¹³C NMR Relaxation Study of Segmental Motion of Poly(*N*^ε-hydroxyethyl L-glutamine) in Aqueous Solution

Shinichi UCHINO,[†] Toshifumi HIRAOKI, and Akihiro TSUTSUMI

*Department of Applied Physics, Graduate School of Engineering, Hokkaido University,
Kita-13 Nishi-8, Kita-ku, Sapporo 060-8628, Japan*

(Received September 11, 2003; Accepted November 29, 2003)

ABSTRACT: To study the segmental motion of poly(*N*^ε-hydroxyethyl L-glutamine) (PHEG) in aqueous solution, the ¹³C spin–lattice relaxation time (*T*₁) and the nuclear Overhauser enhancement (NOE) were measured at six resonance frequencies ($\omega_C/2\pi$) ranging from 15 to 150 MHz and at 100 MHz, respectively, at the temperatures from 20 to 90 °C. For the backbone C α , plots of $\log(T_1/\omega_C)$ against $\log(\omega_C)$ gave the well superposed master curve, showing that the frequency–temperature reduction rule is realized. The shift factor obeyed the Arrhenius type temperature dependence with the activation energy of 29.6 kJ mol^{−1}. Using this activation energy for the temperature dependence of the correlation time, the master curve and NOE were well reproduced by the Dejean–Lauprêtre–Monnerie (DLM) model. One of parameters relating to the segmental motion was $\tau_0/\tau_1 = 5$, where τ_0 and τ_1 are the correlation times for the isolated single and correlated pair conformational transitions, respectively. Comparison of this parameter with that for other vinyl polymers showed that PHEG having planar peptide groups in the backbone belongs to polymer group with smaller values of τ_0/τ_1 . τ_0/τ_1 was discussed in the relation to the distribution width of the correlation time. The spectral density function $J(\omega)$ has the exponent to the correlation time τ_a as $\omega J(\omega) \sim (\omega\tau_a)^{0.61}$ in the region of $\omega\tau_a < 0.2$ which is close to the value of 0.5 from the DLM model.

KEY WORDS ¹³C NMR Relaxation / Poly(*N*^ε-hydroxyethyl L-glutamine) / Segmental Motion / Aqueous Solution / Time–temperature Reduction / DLM Model / Law of Exponent for Power Spectrum /

Molecular conformation and dynamics of synthetic polypeptides have been investigated in various solvents and under various conditions such as the temperature, pH and etc. as the model substances of proteins in order to obtain their functions. For such studies, nuclear magnetic resonance (NMR) spectroscopy gives the fruitful information through the careful examinations on the chemical shift and the relaxation measurements, the spin–lattice relaxation time (*T*₁), spin–spin relaxation time (*T*₂) and nuclear Overhauser enhancement (NOE).^{1,2} Particularly, the motion of polymer backbone was characterized by the ellipsoidal motion for the α -helix and by the isotropic rotation with the distribution of the correlation time for the segmental motion of random coil.^{3–5} Internal rotation in the side chain was investigated by use of several models such as unrestricted rotational diffusion model,⁶ restricted rotations model⁷ and rotational jump model.^{8,9}

For most of the polymers other than polypeptides, the dynamical studies by the NMR relaxation have been performed in the random coil state. The segmental motion of the backbone has been discussed by taking account of the distribution of the correlation time such as Cole–Cole distribution¹⁰ and $\log \chi^2$ distribution.¹¹ The more realistic relaxation model based on

the polymer dynamics has been recently proposed by Dejean, Lauprêtre, and Monnerie as DLM model.^{12–15} This model which includes the effective correlation times for the single conformational transition τ_0 , the correlated pair conformational transition τ_1 and the libration τ_2 as seen below well interprets the experimental relaxation data for various polymers in solution or bulk,^{16–26} assuming that the temperature dependence of the correlation times is Arrhenius type or WLF type whereas the ratios of the three correlation times are temperature independent. However, there have been no reports by the DLM model for polypeptides. It is of interest to apply this model to polypeptides in the random coil state and investigate the more detailed information about the segmental motion.

Poly(L-glutamine)s with alkyl alcohols substituted to the side chain *N*^ε have been investigated to examine the effects of the hydrophobicity of side chains on the polymer conformation.²⁷ Addition of alcohols such as methanol or ethanol to the aqueous solution of these polymers makes the α -helical conformation more stable. However, polymers that have shorter alkyl side chains have a tendency to take the random coil state in aqueous solution even though the concentration of alcohols is more than 60 %. This is the reason why

[†]To whom correspondence should be addressed (Tel: +81-11-706-6642, Fax: +81-11-716-6175, E-mail: schwarz@eng.hokudai.ac.jp).

the aqueous solution of poly(*N*^ε-hydroxyethyl L-glutamine) (PHEG) was selected in this work. Furthermore, when D₂O is selected as a solvent, ¹³C NMR relaxation has the advantage that no influence from the solvent exists on the signals of polymer.

