Raman Spectroscopic Study of Hydrogen Bonding of Polyacrylamide in Heavy Water[†]

Naoki Tanaka

Department of Polymer Science and Engineering, Kyoto Institute of Technology, Kyoto 606, Japan

Kensaku Ito and Hiromi Kitano*

Department of Chemical and Biochemical Engineering, Toyama University, Toyama 930, Japan

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ABSTRACT: The Raman spectra of D_2O solutions of deuterated polyacrylamide, acetamide, propionamide, and butyramide were measured to investigate the self-association of amide groups. Factor analysis and the deconvolution technique were used for the analyses of Raman spectra of these compounds, and the C=O stretching band of the amide groups could be separated into three major peaks. One of the peaks corresponded to a nonassociated amide group, and the others were assigned to different types of self-associated amide groups. Nonlinear least-squares curve fitting suggested the presence of intramolecular hydrogen bonding within a coil of polyacrylamide in aqueous solutions.

Introduction

Hydrogen bonding plays a crucial role in determining the properties of biopolymers. Therefore, investigation of the hydrogen bonding of water-soluble polymers (as model compounds of biopolymers) in aqueous solutions provides a basic knowledge about the properties of biopolymers. In previous studies, we used Raman spectroscopy to investigate the hydrogen bonding of poly(acrylic acid)[1] and poly(vinylpyrrolidinone)[2] in aqueous solutions. Poly(acrylic acid) is known to have the ability to form cross-links by hydrogen bonding,[3–5] and the dimerization constant K_D of the carboxylic group of poly(acrylic acid) in aqueous solutions could be estimated.[1] Poly(vinylpyrrolidinone) was, on the other hand, found to be hydrated in a specific way in aqueous solutions.[2] In the present study we investigated the hydrogen bonding of polyacrylamide in aqueous solutions.

There have been some studies on the vibrational spectrum of polyacrylamide (POAM).[6–8] Gupta and Bansil,[6] for example, suggested that this polymer has a capability to form intra- or intermolecular hydrogen bonds between the amide groups in the solid state. Rheological studies of polyacrylamide in aqueous solution[9–12] suggested the formation of hydrogen bonds between the amide groups in polyacrylamide.

To study the hydrogen bonding of POAM in detail, we analyze here Raman spectra of the C=O stretching mode of N-deuterated polyacrylamide (POAM-d_2) and its monomer analogues (acetamide ACAM-d_2), propionamide (PRAM-d_2), and butyramide (BTAM-d_2)) in D_2O solution by various techniques. We chose heavy water, not light water, as the solvent, because the bending band of H_2O overlaps with the C=O stretching band, which is unfavorable for precise analyses.

A number of studies on the vibrational spectrum of amide groups in monomer analogues of POAM have been carried out.[13–31] Kobayashi *et al.*, for example, investigated hydration of amide groups in aqueous solutions by measuring infrared spectra of the amides in dioxane–D_2O and dioxane–H_2O mixtures.[20–23] They resolved absorption spectra of the C=O stretching band into five Gaussian or Gaussian–Lorentz hybrid functions (Voigt functions) and attributed them to five types of amides with different hydration states. The C=O stretching bands in the Raman spectrum of POAM and its monomer analogues were thought to be the overlaps of several peaks which could be attributed to different hydration states of the amide groups.

We used factor analysis, deconvolution, and least-squares curve fitting to separate the measured spectrum into individual peaks. Factor analysis is a method for estimating the number of individual components in the spectrum.[32–34] Recently, this technique was employed for the analysis of vibrational spectra of acetic acid,[35,36] isomolybdene,[37] phosphate,[38] protein,[39] and polymer blends.[40] The deconvolution technique, which eliminates the distortion by the slit function from the spectroscopic data,[42,43] has been applied in the analysis of vibrational spectroscopy.[44–49] The number of hydration states of amides were estimated by this technique, and the measured spectrum was separated with least-squares curve fitting using the Marquardt method.[41] By using these techniques, we analyzed the association phenomenon of polyacrylamide and its monomer analogues in D_2O solutions.

