Chain Mobility of Pectin in Aqueous Solutions Studied by the Fluorescence Depolarization Method

Jun-ichi Horinaka,*,† Hisanao Mori,‡ Kohei Kani,† and Shiro Maeda‡

Department of Material Chemistry, Graduate School of Engineering, Kyoto University, Nishikyo, Kyoto 615-8510, Japan, and Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Fukui University, Bunkyo, Fukui 910-8507, Japan

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ABSTRACT: The chain mobility of pectin, an anionic polysaccharide containing a carboxyl group in each of its monosaccharide units, in aqueous solutions has been examined by the fluorescence depolarization method. Carboxyl groups of pectin were covalently labeled in part with anthryl groups as the fluorescent probe, and the rotational relaxation time of the probe was estimated as the measure of the mobility of the pectin chain. The activation energy could be estimated from the temperature dependence of the relaxation time and was found to be 1.5 kcal/mol, suggesting the stiff nature of this anionic polysaccharide chain. It is noteworthy that the addition of NaCl to aqueous solutions of up to 1 M does not affect the chain mobility of pectin. The pH dependence of the chain mobility for pectin is clearly different from that for gellan, an anionic polysaccharide containing a carboxyl group in four monosaccharide units. It is proposed that the intramolecular electrostatic repulsions of anionic polysaccharides have two opposing effects with respect to chain mobility: the long-range effect, which increases the degree of chain expansion, enhances the chain motion, and the short-range effect, which acts as the steric hindrance for the conformational transition, decreases the chain mobility.

Introduction

The dynamic properties of polymer chains have been extensively examined using various experimental approaches due to the great importance of such basic information to polymer science.[1−12] The fluorescence depolarization method has been used as a powerful technique to obtain the information regarding the mobility of polymer chains.[1−8] The local mobility of polymer chains has been measured using polymer chains labeled with fluorescent probes in terms of the rotational relaxation time of fluorescent probes, which are allowed to rotate along with the cooperative motion of polymer segments. Because of the fluorescence depolarization studies conducted in previous decades, the local motion of nonelectrolyte polymers in organic solvents as well as in aqueous solutions has been understood in detail; for example, the effects of molecular structure,[5] tacticity,[6] molecular weight,[7] and solvent condition[7] on the chain mobility have been estimated. On the other hand, the chain mobility of polyelectrolytes has been insufficiently examined.[12]

Pectin is a gelling material widely used in the food and pharmaceutical sciences.[13,14] This polysaccharide is also attractive from the point of view of molecular structure—pectin consists of α-D-galacturonic acid units; hence, it contains a carboxyl group in each repeating unit (Figure 1). Pectin exists as an anionic polymer when dissolved in aqueous solutions. Previously, we examined the chain mobility of gellan, a polysaccharide consisting of four monosaccharide units, by the fluorescence depolarization method. Gellan also becomes anionic in aqueous solutions due to the presence of carboxyl groups in the repeating unit. The structural difference between pectin and gellan is due to the density of carboxyl groups in the chain-a carboxyl group exists in each monosaccharide unit of pectin while it exists every four units of gellan. In this sense, pectin may become a model of polymer chains having a high anionic nature, although the carboxyl groups are partly methyl-esterified.[13] Pectin also differs from gellan in that pectin shows no coil−helix transition in aqueous solutions, and this simple behavior of pectin chains may enable an in depth investigation of the chain motion of anionic polyelectrolytes.

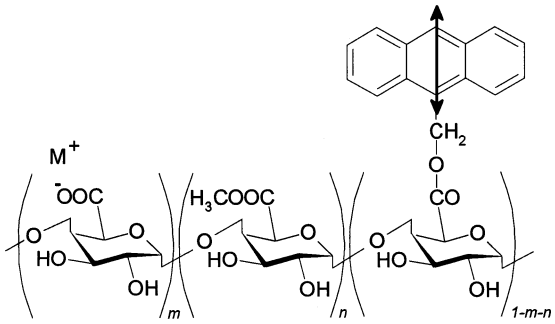

Figure 1. Molecular structure of pectin. The species M is hydrogen or metals such as Na. The arrow represents the transition moments of both excitation and emission of anthryl group.

