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Order-Disorder Conformation Change of Succinoglycan in Aqueous Sodium Chloride as Studied by Static and Dynamic Light Scattering

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Summary

Static and dynamic light scattering measurements have been made on a sodium salt sample of succinoglycan in 0.01 M aqueous NaCl at different temperatures between 25 and 75°C where the polysaccharide undergoes a thermally induced change from an ordered (helical) to disordered conformation with raising temperature *T*. The weight-average molecular weight M_w, the z-average radius of gyration, and the hydrodynamic radius sharply decrease in a relatively narrow *T* range (around 55°C) in which the specific rotation was previously found to change sigmoidally with *T*. In particular, the value of M_w (4.55×10^5) in the ordered state at 25°C is twice as large as that (2.27×10^5) in the disordered state at 75°C, giving decisive evidence that the helical structure of the polysaccharide in aqueous NaCl is composed of paired chains. It is concluded that this structure is a double-stranded helix and breaks directly into two disordered chains with increasing *T*.

Introduction

Succinoglycan, a charged polysaccharide whose repeating unit is shown in Figure 1 [1-3], undergoes an order-disorder conformation change in aqueous salt with raising temperature *T* [4-11]. As shown by chirooptical measurements [6,7,11], the ordered (helical) structure is maintained up to about 40°C in pure water and stable up to a higher *T* in aqueous sodium chloride of a higher salt concentration, but its details are still a matter of debate. In fact, two models, single and double helices, have been proposed and argued on the basis of different pieces of experimental evidence [6,7,9,11]. The crystalline structure of the polymer is unknown probably because of the low crystallinity, so that experimental determination of the ordered conformation in solution is a challenging problem of polymer solution workers.

Very recently [11], we found that the molecular weight dependence of z-average radius of gyration $\langle S^2 \rangle_z^{1/2}$ and zero-shear intrinsic viscosity $[\eta]$ for succinoglycan in 0.1 M aqueous NaCl at 25°C is quantitatively explained by a semi-rigid wormlike chain [12] with a persistence length of about 50 nm and a linear mass density M_L of about 1500 nm^{-1}. This strongly supports the double-helix model since the M_L value is almost twice as large as that (777 nm^{-1}) expected for the single polysaccharide

molecule [7]. Evidently, this model requires the molecular weight to decrease to a half when the polymer is brought to the completely disordered state at an elevated temperature. In addition, dimensional (conformation-dependent) properties such as $<S^2>_z^{1/2}$ and R_H (the hydrodynamic radius) may be expected to decrease sharply (in a cooperative fashion) with raising T.

This paper reports static and dynamic light scattering studies undertaken for a succinoglycan sample in aqueous NaCl over a T range from 25 to 75°C to see how M_w (the weight-average molecular weight), $<S^2>_z^{1/2}$, and R_H vary with T. It was crucial to choose an appropriate concentration of NaCl in water as the solvent for the experiment. The problem was that, when the salt concentration is high enough to suppress polyelecrolyte effects on scattering intensity profiles, the disordered state is attained only at very high temperatures where precise light scattering measurements are infeasible. We chose 0.01 M for the NaCl concentration (instead of 0.1 M) because previous optical rotation data [11] suggested that the entire order-disorder conformation change in the aqueous salt occurs within the above-mentioned T range (25 - 75°C). We note that according to previous [η] data [11] at 25°C, the ordered structures in 0.01 and 0.1 aqueous NaCl hardly differ.

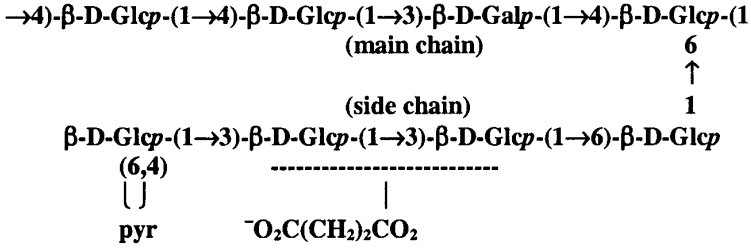

Fig. 1 Repeating unit of succinoglycan. The main chain consists of three glucose (Glc*p*) and one galactose (Gal*p*) residues. A succinate monoester is linked to one or both of the two side chain glucoses, but its content varies depending on bacterial source or cultivation condition [1-3]. A pyruvate (pyr) is attached to the 6,4 positions of the glucose at the side chain end

Experimental

The previously investigated succinoglycan sample S-5 (Na salt) with M_w = 4.76 x 10^5 (in 0.1 M aqueous NaCl at 25°C) [11] was used for the present study. It had been prepared by ultrasonic fragmentation of a commercial sample (Rhöne-Poulenc Rheozan with a D-glucose : D-galactose : pyruvate : succinate ratio of 7 : 1 : 1 : 0.8) at 20 kHz and 0°C, followed by purification and repeated fractional precipitation.