The experimental data covering wide frequency range have been taken to obtain the precise information about the polymer dynamics in the measurements of dynamic mechanical and dielectric relaxations, since polymers undergo generally the molecular motion over a wide frequency range. However, there are few approaches covering such wide frequency range by the NMR relaxation since each NMR spectrometer is fixed at one frequency. In many cases, only two or three frequencies have been used to cover the frequency range even for the studies of polymer dynamics using the DLM model mentioned above. In the DLM model, fixing the ratios of the three correlation time constants seems to imply that the shape of distribution function of correlation times is independent of the temperature. To assess the distribution function, the experimental data covering wide frequency range may be required. For this reason, ¹³C NMR relaxation in the PHEG/D₂O solution system was measured at six frequencies from 15 to 150 MHz in this work. The relaxation data covering the wide frequency range may make possible to apply the scaling law that is well known as the time-temperature reduction law and to estimate the model-independent apparent activation energy of the correlation time. The applicability of the DLM model is investigated and furthermore the law of exponent of the spectrum density functions by segmental motion is discussed based on thus obtained results.

EXPERIMENTAL

Poly(*N*^ε-hydroxyethyl L-glutamine) (PHEG see Figure 1) was prepared from poly(*γ*-benzyl L-glutamate) (polymerized from *N*-carboxyanhydride in dioxane at 40 °C, molecular weight of 48,000 estimated from the intrinsic viscosity in dichloroacetic acid) according to the original procedure of Lupu-Lotan *et*

Figure 1. Chemical structure of poly(*N*^ε-hydroxyethyl L-glutamine).

al.^{28,29} with a few modifications by the reaction with 2-aminoethanol in dioxane at 75 °C.

D₂O (99.96 % D, CEA) solution of 5–7 % (w/v) PHEG was prepared for NMR relaxation measurements. ¹³C spin–lattice relaxation time (*T*₁) was measured by the 180°–*τ*–90° inversion recovery pulse sequence at six resonance frequencies; JEOL FX-60Q, JEOL FX-100, Varian XL-200, JEOL FX-400, Bruker AM-500 and Bruker AMX-600 spectrometers operating at 15 MHz, 25 MHz, 50 MHz, 100 MHz, 125 MHz and 150 MHz, respectively. The delay between the sequences was at least five times of the largest estimated *T*₁ with an accuracy of ±5 %. Temperature range was 20 to 90 °C. NOE was estimated as the ratio of ¹³C signal intensities obtained by proton noise decoupling to those obtained by inverse gate decoupling measured at 100 MHz with an accuracy of ±15 %. Temperature dependence of *T*₁ and the spin–spin relaxation time *T*₂ of the deuteron in D₂O were measured at 41 MHz by use of JEOL FX-270 spectrometer.

THEORETICAL BACKGROUND

Using the assumption of the dipolar interaction with ¹H and ¹³C, *T*₁ and NOE of ¹³C are given by,^{30,31}

$$\frac{1}{T_1} = \frac{\Omega}{10} [J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)], \quad (1)$$

$$\Omega = \left(\frac{\mu_0 \gamma_H \gamma_C \hbar}{4\pi r_{CH}^3} \right)^2 \quad (2)$$

and

$$\text{NOE} = 1 + \frac{\gamma_H}{\gamma_C} \times \left[\frac{6J(\omega_H + \omega_C) - J(\omega_H - \omega_C)}{J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)} \right] \quad (3)$$

where γ_H and γ_C are the gyromagnetic ratios for ¹H and ¹³C, ω_H and ω_C their resonance angular frequencies, respectively, r_{CH} the internuclear distance, μ_0 the permeability in vacuum, $\hbar = h/2\pi$ where h is the Planck's constant. $J(\omega)$ is the spectral density function defined by

$$J(\omega) = \frac{1}{2} \int_{-\infty}^{\infty} G(t) \exp(-i\omega t) dt, \quad (4)$$

where $G(t)$ is the normalized second-order spherical harmonic autocorrelation function. Obtaining the form of $G(t)$ is therefore a decisive step in the interpretation of the relaxation data. For small molecules undergoing the isotropic spherical rotation, $G(t)$ is a single exponential decaying function with the correlation time τ_c ,

Figure 2. ¹³C NMR spectrum of poly(*N*^ε-hydroxyethyl L-glutamine) in D₂O at 100 MHz and 30 °C.

$$G(t) = \exp\left(-\frac{|t|}{\tau_c}\right). \quad (5)$$

Therefore, $J(\omega)$ can be written as

$$J(\omega) = \frac{\tau_c}{1 + \omega^2 \tau_c^2}. \quad (6)$$

This spectral density is derived from BPP (Bloembergen–Purcell–Pound) theory.³² In the case of polymers, due to the constraint resulting from the mobility of the chain, motions that responsible for the NMR relaxation are usually too complicated to be described by such a simple picture. One of the comprehensive pictures of the polymer motions is the Dejean–Lauprêtre–Monnerie (DLM) model that is modified from Hall–Helfand model^{14,15} for the backbone motion. The autocorrelation function of the DLM model is given by

