Dielectric Study on Chain Dynamics of Poly(glutamic acid) in Aqueous Solution Using the Frequency Range 10^7–10^{10} Hz

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ABSTRACT: Dielectric relaxation measurements were performed on poly(glutamic acid)s in aqueous solution by the use of a time domain reflectometry which covers a frequency range from 10 MHz to 10 GHz. Two relaxation peaks were found: one observed at about 20 GHz can be attributed to free water relaxation, judging from its relaxation time and relaxation strength, and the other at about 100 MHz depends drastically on the conformation of the polymer which shows a helix–coil transition if the pH is changed. For $0 \leq f_c < 0.8$, where f_c is the fraction of coil, the relaxation strength is given by a linear function of f_c. Internal motion of the side group has been suggested to give rise to this relaxation peak. For $0.8 < f_c \leq 1$, the dielectric strength is proportional to f_c^5. It is shown that a linkage of five repeat units is required at least for occurrence of micro-Brownian motion of the coiled chain.

Introduction

Poly(glutamic acid) (PGA) has often been used as an analogue for the investigation of electric properties of proteins. It is of particular importance for examination of dielectric properties of polyelectrolyte solution.

Takashima[1] found first a dielectric dispersion in the PGA aqueous solution in the kilohertz region, the relaxation time of which depends strongly on the molecular weight. It was suggested that the dispersion is caused by an overall reorientation of the macromolecule accompanied by counterion displacement. Later Mandel et al.[2] and Nakamura and Wada[3] ascertained the existence of the dispersion and reached the same interpretation.

Mandel et al.[2] found another dispersion in the region 10^5–10^7 Hz in aqueous solution. Its relaxation time and strength depend slightly on the molecular weight and almost linearly depend on the counterion strength. However, the dispersion is not affected by the helix–coil transition at all. This is an interesting contrast with the dispersion in the kilohertz region. They developed a phenomenological theory of counterion processes and suggested that the dispersion in the 10^5–10^7-Hz region is attributed to a counterion migration in a local part of the macromolecule.[4]

Polypeptide has a perpendicular component of electric dipole in each repeat unit to the chain contour. In a random coil, it is expected naturally that micro-brownian motion of the polymer chain occurs and gives rise to a dielectric dispersion. Actually, dielectric dispersion caused by the chain motion was found in synthetic polymers in the frequency region 10^7–10^{10} Hz in organic solvents.[5–7] Nevertheless, in an aqueous solution of biopolymer, dispersion due to the chain motion has not been found yet.

In some poly(α-amino acid)s in organic solvent, where the polymers take on the α-helix form, dielectric dispersion caused by internal motions of polar side groups were discovered in the gigahertz region.[8,9] Discovery of such dispersion has not been reported in aqueous solution so far.

Most of the biological materials including living organisms, proteins, and DNA show a dielectric absorption peak near 100 MHz, which is thought to be caused by reorientation of water molecules bound to the materials.[10] Clusters of water molecules attach to the surface of the biomaterials and the relaxation are caused by breaking and remaking hydrogen bonds in the cluster.[10–12]

In aqueous solution, PGA exhibits a helix–coil transition with varying pH values.[2,3,13] If the pH value is greater than 8, the polymer is randomly coiled. Then the micro-Brownian motion of the polymer chain possibly occurs and will bring about dielectric dispersion. In the transition region, the chain segments are partially α-helix and other parts are randomly coiled. If the helical part is more than 70% of the whole chain, the polymer will be an analogue of globular proteins such as myoglobin and hemoglobin. If the pH value is lower than 4, PGA is completely α-helix, judging from the optical (CD) measurement.[13] The micro-Brownian motion of the chain cannot occur anymore. If the side group has some degrees of freedom for internal motions in the helix, dielectric dispersion is expected to be found. Otherwise if the group participates in the helix and loses freedom, no dispersion will be found.

