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Nuclear Magnetic Resonance Studies on the Copper(II)–Poly(L-lysine) Complex

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ABSTRACT: The structural and dynamical properties of the Cu(II) complex of poly(L-lysine) (PLL) in aqueous solution were investigated by means of proton and ^{13}C NMR of poly(L-lysine) and water proton NMR of aqueous solution. Measurements of chemical shifts, longitudinal relaxation time T_1, and the transverse relaxation time T_2 of side-chain ε protons, δ carbon, and ε carbon indicated that side-chain amino groups are coordinated to Cu(II). It was found that the amino groups undergo a rapid exchange between the complexed and uncomplexed states at alkaline pH and at room temperature. The results of water proton relaxation enhancement showed that water molecules are also in the first coordination sphere of Cu(II). The activation energy of 6 kcal/mol of the lifetime of the complex was estimated from the temperature dependence of the relaxation rates of ε protons at lower temperatures at pH 10.4. The correlation time of tumbling motions of the end of the side chain and its activation energy were also estimated to be 0.5×10^{-10} s and 4 kcal/mol, respectively. No evidence was obtained for the peptide nitrogen to participate in the complex formation at a Cu(II)/PLL molar ratio from 4.6×10^{-4} to 1.3×10^{-3} and at pH 10.4.

In the last few years, studies on the formation, catalytic properties, and structure of the metal complexes of poly(α-amino acids) have been undertaken by many authors, because such compounds can be considered as useful models in order to understand the mode of action of metalloenzymes.[2] In particular, divalent copper (Cu(II)) complexes of poly(α-amino acids) have been investigated in some detail with respect to catalytic and structural properties.[3-13] Among them, the Cu(II)–poly(L-lysine) (PLL) complex has been reported to show a stereospecific catalytic activity for oxidation of 3,4-dihydroxyphenylalanine.[4,6]

Hatano et al.[5,6] investigated the structural properties of the Cu(II)–PLL complex in aqueous solution by means of pH titration, visible and infrared absorptions, optical rotatory dispersion, and circular dichroism. They reported that two types of complex are formed. The first type of complex which is stable at pH <8 contains four side-chain amino groups at the corners of the tetragonal coordination square of Cu(II). The second type of complex stable at pH >8 is an octahedral cupric complex coordinated with four amino groups in a plane which is nearly parallel to the helix axis of PLL and with a hydroxyl ion and one peptide nitrogen at the apical positions.

Recently, Peggion et al.[11-13] and Tosi et al.[9] reinvestigated the structural properties of the Cu(II)–PLL complex. Two research groups also found two types of Cu(II)–PLL complex. In the first type of complex, which has been reported to be stable at pH 8.5 by Peggion et al. and in a pH region of 5.5–7.6 by Tosi et al., only two amino groups are coordinated at the corners of square-planar coordination positions, and the other two corners are occupied by two water molecules. For the second type of complex stable at high pH, Peggion et al. have postulated tetragonal structures involving one amido and three amino nitrogens and/or two amido and two amino nitrogens at the square-planar coordination positions. They concluded that the second type of complex stable at high pH is not compatible with the α-helix conformation and that the binding process causes disruption of the helical structure. Tosi et al. also suggested a model involving two amino nitrogens and two adjacent peptide nitrogens at the corners of the tetragonal.

Nuclear magnetic resonance (NMR) is greatly influenced by the presence of paramagnetic species like the Cu(II) ion.[14-17] In particular, the longitudinal and transverse relaxation rates of NMR are affected by the large magnetic moment of unpaired electrons of paramagnetic species. In some cases, large chemical shifts are induced by the electron paramagnetism. Because NMR can monitor the individual atoms, the application of NMR is thought to be very fruitful in studying the structural and dynamical properties of the Cu(II)–PLL complex. The usefulness of NMR for the investigation of metal complexes has been proven for many metal complexes of low molecular weight compounds. However, very few NMR studies have been done for the polymer complex. We carried out the NMR study on cobalt(II) and nickel(II) complexes with poly(glutamic acid) some years ago,[18] and recently we have carried out such work for Cu(II) and Co(II) complexes of poly(glutamic acid).[19,20]

In this work we extended such NMR studies to the Cu(II)–PLL complex. The longitudinal and transverse relaxation times as well as chemical shifts of proton and ^{13}C NMR were measured as functions of temperature, Cu(II) concentration, and pH. Structural information about the Cu(II)–PLL complex obtained from NMR measurements is presented, and the results are discussed in terms of models of the complex proposed previously. Information about the dynamics of the complex is also presented.