$$G(t) = (1 - a) \exp\left(-\frac{t}{\tau_0}\right) \exp\left(-\frac{t}{\tau_1}\right) \times I_0\left(\frac{t}{\tau_1}\right) + a \exp\left(-\frac{t}{\tau_2}\right), \quad (7)$$

where I_0 is the modified Bessel function of order zero, τ_1 the correlation time associated with correlated jumps responsible for orientation diffusion along the chain, τ_0 the correlation time resulting from the damping which consists either of non-propagating specific motions or of distortions of the chain with respect to its most stable local conformations. τ_2 is the correlation time associated with the local anisotropic reorientation, *i.e.*, the libration proposed by Howarth³³ in terms of a random cone of half-angle θ , the axis of which is the rest position of C–H bond, and

$$1 - a = \frac{1}{4} [\cos \theta (1 + \cos \theta)]^2. \quad (8)$$

The spectral density function is given by Fourier

transform of Eq 7 as

$$J(\omega) = \text{Re} \left[\frac{1 - a}{(\alpha + i\beta)^{1/2}} + \frac{a\tau_2}{1 + i\omega\tau_2} \right] \\ = \frac{1 - a}{(\alpha^2 + \beta^2)^{1/4}} \left[\cos \left[\frac{1}{2} \tan^{-1} \left(\frac{\beta}{\alpha} \right) \right] \right] \\ + \frac{a\tau_2}{1 + \omega^2 \tau_2^2}, \quad (9)$$

where

$$\alpha = \tau_0^{-2} + 2\tau_0^{-1}\tau_1^{-1} - \omega^2 \\ \beta = 2\omega(\tau_0^{-1} + \tau_1^{-1}). \quad (10)$$

RESULTS AND DISCUSSION

¹³C NMR spectrum of PHEG in D₂O at 100 MHz and 30 °C is represented in Figure 2. Horizontal axis is indicated as the chemical shift from TMS in ppm by using dioxane as an internal reference of 67.8 ppm. Signals of backbone C α and C'=O appear at 54.9 and 174.2 ppm, respectively, corresponding to the randomly coiled state of the polymer chain. Dependence of the chemical shift of backbone carbons on the CD₃OD (99.8 % D, CEA) content in D₂O is shown in Figure 3. With increasing CD₃OD content, no change of the chemical shift occurs below 60 % CD₃OD. Another peak appears at 57.2 and 176.9 ppm for C α and C'=O, respectively, at CD₃OD 60 % where the random coil to helix transition occurs.³⁴ The intensity of these peaks increases rapidly with increase of CD₃OD content. It was further observed that the transition region of coil to helix shifts to higher CD₃OD contents with increase of the temperature. It is therefore recognized that the conformation of PHEG is the random coil in D₂O at the temperatures of present experiments.

Figure 4 shows the plots of T_1 for C α measured at

Figure 3. ^{13}C chemical shift plotted against CD_3OD % (v/v) for backbone $\text{C}\alpha$ (○) and $\text{C}'=\text{O}$ (△) in the mixed solvents of D_2O and CD_3OD .

Figure 4. Temperature dependence of the spin lattice relaxation time (T_1) of $\text{C}\alpha$ at the frequencies 15 MHz (●), 25 MHz (■), 50 MHz (▲), 100 MHz (○), 125 MHz (□) and 150 MHz (△).

six frequencies against the inverse of absolute temperature $1/T$. At lower frequencies below 100 MHz, T_1 monotonously decreases against $1/T$. On the other hand, a minimum appears at higher frequencies 125 and 150 MHz. These observations indicate that molecular motion of the backbone $\text{C}\alpha$ is in “fast” to “intermediate” motional region and not “extremely narrow” region compared with the resonance frequencies used in this experiment.

In order to apply the time-temperature reduction law, measured data were superposed according to

Figure 5. Plots of $\log(T_1/\omega_C)$ of $\text{C}\alpha$ against the frequencies at representative temperatures, 20 °C (○), 30 °C (△), 40 °C (□), 50 °C (◇), 60 °C (●), 70 °C (▲), 80 °C (■) and 90 °C (◆).

the following procedures; interpolation was made to obtain the frequency dependence of T_1 at several common temperatures, but no extrapolation was made from the view of reliability, and reduced relaxation times $\log(T_1/\omega_C)$ were plotted against $\log(\omega_C)$ for each temperature. Dependence of $\log(T_1/\omega_C)$ on $\log(\omega_C)$ obtained by these procedures is shown in Figure 5 for representative temperatures. The highest temperature 90 °C was selected as the standard temperature, and data at lower temperatures were shifted in parallel to the horizontal axis ($\log(\omega_C)$ axis) to superpose smoothly on the curve of standard temperature.

According to these procedures, a well superposed master curve of $\log(T_1/\omega_C)$ over the double-digit frequency range was obtained as shown in Figure 6, where the horizontal axis is the product of ω_C and the shift factor a_T . The $\log(a_T)$ is plotted against the inverse of absolute temperature in Figure 7. Fairly well straight line is obtained and its slope gives the activation energy E_a of 29.6 kJ mol $^{-1}$. This fact suggests that the temperature dependence of the correlation time τ_a is the Arrhenius type and/or relaxation mech-

Figure 6. The master curve for $\log(T_1/\omega_C)$ against $\log(\omega_C a_T)$ by the application of the time-temperature reduction procedures to the data obtained from 20 to 90 °C, where a_T is the shift factor. The standard temperature is selected as 90 °C.