In this work, dielectric relaxation measurements have been carried out on PGA in aqueous solution with various pH values over the frequency region 10^7–10^{10} Hz by employing a time domain reflectometry (TDR) method[14–16] in order to discover and investigate the micro-Brownian motion of the polymer chain and the internal motions of the side groups. Recent refinements of the TDR are remarkable, and highly accurate values of complex permittivity within a few percent error can be obtained over 3 decades of frequencies with high speed.[15]

Experimental Section

The sodium salt of poly(L-glutamic acid) (Na-salt PGA) was purchased from Sigma Co. Ltd., the weight average molecular weight of which is 60000. In order to remove the sodium ion, Na-salt PGA solution was dialyzed against distilled water for 144 h. The initial solution before dialysis has 29 mequiv/L of Na^+ ion, but after dialysis it has only about 1 mequiv/L. The PGA solution was freeze dried to obtain the PGA sample. The sample thus obtained was dissolved in 0.1 M NaOH, and 0.1 M HCl was added to regulate the pH value between 3.66 and 11.09 for the use of dielectric and optical measurements.

In order to trace the conformational change, the molar ellipticity at 222 nm $[\theta]_{222}$ was obtained from the CD spectra by using a J-600 CD spectrophotometer (Japan spectroscopy Co. Ltd.).

Dielectric measurements were carried out over a frequency range of 10^7–10^{10} Hz by employing a time domain reflectometry (TDR) method.[14–16]

A step-voltage pulse with a rise time of 25 ps and 200-mV height

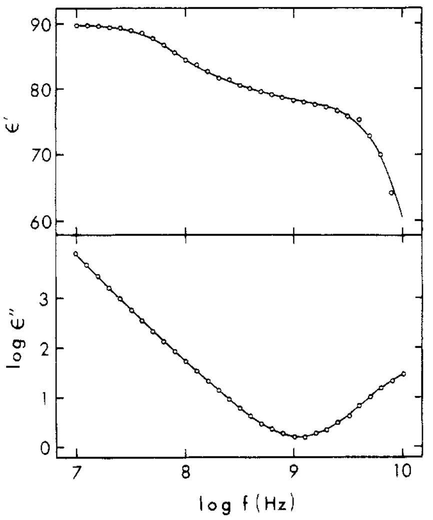

Figure 1. Frequency dependence of complex permittivity of PGA in aqueous solution (5 mg/mL) with pH 9.46 at 25 °C.

Table I
Dielectric Relaxation Parameters for PGA Aqueous Solutions with Various pH Values at 25 °C

	low-frequency process			high-frequency process		
pH	$\Delta\epsilon_l$	τ_l, ns	α	$\Delta\epsilon_h$	τ_h, ps	ϵ_∞
3.92	2.35	3.21	0.795	73.94	8.91	4.75
4.96	3.32	3.11	0.795	73.94	8.91	4.05
5.10	4.95	3.06	0.795	73.85	8.91	4.37
5.42	5.18	2.59	0.764	73.94	8.71	4.44
6.00	8.69	2.64	0.755	73.94	8.91	3.39
6.34	10.37	2.52	0.729	73.94	8.91	4.25
6.95	12.06	2.45	0.701	73.64	8.91	4.13
7.86	12.55	2.44	0.660	73.64	8.91	4.14
9.46	12.47	2.34	0.635	72.39	8.91	4.10
10.96	12.55	2.21	0.649	72.90	8.91	4.28

Table II
Temperature Dependence of Dielectric Relaxation Parameters for PGA Aqueous Solution with pH 9.46

	low-frequency process			high-frequency process		
temp, °C	$\Delta\epsilon_l$	τ_l, ns	α	$\Delta\epsilon_h$	τ_h, ps	ϵ_∞
5	14.43	4.09	0.628	80.87	13.80	4.705
10	13.87	3.58	0.608	76.97	12.59	4.163
15	13.32	3.13	0.632	76.08	11.48	4.424
20	12.96	2.71	0.632	74.12	10.00	4.681
25	12.47	2.34	0.635	72.39	8.91	4.102
30	12.06	2.02	0.609	70.08	7.08	2.297
40	11.29	1.63	0.594	68.71	5.62	4.161

is generated by a tunnel diode (Picosecond Lab, TD-1107B). This pulse passes through a flexible coaxial line (Junko-sha, DGM224, 50 Ω, dc 26.5 GHz) to the sample cell placed at the end of the line, where the pulse reflects. The reflected pulse is measured at a sampling head (Iwatsu, SH-4b, dc 12.4 GHz) of a sampling scope (Iwatsu, SAS-601B, dc 12.4 GHz) and is digitized by a signal analyzer (Iwatsu, SM2100A, 12 bit, 4096 word). A microcomputer (NEC PC-9801XA) is used to calculate numerical Fourier transform of the pulse and to obtain the complex permittivity.

