¹H NMR Study of Hindered Internal Rotation and Hydrogen Exchange of Amide Side Chain of Poly(acrylamide) in Aqueous Solution

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ABSTRACT: Structure and dynamics properties of poly(acrylamide)(PAAm) side-chains are investigated by ¹H NMR methods. The multiplicity of ¹H spectra of NH₂ protons of PAAm side-chains is attributed to the tacticity splitting at the triad level. The proton exchange and the hindered internal rotation of the NH₂ group are studied by examining pH and temperature dependences of the ¹H spectra. It is found that the proton exchange rates of the two NH₂ protons with solvent water are almost the same. The activation energy of hindered internal rotation is found to be about $58 \pm 9 \text{ kJ mol}^{-1}$. The present results indicate that there is no hydrogen bonding between amide groups of neighboring units.

KEY WORDS High-Resolution NMR / Poly(acrylamide) / Nuclear Overhauser Effect / Two Dimensional NMR / Hindered Internal Rotation / Proton Exchange /

It has been widely pointed out that proton exchange of amide groups of protein¹ and synthetic polymer amides^{2,3} is strongly influenced by the secondary and tertiary structure. In this work, we studied poly(acrylamide) (PAAm), which has an amide group as the side chain. The micro-structure of PAAm has been studied by Raman, infrared,⁴ and NMR spectroscopies.^{5,6,7} A two-dimensional NMR (2D NMR) study established the assignment of ¹H signals of the PAAm main chain at the triad-tetrad level, and ¹³C signals at the pentad-hexad level.⁶ In this work, firstly, we studied the micro-structure of side chain NH₂ protons by using the two-dimensional nuclear Overhauser enhancement (2D NOE) method. Secondly, we studied the exchange of the NH₂ protons with solvent water protons. The

hindered internal rotation of the NH₂ group about its C–N bond was also investigated.

EXPERIMENTAL

PAAm(molecular weight of 74000) purchased from Polyscience, Inc. was dissolved in water (D₂O/H₂O=10/90) at a concentration of 10 wt%. The pH was adjusted to 5.0 by HCl and NaOH solution. NMR experiments were performed on a JEOL JNM-GX400 spectrometer operating at 400 MHz for ¹H at 298 K unless otherwise stated. The temperature was controlled within 1 K using a JEOL temperature controller. Sodium trimethylsilylpropionate is used as an internal reference. The 2D NOE spectrum was obtained with a mixing time of 200 ms. The time domain data matrix

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of 256×2048 points was obtained. The data matrix was zero-filled to 512×4096 . Sinebell-sinebell window functions were multiplied in both dimensions prior to Fourier transformation.

RESULTS AND DISCUSSION

2D NOE experiment

Figure 1 shows the amide region of the ^1H NMR spectrum of PAAm. Two groups of resonances appear in this region, indicating that the two NH_2 protons are magnetically nonequivalent. This is attributed to restricted rotation of the NH_2 group about the $\text{C}-\text{N}$ bond. The amide group prefers a planar structure. One of the two resonances is due to the proton *trans* to the $\text{C}=\text{O}$ oxygen, and the other is *cis* to the $\text{C}=\text{O}$ oxygen. Several approaches have been proposed to assign the *cis/trans* signals of NH_2 protons; such as utilizing the inequality of *cis* and *trans* spin-spin coupling constants,^{8,9} different solvent shifts,¹⁰ and nuclear Overhauser effects (NOE).¹¹ In this work, we adopted the NOE approach.