Figure 7. Temperature dependence of $\log a_T$ against $1/T$. The straight line is obtained by the least squares method and square of the correlation coefficient is 0.998. The slope gives the activation energy of 29.6 kJ mol^{-1} .

anism is Orbach process,³⁵ since the following relation holds among a_T , τ_a and T

$$a_T \propto \tau_a = \tau_\infty \exp\left(\frac{E_a}{RT}\right), \quad (11)$$

where R is the gas constant and τ_∞ the pre-exponential factor as a constant independent of T . Therefore, $\log(\omega_C a_T)$ can be replaced to $\log(\omega_C \tau_a)$ which contains quantities equivalent to the logarithm of frequency and the inverse of the absolute temperature, that is,

$$\log(\omega_C \tau_a) = \log(\tau_\infty) + \log(\omega_C) + 0.4343 \frac{E_a}{RT}. \quad (12)$$

This means that the master curve of ω_C/T_1 can be transformed to a function of the product of the frequency and the correlation time. Using T_1 in Eq 1 and $J(\omega)$ for the single distribution in Eq 6, ω_C/T_1 can be expressed as a function of only $\omega_C \tau_c$ as

$$\frac{\omega_C}{T_1} = \frac{\Omega}{10} \left[\frac{(\omega_C \tau_c)}{1 + \left(\frac{\gamma_H}{\gamma_C} - 1\right)^2 (\omega_C \tau_c)^2} + \frac{3(\omega_C \tau_c)}{1 + (\omega_C \tau_c)^2} + \frac{6(\omega_C \tau_c)}{1 + \left(\frac{\gamma_H}{\gamma_C} + 1\right)^2 (\omega_C \tau_c)^2} \right]. \quad (13)$$

In the case of DLM model, when $\tau_1/\tau_0 \equiv k_1$ and $\tau_2/\tau_1 \equiv k_2$ are referred to as constant, $\omega J(\omega)$ is expressed as

$$\omega J(\omega) = \frac{1-a}{(A^2+B^2)^{1/4}} \left[\cos\left(\frac{1}{2} \tan^{-1} \frac{B}{A}\right) \right] + \frac{ak_2(\omega\tau_1)}{1+k_2^2(\omega\tau_1)^2}, \quad (14)$$

where

$$\begin{aligned} A &= k_1^2(\omega\tau_1)^{-2} + 2k_1(\omega\tau_1)^{-2} - 1 \\ B &= 2(k_1+1)(\omega\tau_1)^{-1}. \end{aligned} \quad (15)$$

It is also recognized that ω_C/T_1 is expressed as a function of only $\omega_C \tau_1$ as well as Eq 13. For the Cole–Cole distribution which has been frequently used in the analysis of relaxation behaviors of polymers, $\omega J(\omega)$ is written as^{10,36}

$$\omega J(\omega) = \frac{1}{2} \frac{\sin \frac{\gamma\pi}{2}}{\cosh(\gamma \ln \omega\tau_m) + \cos \frac{\gamma\pi}{2}}, \quad (16)$$

where τ_m is the most probable correlation time, and γ is the parameter of this distribution ($0 < \gamma < 1$). ω_C/T_1 is again expressed as a function of $\omega_C \tau_m$.

Using the activation energy of 29.6 kJ mol^{-1} for the correlation time, the applicability of the DLM model to present NMR data was investigated. In addition, calculations were also made for the Cole–Cole distribution and the single distribution for comparison. The C–H internuclear distance r_{CH} was 0.109 nm .³⁹ For the single distribution, the modified form of the spectral density,

$$J(\omega) = \frac{A\tau_c}{1 + \omega^2\tau_c^2} \quad (17)$$

was used in the calculation, where the parameter A was introduced for bottom raising of the T_1 minimum. Although further discussions are not given below, this modification corresponds to the model-free approach,^{37,38} assuming the case where the effective correlation time τ_c for the isotropic motion is large enough compared to the correlation time for the rapid internal motion, A being a function of the generalized order parameter for the internal motion.

The calculated curves of T_1 and NOE for three models are shown in Figures 8 and 9, respectively. The pre-exponential factor τ_∞ was estimated as $1.0 \times 10^{-14} \text{ s}$ from the position of the T_1 minimum for the three models. Other fitting parameters are listed in Table I. The fitting parameters for the DLM model are $\tau_0/\tau_1 = 5$, $\tau_1/\tau_2 = 100$ and $\theta = 21^\circ$, and express fairly well both of T_1 and NOE simultaneously. For the Cole–Cole distribution, although NOE was

Figure 8. $\log(T_1/\omega_C)$ against $\log(\omega_C \tau_a)$. The solid, broken and dotted lines represent the DLM model ($\tau_a = \tau_1$), the Cole–Cole distribution ($\tau_a = \tau_m$) and the single distribution ($\tau_a = \tau_c$), respectively. The fitting parameters in the calculated curve to observed results for each model are listed in Table I.