If a cell with length d and effective length γd is employed and two measurements of the sample with unknown complex permittivity ϵ^* and reference sample with ϵ_s are performed, the permittivity (ϵ^*) is given by[15,16]

$$\epsilon^* = \epsilon_s \frac{1 + \{(cf_s)/[j\omega(\gamma d)\epsilon_s]\}\rho}{1 + \{[j\omega(\gamma d)\epsilon_s]/(cf_s)\}\rho} \frac{f_x}{f_s} \qquad (1)$$

$$\rho = \frac{r_s - r_x}{r_s + r_x} \qquad (2)$$

$$f = Z \cot Z \qquad (3)$$

and

$$Z = (\omega d/c)\epsilon^{1/2} \qquad (4)$$

where r_s and r_x are the Fourier transform of the reflected pulse from the reference sample $R_s(t)$ and that from the unknown sample $R_x(t)$, j is the imaginary unit, ω is the angular frequency, and c is the speed of propagation in vacuo (0.300 mm/ps).

In this measurement, we used a cell with d = 0.38 mm and γd = 0.283 mm. An aqueous solution of 0.01–0.02 M NaCl was used as the reference sample.[17,18] Dispersion and absorption of the solution are nearly the same as those of pure water except for the dc conductivity. Permittivity of the solution is given by

$$\epsilon^*_{\mathrm{NaCl}} = \epsilon^*_w - j\frac{\sigma}{\omega\epsilon_0} \qquad (5)$$

where σ is the conductivity, ϵ^*_w is the permittivity of pure water, and ϵ_0 is the dielectric permittivity in vacuo.

In the case where the unknown sample has a dispersion in a frequency region far lower than that concerned, the contribution of the dispersion to the time domain signal of $R_s - R_x$ remains constant within the whole range of times used and cannot be distinguished from that of dc conductivity. In the present case, the neighboring dispersion to the dispersion around 100 MHz was reported around 1 MHz.[2] Therefore, its contribution to the time domain signal cannot be distinguished from that of dc conductivity within the time range used here.

Experimental Results

Typical dielectric dispersion and absorption are shown in Figure 1 for PGA solution (5 mg/mL) with pH 9.46 at 25 °C. The absorption curve includes a contribution of dc conductivity. If the contribution is estimated to be proportional to $1/\omega$ and eliminated properly from the absorption curves, two absorption peaks can be observed definitely around 100 MHz and 20 GHz. The absorption curves thus obtained for PGA solutions at various pH's are shown in Figure 2, together with the dispersion curves. These curves are explained satisfactorily by assuming two relaxation processes

$$\epsilon^* = \epsilon_\infty + \frac{\Delta\epsilon_l}{[1 + j\omega\tau_l]^\alpha} + \frac{\Delta\epsilon_h}{1 + j\omega\tau_h} \qquad (6)$$

as shown in Figure 2, where τ is the relaxation time and $\Delta\epsilon$ is the relaxation strength. The relaxation parameters in eq 6 determined by a least-squares fitting procedure are listed in Table I.

The high-frequency process is concluded to be due to reorientation of free water molecule, judging from the values of the relaxation time and relaxation strength. The literature values of τ and $\Delta\epsilon$ for pure water at 25 °C are 8.71 ps and 73.0, respectively,[19] and in good agreement with those of τ_h and $\Delta\epsilon_h$, respectively, listed in Table I.