Static scattering intensities were measured on a Fica-50 light scattering photometer in an angular range from 30 to 150°, using vertically polarized incident light of 436-nm wavelength. The temperature was successively raised from 25 to 75°C, because the thermally induced conformation change of succinoglycan is not always reversible as detected by optical rotation and viscosity measurements on heat-treated solutions [6,11] (thermal hysteresis is more pronounced at a lower salt concentration). Calibration of the apparatus and optical clarification of test solutions were carried out in the manner described previously [11]. The concentration dependence of scattering

intensity at fixed scattering angles θ was analyzed by the square-root plot and the angular dependence at fixed polymer mass concentrations *c*, by the linear plot.

The specific refractive index increment $(\partial n/\partial c)_\mu$ for succinoglycan in 0.01 M aqueous NaCl at fixed chemical potentials μ of diffusible components was determined at 25, 45, and 65°C for wavelengths λ_0 of 436 and 546 nm using a modified Schulz-Cantow type differential refractometer. Solutions were dialyzed at the respective temperatures using the apparatus described elsewhere [13]. The values of $(\partial n/\partial c)_\mu$ obtained were 0.146, 0.144, and 0.141 $cm^3\ g^{-1}$ at 25, 45, and 65°C, respectively, for 436 nm and 0.144, 0.142, and 0.139 $cm^3\ g^{-1}$ at 25, 45, and 65°C, respectively, for 546 nm. Necessary values at other temperatures were interpolated from these data.

Dynamic light scattering measurements were made on an ALV/DLS/SLS-5000 light scattering photometer equipped with an ALV-5000E/WIN correlator, immediately after the static intensity measurement at each *T*. A Yag laser of λ_0 = 532 nm was used as the light source. The normalized autocorrelation function $g^{(2)}(t)$ at time *t* was determined at scattering angles between 30 and 90°.

Results and Discussion

Molecular Weight and Statistical Radius

Figure 2 illustrates the angular dependence of $(Kc/R_\theta)_{c=0}$ (i.e., Kc/R_θ at infinite dilution) for succinoglycan sample S-5 in 0.01 M aqueous NaCl at all the temperatures studied. Here, *K* is the optical constant, R_θ is the excess reduced scattering intensity at θ, and *k* is the magnitude of the scattering vector defined by $k = (4\pi n_0/\lambda_0) \sin(\theta/2)$, with n_0 being the solvent refractive index. For clarity, the ordinate values at the respective temperatures are shifted by *A* as indicated in the graph.

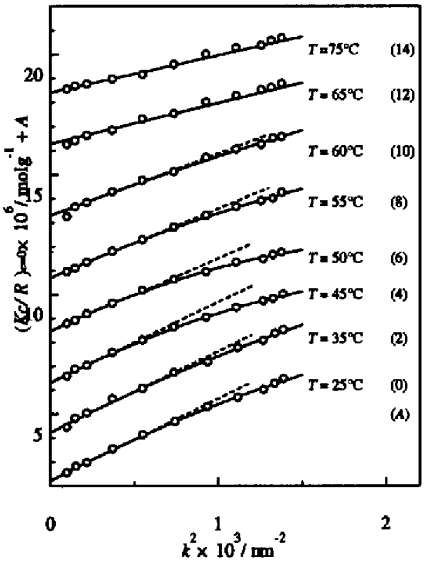

Fig. 2 Angular dependence of $(Kc/R_\theta)_{c=0}$ for succinoglycan sample S-5 in 0.01 M aqueous NaCl at the indicated temperatures. The ordinate values at the respective temperatures are shifted by *A* as indicated

The values of M_w and $\langle S^2\rangle_z^{1/2}$ evaluated from the indicated dashed lines in Figure 2

are plotted against T in Figures 3 and 4, respectively. As T increases, M_w and $\langle S^2 \rangle_z^{1/2}$ decrease first gradually and then sharply (around 55°C) and finally level off above 65°C. Thus, we find that the chirooptically detected (local) conformation change of succinoglycan is accompanied by sharp decreases in both molecular weight and statistical radius. Importantly, the molecular weight at 75°C (in the disordered state) is almost one half that at 25°C (in the ordered state), giving decisive evidence that the ordered (helical) structure of the polysaccharide is composed of two chains. The numerical data for M_w and $\langle S^2 \rangle_z^{1/2}$ are summarized in Table I, along with those for the second virial coefficient A_2. It can be seen that A_2 is rather insensitive to T and hence to the conformational state of succinoglycan. We also note that, as expected from the previous $[\eta]$ data, $\langle S^2 \rangle_z^{1/2}$ at 25°C is only slightly larger than the value of 66 nm in 0.1 M aqueous NaCl [11] at the same T.