Experimental Section

Materials. POAM-d_2 of M_w = 1500 (POAM1500) was obtained by deuteration of polyacrylamide (M_w = 1500) from Polysciences, Warrington, PA. The polymer had been purified by ultrafiltration beforehand. POAM-d_2's of M_w = 100000 (POAM100000) and M_w = 700000 (POAM700000) were prepared by fractionation and subsequent deutration of a commercial sample from Wako Pure Chemicals, Osaka, Japan. The molecular weights of these polymers were determined by GPC. ACAM and PRAM were purchased from Nacalai Tesque, Kyoto, and BTAM was supplied by Tokyo Kasei Co., Tokyo. These three samples were recrystallized from chloroform and deuterated to ACAM-d_2, PRAM-d_2, and BTAM-d_2. Heavy water was obtained from CEA, Gif-sur-Yvette, France, and used for preparation of sample solutions and deuteration.

Spectroscopic Measurements. Raman spectra were recorded using an NR-1100 Raman spectrophotometer (Japan Spectroscopic Co., Tokyo, Japan) with a resolution of 5 cm^{-1}. The 488-nm line of an argon ion laser (GLG 3200, NEC, Tokyo)

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[*] To whom correspondence should be addressed.

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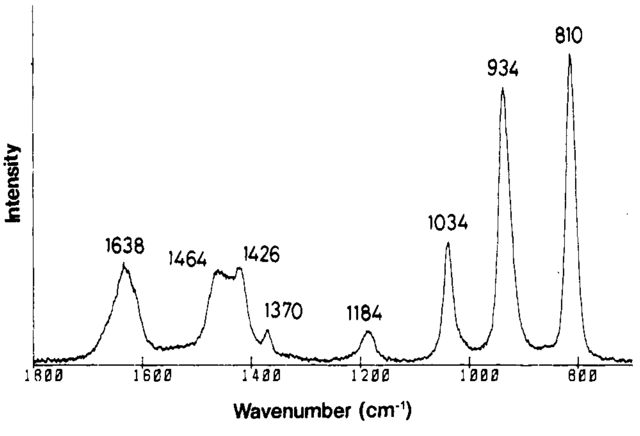

Figure 1. Raman spectrum of 70 wt % D_2O solution of acetamide at 25 °C in the region from 700 to 1800 cm^{-1}.

at 300–500 mW was chosen as the excitation light. The depolarization ratio was measured with a system consisting of a half-wave plate, lens, and a polarizer. All measurements were carried out in a thermostated chamber controled at 25 ± 0.5 °C using a Peltier device (Model RT-IC, Japan Spectroscopic Co.).

Computations. Factor analysis of the spectral data was performed with the program FCCPM written in C language by Drs. H. Kihara and T. Ozeki, Hyogo University of Teacher Education, Hyogo Prefecture, Japan. A deconvolution program employing the Gauss–Seidel method was used for the analyses (the program was written by Y. Senga[42] in BASIC). A deconvolution function in this analysis has a trianglular shape with a half-width of 5 cm^{-1}, which is equal to the spectral slit width of the present measurements. The spectra were resolved with the data decomposition program using the Marquardt method described previously.[41] All calculations were performed by an NEC 9801 RA microcomputer.

Results and Discussion

A. Raman Spectra of Monomer Analogues. Figure 1 shows the Raman spectrum of a 70 wt % D_2O solution of ACA-d_2 at 25 °C. According to previous studies,[13–17] the band at 1638 cm^{-1} corresponds to the C=O stretching band, and the complex bands in the region 1350–1480 cm^{-1} are composed of the C–N stretching band and the CH_3 deformation bands. The bands at 810, 934, and 1034 cm^{-1} are assigned to the C–C stretching band, the ND_2 rocking band, and the CH_3 rocking band, respectively. The C–C stretching mode at 810 cm^{-1} was used as an internal standard. The advantages of using an internal standard were described elsewhere.[36] We believe that the use of the C–C stretching mode at 810 cm^{-1} as the internal standard is valid, because the intensities of the normalized peaks of the CH_3 rocking band at 1034 cm^{-1} did not change with the concentration.