Figure 9. Temperature dependence of NOE at 100 MHz. The solid, dotted and broken lines represent the DLM model, the Cole–Cole distribution and the single distribution, respectively. The fitting parameters in each model are listed in Table I.

Table I. Values of parameters used in the calculations of T_1 and NOE for three models

Model	Fitting parameter
DLM	τ_0/τ_1 5
	τ_1/τ_2 100
	$1 - a$ 0.82
Cole–Cole	γ 0.73
Single	A 0.75

well in agreement with the experimental result, T_1 was not. For the single distribution, both of T_1 and NOE were not able to fit the experimental results. Here, it is emphasized again that all of these curves were obtained using the activation energy E_a of 29.6 kJ/mol estimated from the time–temperature reduction procedure. Instead of using only this activation energy, it was also tried to adjust E_a and τ_∞ giving the best fitted curve in each model. $E_a = 35$ kJ/mol and $\tau_\infty = 1.0 \times 10^{-15}$ s for the Cole–Cole distribution with $\gamma = 0.73$, and $E_a = 24.5$ kJ/mol and $\tau_\infty = 8.0 \times 10^{-14}$ s for the single distribution with $A = 0.75$ gave the best fitted curves of T_1 , although it was difficult to

fit the NOE curve simultaneously. On the other hand, for the DLM model, $E_a = 29.6$ kJ/mol and $\tau_\infty = 1.0 \times 10^{-14}$ s which are identical with the values from the time–temperature reduction procedure gave the best fitted curves of T_1 and NOE.

These facts indicate that NMR relaxations in PHEG/D₂O cannot be simply expressed by the semi-empirical Cole–Cole distribution where the shape of distribution in the logarithmic scale is symmetric around the most probable correlation time τ_m . The DLM model describes the segmental motion using the effective correlation times for isolated single conformational transition and the correlated pair conformational transition. In this connection, it seems important to understand more clearly the spectral density function from the viewpoint of the distribution of the correlation time. The distribution function is given by inverse Laplace transform⁴⁰ of the autocorrelation function 7 as follows;

$$F(s) d \ln s = \text{Re} \frac{1 - a}{\pi \left[\left(\frac{1}{s\tau_0} + \frac{2}{s\tau_1} - 1 \right) \left(1 - \frac{1}{s\tau_0} \right) \right]^{1/2}} d \ln s. \quad (18)$$

The contribution from the 2nd term of Eq 7 is transformed into the delta function and is not written here. s is the inverse of the correlation time τ and must be

$$\frac{1}{\tau_0} < s = \frac{1}{\tau} < \frac{1}{\tau_0} + \frac{2}{\tau_1}, \quad (19)$$

in order for $F(s)$ not to be zero. The shape of this distribution function is shown in Figure 10, where the curves for $\tau_0/\tau_1 = 2, 5, 10$, and 30 are drawn. The distribution has two horns at the outer-most part and spreads with increasing the ratio of τ_0/τ_1 . It is clear that the shape of the distribution function is essentially

Figure 10. The correlation time distribution for the DLM model. The solid, dotted, broken and chained curves represent the cases for $\tau_0/\tau_1 = 2, 5, 10$ and 30, respectively.

Table II. Values of parameters in DLM model for various polymers

Polymer/Solvent	τ_0/τ_1	τ_1/τ_2	$1 - a$	θ/deg	$E_a/\text{kJ mol}^{-1}$	τ_∞/fs	References
PVC/DMSO	15	40	0.63	31	35.9*	0.76*	16
PVC/dioxane- <i>d</i> ₈	15	30	0.63	31	33.9*	1.4*	
PVC/CDCl ₃	30	90	0.65	30	24.1*	36*	
PNA/TCE	3	30	0.78	22.9	24	600	17–19
PNMA/TCE	5	50	0.85	18.9	23	500	
PNEA/TCE	7	70	0.84	19.2	21	1100	
PNMA/PCE	7	100	0.77–0.86	18.2–23.6	25	300	
PNMA/CDCl ₃	7	80	0.78–0.84	19.3–23.1	20	500	
PNVC/CDCl ₃	20	280	0.73–0.79	22.5–26	17.5*	1100*	20, 21
PNVC/THF	30	300	0.75–0.80	22–25	17.5*	1100*	
PNVC/dioxane- <i>d</i> ₈	20	300	0.71–0.80	22–27	23*	340*	
PNVC/TCE	25	200	0.73–0.82	21–26	20.5*	950*	
PNVC/PCE	30	300	0.70–0.79	22.5–27.5	21.5*	1000*	
PHEG/D ₂ O	5	100	0.82	21	29.7	10	This work
PIBM/toluene- <i>d</i> ₈	2	50	0.64	30.7	36.4	7.39	22, 23
P2VP/CDCl ₃	8	100	0.73–0.81	21.3–25.7	18.5	395	
PVC/DBP	7	600	0.61–0.71	27–32	33.4	7.294	24
PVC/TCE	7	200	0.59–0.65	30–33	23.9	72.0	
PVA/D ₂ O	10	200	0.67–0.79	22.5–29.1	30.9	2.1	25
iso-P2VPCl/D ₂ O	5	100	0.83	20	18.2	500	26
iso-P2VP/ dioxane- <i>d</i> ₈	8	100	0.71–0.76	24–27	19.3	500	

*Apparent activation energy at 300 K assuming the WLF type temperature dependence of the correlation time.

