Polymer Journal, Vol. 11, No. 11, pp 901—904 (1979)

NOTE

Curdlan (Bacterial β-1,3-Glucan) in a Cadoxen–Water Mixture

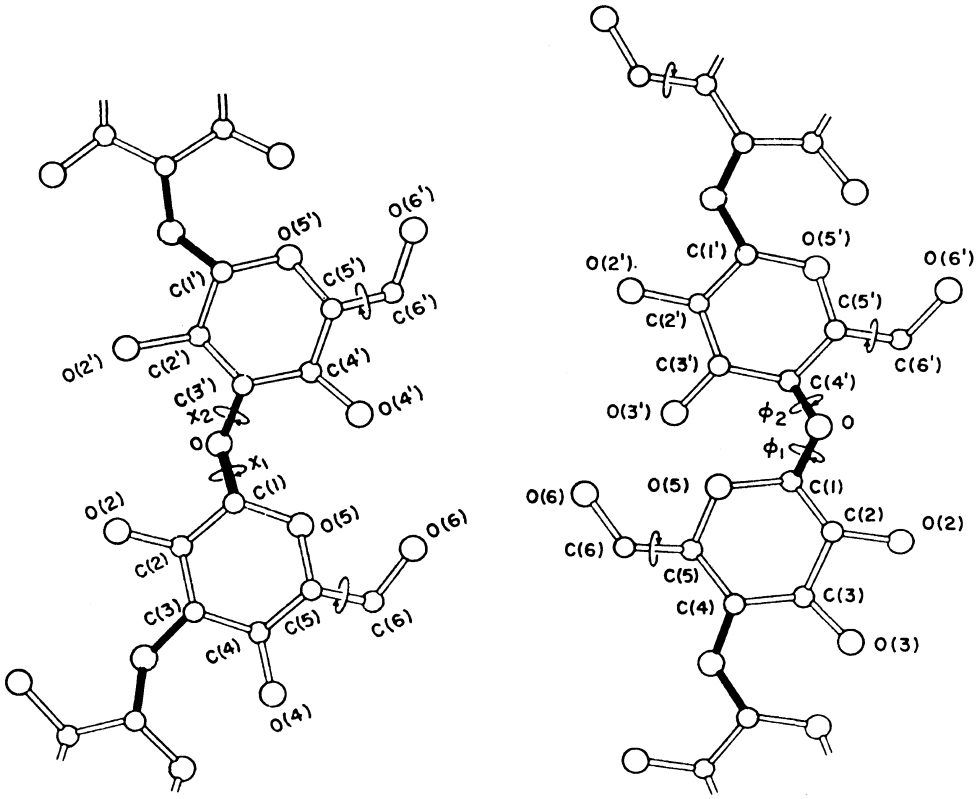
Isamu HIRANO, Yoshiyuki EINAGA, and Hiroshi FUJITA

Department of Polymer Science, Osaka University, Toyonaka, Osaka 560, Japan.

(Received July 14, 1979)

KEY WORDS Curdlan / Cadoxen / Light Scattering / Weight-Average Molecular Weight / Radius of Gyration / Intrinsic Viscosity /

Curdlan is the general name for a series of very similar, microbial β-1,3-glucans which form firm, resilient, and heat-irreversible gels when heated in aqueous suspension.[1,2] The β-1,3-glucosidic linkages in these polymers are, more than 99%.[1,2] Thus, curdlan differs, only with respect to the linking patterns of repeat units, from cellulose which is a β-1,4-glucan (Figure 1). The lack of a convenient solvent has hampered the molecular characterization of this polysaccharide in terms of dilute solution properties. Recently, we found that cadoxen (triethylene diamine cadomium hydroxide) known as a solvent for cellulose[3] can also dissolve curdlan; measurement of molecular weights, radii of gyration,



β-1,3-glucan (curdlan) β-1,4-glucan (cellulose)

Figure 1. Chemical structures of curdlan and cellulose.

and intrinsic viscosities of a series of curdlan fractions in this solvent at 25°C has been carried out.