The C=O stretching band exhibited a remarkable change with the concentration of ACAM in D_2O solutions. Previously, Kobayashi *et al.* investigated the hydration of amides in aqueous and D_2O solutions by measuring infrared spectra of amides in dioxane–D_2O and dioxane–H_2O mixtures. They found that the absorption due to the C=O stretching vibration showed a remarkable red shift accompanied by a characteristic change of the band shape as the water content in the medium was increased. They attributed this phenomenon to the change of the hydration state at the carbonyl oxygen in equilibrium, which depends on the water content. On the other hand, the amides self-associate through the hydrogen bonding between an oxygen atom of the carbonyl group and a hydrogen atom of the amino group in an argon matrix.[29] Kobayashi *et al.* also reported that the amides self-associate in dioxane solutions at high concentrations.[20,23]

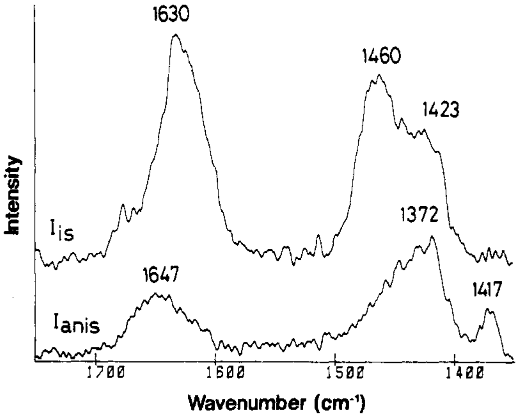

Figure 2. Raman spectrum of 70 wt % D_2O solution of acetamide at 25 °C in the region from 1350 to 1800 cm^{-1}: (A) isotropic spectrum; (B) anisotropic spectrum.

B. Polarization Measurements. Raman spectra of ACAM-d_2 in D_2O solution under parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) polarizations were measured by means of an analyzer in front of the slit and a polarization rotator for the excitation radiation using 90° geometry. The isotropic and anisotropic spectra were calculated using the equations

$$I_{\mathrm{is}} = I_{\parallel} - {}^{4}/_{3} I_{\perp}$$

and

$$I_{\mathrm{anis}} = {}^{4}/_{3} I_{\perp}$$

Figure 2 shows the isotropic and anisotropic spectra of the carbonyl stretching mode for a D_2O solution of ACAM-d_2. The figure shows that the frequencies of the peak tops of the isotropic and anisotropic spectra are different from each other, which means that there should be at least two components which have different depolarization ratios in the carbonyl stretching mode. One is almost at the same frequency of 1631 cm^{-1}, and the other is at a higher frequency than the former band. The same was true for the spectra in higher concentration regions and D_2O solutions of PRAM-d_2.

C. Deconvolution. Deconvolution is a procedure for obtaining an essential spectrum from the observed spectrum. The observed spectrum is a convolution from the original signal spectrum and the slit function of the spectrophotometer. The slit function affects information about the resolution, peak position, and intensity in the observed spectrum. In the deconvolution procedure, a slit function was eliminated from the observed spectrum, and the original signal spectrum is presumed. The Gauss–Seidel method is a procedure which isolates the essential spectrum vector **x** by repeating calculations of the equation

$$\mathbf{y} = \mathbf{H}\mathbf{x}$$

to fit the observed spectrum vector **y**. In this equation, **H** is the slit function matrix.[43] The row of **H** is the slit function of the spectroscope. In this case, a function of a triangular shape with a half-width of 5 cm^{-1} (equal to the spectral slit width) was adopted as the slit function. Figure 3 shows Raman spectra of D_2O solutions of ACAM-d_2 (70 wt %) before and after deconvolution. The spectra after the deconvolution showed the existence of shoulders which had frequencies below and above 1631 cm^{-1}. We found the same shoulder in the deconvoluted spectrum of PRAM-d_2 in D_2O solution (data are not shown).