In this study, the mobility of pectin chains in dilute aqueous solutions is examined by making fluorescence depolarization measurements in terms of the effects of temperature, concentration of cation species added to the solution, and the pH of the solvent. Using an originally synthesized sample of pectin, which is labeled with anthryl groups as the fluorescent probe, we discuss the dynamic properties of this anionic polysaccharide in aqueous solutions.

Experimental Section

Labeling Reaction. The fluorescent probe-labeled pectin (labeled-pectin) was prepared as follows. Pectin (from citrus, Wako) was purified thrice before the labeling reaction by

† Kyoto University.
‡ Fukui University.

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reprecipitation from water to 2-propanol. Purification was also carried out for 9-anthracenemethanol (An−MeOH) (Aldrich) by recrystallization using an equivalent-volume mixture of water and methanol. The purified pectin (1.03 g) was dissolved in water (100 cm^3) of pH = 2, which is adjusted using sulfuric acid along with An−MeOH (0.236 g) and 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (WSC) (Wako) (0.327 g). WSC was used without further purification. Water that was deionized after distillation was used in this study. The aqueous reactant was stirred for 4 h at 40 °C to allow the hydroxyl groups of An−MeOH to esterify with the carboxyl groups of pectin. The product was poured into 2-propanol, and the precipitate obtained was purified thrice by reprecipitation from water to 2-propanol before being used as the labeled-pectin. The weight-average molecular weight M_w and the number-average molecular weight M_n of labeled-pectin were determined by GPC with poly(ethylene glycol) standards to be $M_w = 11.5 \times 10^4$ and $M_n = 3.2 \times 10^4$.

Sample Preparation. The concentration of aqueous solutions of labeled-pectin was fixed at 0.1 wt %. The labeled-pectin was dissolved into water with stirring for 1 h at 40 °C. The aqueous solutions were filtered through membranes of 1 μm pore size immediately before being filled into quartz cells for optical measurements.

Measurements. Fluorescence polarization and fluorescence spectra were measured using a spectrophotometer (850, Hitachi). The fluorescence polarization was estimated from the steady-state fluorescence intensities obtained using polarizer and analyzer. The fluorescence anisotropy ratio, which is a measure of fluorescence polarization, is defined as

$$\bar{r} = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}, \tag{1}$$

where

$$G = \frac{I_{HV}}{I_{HH}}, \tag{2}$$

I_{XY} is the fluorescence intensity, and the subscripts represent the directions of linear polarization of the incident light (X) and emission (Y); for example, $I_{VH}$is the intensity of the horizontally polarized component of fluorescence from the sample excited with vertically polarized light. The factor G accounts for the polarization bias of the detection system. The excitation and emission band-passes were fixed at 365 and 415 nm, respectively.

The relaxation time for the rotational diffusion of the fluorescent probes can be estimated using the fluorescence anisotropy ratio and fluorescence lifetime according to eq 3, given as

$$\frac{1}{\bar{r}} = \frac{1}{r_0}\left(1 + \frac{\tau}{\tau_r}\right), \tag{3}$$

where r_0, τ, and τ_r are the initial anisotropy ratio, fluorescence lifetime, and rotational relaxation time, respectively. We employed $r_0 = 0.25$ in this study; this value was obtained previously for an anthryl group-labeled polymer dissolved in aqueous solutions.[8] Fluorescence lifetime was measured using a time-resolved fluorescence spectrophotometer (Hamamatsu, OB920) by the time-correlated single photon counting method. The fluorescence decay was deconvoluted according to the instrumental response function, and it was fitted with a double-exponential function. The value of τ was determined as the weighed mean value of the two lifetime components.

To confirm that the coil−helix transition of pectin chains in aqueous solutions does not occur, the optical rotation was measured with a polarimeter (J-500A, JASCO).[15] The aqueous solutions of pectin were filled in a cylindrical quartz cell that had a path length of 10 mm. Unlabeled pectin was used for the optical rotation measurements.

