Macromolecules 1987, *20*, 3003-3006 **3003**

Local Conformation of Polysaccharides in Solution Investigated by Small-Angle X-ray Scattering

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ABSTRACT: In order to investigate the effect of solvents on the local conformation of polysaccharides, the changes in the persistence lengths of cellulose diacetate, cellulose triacetate, and pullulan with solvent, and also those of sodium (carboxymethyl)cellulose with concentration of added salt, were examined by small-angle X-ray scattering. It is shown that pullulan behaves as a much more flexible chain than cellulose derivatives. The chain flexibility of cellulose diacetate is more sensitively affected by solvents, compared with that of cellulose triacetate. However, the persistence length of (carboxymethyl)cellulose could not be unambiguously determined by this method.

Introduction

The overall conformations of linear polymers in solution are markedly affected by solvent, temperature, and other effects due to the so-called excluded volume effect. In two-parameter theories, it is assumed that these changes in the overall conformation of polymer chains are not accompanied by a significant change in the local conformation. In our previous paper,[1] in fact, it was confirmed that the local conformation or the persistence length of poly(sodium acrylate) is almost fixed because of the high steric hindrance between side grups, even when the overall conformation is changed by varying the concentration of added salts, C_s, or the degree of neutralization, i. Strictly speaking, however, this assumption should be checked case by case. According to Kamide et al.,[2-4] the local conformation of polysaccharides (cellulose diacetate, CDA, and triacetate, CTA) may be affected by solvents or temperature.

It was reported in our previous papers[1,5] that, in the Kratky plot obtained from the small-angle X-ray scattering, SAXS, the highest scattering angle region is free from the excluded volume effect and the Kratky method can be effectively used to estimate the persistence length of the polymer chain y as an index of its inherent chain stiffness, even when the excluded volume effect cannot be neglected. In this work, we study the effects of solvents upon the persistence lengths of cellulose acetates (CDA and CTA) and also the effect of added neutral salt on that of (carboxymethyl)cellulose (NaCMC) by applying the Kratky method to their SAXS data.

As a supplementary purpose, SAXS of pullulan, which has a different glucosidic linkage, is also studied for comparison with cellulose derivatives. It is well-known that the solution properties of polysaccharides depend significantly on the kind of glucosidic linkage which links the adjacent pyranose units of the polysaccharides. For example, the relation of the radius of gyration to the molecular weight of cellulose,[6] linked by the β-1,4-glucosidic linkage, in an equal volume ratio mixture of cadoxen and water is clearly different from that of amylose,[7] linked by the α-1,4-glucosidic linkage, in dimethyl sulfoxide, and also quite different from that of pullulan,[8] linked by the α-1,4- and α-1,6-glucosidic linkages, in water. From studies of the dilute solution properties, it was concluded that such differences can be explained in terms of the difference in the characteristic ratios of the polymer chains, which reflect the chain stiffness.

Experimental Section

1. Samples. Two cellulose acetates, having low and high degrees of esterification (cellulose diacetate, CDA, and cellulose triacetate, CTA, respectively) and one NaCMC were kindly supplied from Daicel Chemical Ind. Co. Ltd. Cellulose acetates were purified by precipitating them from dichloromethane solution by adding *n*-hexane to the solution. NaCMC was purified and fractionated by the following procedure: after removing microgel in the sample by ultracentrifuging its aqueous solution, it was dissolved in 1 N aqueous NaOH. To the resultant solution was added isopropyl alcohol at 30 °C in an atmosphere of nitrogen until a slight precipitation is effected under stirring. After the solution was allowed to stand for several days, the concentrated phase was separated from the dilute phase, to which isopropyl alcohol was added to obtain another concentrated phase. This procedure was repeated several times. Two middle fractions were combined and used as a sample for SAXS. The ratio of M_w to M_n of the sample was 1.1, sufficiently small for the present purpose. Pullulan was purchased from Hayashibara Biochemical Laboratories, Inc., and was used without further purification.

Table I
Molecular Characteristics of the Samples

sample	deg of substitutn	M_n	M_w	M_w/M_n
pullulan		6.5×10^4 [a]		
CDA	2.43	7.0×10^4	19.4×10^4	2.8
CTA	2.92		8.0×10^4 [b]	
NaCMC	2.92	4.8×10^5	5.4×10^5	1.1

[a] Determined by the supplier. [b] Determined from intrinsic viscosity. [c] The other molecular weights were calculated from GPC by using calibration curves obtained with standard polystyrene and pullulan.