EXPERIMENTAL

A sample of curdlan 13140 (Lot No. 20) was supplied from Takeda Chemical Industries by the courtesy of Professor T. Harada of Osaka University, Institute for Scientific and Industrial Research. The average degree of polymerization (DP_n) of the original preparation (Lot No. 20) was said to be 470 by the supplier who determined it by the method of Manners *et al.*[4] The sample was divided into nine fractions by fractional precipitation at 25°C using cadoxen as the solvent and a 1-propanol–water mixture (3:1 by volume) as the precipitant. All fractions except the initial one were washed thoroughly with methanol, freeze-dried from dispersions in benzene, and used for the present measurements.

The solvent cadoxen was prepared at one time in a large amount, by saturating a 28 wt% aqueous solution of ethylene diamine with cadomium oxide at 3°C and then adding sodium hydroxide.[3] The solution so prepared was clear and stable at room temperature. Until use, it was stored at about 5°C in a refrigerator. Its composition was 4.5 wt% in cadomium, 26 wt% in ethylene diamine, and 0.33 N in sodium hydroxide, and its density at 25°C was 1.0670 g cm^{-3}

Cadoxen is a multicomponent system. Hence, the refractive index increment of a polymer in this solvent for use in analyzing light scattering data must be determined at constant chemical potentials of the diffusible components. However, since no semipermeable membrane resistant to cadoxen was available, Henley's procedure[3] was employed. Thus, the polymer was first dissolved in cadoxen, diluted with water to a 1:1 volume ratio, and dialyzed against a 1:1 water-diluted cadoxen. This diluted cadoxen does not dissolved the cellulosic semipermeable membrane, but curdlan is still stable in it. For dialysis, an osmometer of the Zimm–Myerson type was used; all metal parts were stainless steel to prevent corrosion by the solvent. Ten days were required to attain the dialysis equilibrium. The specific refractive index increment $(\partial n/\partial c)_{T,\mu}$ thus determined for the dialyzed curdlan solution at 25°C and 436 nm was 0.163 cm^3 g^{-1}, while the corresponding figure for the undialyzed solution was 0.107 cm^3 g^{-1}. The refractive index and density of the 1:1 water-diluted cadoxen were 1.370 and 1.031 g cm^{-3}, respectively.

Light scattering and viscosity were measured in the 1:1 water-diluted cadoxen at 25°C. For the former, a Fica 50 photogoniometer was used, and the angular dependence of scattered light intensity was determined in an angular range from 22.5 to 150°, with vertically polarized light of 436 nm as the incident beam. For the latter conventional capillary viscometers of the Ubbelohde type were used.

RESULTS AND DISCUSSION

Figures 2 and 3 show, respectively, the angular dependence of infinite-dilution values of reduced scattering intensity $(Kc/R_\theta)_{c\to 0}$ and the concentration dependence of zero-angle values of reduced scattering intensity $(Kc/R_\theta)_{\theta\to 0}$, on Berry's square-root plot.[5] The values of weight-average molecular weight M_w, second virial coefficient A_2, and z-average mean-square radius of gyration $\langle S^2\rangle_z$ determined from the intercepts and slopes of the straight lines in these figures are given in Table I. These values of M_w are probably the first reported for β-1,3-glucan, includ-

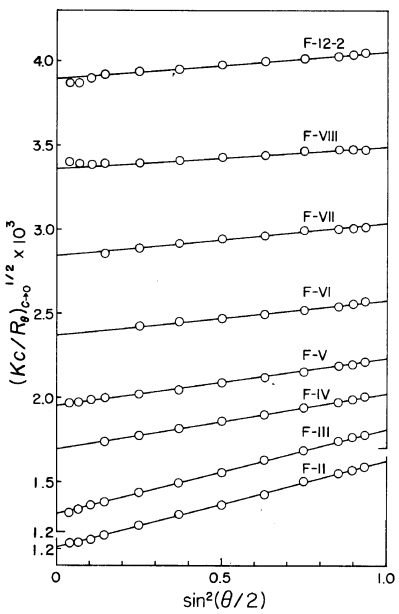


Figure 2. Angular dependence of $(Kc/R_\theta)_{c\to 0}^{1/2}$ for curdlan fractions in the 1:1 water-diluted cadoxen at 25°C.