Experimental Section

Poly(ε-*N*-(benzyloxycarbonyl)-L-lysine) was prepared by polymerization of α-*N*-carboxy-ε-*N*-(benzoyloxycarbonyl)-L-lysine anhydride in dioxane, using a trace of triethylamine as an initiator. Poly(L-lysine·HBr) was obtained by passing anhydrous hydrogen bromide through a dioxane solution of poly(ε-*N*-(benzyloxycarbonyl)-L-lysine). The intrinsic viscosity measured in an aqueous

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solution of 0.2 M NaCl at pH 6.7 was 0.84 dL/g, which yields the degree of polymerization of 250.

The polymer was dissolved in D_2O obtained from Commissariat à l'Énergie Atomique or in H_2O which was distilled and deionized. Reagent grade anhydrous cupric chloride (Nakarai Chemicals Ltd.) was used without further purification. Aliquots of $CuCl_2$ aqueous solution were added to the PLL solution.

Adjustments of pH were made with NaOD and DCl, obtained from Merck, or NaOH and HCl, obtained from Wako Chemical Industries Ltd. The pH was measured on a Hitachi-Horiba M-7 pH meter equipped with a combination electrode. The pH values reported here are direct meter readings without a correction of the deuterium isotope effect for the D_2O solution.

Proton and ^{13}C nuclear magnetic resonance spectra of PLL at 100 and 60 MHz and 15.04 MHz were obtained on JEOL-PS-100 and FX-60Q spectrometers. The longitudinal relaxation times (T_1) for protons and carbons of PLL were measured at frequencies of 60 and 15.04 MHz, respectively, by the inversion recovery method, using a JEOL-FX-60Q. The transverse relaxation times (T_2) were estimated, using the relaxation $T_2^{-1} = \pi\Delta\nu_{1/2}$, where $\Delta\nu_{1/2}$ is the half width of the signal.

The longitudinal relaxation time of water protons was measured by a Bruker SXP-4/100 spectrometer operating at a frequency of 90 MHz in a mode of the external (H_2O) lock for the H_2O solution.

The nuclear Overhauser enhancement (NOE) for protonated carbons was obtained by the comparison of integrated intensities of completely decoupled and gated decoupled spectra.[21,22]

Theoretical Section

The paramagnetic contribution to the relaxation rates is defined as[23,24]

$$T_{ip}^{-1} = T_i^{-1} - T_{i0}^{-1} \qquad (i = 1 \text{ and } 2) \tag{1}$$

where T_i^{-1} and T_{i0}^{-1} are the relaxation rates in the presence and the absence of the paramagnetic metal ion, and i = 1 and 2 correspond to the longitudinal and transverse relaxation rates, respectively.

If chemical exchange between the complexed and uncomplexed states occurs, the longitudinal relaxation rate is written as[24]

$$T_{1p}^{-1} = fq/(T_{1M} + \tau_M) \tag{2}$$

where τ_M is the lifetime of the observing ligand in the complexed state, f is the molar ratio of the paramagnetic metal ions to the PLL residue, q is the average number of the observing ligands in the first coordination sphere of the paramagnetic metal ion, and T_{1M} is the longitudinal relaxation time of the ligand in the complexed state. T_{1M} is given by the Solomon–Bloembergen equation:[25,26]

$$T_{1M}^{-1} = \frac{2}{15}\frac{S(S+1)\gamma_I^2 g^2\beta^2}{r^6}\left(\frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{7\tau_c}{1+\omega_S^2\tau_c^2}\right) + \frac{2}{3}\frac{S(S+1)A^2}{\hbar^2}\frac{\tau_e}{1+\omega_S^2\tau_e^2} \tag{3}$$