PVC: polyvinyl chloride, DMSO: dimethylsulfoxide-*d*₆, PNA: poly(1-naphthyl acrylate), TCE: 1,1,2,2-tetrachloroethane-*d*, PNMA: poly(1-naphthylmethyl acrylate), PCE: pentachloroethane, PNEA: poly[2-(1-naphthyl)methyl acrylate], PBMA: poly(butyl methacrylate), PHMA: poly(hexyl methacrylate), PNVC: poly(*N*-vinylcarbazole), THF: tetrahydrofuran-*d*₈, PEO: poly(ethylene oxides), PIBM: poly(isobutyl methacrylate), PVME: poly(vinyl methyl ether), P2VP: poly(2-vinyl pyridine), DBP: dibutyl phthalate, PVA: poly(vinyl alcohol), P2VPCl: poly(4-vinylpyridinium chloride)

different form that of the Cole–Cole distribution.

For comparison, fitting parameters of the DLM model obtained for other polymer solutions are listed in Table II. The parameter τ_0/τ_1 is in the region of $2 \leq \tau_0/\tau_1 \leq 30$ and depends on polymer structure rather than the kinds of solvents. τ_0/τ_1 is mostly in the region of ≥ 10 for the polymers having no side chains or shorter rigid side chains such as PVC or PNVC, and mostly in the region ≤ 5 for the polymers having longer flexible side chains such as PIBM or PHEG. PHEG with longer side chains has $\tau_0/\tau_1 = 5$ and belongs to the group having smaller values, indicating that the distribution of the correlation time is relatively narrow. A recent study⁴¹ pointed out that τ_0/τ_1 varies with the chain stiffness and the polymer chain is probably stiffer if τ_0/τ_1 is smaller. Therefore the backbone of PHEG is rather stiffer comparing with vinyl polymers presumably due to the planar structure of peptide groups and longer pendent side chains.

The parameter τ_1/τ_2 does not depend on the kinds of solvents and is mostly in the region of $50 \leq \tau_1/\tau_2 \leq 300$, but any conspicuous feature cannot be found out relevant to polymer structure. Since the T_1 minimum by the libration was not observed for

PHEG, τ_2 was estimated at least 100 times as fast as τ_1 in the calculation similarly to the case of other vinyl polymer solutions.

The parameter $(1 - a)$ was introduced as a quantity relevant to the libration angle θ given by Eq 8 and can be considered also as the fractional contribution of the segmental motion to the relaxation, playing the role of a kind of bottom raising parameter. This quantity is almost in the same range of 0.80 ± 0.05 ($\theta = 22 \pm 3^\circ$) except PVC and PIBM, and is unrelated to the kinds of solvents. For PHEG, $\theta = 21^\circ$ was obtained and it is a typical value found in other polymers. The reason why PVC and PIBM have deviant values compared with other polymers is unclear.

The activation energy E_a and the pre-exponential factor τ_∞ strongly depend on the kinds of solvents. This can be understood from the fact that the activation energy contains two contributions, viscosity of solvent and conformational transition of polymer. For PHEG, the activation energy of D₂O determined from the deuteron quadrupole relaxation was 16 kJ mol^{-1} , and as a result the contribution from the conformational transition is derived as 13.6 kJ mol^{-1} . Therefore, solvents affect strongly the ac-

tual temperature dependence of the correlation times. However, in the DLM model, the ratios of the correlation times are important to express the feature of the segmental motion. For example, although E_a and τ_∞ of PHEG/D₂O and iso-P2VPCl/D₂O are quite different each other, the other parameters are almost equivalent. This means that the features of segmental motion in the two polymers are equivalent in spite of the different temperature dependence of the correlation time.

Finally, the exponent to the spectral density function by the segmental motion is examined. In the region of $\omega_C \tau_a \ll 1$, the following relation is derived

$$T_1/\omega_C \propto [\omega_C J(\omega_C)]^{-1} \propto (\omega_C \tau_a)^{-\nu}, \quad (20)$$

and for an arbitral number λ ,

$$(\lambda \omega) J(\lambda \omega, \tau_a) = \lambda^\nu J(\omega, \tau_a) \quad (21)$$

and/or

$$\omega J(\omega, \lambda \tau_a) = \lambda^\nu J(\omega, \tau_a) \quad (22)$$

are indicated. Note the spectral density function $J(\omega)$ is given by Fourier transform of autocorrelation function $G(t)$. If τ_0/τ_1 and τ_1/τ_2 approach to the infinity, the autocorrelation function of the DLM model is approximated to