Molecular weights and molecular weight distributions of NaCMC and CDA were roughly estimated by gel permeation chromatography (Toyo Soda GPC HLC-802UR and HLC-802A), using pullulan in an aqueous solution and polystyrene in organic solvents as standard samples, respectively. The molecular weight of CTA was determined from the intrinsic viscosity according to eq 1.[9] The degrees of substitution of CDA, CTA, and NaCMC

$$[\eta] = 14.9 \times 10^{-5} M^{-0.82} \qquad (\mathrm{dL{\cdot}g^{-1}}, \text{acetone}, 25\ ^\circ\mathrm{C}) \qquad (1)$$

were determined by the supplier. All data of the samples are listed in Table I.

2. Measurements of Small-Angle X-ray Scattering and Numerical Computations. Measurements were performed with a Kratky U-slit camera from Anton Paar Co. X-rays from the Cu Kα line with a 1.54-Å wavelength (λ) were employed. The width of the entrance and counter slits were 100 and 250 μm, respectively, and the distance between the sample and the plane of registration was 21 cm.

In the exactly same way as reported in our previous papers,[1,5] four corrections were made on the observed intensities to obtain final desmeared intensities; corrections were made for the difference in the absorption coefficients between samples, for the finite slits dimension, for the fluctuation in the electron density within the particle, and for finite cross-sectional area of a polymer chain. The details of the experimental conditions, procedures, and numerical computations are given in previous papers.[1,5]

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Table II
Experimental Results[e]

sample	solvent	C_p, g/dL	C_p^*, g/dL	y, Å	y_K, Å	ξ, Å
PTBM	toluene	3.3	1.4	8–12		40–60
pullulan	H_2O	3.0	3.5	12–19		40–60
CTA-1	acetone	2.6		44–66		110–130
CTA-2	DMAc	3.6	0.26	47–71	59	80–100
CTA-3	DMAc	2.3		51–77		100–120
CTA-4	DMAc	1.0		53–80		110–130
CDA-1	THF	2.3	0.41	34–52	39	120–140
CDA-2	acetone	2.0	0.25	48–72	77	140–160
CDA-3	DMAc	1.9	0.10	67–101	139	240–280
PNaA	H_2O[a]	1.6	0.16	9–13		30–40
NaCMC-1	H_2O[b]	1.4	0.1	87–131		20–40
NaCMC-2	H_2O[c]	1.3		96–144		
NaCMC-3	H_2O[d]	1.6	0.03	80–120		

[a] Degree of neutralization i = 0.80, concentration of added salt C_s = 0.10 N. [b] i = 0.90, C_s = 0.10 N. [c] i = 0.10, C_s = 0.10 N. [d] i = 0.10, C_s = 0.01 N. [e] C_p, polymer concentration; C_p^*, critical polymer concentration; y, persistence length of polymer chain; y_K, y estimated from the data by Kamide et al.; ξ, correlation length.

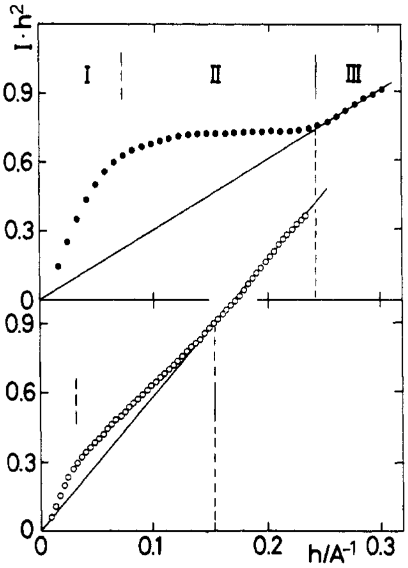

Figure 1. Kratky plots of pullulan in water (○) (C_p = 3.0 g/dL) and poly(*tert*-butyl methacrylate) in toluene (●) (C_p = 3.3 g/dL).