where S is the spin quantum number of the paramagnetic metal ion, γ_I is the gyromagnetic ratio of the nuclear spin, g is the electronic g factor, β is the Bohr magneton, A is the hyperfine coupling constant, r is the distance between the metal ion and the nucleus, ω_I and ω_S are the Larmor angular precession frequencies of nuclear and electron spins, respectively, $\hbar$ is Planck's constant divided by 2π, and τ_e and τ_c are the correlation times of hyperfine and dipolar interactions, respectively. The correlation times τ_c and τ_e are defined by

$$\tau_c^{-1} = \tau_R^{-1} + \tau_M^{-1} + \tau_s^{-1} \tag{4}$$

$$\tau_e^{-1} = \tau_M^{-1} + \tau_s^{-1} \tag{5}$$

where τ_R is the correlation time of the tumbling motion of the metal–ligand bond, and τ_s is the electron-spin relaxation time.

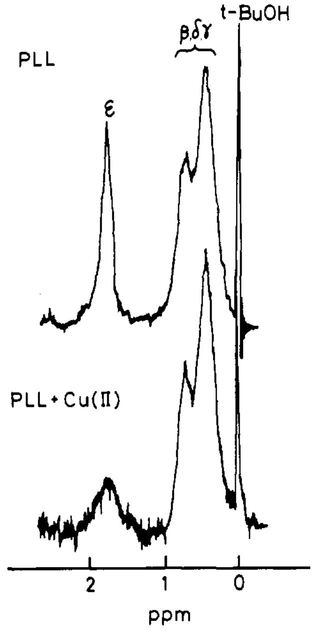

Figure 1. The high-field portion of the 100-MHz ^1H NMR spectra of poly(L-lysine) in D_2O at a residual concentration of 4.82×10^{-2} M at pH 10.4 and at 310 K: upper, no Cu(II) is added; bottom, Cu(II) of 6.22×10^{-5} M is added.

On the other hand, the paramagnetic contribution to the transverse relaxation rate is given by[23,24]

$$T_{2p}^{-1} = \frac{fq}{\tau_M}\frac{(1/T_{2M})(1/T_{2M} + 1/\tau_M) + \Delta\omega_M^2}{(1/T_{2M} + 1/\tau_M)^2 + \Delta\omega_M^2} \tag{6}$$

where $\Delta\omega_M$ is the paramagnetic shift in units of radians/s, and T_{2M} is the transverse relaxation time of the ligand in the complexed state which is given by[25,26]

$$T_{2M}^{-1} = \frac{1}{15}\frac{S(S+1)\gamma_I^2 g^2\beta^2}{r^6}\left(4\tau_c + \frac{3\tau_c}{1+\omega_I^2\tau_c^2} + \frac{13\tau_c}{1+\omega_s^2\tau_c^2}\right) + \frac{1}{3}\frac{S(S+1)A^2}{\hbar^2}\left(\tau_e + \frac{\tau_e}{1+\omega_s^2\tau_e^2}\right) \tag{7}$$

In case of negligible paramagnetic shift, as is the case in the present work, eq 6 is reduced to

$$T_{2p}^{-1} = fq/(T_{2M} + \tau_M) \tag{8}$$

Results and Discussion

^1H NMR of PLL. Figure 1 shows the high-field portion of the 100-MHz ^1H NMR spectra of PLL in aqueous (D_2O) solution at pH 10.4 and at a temperature of 310 K in the absence (upper) and the presence (bottom) of Cu(II). Concentrations of PLL residues and Cu(II) are 4.82×10^{-2} and 6.22×10^{-5} M, respectively, i.e., the molar ratio of Cu(II) to the PLL residue f is 1.29×10^{-3}. Assignments of signals follow from those of Bradbury et al.[27] A drastic line broadening was apparently observed for the signal of the side-chain ϵ protons when Cu(II) was added.