$$G(t) \approx (1 - a) \exp\left(-\frac{t}{\tau_1}\right) I_0\left(\frac{t}{\tau_1}\right) \quad (23)$$

from Eq 7, and the following relation is found for $t/\tau_1 \gg 1$,¹³

$$G(t) \approx (1 - a) \left(\frac{\tau_1}{2\pi t}\right)^{1/2}. \quad (24)$$

Therefore,

$$G(\lambda t) = \lambda^{-1/2} G(t) \quad (25)$$

The same approximation to Eq 18 gives the distribution function of τ as follows

$$F(s) d \ln s = \text{Re} \frac{1 - a}{\pi \left[\left(\frac{2}{s\tau_1} - 1 \right) \right]^{1/2}} d \ln s. \quad (26)$$

This relation corresponds to Davidson–Cole distribution with the index of distribution as 0.5 and $\tau_1/2$ as cut off value.^{36,42} If τ_0/τ_1 is finite, such scaling rule as described above will no longer be realized, but other type of characteristic region appears in the spectral density function. Figure 11 shows shapes of the spectral density function $\log[\omega J(\omega)]$ against $\log(\omega \tau_1)$ of the DLM model for $\tau_0/\tau_1 = 10^3$, $\tau_0/\tau_1 = 10^2$, $\tau_0/\tau_1 = 10$, $\tau_0/\tau_1 = 1$, and the single distribution (BPP) for comparison. In a relatively narrow region of $1/(1 + 2\tau_0/\tau_1) < \omega \tau_1 < 1$ (region 1) which ex-

Figure 11. $\log[\omega J(\omega)]$ against $\log(\omega \tau_1)$ for the DLM model. Calculated curves for $\tau_0/\tau_1 = 10^3$, $\tau_0/\tau_1 = 10^2$, $\tau_0/\tau_1 = 10$, and $\tau_0/\tau_1 = 1$ are represented by the leftward solid, dotted, broken and chained curves, respectively. The centered solid curve is drawn for the single distribution (BPP) for comparison.

Figure 12. Plots of $\log(T_1/\omega_C)$ against $\log(\omega_C \tau_a)$ in the region of $\omega_C \tau_a < 0.2$. The straight line is obtained by the least squares method, and gives the slope of 0.61 and square of the correlation coefficient of 0.981.

presses the feature of the segmental motion, the spectral density has a scaling rule having the slope of 0.5 corresponding to ν in Eq 21 and/or 22. Whereas in the region of $\omega \tau_1 < 1/(1 + 2\tau_0/\tau_1)$ (region 2), the slope is 1. The value of $1/(1 + 2\tau_0/\tau_1)$ is 0.09 for the present case of PHEG. On the other hand, for $\log(T_1/\omega_C)$ against $\log(\omega_C \tau_a)$, the region corresponding to the region 1 is derived as $0.04 < \omega \tau_a < 0.2$ estimated from Eq 1. Figure 12 shows $\log(T_1/\omega_C)$ against $\log(\omega_C \tau_a)$ at the region of $\omega_C \tau_a < 0.2$ where the linear relation sufficiently holds and is recognized as the region 1 from Figure 6. The slope gives the exponent $\nu = 0.61$. Although this exponent is somewhat larger, it is close to 0.5 from the DLM model taking into account of experimental errors. These results also indicate that the DLM model expresses the feature of segmental motion in PHEG.

CONCLUSION

To investigate the segmental motion in polypepti-

des, ¹³C NMR relaxation at six frequencies from 15 to 150 MHz were measured for PHEG in aqueous solution. The superposition of T_1/ω_C applying the time-temperature reduction rule gave the master curve over the double-digit frequency range. The unique activation energy of 29.6 kJ mol⁻¹ was obtained from the temperature dependence of the shift factor. Using this activation energy for the correlation time, calculated curves by the DLM model were well fitted the experimental T_1 and NOE. This fact indicates that the feature of segmental motion of PHEG is well expressed by the DLM model. It was shown that the parameter τ_0/τ_1 represents broadness of the distribution of correlation time. Comparing this parameter in polymers reported, it was found that PHEG with $\tau_0/\tau_1 = 5$ belongs to the polymer group having smaller value of τ_0/τ_1 indicating that the distribution of the correlation time is relatively narrow and the backbone is rather stiffer presumably due to the planar structure of peptide groups. Another fact obtained from present wide frequency experiment is that the spectral density function $J(\omega)$ has the exponent to the correlation time τ_a as $\omega J(\omega) \sim (\omega\tau_a)^{0.61}$ in the region of $\omega_C\tau_a < 0.2$ which is close to 0.5 from the DLM model. Such a discussion has not been given in other reports yet.

Acknowledgment. Authors express their gratitude to Prof. Tatsuo Hamada of Graduate School of Pharmaceutical Science and Mr. Eiji Yamada in the NMR Laboratory of Graduate School of Engineering at Hokkaido University for the measurements of T_1 at 150 MHz and 50 MHz, respectively.