Assessment of the Labeling Reaction. The labeling reaction was assessed using fluorescence techniques.[16] We compared the fluorescence spectra as well as $\bar{r}$ between an aqueous solution of labeled-pectin and an unlabeled pectin containing a trace of free An−MeOH. The fluorescence spectrum for the solution of labeled-pectin was 5 nm red-shifted from that for the solution of unlabeled pectin while maintaining the fingerprint characteristics of the anthryl group. It is usually observed that the fluorescence spectra for anthryl groups that are covalently labeled with polymer chains are red-shifted by several nanometers in comparison with those for free anthryl groups. Hence, these results indicate that the anthryl group was introduced into the pectin chain. The value of $\bar{r}$ for the labeled-pectin solution was 0.063 while that for the free An−MeOH solution was 0.003. This large value of $\bar{r}$ for the labeled-pectin solution implies that the rotational relaxation of anthryl groups in this solution is considerably suppressed in comparison with that of the free one. When covalently bonded with the polymer chain, the rotational relaxation of the anthryl group is permitted as the cooperative motion with the neighboring segments of the polymer chain. It is expected that such a cooperative motion has a lower mobility than the free rotation of An−MeOH. On the basis of the above results obtained, we concluded that the labeling reaction was occurred successfully, and that the labeled-pectin contained anthryl groups attached to the carboxyl groups. The fraction of labeled carboxyl groups estimated from the absorbance of the aqueous solution was less than 10^{-4}, implying that the reduction of carboxyl groups in the pectin chain by the labeling reaction was negligibly small and that the interaction between probes was almost impossible.

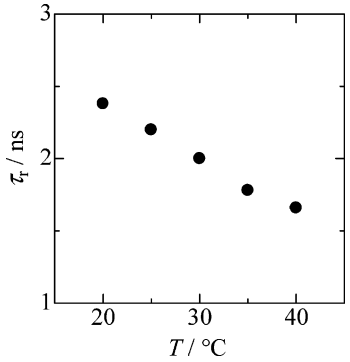

Figure 2. Temperature dependence of τ_r for pectin in 0.1 wt % aqueous solution without added salt.

Results and Discussions

Local Motion of Pectin. Figure 2 shows the plots of τ_r with respect to temperature. The value of τ_r decreases with increasing temperature; namely, the chain mobility becomes high on heating. The optical rotation measurement indicates that the coil−helix transition of pectin chain does not occur within the studied temperature range. Therefore, the increase in the chain mobility can be easily interpreted as an enhancement of the thermal motion of the chain segments as well as that of the surrounding solvent molecules, as is usually obtained for nonelectrolyte polymers in solutions.[2−7,10] On the basis of this interpretation, we estimated the activation energy of the local motion, E^*, for pectin.[17] The velocity, k, of a particle with a frictional coefficient, ζ, passing over an energy barrier of height E is represented as

$$k \propto \zeta^{-1} \exp(-E/RT), \tag{4}$$

where R is the gas constant and T is the absolute temperature. In the case of a local motion, the rotational relaxation time, τ_r, is proportional to the reciprocal of k. The problem under discussion is to assess the

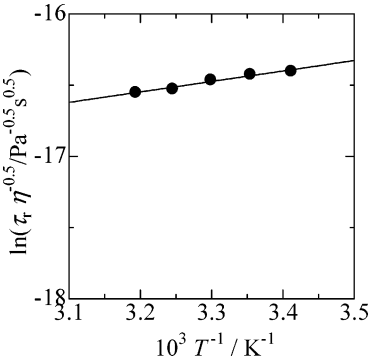

Figure 3. Plot of $\ln(\tau_r/\eta^{0.5})$ for pectin in 0.1 wt % aqueous solution with respect to $1/T$. The activation energy for the local motion of pectin can be estimated from the slope of linear dependence.