The variation in chemical shift of the ^1H NMR of PLL with the addition of Cu(II) was less apparent compared with the line broadening. Figure 2 shows the chemical shifts of α and ϵ protons as a function of pH in the absence and the presence of Cu(II) at 310 K. The addition of Cu(II) did not significantly alter chemical shifts of the ^1H NMR of PLL. The upfield shifts with an increase in pH observed in the pH region of 9.5–11 are the manifestation

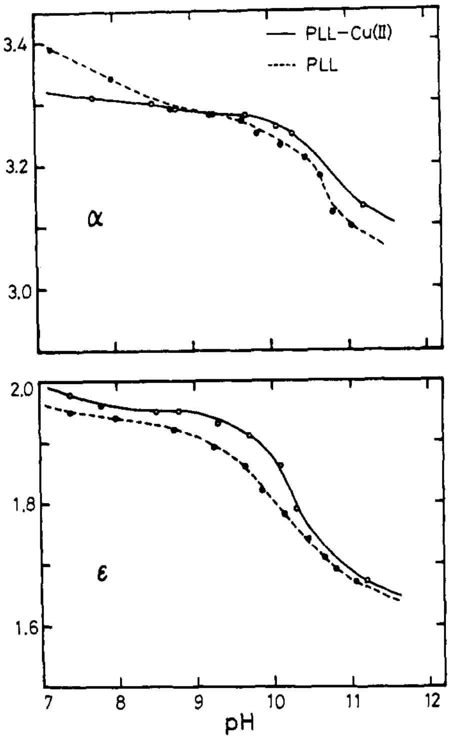

Figure 2. pH dependence of chemical shifts of α and ϵ protons of poly(L-lysine) at 310 K in the absence (●) and the presence (○) of Cu(II). The concentrations of PLL residues and Cu(II) are 4.82×10^{-2} and 6.22×10^{-5} M, respectively. Chemical shifts were measured from the external reference of *tert*-butyl alcohol in ppm.

of the deprotonation of the amino group of the side chain and/or the coli-to-helix transition. It appears that the transition region slightly moves to higher pH upon the addition of Cu(II).

The remarkable line broadening observed for the ϵ proton signal suggests that Cu(II) is bound to sites near the ϵ protons because the line broadening occurs if a paramagnetic species like Cu(II) is located in the vicinity of the observing nucleus. This finding does not conflict with earlier results that the side-chain amino groups are coordinated to Cu(II).[6,9,11,12]

The paramagnetic contribution to the transverse relaxation rate T_{2p}^{-1} for ϵ protons was estimated from the line width. It was found that T_{2p}^{-1} is proportional to the Cu(II) concentration f, indicating that PLL residues undergo exchange between the complexed and uncomplexed states. Thus, T_{2p}^{-1} is described by eq 8.

Figure 3 shows the T_2^{-1} of ϵ protons in the absence of Cu(II) and $(fT_{2p})^{-1}$ in the presence of Cu(II) at 310 K as functions of pH. The concentrations of PLL and Cu(II) are 4.83×10^{-2} and 2.22×10^{-5} M, respectively ($f = 4.6 \times 10^{-4}$). There appears to be an increase in T_2^{-1} at alkaline pH, which is likely due to the conformational transition from coil to helix. The paramagnetic contribution $(fT_{2p})^{-1}$ also shows a remarkable increase at about pH 10. Below neutral pH, little effect of the addition of Cu(II) on proton spectra of PLL was observed. These results indicate that the PLL residue does not bind to Cu(II) at acidic pH.

In Figure 4, T_2^{-1} in the absence of Cu(II) and $(fT_{2p})^{-1}$ in the presence of Cu(II) for ϵ protons at pH 10.4 are plotted against the reciprocal of the temperature. It appears that T_2^{-1} remains almost constant with changing temperature, although PLL has been reported to undergo the conformational transition from α helix to β form at elevated temperatures and at alkaline pH. The experimental values of T_2^{-1} in this work are probably determined in large part by line broadening due to unresolved multiplets.