Results

In Figure 1 is shown the Kratky plot, i.e., the plot of Ih^2 versus h for pullulan in water, where h is the magnitude of the scattering vector, defined by $(4\pi/\lambda)\sin(\theta/2)$, where θ is the scattering angle. In agreement with theory, the plot consists of three regions, the Guinier (I), Debye (II), and rod (III) regions, and exhibits a break between regions II and III. In region II the plot will be horizontal if the conformation of a polymer is Gaussian, while in region III the plot will be a straight line passing through the origin if a polymer chain is represented by a rod. In Figure 1 is also shown for comparison the plot of poly(*tert*-butyl methacrylate), PTBM, in toluene, as a typical flexible polymer, reproduced from a previous paper.[5] In the plots of pullulan and PTBM, there is a clear difference in region II. The plot is horizontal for PTBM, but nonhorizontal for pullulan. It is well-known that the plot of Ih^2 versus h in region II becomes nonhorizontal if the excluded volume effect works between segments so that the distribution of segments may be non-Gaussian. Judging from the difference in region II between PTBM and pullulan in Figure 1, it may be suggested that the distribution of segments in a pullulan molecule is not Gaussian, though the reason is not yet clear.

In a previous investigation[1], however, it was ascertained that the abscissa of a break position, h^*, between regions II and III is almost free from excluded volume effects working between segments, in accordance with the theoretical prediction derived by Koyama.[10] Therefore, it may be reasonable to assume that under all present experimental conditions h^* can be related to the persistence length of a polymer chain, y, through eq 2[11,12] or 3,[13] which was derived assuming no excluded volume effect.

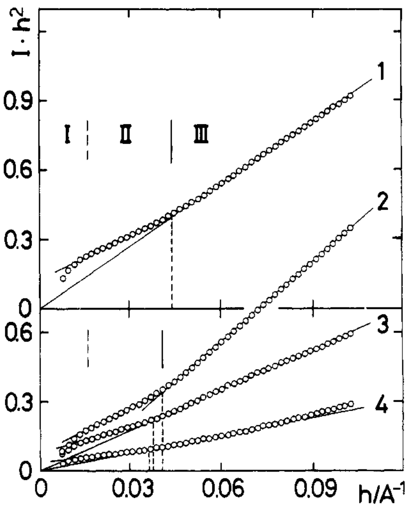

Figure 2. Kratky plots of cellulose triacetate in (1) acetone and (2–4) *N,N*-dimethylacetamide. C_p: (1) 2.6; (2) 3.6; (3) 2.3; (4) 1.0 g/dL.

$$y = 1.91/h^* \qquad (2)$$

$$y = 2.87/h^* \qquad (3)$$

The persistence length y of pullulan estimated from the data in Figure 1 is in the range 12–19 Å (Table II), which is satisfactorily coincident with the theoretical value, 13 Å, obtained by Brant and Burton.[14] This value of y for pullulan is comparable to that for a typical flexible polymer: 9–10 Å for poly(styrene) in cyclohexane,[15] 8–12 Å for poly(*tert*-butyl methacrylate) in toluene,[5] 10–15 Å for poly(*tert*-butyl acrylate) in toluene,[5] and, therefore, it may be concluded that, in aqueous solution, pullulan behaves as a flexible chain. This conclusion is in agreement with the conclusion derived by Kato et al.[8] from a study of its solution properties.

In Figures 2 and 3 are shown the Kratky plots of CTA and CDA in organic solvents, respectively. It is observed that all values of h^* for both samples are much smaller than that of pullulan in water (Figure 1) and that, consequently, y's of CDA and CTA are several times longer than that of pullulan (Table II). That is, CDA and CTA behave as a semiflexible chain in these solvents, in contrast

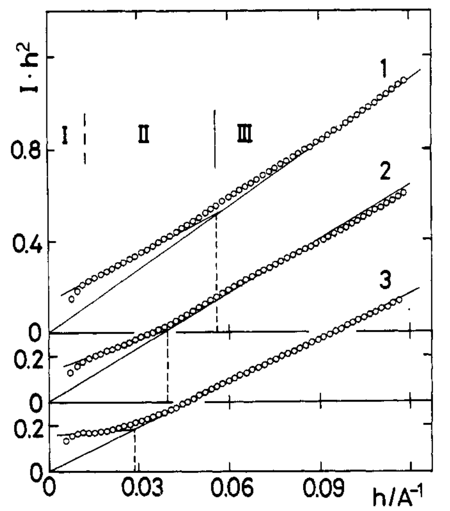

Figure 3. Kratky plots of cellulose diacetate in (1) THF, (2) acetone, and (3) *N,N*-dimethylacetamide. C_p: (1) 2.3; (2) 2.0; (3) 1.9 g/dL.

