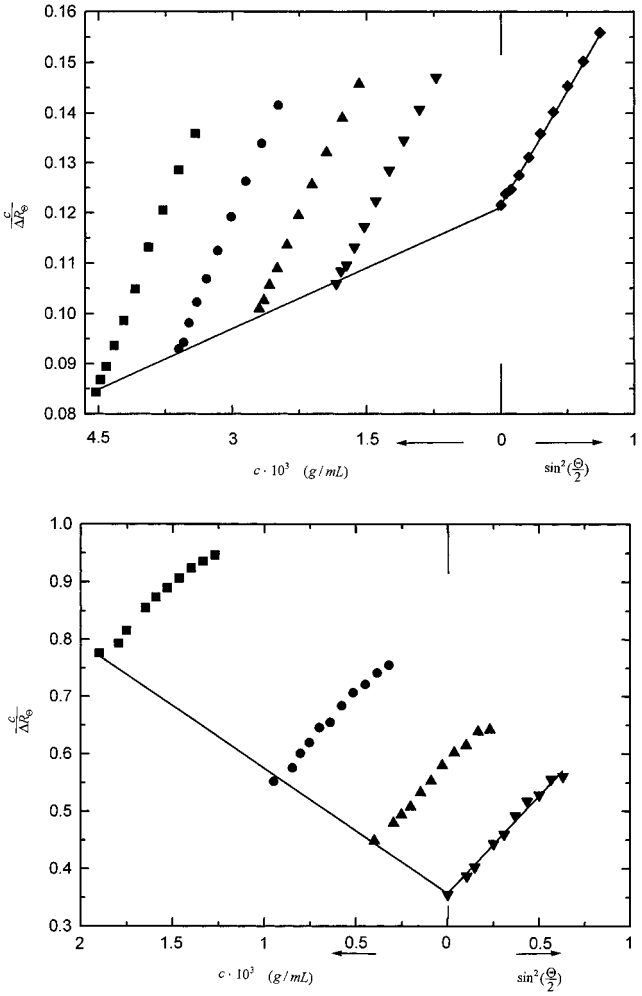

Figure 1. Zimm plots for FC16-2-96 at 25 °C: (A) in 0.01 N NaOH (M_w = 148 000 ± 1000) and (B) in 1 N NaOH (M_w = 49 000 ± 2200). ΔR_Θ is the Rayleigh ratio difference between the solution and the pure solvent, c the polysaccharide concentration, and Θ the scattering angle.

(1−3 h after adding the NaOH) ΔR_Θ decreases in a sharp step. The nature of this change is still unclear to us. We asume that it is an internal change of the triple helix in analogy to the change found for scleroglucan in small NaOH concentrations with viscometry and optical rotation.[15] The helix disentanglement starts after about 7 h and shows a relatively sharp step at about 15 h. After 45 h ΔR_Θ and hence the molecular weight stay stable, indicating that the helix has been entirely disentangled. As a microscopic mechanism for the disentanglement in NaOH, it is assumed that the polysaccharide triple helix is stabilized by hydrogen bonds, i.e., intermolecular bonds between single strands and intramolecular bonds. The OH^- ions replace these bonds, so that the helix disentangles into the single strands.[5] When this process is completed, the three single chains that formed a helix would get apart from one another. It is at this stage that light-scattering detection becomes feasible. Therefore the disentanglement of each triple helix must be a chain-length-dependent slow process that takes tens of hours in certain conditions.

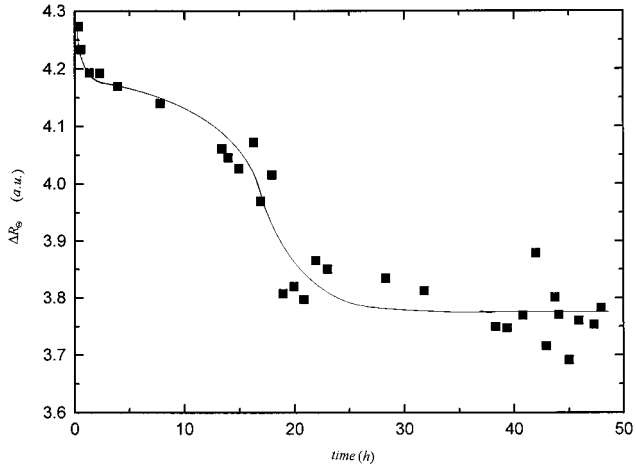

Figure 2. Kinetics of the disentanglement of the cinerean triple helix in 0.5 N NaOH followed by light scattering: change of the Rayleigh ratio for 150° with time (at 25 °C). For small times the value decreases in a sharp step because the agglomerates existing in pure H_2O are dissolved. The helix disentanglement starts after about 7 h and shows a relatively sharp step at about 15 h.

Conclusions

Samples of sonicated cinerean have rather broad molecular weight distributions that can be described by Schulz−Zimm distributions. In pure water cinerean tends to form agglomerates. With light scattering the molecular weight of the multihelical rods in 0.01 N NaOH and of the single chains in 1 N NaOH have been determined. The comparison shows that cinerean in water is a triple helix. The knowledge of the helicity was used to determine indirectly the molecular weight of the intact native cinerean. The kinetics of the disentanglement in 0.5 N NaOH shows a relatively sharp step after 15 h.

Acknowledgment. We thank N. Ide and K.-P. Stahmann for technical support and helpful discussions.

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MA970433V