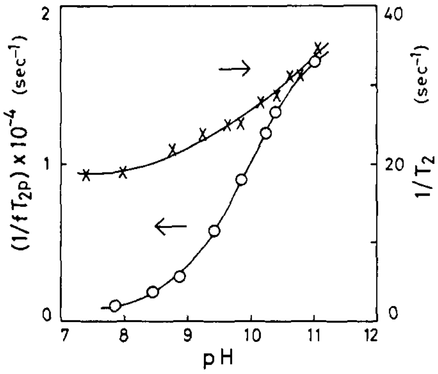

Figure 3. pH dependence of T_2^{-1} of the ϵ protons in the absence of Cu(II) and $(fT_{2p})^{-1}$ in the presence of Cu(II) at 310 K. The concentrations of the PLL residues and Cu(II) are 4.83×10^{-2} and 2.22×10^{-5} M, respectively ($f = 4.6 \times 10^{-4}$).

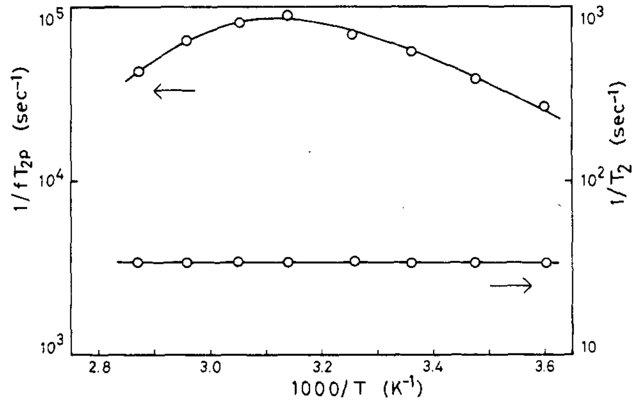

Figure 4. T_2^{-1} in the absence of Cu(II) and $(fT_{2p})^{-1}$ in the presence of Cu(II) for ϵ protons at pH 10.4 as functions of the reciprocal of the temperature. The concentrations are the same as those in Figure 3.

The $(fT_{2p})^{-1}$ curve can be divided into two regions: the low-temperature range where $(fT_{2p})^{-1}$ increases with increasing temperature, and the high-temperature range, where $(fT_{2p})^{-1}$ decreases with increasing temperature. In the low-temperature range, the exchange between the complexed and uncomplexed states will be slow, i.e., $T_{2M} < \tau_M$. Thus, in this temperature range eq 8 is written as

$$(fT_{2p})^{-1} = q/\tau_M \tag{9}$$

Since τ_M decreases with increasing temperature, eq 9 suggests that $(fT_{2p})^{-1}$ increases with increasing temperature. From the slope of the curve in the low-temperature range, the activation energy of the exchange process for the PLL residue between the complexed and uncomplexed states can be estimated. A value of 6 kcal/mol was obtained at pH 10.4. This value is a little greater compared with a value found for the water proton of the aqua complex of Cu(II) which has been reported to be 2–3 kcal/mol by Bernheim et al.[28]

In the high-temperature range, the exchange will become fast, i.e., $T_{2M} > \tau_M$, and eq 8 is simplified as

$$(fT_{2p})^{-1} = q/T_{2M} \tag{10}$$

Therefore, the temperature dependence of $(fT_{2p})^{-1}$ at higher temperatures corresponds primarily to that of T_{2M}^{-1}, which is in most cases a decreasing function of temperature.

Figure 5 shows the longitudinal relaxation rate T_1^{-1} for ϵ protons in the absence of Cu(II) and the paramagnetic contribution to it, $(fT_{1p})^{-1}$, as functions of the reciprocal

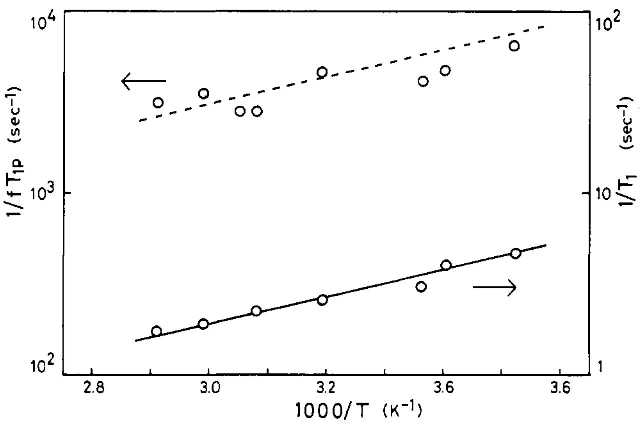

Figure 5. T_1^{-1} in the absence of Cu(II) and $(fT_{1p})^{-1}$ in the presence of Cu(II) for ϵ protons at pH 10.4 as functions of the reciprocal of the temperature. The concentrations are the same as those in Figure 3.