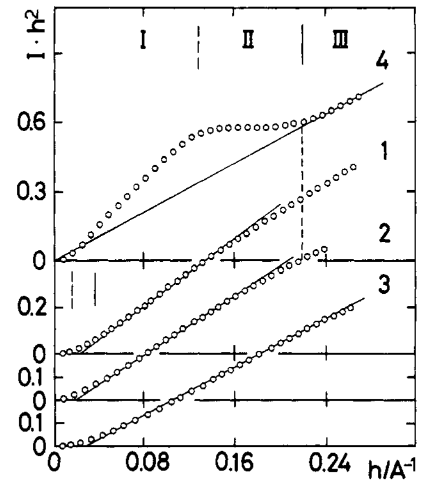

Figure 4. Comparison between the Kratky plots of (1–3) sodium (carboxymethyl)cellulose and (4) poly(sodium acrylate) in aqueous solution. *i*: (1) 0.9; (2) 0.1; (3) 0.1; (4) 0.8. C_s: (1) 0.1; (2) 0.1; (3) 0.01; (4) 0.1 N. C_p: (1) 1.4; (2) 1.3; (3) 1.6; (4) 1.6 g/dL.

with pullulan in water. There is, however, a clear distinction between the solvent effects on chain flexibilities of CDA and CTA. The chain flexibility of CDA is markedly sensitive to solvent, compared with that of CTA. Such unique solvent effects on chain flexibility of cellulose acetates have already been pointed out by Kamide et al.[2-4] as explained later.

In Figure 4 is shown the Kratky plot of NaCMC, in comparison with that of poly(sodium acrylate), PNaA, which is taken from a previous paper.[1] Regions I and II of NaCMC are not clearly distinguished from region III, in contrast with that of PNaA. Moreover, in region III the plot does not pass through the origin. Such a unique Kratky plot was also observed in the small-angle neutron scattering of (carboxymethyl)cellulose, CMC in D_2O without added salt.[16] Although the reason why the Kratky plot of NaCMC in region III does not pass through the origin is not clear, it is understandable that the Kratky plot for NaCMC does not show an ideal form, considering that the experiments for NaCMC are carried out at such high concentrations that the correlation lengths are shorter than the persistence length of NaCMC. The critical concentration C_p^* and correlation length ξ of NaCMC are discussed in relation with those of the other samples in the Discussion section. Therefore, let us assume, tentatively, that the intercept of the straight line with the abscissa is a break position between the Debye (II) and the rod region (III), as was done by Moan et al.[16] The values for y of NaCMC determined on this assumption are listed in Table II and are found to be several times longer than that of PNaA (Table II). Judging from the fact that CTA or CDA, which have the same backbone chain as NaCMC, behaves as a semiflexible chain (Table II) and from the expectation that the introduction of ionizable group would not soften the original polymer chain, it seems certain, at least, that y for NaCMC would not be smaller than those for CTA and CDA, as observed in Table II.

The conclusion that NaCMC behaves as a semiflexible polymer is supported by the experimental result that its potentiometric titration curve[17] can be well represented by the theoretical curve for a rod molecule with a constant radius of cross section over a wide range of *i*, whereas the potentiometric titration curve of PNaA[18] cannot, because of its chain flexibility. From the experimental data in Figure 4, however, it is difficult to judge whether the degree of neutralization of CMC *i* or the concentration of added salt C_s affects the local conformation of NaCMC or not. Moan et al.[16] suggested that y of CMC in D_2O without added salt increases with dilution and also with charge density.

Discussion

Needless to say, eq 2 or 3 should be applied to a sufficiently dilute solution, the concentration of which, C_p, is lower than the critical polymer concentration, C_p^*, where polymer coils begin to overlap. The C_p^* for all samples except NaCMC was evaluated by using eq 4–9, where N_A is Avogadro's number, and M and $\langle S^2\rangle$ are the molecular weight and the mean square radius of gyration of polymer in nm at 25 °C, respectively.