Figure 2 shows a cross-peak region of the NH_2 protons and the CH proton of the $^1\text{H}-^1\text{H}$ 2D NOE spectrum. The intensity of cross-peak between the low-field NH_2 and the CH signals is larger than those between the high-field NH_2 and the CH signals. This indicates that the proton resonating at the low field is closer to the main-chain CH proton than that at the high field. By examining molecular model, it can be shown that the NH_2 proton *trans* to the $\text{C}=\text{O}$ oxygen is closer to the main chain. The low-field peak is, therefore, assigned to the NH_2 proton *trans* to the $\text{C}=\text{O}$ oxygen. This assignment is in accord with those of small molecules such as acetamide.¹²

A closer examination of the NH_2 signals shows that the *trans* signal splits into a triplet, and that the *cis* signal has an asymmetric broad line-shape (Figure 1). The splitting is attributed to the stereo-configurations of the main-

Figure 1. ^1H NMR spectrum of NH_2 protons of PAAm at 298 K and chemical structure of a monomer unit.

Figure 2. Expanded view of a cross-peak region of NH_2/CH peaks of 2D NOE spectrum.

chain.⁶ As shown in Figure 2, each of the triplet signals has cross-peaks with each of the CH triplet signals. The low-, middle-, and high-field triplet resonances of the CH proton have been assigned to the *mm*, *mr*, and *rr* configurations, respectively.⁶ On the basis of the cross-peak pattern, the low-, middle-, and high-field resonances of the *trans* NH_2 signals are, therefore, assigned to the *rr*, *mr*, and *mm* configurations, respectively. However, it must be noted that their relative intensities are not consistent with the previous results,⁵⁻⁷ which indicated that the *mr* configuration is more populated. At present, we can not explain the inconsistency. Further study is needed.

The asymmetric broad line-shape of the *cis*

Figure 3. Expanded view of a diagonal/cross-peak region of NH₂ peaks of 2D NOE spectrum.

Table I. Assignment and chemical shift value of the NH₂ signals of PAAm at 298 K

	Configuration	Chemical/shift/ppm
<i>cis</i>	<i>rr</i>	7.03
	<i>mr</i>	6.99
	<i>mm</i>	6.96
<i>trans</i>	<i>rr</i>	7.77
	<i>mr</i>	7.70
	<i>mm</i>	7.60

NH₂ signal is probably due to the unresolved signals of stereo-configurations. Cross-peaks are also observed between the *cis* NH₂ signals and the triplet CH signals. The cross-peaks are not so well resolved to be assigned.

Figure 3 shows a diagonal/cross-peak region of the two NH₂ signals. Several cross-peaks are observed between the *trans* NH₂ signals and the *cis* NH₂ signals. On the basis of the cross-peak pattern, we assign the low-, middle-, and high-field resonances of the *cis* NH₂ signal to the *rr*, *mr*, and *mm* configurations, respectively. Table I lists the results of assignment of the NH₂ resonances of PAAm at 298 K.

pH Dependence

The proton exchange between NH₂ protons and water has been examined for several amide compounds. Bovey and Tiers showed that the

Figure 4. pH Dependence of spectra of PAAm NH₂ protons at 298 K.

exchange of NH₂ protons of PAAm with water is catalyzed by both acid and base.¹³ Perin showed that the proton exchange between water and the *trans* proton is faster than that between the *cis* proton for several amides. The relative ratio of exchange rates (k_{trans}/k_{cis}) is about 5.1 for acetamide and 1.7 for isobutylacrylamide. They concluded that the difference is due to the steric hindrance of substituent at the C=O carbon.¹⁴

For PAAm, one may expect hydrogen bonding between the side-chain amide groups of the neighboring units. Such extra interaction would affect the exchange rate. In order to investigate possible hydrogen bonding between side chain amide groups, we observe pH dependence of the NH₂ signals. The results are shown in Figure 4. The NH₂ signals are found

to broaden with increasing pH.