of the temperature at pH 10.4. T_1^{-1} in the absence of Cu(II) decreases with increasing temperature, indicating that the extreme narrowing conditions are satisfied. Therefore, T_1^{-1} is approximately given by the following equation[29]

$$T_1^{-1} = \frac{2\gamma_H^4\hbar^2 I(I+1)}{r_{H-H}^6}\tau_R \tag{11}$$

where r_{H-H} is the distance between two ϵ protons, γ_H is the gyromagnetic ratio of the proton, I is the nuclear spin quantum number of the proton, and τ_R is the rotational correlation time of the tumbling motion of the ϵ protons. We neglect relaxation effects due to the δ protons. This approximation gives 30% error at worst to the estimated T_1 value. A τ_R value of 1×10^{-10} s was obtained from the observed value of T_1 in the absence of Cu(II) at 297 K and pH 10.4, using eq 11 with an assumption of $r_{H-H} = 1.78$ Å. Taking account of ^{13}C data by Saito and Simth[30] and also the present ^{13}C data shown in the next section, the order of magnitude of the τ_R value seems reasonable. The temperature dependence of T_1^{-1} corresponds to that of the correlation time of the tumbling motions of ϵ protons τ_R, and the slope of the straight line shown in the figure yields the activation energy for the tumbling motions of ϵ protons. We obtained a value of 4 kcal/mol, which was compared with a value of 4.6 kcal/mol obtained for the tumbling motions of the γ-carbon atom in the side chain of poly(D-glutamic acid).[31]

The paramagnetic contribution $(fT_{1p})^{-1}$ decreases with increasing temperature in the entire temperature range studied. This suggests that the lifetime of a PLL residue in the coordination position of Cu(II), τ_M, is less than the longitudinal relaxation time in the complexed state T_{1M}, i.e., the exchange process is fast. Thus, eq 2 can be simplified as

$$(fT_{1p})^{-1} = q/T_{1M} \tag{12}$$

We assume that the correlation time for the tumbling motions of the internuclear vector pointing from the ϵ proton to Cu(II) is approximated by the rotational correlation time for the internuclear vector joining two ϵ protons τ_R, which is in a range of 10^{-10} s as estimated above. The electron spin relaxation time τ_s of Cu(II) is reported to be in the order of 10^{-8} s,[23] which is greater than τ_R. Thus, eq 3 is reduced to

$$T_{1M}^{-1} = \frac{2}{5}\frac{\gamma_H^2 g^2\beta^2 S(S+1)}{r_{M-H}^6}\tau_R \tag{13}$$

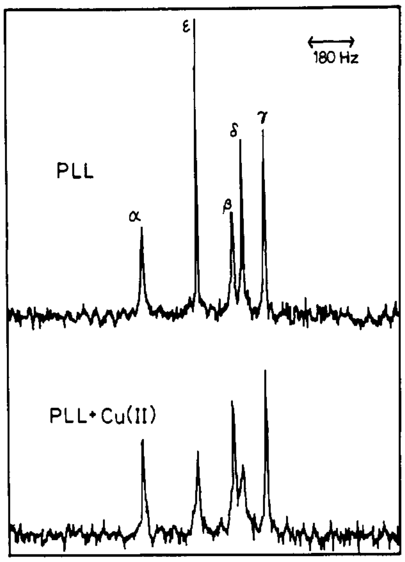

Figure 6. The high-field portion of the 15.04-MHz ^{13}C NMR spectra of poly(L-lysine) in D_2O at a residual concentration of 2.73×10^{-1} M at pH 10.5 and at 297 K: upper, no Cu(II) is added; bottom, Cu(II) of 4.69×10^{-4} M is added.

where r_{M-H} is the distance between the Cu(II) and ϵ protons. Therefore, the temperature dependence of $(fT_{1p})^{-1}$ is similar to that of T_1^{-1}. A best fitted line to the data of $(fT_{1p})^{-1}$ parallel to the straight line of T_1^{-1} is depicted as a dotted line in Figure 5.