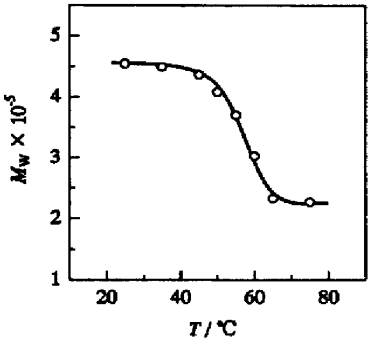

Fig. 3 Change in M_w with T for sample S-5 in 0.01 M aqueous NaCl

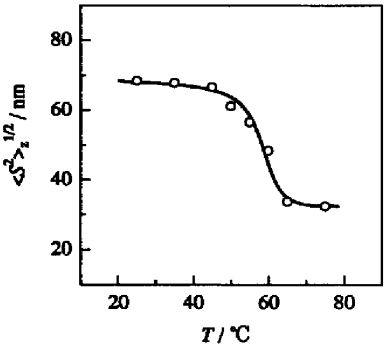

Fig. 4 Change in $\langle S^2 \rangle_z^{1/2}$ with T for sample S-5 in 0.01 M aqueous NaCl

Table I. Results from static and dynamic light scattering measurements on succinoglycan sample S-5 in 0.01 M aqueous NaCl at different temperatures

T (°C)	$M_w \times 10^{-5}$	$A_2 \times 10^3$ (mol g^{-2} cm^3)	$\langle S^2 \rangle_z^{1/2}$ (nm)	$D \times 10^7$ (cm^2 s^{-1})
25	4.55	1.52	68.5	0.960
35	4.50	1.62	67.9	1.23
45	4.37	1.52	66.7	1.54
50	4.08	1.38	61.2	1.72
55	3.70	1.32	56.6	1.96
60	3.03	1.67	48.5	2.30
65	2.33	1.69	34.3	2.85
75	2.27	1.65	32.4	3.40

Hydrodynamic Radius

Figure 5 shows plots of ln $[g^{(2)}(t) - 1]$ against k^2t at fixed scattering angles for a 0.01 M aqueous NaCl solution of the succinoglycan sample with c = 1.891 x 10^{-3} g cm^{-3} at 25°C. The values of Γ/k^2 (the first cumulant Γ relative to k^2) evaluated from the slopes of the straight lines were independent of k in the range studied. This was also the case for all other polymer concentrations and temperatures.

Figure 6 illustrates the concentration dependence of $(\Gamma/k^2)_{k=0}$, i.e., the zero angle value of Γ/k^2, at all the temperatures studied. The translational diffusion coefficients D obtained from the intercepts of the straight lines are presented in the fifth column of Table I. The monotonic increase in D with an increase in T reflects a decrease in chain dimension accompanying the order-disorder conformation change, but since D contains the effects of T and solvent viscosity η_0, R_H defined by the relation $R_H = k_B T/6\pi\eta_0 D$ is relevant to the present purpose. Here, k_B denotes the Boltzmann constant.

The calculated values of R_H are plotted against T in Figure 7. The curve resembles those observed above for M_w and $\langle S^2\rangle_z^{1/2}$, confirming that the order-disorder conformation change of succinoglycan is accompanied by a sharp decrease in molecular size.

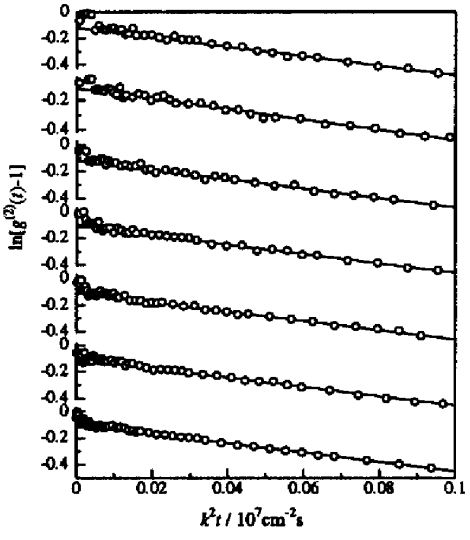

Fig. 5 Plots of ln $[g^{(2)}(t) - 1]$ against k^2t at fixed scattering angles for a 0.01 M aqueous NaCl solution of succinoglycan sample S-5 with $c = 1.891 \times 10^{-3}$ g cm^{-3} at 25°C