The relaxation strength ($\Delta\epsilon_l$) for the low-frequency process shows a transition in the vicinity of pH 6 as shown in Figure 3. It remains constant above pH 7 but decreases quickly as the pH value decreases.

The molar ellipticity $[\theta]_{222}$ obtained is shown in Figure 3 too, which shows a definite helix–coil transition in the vicinity of pH 5. It is obvious that the dielectric transition observed reflects the helix–coil transition. However, the center of the dielectric transition is slightly but definitely different from that of the transition in the molar ellipticity.

The temperature dependence of τ_l for pH 9.46 where PGA is randomly coiled is shown in Figure 4 which gives an apparent activation energy of 4.7 ± 0.2 kcal/mol. The relaxation parameters for the high- and low-frequency processes at various temperatures are listed in Table II. The parameters τ_h and $\Delta\epsilon_h$ are in good agreement with those of pure water at these temperatures.

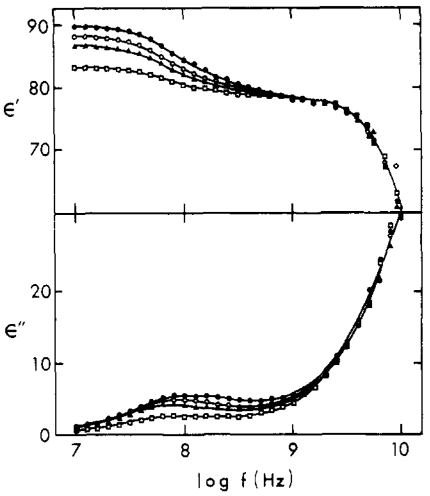

Figure 2. Dielectric dispersion and absorption curves of PGA in aqueous solutions (5 mg/mL) with various pH values at 25 °C: (●) pH 9.46, (○) pH 6.34, (△) pH 6.00, (□) pH 5.42. Contribution of dc conductivity has been subtracted from the permittivity of each solution.

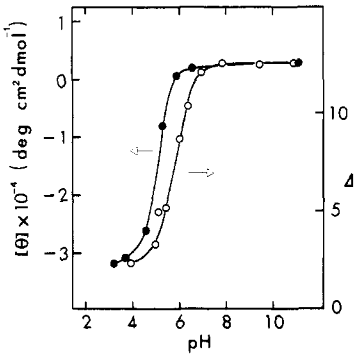

Figure 3. Plots of relaxation strength ($\Delta\epsilon_1$) and molar ellipticity ($[\theta]_{222}$) against pH at 25 °C. The strengths ($\Delta\epsilon_1$) at pH 3.92 and 4.96 were obtained by using solutions with a concentration of 0.5 mg/mL and multiplying by a factor of 10.

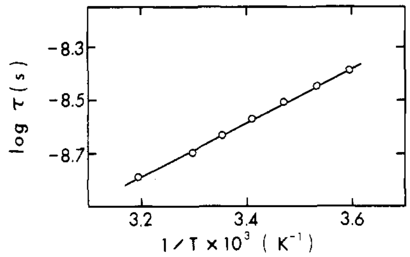

Figure 4. Plot of log τ_1 for the low-frequency process against $1/T$.

Discussion

In general, dielectric dispersion caused by chain motions of randomly coiled polymers is observed in a frequency range similar to that of the low-frequency dispersion of the present coiled PGA.[5-7] It has been established that distribution of the relaxation time is asymmetric. The value of α is usually 0.6–0.8 and nearly the same as that of the present coiled PGA.[5-7] Furthermore, the apparent activation energy concerned, 4.9 ± 0.2 kcal/mol, is also reasonable, if compared to that of other polymers in dilute solution.