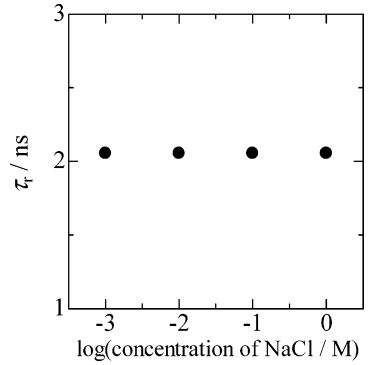

Figure 4. Plot of τ_r for pectin in 0.1 wt % aqueous solution containing NaCl with respect to the logarithmic value of the concentration of NaCl.

influence of solvent viscosity, η, on the local motion of a polymer chain-the power law, $\tau_r \propto \eta^{\alpha}$, has been proposed for the local motion of polymers in low viscosity solvents; however, the value of α is unsettled from 0.5 to 1.0.[2,4,7,10,18] For aqueous solutions of polysaccharide, it has been recently reported that $\alpha = 0.5$ is appropriate to represent the rotational relaxation of a fluorescent probe covalently labeled with a polysaccharide chain.[18] Hence, $\alpha = 0.5$ is employed in this study, and the following equation is obtained from eq 4

$$\tau_r/\eta^{0.5} = A \exp(E^*/RT) \tag{5}$$

Equation 5 implies that the activation energy for the local motion is estimated from the slope of the plot of the logarithm of $\tau_r/\eta^{0.5}$ as a function of $1/T$. Figure 3 shows that $\ln(\tau_r/\eta^{0.5})$ has a linear dependence on $1/T$, yielding the value of E^* evaluated from the slope to be 1.5 kcal/mol. The solvent viscosity was estimated from the value reported in the literature.[19] To the best of our knowledge, no study has been conducted on the fluorescence depolarization with regard to E^* for a polysaccharide chain in solution. The value of $E^* = 1.5$ kcal/mol is close to that for the main chain-labeled vinyl polymers in good solvents obtained by the fluorescence depolarization.[5,7] Considering that the pectin chain used in this study is labeled with anthryl groups attached as a pendant to the side group, pectin may be stiff in nature in comparison with vinyl polymers. NMR study has reported that the segmental motion of amylose and inulin, nonelectrolyte polysaccharides, in aqueous solutions have values of E^* similar to those for typical vinyl polymers.[20] This result implies that the stiffness of the pectin chain results from the electrostatic repulsion between intramolecular segments rather than from its molecular structure including the piranose ring.

Effect of Cation Species. Figure 4 shows the plots of τ_r with respect to the concentration of NaCl added to the pectin solutions. The abscissa represents the logarithmic value of the NaCl concentration. The figure clearly illustrates that τ_r is independent of the NaCl concentration up to 1 M. The possible effect of cation species from the added salt, such as Na^+, is the shielding effect on the electrostatic repulsions between the anionic groups in polyelectrolytes.[21,22] This may lead to the suppression of the electrostatic repulsion between intramolecular pectin segments dissolved in an aqueous solution. It has been determined that the intrinsic viscosity for electrolyte polysaccharides decreases with an increasing concentration of salt contained in the solution. This indicates that the electrolyte polysaccharide chains assume less-expanded conformations at high salt concentrations.[23−25] It has been reported for nonelectrolyte polymers that the chain mobility increases with an increase in the degree of chain expansion due to the suppression of steric hindrance for local conformational transition and vice versa.[4,7] These results imply that the chain mobility of pectin depends on the concentration of NaCl. However, the cation species coexisting with pectin chains in an aqueous solution have no effect on the chain mobility. Recently, similar results have been obtained for gellan[16]—the chain mobility of the random-coiled gellan in aqueous solutions was not affected by the addition of a cation species, although the coil−helix transition temperature as well as the gelation temperature for gellan changed with the concentration of the cation species. These results imply that cation species do not affect the chain mobility of anionic polysaccharides. Since no rigorous method to explain the independence of the chain mobility from the salt concentration, that is obtained in this study, has yet been established, we suggest an interpretation as follows. Fluorescence depolarization studies on the nonelectrolyte polymers have previously reported that the chain mobility is affected by the local potentials for the conformational transition of the main chain bonds rather than by the density of the surrounding segments apart from the fluorescent probe along the main chain; namely, it appears that the chain mobility is determined by the local condition. It might be considered that the cation species shield the electrostatic repulsions between isolated segments while they do not shield those between neighboring segments. The fact that the chain mobility of pectin as well as that of gellan obviously depend on pH, which is described in the following section, indicate the possibility that the chain mobility of electrolyte polysaccharides is affected by the change in the electrostatic repulsions between the intramolecular carboxyl groups.