From the results of the polarization measurements and deconvolution, we conclude that the carbonyl stretching modes of D_2O solutions of ACAM-d_2 and PRAM-d_2 are

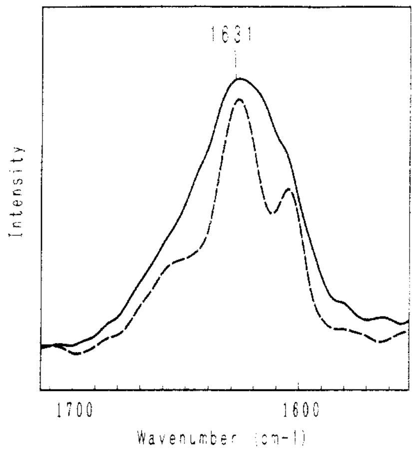

Figure 3. Deconvoluted Raman spectra of the carbonyl stretching band of D_2O solution of acetamide in 70 wt % D_2O solution: solid line, original spectrum; broken line, deconvoluted spectrum.

Table 1. Factor Analysis of D_2O Solution of Amides[a]

	eigenvalue λ		
m	ACA	PRA	POAM1500
1	2276220.0	2273930.0	2072620.0
2	4969.5	7877.0	15648.4
3	1252.4	2362.1	3217.3
4	510.2	344.8	1828.2
5	335.4	256.8	1273.0
6	220.8	142.4	1146.3
7	0.1	112.6	

[a] Seven solutions (10–70 wt %) for ACA and PRA and six solutions (10–60 wt %) for POAM were obtained by the digitization at 201 data points.

convoluted by at least three components located around 1610, 1630, and 1650 cm^{-1}.

D. Factor Analysis. Factor analysis is a mathematical technique for determining the number of linearly dependent components in a composite system. The details of the calculations can be found in the literature.[32–38] We applied this method to seven Raman spectra of D_2O solutions of ACAM-d_2 and PRAM-d_2 in the concentration range 10–70 wt % and to six Raman spectra of a D_2O solution of POAM-d_2 in the concentration range 10–60 wt %. The spectra were digitized at 201 data points between 1550 and 1750 cm^{-1} and normalized with the C–C stretching band. In this region, the spectrum includes only the C=O stretching band. The data sets are expressed by the data matrix **D**, which is the product of spectrum matrix **R** and component matrix **C**.

$$\mathbf{D} = \mathbf{RC} \qquad (1)$$

In essence, we diagonalize the covariance matrix **Z** which is obtained using eq 2 by the Jacobi method and get the eigenmatrix **Q** and eigenvector **E**.

$$\mathbf{Z} = {}^t\mathbf{D}\mathbf{D} = \mathbf{Q}\mathbf{E}\,{}^t\mathbf{Q} \qquad (2)$$

The **Z** matrix is also expressed as

$$\mathbf{Z} = {}^t\mathbf{D}\mathbf{D} = {}^t(\mathbf{RC})(\mathbf{RC}) = {}^t\mathbf{C}({}^t\mathbf{R}\mathbf{R})\mathbf{C} \qquad (3)$$

Comparing eqs 2 and 3, we obtain

$${}^t\mathbf{Q} = \mathbf{C}, \quad ({}^t\mathbf{R}\mathbf{R}) = \mathbf{E} \qquad (4)$$

The resulting eigenvalue λ in eigenmatrix **Q** represents a relative importance in the relevancy of the associated eigenvector; the number of larger eigenvalues corresponds to the number of independent components. Table 1 shows λ values against *m*, the number of factors assumed for ACAM-d_2, PRAM-d_2, and POAM-d_2 in D_2O solution.