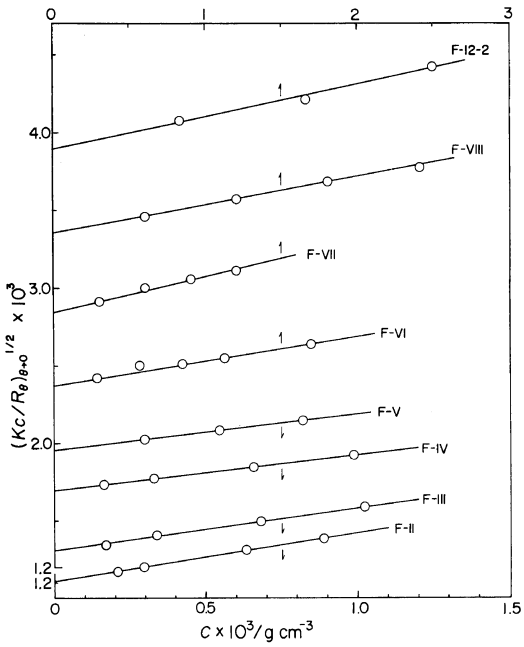


Figure 3. Concentration dependence of $(Kc/R_\theta)_{\theta\to 0}^{1/2}$ for curdlan fractions in the 1 : 1 water-diluted cadoxen at 25°C.

Table I. Numerical results from light scattering measurements on curdlan fractions in the 1 : 1 water-diluted cadoxen at 25°C

Sample code	$M_w \times 10^{-5}$	$\langle S^2\rangle_z \times 10^{11}$ / cm^2	$A_2 \times 10^4$ / cm^3 mol g^{-2}
F-II	6.8_3	1.6_2	3.8_1
F-III	5.8_5	1.4_9	3.5_4
F-IV	3.4_8	0.75_1	3.8_5
F-V	2.6_0	0.53_4	4.3_8
F-VI	1.7_8	0.35_0	3.7_1
F-VII	1.2_4	0.26_2	6.5_8
F-VIII	0.88_8	0.15_9	6.1_1
F-12-2[a]	0.65_9	0.15_6	8.1_3

[a] Sample fractionated by Dr. J. Kakinuma of Takeda Chemical Industries, who obtained a DP_n of 131 for this sample by the method of Manners *et al.*[4]

ing curdlan, and, interestingly, are about one order of magnitude larger than the curdlan molecular weights determined up to now by an enzymatic method.[6]

Figure 4 shows the molecular weight dependence

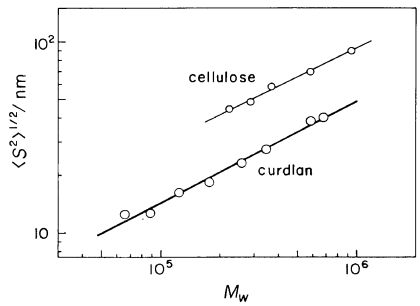


Figure 4. Molecular weight dependence of $\langle S\rangle_z^{1/2}$ for curdlan (large circles) and cellulose (small circles, Henley[3]) in the 1 : 1 water-diluted cadoxen at 25°C.

of $\langle S^2\rangle_z^{1/2}$ for curdlan in the 1 : 1 water-diluted cadoxen at 25°C. The data points may be fitted by a straight line as illustrated, yielding the relation

$$\langle S^2\rangle_z^{1/2} = 3.2 \times 10^{-2} M_w^{0.53} \quad \text{(in nm)} \quad (1)$$

The other points shown refer to Henley's measurements on cellulose in the same solvent.[3] The line fitting these points has a slope of 0.50. At fixed M_w, the curdlan values of $\langle S^2\rangle_z^{1/2}$ are about one half the cellulose values, indicating that the curdlan molecule assumes a more coiled form than the cellulose molecule in the 1 : 1 water-diluted cadoxen.