The fraction of the coil in PGA can be estimated from molar ellipticity as

$$f_c = \frac{[\theta]_{222} - [\theta]_{222}^{\mathrm{h}}}{[\theta]_{222}^{\mathrm{c}} - [\theta]_{222}^{\mathrm{h}}} \tag{7}$$

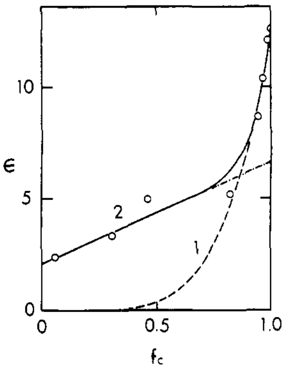

Figure 5. Dependence of $\Delta\epsilon_1$ on f_c at 25 °C. Curve 1, calculated by eq 14 with $m = 5$. Curve 2, calculated by eq 15.

where $[\theta]_{222}^{\mathrm{c}}$ and $[\theta]_{222}^{\mathrm{h}}$ are the limiting molar ellipticity of random coil and that of helix, respectively. The relaxation strength ($\Delta\epsilon_1$) is plotted against f_c in Figure 5. In the region $0.8 < f_c \le 1$, $\Delta\epsilon_1$ decreases radically as f_c is decreased. On the other hand, in the region $0 \le f_c < 0.8$, it increases linearly with f_c.

Micro-Brownian motions of the polymer chain occur only in the coiled part, and the motion requires simultaneous or compensating movements of several neighboring repeat units.[20-22]

The total dipole moment of a polymer chain is written as

$$M = \sum_{i=1}^{n} \mu_i \tag{8}$$

where μ_i is the moment of the ith repeat unit and n is the degree of polymerization. The time-dependent dipolar correlation is given by

$$\langle M(0)M(t)\rangle = \mu_0^2\Big[\sum_i \varphi_i(t) + \sum_i \sum_{\substack{j \\ (i\neq j)}} \varphi_{ij}(t)\Big] = n\mu_0^2 g(t) \tag{9}$$

where $\varphi_i(t) = \langle \mu_i(0)\mu_i(t)\rangle/\mu_0^2$ is the autocorrelation function, $\varphi_{ij}(t) = \langle \mu_i(0)\mu_j(\mathrm{t})\rangle/\mu_0^2$ is the cross-correlation function, and μ_0 is the magnitude of μ_i. The function $g(t)$ at $t = 0$ gives the Kirkwood g factor, usually taken as a value between 0.4 and 0.9 for randomly coiled polymers. For $|i - j| > 5$–6, $\varphi_{ij}(t)$ is negligibly small.[20-22] This means that the dipolar correlation extends to only several repeat units along the chain.

Complex permittivity (ϵ^*) is related to eq 9 as

$$\epsilon^* - \epsilon_\infty = \frac{4\pi N n g(0)\mu_0^2}{3KT} F \int_0^\infty e^{-j\omega t}[-\dot{\varphi}(t)]\,\mathrm{d}t \tag{10}$$

where $\varphi(t) = g(t)/g(0)$ is a dielectric response function independent of the chain length and N is the number of polymers in unit volume. The Kirkwood formula of the internal field[23] gives

$$F = \frac{\epsilon_0(\epsilon_\infty + 2)^2}{3(2\epsilon_0 + \epsilon_\infty)} \tag{11}$$

Fourier transform of $-\dot{\varphi}(t)$ is described empirically by[24,25]

$$\int_0^\infty e^{-j\omega t}[-\dot{\varphi}(t)]\,\mathrm{d}t = \frac{1}{[1 + (j\omega\tau)^\beta]^\alpha} \tag{12}$$

Ramdonly coiled polymers take a value of 0.5–0.7 for α and 0.8–1 for β in dilute solution.

If it is assumed that chain motion possibly occurs only when a linkage of more than m repeat units in the coiled part takes place, the dipolar correlation for partially coiled polymer is given by

$$\langle M(0)M(t)\rangle = n f_c^{\,m} \mu_0^2 g_m(t;f_c) \tag{13}$$

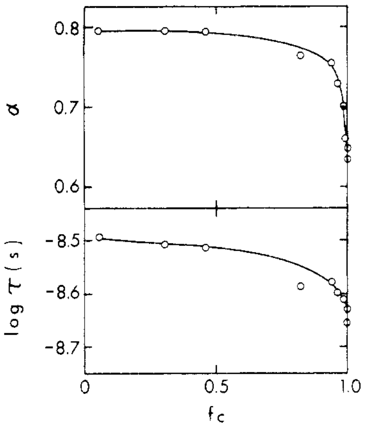

Figure 6. Dependences of log τ_1 and α on f_c at 25 °C.