REFERENCES

1. Y. Izumi, H. Takezawa, N. Kikuta, S. Umeura, and A. Tsutsumi, *Macromolecules*, **31**, 430 (1998).
2. N. Morikoshi, T. Hiraoki, and A. Tsutsumi, *Rep. Prog. Polym. Phys. Jpn.*, **42**, 477 (1999).
3. T. Hiraoki and K. Hikichi, *Polym. J.*, **11**, 299 (1979).
4. A. Tsutsumi, *Prog. Polym. Sci.*, **18**, 651 (1993).
5. B. Perly, C. Chachaty, and A. Tsutsumi, *J. Am. Chem. Soc.*, **99**, 2463 (1977).
6. D. E. Woessner, *J. Chem. Phys.*, **1**, 36 (1962).
7. R. J. Wittevert and A. Szabo, *J. Chem. Phys.*, **69**, 1722 (1978).
8. R. E. London and J. Avitavile, *J. Chem. Phys.*, **65**, 2443 (1976).
9. A. Tsutsumi, *Mol. Phys.*, **37**, 11 (1979).
10. K. S. Cole and R. H. Cole, *J. Chem. Phys.*, **9**, 341 (1941).
11. L. J. Burnett and B. H. Muller, *J. Chem. Phys.*, **55**, 5829 (1971).
12. T. F. Schatzki, *Polym. Prepr.*, **6**, 464 (1965).
13. E. Helfand, *J. Chem. Phys.*, **54**, 4651 (1971).
14. C. K. Hall and E. Helfand, *J. Chem. Phys.*, **77**, 3275 (1982).
15. R. Dejean de la Batie, F. Lauprêtre, and L. Monnerie, *Macromolecules*, **21**, 2045 (1988).
16. E. I. Tylianakis, P. Dais, and F. Heatley, *J. Polym. Sci., Part B: Polym. Phys.*, **35**, 317 (1997).
17. A. Spyros, P. Dais, and F. Heatley, *Macromolecules*, **27**, 5845 (1994).
18. A. Spyros, P. Dais, and F. Heatley, *Macromolecules*, **27**, 6207 (1994).
19. A. Spyros, P. Dais, and F. Heatley, *J. Polym. Sci., Part B: Polym. Phys.*, **33**, 353 (1995).
20. A. Karali, P. Dais, E. Mikros, and F. Heatley, *Macromolecules*, **34**, 5547 (2001).
21. A. Karali, P. Dais, and F. Heatley, *Macromolecules*, **33**, 5524 (2000).
22. S. Ravindramathan and D. N. Sathyanarayana, *Macromolecules*, **29**, 3525 (1996).
23. S. Ravindramathan and D. N. Sathyanarayana, *Macromolecules*, **28**, 2396 (1995).
24. T. Radiotis, G. R. Brown, and P. Dais, *Macromolecules*, **26**, 1445 (1993).
25. J. M. Petit and X. X. Zhu, *Macromolecules*, **29**, 2075 (1996).
26. S. Yamazaki, E. Okada, Y. Muroga, I. Noda, and A. Tsutsumi, *Polym. J.*, **31**, 614 (1999).
27. F. J. Jouvert, N. Lotan, and H. A. Scheraga, *Biochemistry*, **9**, 2197 (1970).
28. B. Perly, C. Chachaty, and A. Tsutsumi, *J. Am. Chem. Soc.*, **27**, 1521 (1980).
29. N. Lupu-Lotan, A. Yaron, A. Berger, and M. Sela, *Biopolymers*, **3**, 625 (1965).
30. A. Abragam, "Principles of Nuclear Magnetism," Oxford University Press, New York, N.Y., 2002, pp. 289–305.
31. S. Glowinkowski, D. J. Gisser, and M. D. Ediger, *Macromolecules*, **23**, 3520 (1990).
32. N. Bloembergen, E. M. Purcell, and R. V. Pound, *Phys. Rev.*, **73**, 679 (1948).
33. O. Howarth, *Chem. Soc. Faraday Trans. 2*, **7**, 1031 (1977).
34. R. Diblasi, and A. S. Verdini, *Biopolymers*, **13**, 765 (1974).
35. P. L. Scott and C. D. Jefferies, *Phys. Rev.*, **127**, 32 (1962).
36. P. A. Beckmann, *Phys. Rep.*, **171**, 85 (1988).
37. G. Lipari and A. Szabo, *J. Am. Chem. Soc.*, **104**, 4546 (1982).
38. G. Lipari and A. Szabo, *J. Am. Chem. Soc.*, **104**, 4559 (1982).
39. A. Guillermo, R. Dupeyre, and J. P. Cohen-Addad, *Macromolecules*, **23**, 1291 (1990).
40. D. J. Gisser, S. Glowinkowski, and M. D. Ediger, *Macromolecules*, **24**, 4270 (1991).
41. S. Yamazaki, E. Okada, Y. Muroga, I. Noda, and A. Tsutsumi, *Polym. J.*, **31**, 542 (1999).
42. D. W. Davidson and R. H. Cole, *J. Chem. Phys.*, **9**, 1484 (1951).