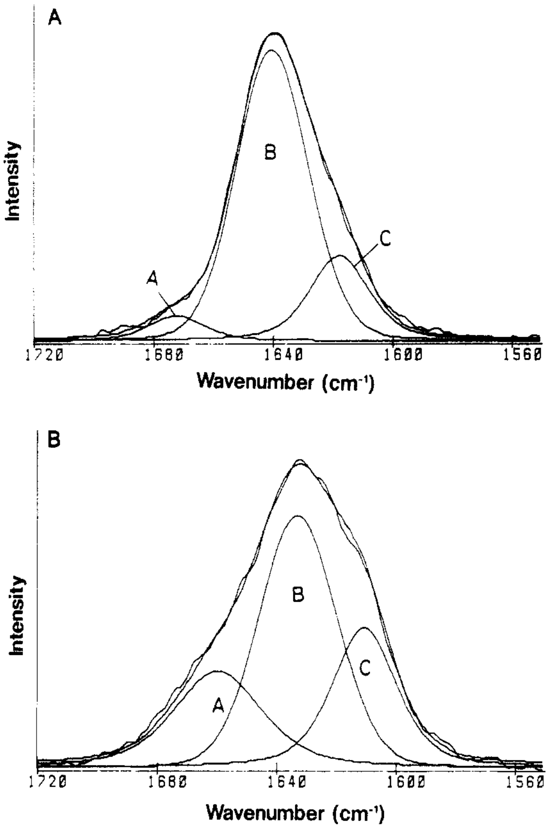

Figure 4. Separation of the carbonyl stretching band of acetamide in D_2O solution into Voigt components: (A) 10 wt %; (B) 70 wt %.

Among the seven values of λ (six values for POAM-d_2), three values are by far larger than the other λ values. Hence, the number of components was concluded to be three for all these solutions. This is consistent with the result from the polarization measurements and deconvolution.

E. Least-Squares Curve Fitting. According to the results from the polarization measurements, deconvolution, and factor analysis, we divided the stretching C=O band of ACAM-d_2, PRAM-d_2, and POAM-d_2 into three individual peaks given by a linear combination of Gaussian and Lorentzian functions. A resolved spectrum in the C=O stretching region for the 10 wt % D_2O solution of ACAM is shown in Figure 4A. In the concentrated region (70 wt %), the intensity ratio of three peaks varied as shown in Figure 4B. The intensity of the central peak decreased, whereas those at both ends increased. The same was found for the peaks in the C=O stretching region for the D_2O solutions of PRAM-d_2 and POAM-d_2.

Self-association of the amides through hydrogen bonding might be remarkable under the present experimental conditions, because the mole fraction of the amides was relatively high (0.035–0.433 for ACAM-d_2 and 0.029–0.383 for PRAM-d_2) and the number of water molecules which surrounded the amide molecules was not sufficient to prevent the amides from self-associating. Two kinds of self-association were proposed for alkyl amides. One is a linear hydrogen bonding between a hydrogen atom of an NH_2 group and an oxygen atom of a carbonyl group of another molecule. The other was a closed dimer formed by two hydrogen bonds like carboxylic acids.[50–52] Since bands A and C increased their areas with concentration (Figure 4A,B), these two bands correspond to the stretching of the carbonyl group of the associated amide. Band B corresponds to the C=O stretching mode of a non-self-associated amide molecule.