Combining eq 11, 12, and 13, we obtain

$$(fT_{1p})/T_1 = \frac{5}{q}\left(\frac{\gamma_H\hbar}{g\beta}\right)^2\left(\frac{r_{M-H}}{r_{H-H}}\right)^6 \tag{14}$$

From this equation, the distance between the Cu(II) and ϵ protons can be estimated, using experimental values of T_1 and fT_{1p}. An estimated value of r_{M-H} was found to be in a range of 3.4–4.3 Å with assumptions involved in eq 11 and of $q = 1$–4. This value seems reasonable, taking into account the fact that the side-chain amino group binds to Cu(II).

A comparison of values of $(fT_{2p})^{-1}$ and $(fT_{1p})^{-1}$ shown in Figures 4 and 5 shows that $(fT_{2p})^{-1}$ is an order of magnitude greater than $(fT_{1p})^{-1}$. This indicates that T_{2M}^{-1} is much greater than T_{1M}^{-1}, suggesting a predominant contribution of the hyperfine interaction term to the transverse relaxation rate T_{2M}^{-1}. The implication of this finding is that a significant amount of electron spin is delocalized from Cu(II) to the side-chain ϵ protons. Taking account of the large contribution of the hyperfine interaction term to T_{2M}^{-1}, eq 7 can be written as

$$T_{2M}^{-1} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\tau_e \tag{15}$$

Thus, the temperature dependence of $(fT_{2p})^{-1}$ in the high-temperature range of Figure 4 is that of the correlation time of the hyperfine interaction τ_e, which in general varies in a complicated fashion.[32]

^{13}C NMR of PLL. Figure 6 shows the high-field portion of the 15.04-MHz ^{13}C NMR spectra of PLL in aqueous (D_2O) solution at pH 10.5 and at a temperature of 297 K in the absence (upper) and the presence (bottom) of Cu(II). The concentrations of the PLL residues and Cu(II) are 2.73×10^{-1} and 4.69×10^{-4} M, respectively. The signal of the peptide carbonyl carbon appears at lower fields and is not shown in this figure. Assignments of signals were based on the results by Saito and Smith.[30]

Table I
Paramagnetic Contributions of Cu(II) to Relaxation Rates for Side-Chain Carbons of PLL

	$(fT_{2p})^{-1}$, s^{-1}			$(fT_{1p})^{-1}$, s^{-1}	
pH	γ	δ	ϵ	δ	ϵ
7.0	0	0	0	0	0
8.7	0	6.5×10^3	5.7×10^3	0.6×10^3	1.1×10^3
10.5	6×10^3	24×10^3	15×10^3	1.2×10^3	2.7×10^3

As can be seen in Figure 6, the addition of Cu(II) does not have any appreciable effects upon the signals of α and β carbons, but remarkable line broadening is observed for ϵ and δ carbons. No effects are observed at neutral and acidic pH. These results as well as the proton NMR results described above strongly suggest that the side-chain amino group is coordinated to Cu(II).

The paramagnetic contributions to the transverse and longitudinal relaxation rates were measured as functions of pH and temperature. The concentrations of the PLL residues and Cu(II) were 2.73×10^{-1} and 3.47×10^{-4} M, respectively, i.e., $f = 1.27 \times 10^{-3}$. Table I shows the results of $(fT_{1p})^{-1}$ for ϵ, δ, and γ carbons at three different pHs and at 297 K. $(fT_{1p})^{-1}$ as well as $(fT_{2p})^{-1}$ increases with increasing pH from 7 to 10.5, indicating that the binding occurs at alkaline pH.

As in the case of ^{1}H NMR, $(fT_{2p})^{-1}$ for ^{13}C NMR is an order of magnitude greater than $(fT_{1p})^{-1}$, suggesting that a large amount of electron is delocalized from Cu(II) to ϵ, δ, and even γ carbons. It is of interest to note that $(fT_{2p})^{-1}$ for the δ carbon is greater than that for the ϵ carbon. This may imply that a larger amount of electron spin exists on the ϵ-carbon atom than on the δ-carbon atom.