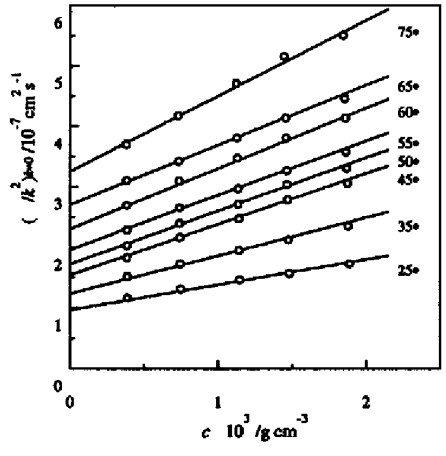

Fig. 6 Concentration dependence of $(\Gamma/k^2)_{k=0}$ for succinoglycan sample S-5 in 0.01 M aqueous NaCl at the indicated temperatures

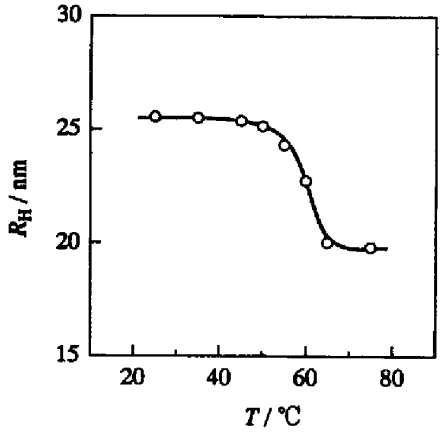

Fig. 7 Change in R_H with T for sample S-5 in 0.01 M aqueous NaCl

Ordered Structure and Its Thermal Breaking

As mentioned in the Introduction, our previous analysis [11] of the molecular weight dependence of $\langle S^2 \rangle_z^{1/2}$ and $[\eta]$ for succinoglycan in 0.1 M aqueous NaCl at 25°C suggested that the ordered conformation should be a double-stranded helix. In contrast to the single helix model, this does not contradict the observed irreversible thermal changes in specific rotation and relative viscosity [11]. However, we did not preclude a possibility that the predominant molecular species in the ordered state could be lateral aggregates composed of two single helices on the average. Although the molecular-weight change observed in the present work substantiates that the order-disorder change is an event associated with two chains, the molecular weight data alone do not rule out the aggregate model [6].

The specific rotation [11] for a succinoglycan sample in 0.01 M aqueous NaCl sigmoidally changed in the same temperature range as that found here for the changes in M_w, $\langle S^2 \rangle_z^{1/2}$, and R_H (see Figures 3, 4, and 7). This coincidence implies that the local conformation change, the dimensional change, and the dissociation of paired chains all simultaneously occur. Hence, the lateral aggregate model is irrelevant. Note that in this model, the dissociation of semi-rigid aggregates into single helices in a certain temperature region causes no remarkable change in specific rotation and chain dimension, whereas it leads to a substantial lowering of M_w. Furthermore, the aggregate model can hardly be reconciled with the large A_2 values in the ordered state that are comparable to those in the disordered state (see Table I). In conclusion, the ordered structure of the polysaccharide in aqueous NaCl is a double-stranded helix and breaks directly into two disordered chains without passing through intermediate conformations of partially broken double helices.

According to our previous analysis [11] based on the wormlike chain, the double helix of succinoglycan (in 0.1 M aqueous NaCl) is characterized by $M_L \approx 1500$ nm^{-1}, q (the persistence length) ≈ 50 nm, and a helix diameter of about 2.5 nm. The parameters in 0.01 M aqueous NaCl should be essentially the same as these values, though q can be slightly larger at the lower salt concentration. We note again that no significant difference in $[\eta]$ (at 25°C) between the two aqueous salts was previously observed over a wide range of molecular weight from 1.0×10^5 to 8.7×10^6 [11]. The M_L of 1500 nm^{-1} with the molar mass of the repeating unit (1491) yields 2.0 nm for the helix pitch per repeating unit [11]. This pitch suggests that the double helix of the polysaccharide should be fairly extended along the helix axis. The q value of 50 nm indicates that the rigidity of this helix is comparable to that of double-stranded DNA at high ionic strength [14].

On the other hand, the disordered conformation of succinoglycan in 0.01 M aqueous NaCl at 75°C is left unexplored. We wish to determine it when we obtain data of $\langle S^2 \rangle_z$, R_H, and $[\eta]$ at this temperature as functions of molecular weight.

Conclusions

As the temperature is increased from 25 to 75°C, the molecular weight and the statistical and hydrodynamic radii for a succinoglican sample (Na salt) in 0.01 M aqueous NaCl sharply decrease around 55°C where the polysaccharide undergoes a change from a helical to disordered conformation. The molecular weight at 25°C, i.e., in the helical state, is twice as large as that in the disordered state at 75°C. Thus, the thermally induced order-disorder conformation change involves dissociation of paired

helical chains, and the ordered conformation in aqueous NaCl must be a double-stranded helix, as previously deduced from analyses of the molecular weight dependence of radius of gyration and intrinsic viscosity [11]. This helix directly melts into two disordered chains without passing through intermediate conformations.

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