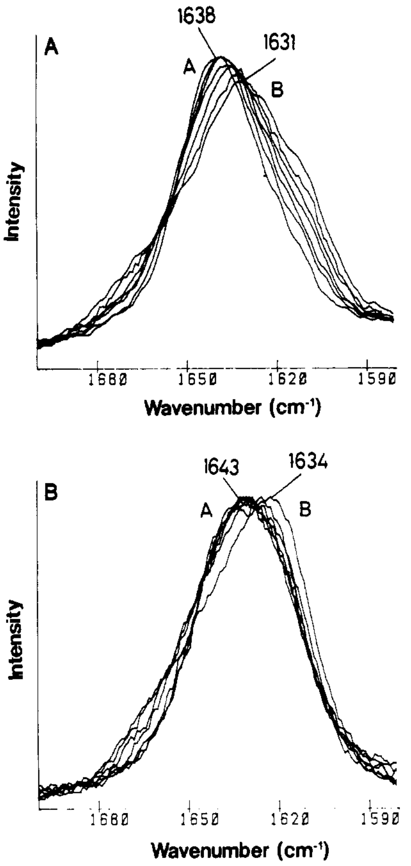

Figure 5. (A) Raman spectra of D_2O solution of acetamide at 25 °C in the region from 1600 to 1700 cm^{-1} (concentration range 10 (B) to 70 wt % (A)). (B) Raman spectra of D_2O solution of propionamide at 25 °C in the region from 1600 to 1700 cm^{-1} (concentration range 10 (B) to 70 wt % (A)).

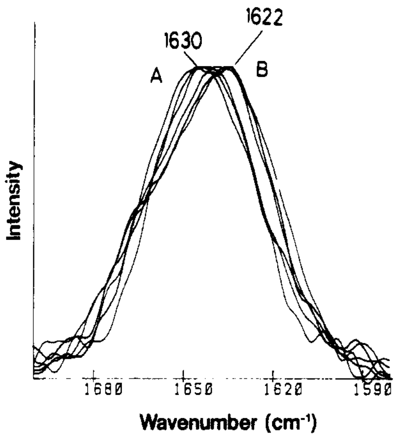

Figure 6. Raman spectra of D_2O solution of polyacrylamide (M_w = 1500) at 25 °C in the region from 1600 to 1700 cm^{-1} (concentration range 10 (B) to 60 wt % (A)).

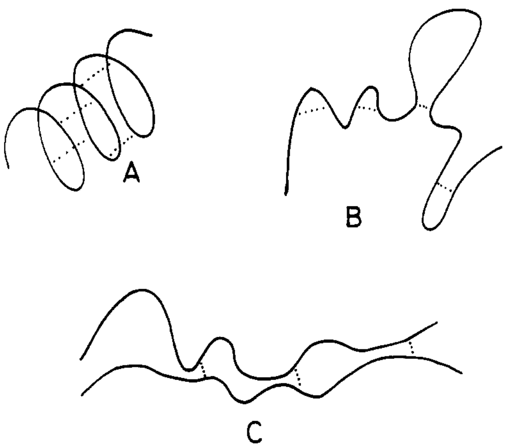

Figure 7. Schematic diagram showing three types of hydrogen bonding expected in D_2O solution of polyacrylamide: (A) intramolecular hydrogen bonding in the helical structure; (B) intramolecular hydrogen bonding in extended segments; (C) intermolecular hydrogen bonding.

According Kobayashi *et al.*,[20-23] the hydration at the carbonyl oxygen is in an equilibrium state among the various states of hydration depending on the number of hydrating water molecules. The C═O stretching modes of the different hydration states were separated into five Gaussian or Voigt functions. Their result is contradictory to our results, which were evaluated by the factor analysis, which indicated the predominance of the three components. The other two components found by Kobayashi *et al.* did not appear, probably because they were too small compared to the two components of the self-association.

F. Self-Association of Alkyl Amides. Figure 5 shows the comparison of the spectral change of the C═O stretching bands of ACAM-d_2 and PRAM-d_2 in D_2O solutions. Both of these bands similarly changed their shapes as the concentration varied. The half-width of the carbonyl stretching band of PRAM was, however, always larger than that of ACAM at the same concentration. The curve resolution clarified that the areas of band A and band C for PRAM-d_2 in Figure 5B are larger than those for ACAM-d_2 in Figure 5A. This shows that the self-association of PRAM in D_2O solution is more significant than that of ACAM. The same procedure was used for BTAM-d_2 in D_2O solutions at 10 and 20 wt %. BTAM-d_2 could not be dissolved above *ca.* 30 wt %.