Effect of pH. Figure 5 shows the pH dependence of τ_r for pectin in aqueous solutions. Aqueous solutions of HCl and NaOH were used in order to adjust the pH of pectin solutions. The figure shows that the chain mobility of pectin depends on pH and increases with decreasing pH. The change in pH by the addition of HCl and NaOH accompanies the change in the concentration of cation species; however, this change does not affect the chain mobility, as stated in the previous section. The value of pH affects the degree of dissociation of carboxyl groups, because the carboxyl group has a weak acidity; namely, the carboxyl groups dissociate to become nega-

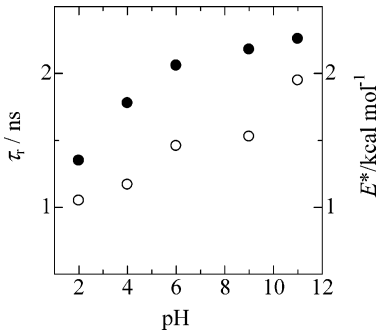

Figure 5. pH dependence of τ_r (filled circle) and E^* (unfilled circle) for pectin in 0.1 wt % aqueous solution without salt. The value of pH was adjusted by using aqueous solutions of HCl and NaOH.

tively charged under high pH conditions, while they exist as neutral groups under low pH conditions. The pH dependence in Figure 5 implies that the mobility of the neutral pectin chain is higher than that of the negatively charged chain. We note that the pH dependence of pectin is obviously different from that for gellan obtained in our previous study.[26] In the case of gellan, the chain mobility is suppressed with decreasing pH. Gellan is also an anionic polysaccharide because of the presence of carboxyl groups; therefore, the anionic nature of gellan determined by the degree of dissociation of the carboxyl groups must depend on pH in a similar manner as it does for pectin. The pH dependence of gellan has been attributed to the change in the degree of chain expansion-negatively charged gellan chains under high pH conditions take more expanded conformations than those neutral under low pH conditions, because of the electrostatic repulsion between the intramolecular segments of the charged gellan chains. Thus, the relationship between the chain expansion and the mobility, as stated above, explains the pH dependence of gellan, but it is inapplicable to the pH dependence of pectin. We attribute the difference in the pH dependence between pectin and gellan to the arrangement of carboxyl groups in a chain. The pectin chain containing a carboxyl group in each repeating unit may have negative charges at neighboring repeating units under high pH conditions. In the case of gellan, negative charges exist at the interval of at least four saccharide units. The intramolecular electrostatic repulsion between charged units affects the mobility of polymer chains in two ways. The electrostatic repulsion between units apart from each other along the chain, the long-range effect, expands the chain conformations to increase the chain mobility. On the other hand, the electrostatic repulsion between neighboring units—the short-range effect—may become a steric hindrance for the internal rotation of single bonds therein, and may decrease the chain mobility. The pH dependence of pectin shown in Figure 5 suggests that the short-range effect for pectin is predominant over the long-range effect due to its molecular structure. Figure 5 also shows the pH dependence of E^*—the value of E^* increases with increasing pH. This pH dependence may be attributed to the steric hindrance for the conformational transition resulting from the short-range effect.

Conclusion

By employment of anthryl group-labeled pectin, the chain mobility of pectin in aqueous solutions was examined by the fluorescence depolarization method. The value of E^* for the local motion of the pectin chain was estimated from the T dependence of τ_r to be 1.5 kcal/mol, suggesting that the pectin chain in aqueous solutions has a stiff nature in comparison with vinyl polymers in organic solvents. The chain mobility of pectin is independent of the concentration of cation species. We interpreted that the cation species may shield the electrostatic repulsion between the charged groups apart from each other along the chain, while the mobility is determined by the interaction between neighboring segments. The pH dependence of pectin is significantly different form that of gellan. This difference is explained by the location of carboxyl groups along the chain, where the long- and short-range effects of electrostatic repulsions change the chain mobility in an opposite manner.

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