since the probability of linkage of m repeat units will be given by f_c^m. The relaxation strength ($\Delta\epsilon_1$) is then given by

$$\Delta\epsilon_1 = \Delta\epsilon_1^c f_c^m \qquad (14)$$

where $\Delta\epsilon_1^c$ is the strength at $f_c = 1$, if $g_m(0;f_c)$ is assumed not to be different from $g(0)$ in eq 9. The dependence of ϵ_1 on f_c is explained quite well by eq 14 with $m = 5$ for $0.8 < f_c \leq 1$ as shown in Figure 5, and the value of m is quite reasonable for the occurrence of the chain motion.

In the region $0 \leq f_c < 0.8$, $\Delta\epsilon_1$ is described approximately by a linear function of f_c as

$$\Delta\epsilon = 2.1 + 3.9f_c \qquad (15)$$

It is highly unlikely that the chain motion occurs within a repeat unit. Internal motion of the side group possibly occurs within the unit. It has been thought that the side groups participate in the helix as well as the main chain. However, recent studies of polypeptide in dioxane, where the polymer is in the α-helix, have revealed dielectric dispersions caused by the side groups in the frequency range 300 MHz to 3 GHz.[8,9] The dispersion of the present PGA for $1 \leq f_c < 0.8$ appears around 100 MHz, which is not far from those for polypeptides in organic solvent. The side group motions possibly occur to some extent even in helix PGA. Therefore, it is very natural that the relaxation strength increases as f_c is increased, since the degree of freedom for the motions will increase linearly with f_c.

The dipole moment for the side group dispersion calculated from eq 10–12 and using a value of $\Delta\epsilon$ in eq 15 extrapolated to $f_c = 1$ is 1.7 D. This is quite reasonable for the dipole moment of the side group of glutamic acid.

The chain motion will be hindered if the helix part increases. This is in good conformity with the experimental result. Relaxation time for the chain motion observed for $0.8 < f_c \leq 1$ increases as f_c is decreased, as is seen in Figure 6. Simultaneously α becomes large when f_c is decreased. This may mean that the correlation length of the chain motion decreases with an increase of the helix part.[27]

The chain motion for $0.8 < f_c \leq 1$ is thought to be cooperative with the motion of the side group. Cooperative motion like this is observed often in the synthetic polymers such as poly(alkyl methacrylate)s in solution[27,28] and also in solid state.[29]

We denote the relaxation time for the main chain motion as τ_m and that for the side group motion as τ_s. In the case where $\tau_s > \tau_m$, the cooperative motion is solely observed and its relaxation time is near to or the same as τ_m.[5] The motion of PGA observed for $0.8 < f_c \leq 1$ corresponds to this case. For $\tau_s \ll \tau_m$, two kinds of dielectric dispersions will be observed independently.[5] However, in the present case, the relaxation strength for the main chain motion decreases quickly, proportionally to f_c^5. Therefore, for $f_c < 0.5$, the dispersion due to the main chain motion will be negligibly small.

The effective dipole moment of the repeat unit for the cooperative motion calculated from eq 10 and 11, $[g(0)\mu_0]^{1/2}$, is 2.7 D. The dipole moment (μ_0) reported for the perpendicular component to chain contour is 3.4–6.0 D.[30] Then the g factor takes a value between 0.2 and 0.6, very reasonable for randomly coiled polymer.

Discrepancy of the center of transition region between dielectric relaxation strength and molar ellipticity, which is clearly seen in Figure 3, can be thus explained quite satisfactory by the chain dynamics described above. It is due to the existence of correlated motions among five repeat units along the polymer chain.

Water molecules can be bound to the main chain and the side groups too. However, the bound water will move together with the chain and the side groups. Therefore, dielectric relaxation due to the bound water cannot be observed independently of those motions.

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Registry No. PGA (homopolymer), 25513-46-6; PGA (SRU), 24991-23-9.

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