$$C_p^* = 3M/(4\pi\langle S^2\rangle^{3/2}N_A) \quad (4)$$

$$\langle S^2\rangle^{1/2} = 1.64 \times 10^{-2}\, M^{0.57} \quad \text{(pullulan in water)}^{8} \quad (5)$$

$$\langle S^2\rangle^{1/2} = 4.63 \times 10^{-2}\, M^{0.55} \quad \text{(CTA in DMAc)}^{3} \quad (6)$$

$$\langle S^2\rangle^{1/2} = 7.39 \times 10^{-1}\, M^{0.308} \quad \text{(CDA in acetone)}^{2} \quad (7)$$

$$\langle S^2\rangle^{1/2} = 2.99 \times 10^{-2}\, M^{0.558} \quad \text{(CDA in THF)}^{2} \quad (8)$$

$$\langle S^2\rangle^{1/2} = 6.8 \times 10^{-2}\, M^{0.53} \quad \text{(CDA in DMAc)}^{4} \quad (9)$$

The Cp* for NaCMC was estimated on the basis of the experimental results in ref 19, 20. As shown in Table II, C_p^*'s for all samples are lower than C_p, except for pullulan in water. Even when C_p is higher than C_p^*, however, eq 2 and 3 may be utilized to estimate y, if C_p is not so high that the distance between two entanglement points, that is, the correlation length ξ, becomes shorter than y. By employing the same procedure as in the previous work,[5] the values for ξ were obtained as shown in Table II. These correlation lengths are several times longer than the corresponding persistence lengths, except NaCMC. The fact that ξ is shorter than y for NaCMC may be a reason why its Kratky plots in regin III are not on the line passing through the origin (Figure 4).

Kamide et al.[2-4] estimated the unperturbed dimension of cellulose acetates from their solution properties and calculated a characteristic length, A, from eq 10,

$$A = (\langle R^2\rangle_0/M)^{1/2} \quad (10)$$

where $\langle R^2\rangle_0$ is the mean square end-to-end distance in the unperturbed state. A is related to y through eq 11, where

$$y = (A^2M_L)/2 \quad (11)$$

M_L is the molecular weight per unit contour length. In

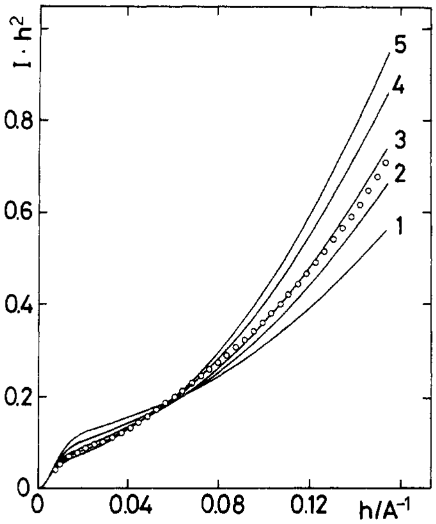

Figure 5. Comparison between the observed Kratky plot of cellulose triacetate in *N,N*-dimethylacetamide (C_p = 1.0 g/dL) and the theoretical curve, obtained on the basis of the wormlike chain model with assumed persistence lengths: (1) 40; (2) 50; (3) 60; (4) 70; (5) 80 Å.

Table II, y's of CDA and CTA are compared with those obtained by Kamide et al.,[2-4] y_K, which was calculated from A through eq 11, assuming that M_L are 51.5 and 55.0 $Å^{-1}$ for CDA and CTA, respectively, on the basis of the crystal structure.[21] There is a satisfactory agreement between both values. Especially, it should be noted that y of CDA shows the same solvent dependence as that of Kamide et al. reported. The solvent effect on y of CTA also was not found in the work of Kamide et al.

As is well-known, the wormlike chain model has been successfully used to elucidate the behavior of a semiflexible chain.[22] The scattering function for wormlike chains was given by Sharp and Bloomfield.[23] In Figure 5, as an example, the observed scattering curve of CTA in *N,N*-dimethylacetamide is compared with the theoretical curves, with y as an adjustable parameter. The observed scattering curve seems to be well represented by the theoretical curve, using y determined from h^*, 50–70 Å (Table II). However, it should be pointed out that the overall profile of the observed curve may be more markedly affected by the corrections, discussed in the experimental section, than h^* in the Kratky plot. For estimating y, it seems more reliable to use h^*, which is insensitive to these corrections, than to use an overall profile of observed curves.

Registry No. CDA, 9035-69-2; CTA, 9012-09-3; NaCMC, 9004-32-4; pullulan, 9057-02-7.

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