The values of intrinsic viscosity $[\eta]$ and Huggins' constant k' for the curdlan fractions in the 1 : 1 water-diluted cadoxen at 25°C are given in Table II, and the former are plotted double-logarithmically against M_w in Figure 5. Here the data of Henley[3] and of Brown and Wilkstrom[7] for cellulose in the same solvent are also plotted. The points for either polymer exhibit a slight downward curvature at

Table II. Numerical results from viscosity measurements on curdlan fractions in the 1 : 1 water-diluted cadoxen at 25°C

Sample code	$[\eta] \times 10^{-2}$ / cm^3 g^{-1}	k'
F-II	2.1^1	0.31
F-III	1.7_9	0.33
F-IV	1.3_1	0.35
F-V	1.0_9	0.31
F-VI	0.82_0	0.49
F-VII	0.57_0	0.39
F-12-2	0.37_2	0.38

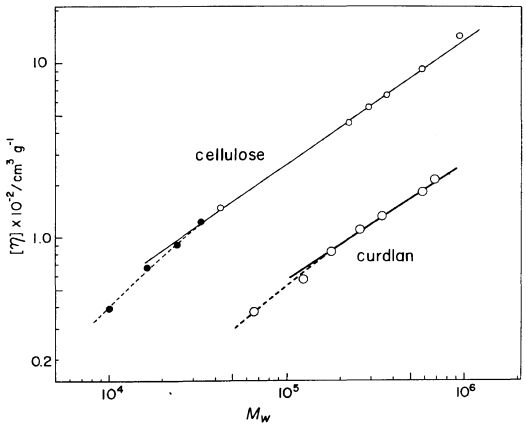


Figure 5. Molecular weight dependence of $[\eta]$ for curdlan (large circles) and cellulose (small circles; open circles, Henley[3]; closed circles, Brown and Wikstrom[6]) in the 1 : 1 water-diluted cadoxen at 25°C.

lower M_w. This feature suggests that these polymers are not quite flexible.[8] The straight line fitting the curdlan points for M_w above 2×10^5 is described by

$$[\eta] = 2.5 \times 10^{-4} M_w^{0.65} \quad (\text{in cm}^3\,\text{g}^{-1}) \qquad (2)$$

The two curves in Figure 5 indicate that the hydrodynamic volume of curdlan is about one fifth that of cellulose of the same molecular weight. This difference is consistent with the relation that the statistical radius of curdlan is about one half that of cellulose.

From computer calculations, Rees and Scott[9] have shown that the most energetically stable conformation of β-1,3-glucan(curdlan) and that of α-1,4-glucan(amylose) are close to the 6/1 helix, whereas the corresponding conformation of β-1,4-glucan(cellulose) is the 2/1 helix. These calculated results are consistent with our experimental finding that both $\langle S^2 \rangle_z^{1/2}$ and $[\eta]$ of curdlan are significantly smaller than those of cellulose of the same molecular weight. However, this consistency should not be taken to mean that these polymers in solution are predominantly helical. Rather, their overall conformations may be random coil and contain interrupted sequences of helical loops. Jordan *et al.*[10] have simulated, by Monte Carlo calculations, chain models of amylose containing a significant amount of "pseudohelical backbone trajectory" sequences. The same model may be a good representation of the conformation of the curdlan molecule in solution.

Acknowledgments. Our thanks are extended to Professor T. Harada of Osaka University Institute for Scientific and Industrial Research and also to Takeda Chemical Industries for supplying the curdlan.

REFERENCES

1. T. Harada, ACS Symposium Series, No. 45, 1977, p 265.
2. T. Harada and S. Sato, *Ferment. Ind.*, **36**, 86 (1978).
3. D. Henley, *Arkiv Kemi*, **18**, 327 (1961).
4. D. J. Manners, A. J. Masson, and R. J. Sturgeon, *Carbohydr. Res.*, **17**, 109 (1971).
5. G. C. Berry, *J. Chem. Phys.*, **44**, 4550 (1966).
6. I. Nakanishi, K. Kimura, S. Kusi, and E. Yamazaki, *Carbohydr. Res.*, **32**, 47 (1974).
7. W. Brown and R. Wilkstrom, *Eur. Polym. J.*, **7**, 1 (1965).
8. M. Motowoka, T. Norisuye, and H. Fujita, *Polym. J.*, **7**, 558 (1975).
9. D. A. Rees and W. E. Scott, *J. Chem. Soc., Sect B*, 469 (1971).
10. R. C. Jordan, D. A. Brant, and A. Cesaro, *Biopolymers*, **17**, 2617 (1978).