The rate of exchange between the NH_2 protons and water protons k is deduced from linewidth as follows.¹⁵

$$k = \pi[v_1 - v_0], \quad (1)$$

where v_1 denotes the full linewidth at each pH and v_0 denotes the full linewidth without proton exchange. It has been shown that the exchange rate is negligibly small at about pH 5.0 for most amide compounds.^{2,3,14} Hence, we adopt the full linewidth observed at pH 5.0 as v_0 . The resolution of the spectra is insufficient to analyze each exchange rate for the protons of different stereo-configurations. For simplicity, we assume that the *trans* and *cis* signals are single resonances, and that v_0 of the *cis* and *trans* peaks are 20 Hz and 40 Hz, respectively. A detailed procedure to define v_0 will be described in the following section. The exchange rate was evaluated for the *cis* peak (k_{cis}) and for the *trans* peak (k_{trans}). The $k_{\text{trans}}/k_{\text{cis}}$ were calculated in a range of pH 8.2 to 9.1. It is found that the relative ratios remain almost constant in this pH range. The average value of $k_{\text{trans}}/k_{\text{cis}}$ is 1.3 ± 0.1 , smaller than that of isobutylacrylamide. The results indicate that the proton exchange rates of *trans* and *cis* protons are not significantly different. If one of the NH_2 protons were intramolecularly hydrogen-bonded to a neighboring carbonyl group and the other to water, the exchange rate would markedly differ. This present result suggests hydrogen bond cannot be formed between NH_2 protons and neighboring $\text{C}=\text{O}$ oxygen. This may be due to the steric hindrance of the PAAm main-chain.

Temperature Dependence

The line shapes are influenced not only by the hindered internal rotation but also the proton exchange with water. As the temperature is raised, the rate of these processes increase. It is difficult to separate the hindered internal rotation and the proton exchange from line-shape data. Bovey and Tiers showed that the

Figure 5. Temperature dependence of spectra of PAAm NH_2 protons at pH 5.0.

rate of hindered internal rotation of PAAm is faster than that of proton exchange at about pH 4.5; the line-shape change was dominated by the hindered rotation up to about 400 K.¹³ We adjusted the pH to about 5.0 to minimize line broadening due to the proton exchange.^{2,3,14}

Figure 5 shows the temperature dependence of the NH_2 signals. The figure indicates that there is an apparent upfield shift of the whole NH_2 signals with increasing temperature below 348 K. Above 348 K, the resonances of the NH_2 protons become broad with increasing temperature. The upfield shift of NH_2 signals is ascribed to breaking of hydrogen bond between the NH_2 protons and water. The broadening is attributed to motional broadening due to the hindered internal rotation of the NH_2 group about its C–N bond.

In order to estimate the activation energy

Figure 6. The observed ^1H spectra of the NH_2 group at 348–368 K (the left-hand side) and the simulated spectra (the right-hand side) together with the best-fit exchange rates.

of hindered internal rotation, the line-shape analysis was done for spectra at 348 K–368 K by using DNMR5 program.¹⁶ The NH_2 rotation is assumed to be the two-site π -flip. For simplicity, we assume that each of *trans* and *cis* peaks is a singlet. The natural linewidth for the *cis* peak was taken from the spectrum at 298 K to be 20 Hz. The natural linewidth for the *trans* peak was taken as a fitting parameters in DNMR5. Other parameters are chemical shift, exchange rate, baseline height, and baseline tilt. The “best-fit” linewidth for the *trans* peak at 348 K was found to be 40 Hz. This value was used as the natural line-width for the *trans* peak in the analysis of the temperature and pH dependence experiments in the previous section.

The simulation was executed from 348 K to 368 K. The observed spectra are shown at the left-hand side of Figure 6. The simulated spectra with the best-fit exchange rate are shown at the right-hand side of Figure 6. Assuming an Arrhenius type of temperature

dependence for the exchange rate, we deduce an activation energy E_a to be $58 \pm 9 \text{ kJ mol}^{-1}$. It has been reported that E_a of hindered internal rotation of the NH_2 group of acetamide in acetone is about 70 kJ mol^{-1} .¹² The NH_2 group of PAAm are easier to rotate than that of acetamide in acetone. This should be reversed if hydrogen bonding exists between neighboring units of PAAm.

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