If hydrophobic interaction influences the self-association of alkyl amide as found in the self-association of carboxylic acid in aqueous solutions,[50-52] BTAM-d_2 must self-associate more strongly than PRAM-d_2. The spectra of the C═O stretching band of BTAM-d_2 at these concentrations were, however, almost identical to that of PRAM-d_2 at the same concentration. Therefore, the quantitative difference between the self-associations of ACAM-d_2 and PRAM-d_2 may be due to the difference in their structures.

G. Association between Amide Groups of POAM. By the similar method adopted for ACAM-d_2 and PRAM-d_2, we investigated the C═O stretching band of POAM-d_2 in D_2O solutions. As shown in Figure 6, the C═O stretching band of POAM1500 shifted to a higher frequency as the concentration was increased. These bands are also composed of three individual peaks like ACAM-d_2 and PRAM-d_2 as clarified by the factor analysis (Table 1). The curve fitting of this band by the Voigt function showed that areas of the bands which were assigned to the associated C═O stretching band were larger than those of PRAM-d_2. Therefore, the association of an amide group with another amide was enhanced in the order of ACAM-d_2, PRAM-d_2, and POAM-d_2.

POAM-d_2 can have two kinds of cross-linkings: intra- and intermolecular cross-linkings through hydrogen bonding. We compared the C═O stretching bands of POAM1500, POAM100000, and POAM700000 in D_2O solutions of 10 wt %. The spectra of these solutions were almost the same, which means that the molecular weight does not affect the cross-linking.

Susceptible forms of the cross-linking of POAM-d_2 are schematically presented in Figure 7. The helical structure of type A and the extended structure of type B show intramolecular cross-linking, and type C is due to intermolecular cross-linking. Polymer complexes through hydrogen bonding (such as poly(acrylic acid)–polyacrylamide, poly(acrylic acid)–poly(vinylpyrrolidinone), and poly(acrylic acid)–poly(ethylene glycol)) are formed by the cooperative interaction between two macromolecules, and drastic chain-length dependences are found in this phenomenon.[53] If we assume cross-linking of type C for the association of POAM-d_2 in D_2O solutions, the association should increase with molecular weight due to the cooperative interaction between two macromolecules.

As mentioned above, however, the degree of intramolecular hydrogen bonding of POAM1500 in D_2O solutions was the same as those of higher molecular weight samples which can easily form the helical structure. The degree of polymerization of POAM1500 is *ca.* 20, and the polymer of such a small degree of polymerization cannot form the helical structure of type A, which shows that the hydrogen bond of type B is relatively more plausible for POAM.

The molecular weight dependence of the hydrogen bonding of poly(acrylic acid) is the same as that of POAM: the K_D values (dimerization constant for carboxylic groups) of poly(acrylic acid) of $M_w = 1000$ and that of $M_w = 90000$ were almost the same.[1] The hydrogen bond in the extended structure may be formed for both poly(acrylic acid) and POAM in aqueous solutions. In the case of poly(acrylic acid), the amount of hydrogen bonding of the polymer was smaller than that of the monomer analogue,[1] whereas the reverse was found for the hydrogen bonding of POAM. Most of the hydrogen bonds between carboxyl groups of poly(acrylic acid)s in aqueous solution are of a "closed" type in which two carboxyl groups form a ring structure through the two hydrogen bondings between oxygen atoms of carbonyl groups and hydrogen atoms of hydroxyl groups.[1] The amount of the hydrogen bonding of poly(acrylic acid) is, therefore, smaller than that of its monomer analogue because of a steric restriction. "Closed" type and "linear" type are, on the other hand, proposed for the forms of the hydrogen bonding of POAM in D_2O solutions. The steric restriction for the hydrogen bonding is small in this case, which may be the reason for the difference between the hydrogen bondings of poly(acrylic acid) and that of POAM.

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