$(fT_{2p})^{-1}$ at pH 10.5 as well as T_2^{-1} in the absence of Cu(II) at the same pH for the ϵ carbon is plotted against the reciprocal of the temperature in Figure 7. T_2^{-1} remains constant with changing temperature, suggesting that T_2^{-1} is in large part determined by the instrumental broadening. On the other hand, $(fT_{2p})^{-1}$ decreases with increasing temperature, indicating that the exchange process is fast and that a dominant contribution to $(fT_{2p})^{-1}$ is T_{2M}. It seems likely that a maximum of $(fT_{2p})^{-1}$ appears at lower temperatures compared to $(fT_{2p})^{-1}$ for ϵ protons shown in Figure 4. This suggests that the transverse relaxation time for ϵ protons in the complexed state T_{2M} (ϵ-H) is less than the one for the ϵ carbon $T_{2M}(\epsilon\text{-C})$. This means that the hyperfine coupling constant of ϵ protons-Cu(II) interaction is greater than that of ϵ carbon-Cu(II).

Figure 8 shows T_1^{-1} values for protonated carbons at three different pHs in the absence of Cu(II) as functions of the reciprocal of the temperature. T_1^{-1} values in the absence of Cu(II) decrease with increasing temperature, indicating that the extreme narrowing conditions are fulfilled. This was confirmed by the nuclear Overhauser enhancement (NOE) measurements. NOE values for all protonated carbons were found to be greater than 2. Therefore, T_1^{-1} is approximately given by

$$T_1^{-1} = \frac{N\gamma_H^2\gamma_C^2\hbar^2}{r_{C\text{-}H}^6}\tau_R \qquad (16)$$

where N is the number of protons directly attached to the observing carbon. The correlation times estimated from eq 16, using observed values of T_1, are lisited in Table II.

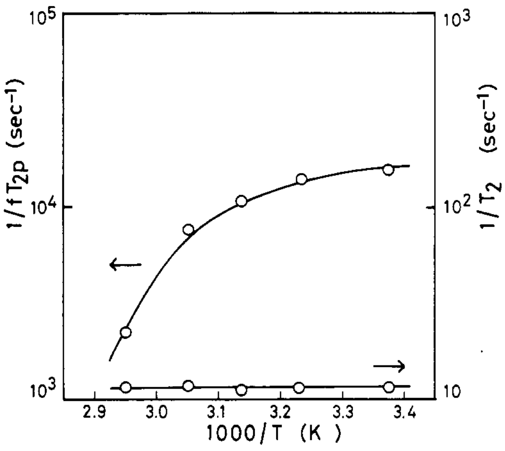

Figure 7. T_2^{-1} in the absence of Cu(II) and $(fT_{2p})^{-1}$ in the presence of Cu(II) for the ϵ carbon of poly(L-lysine) at pH 10.5 as functions of the reciprocal of the temperature.

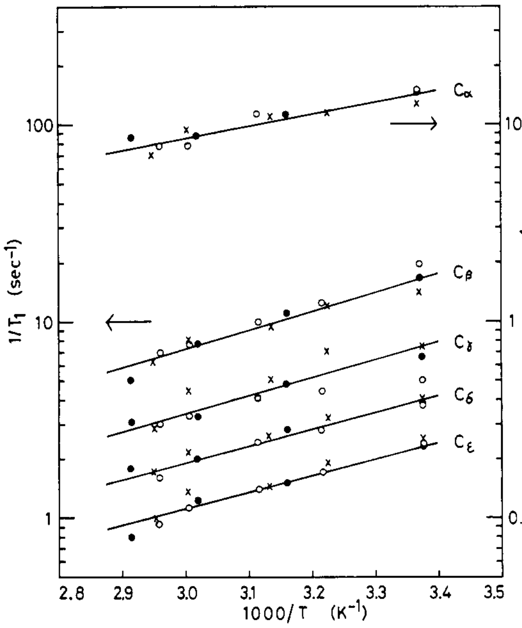

Figure 8. T_1^{-1} values for protonated carbons of poly(L-lysine) as functions of the reciprocal of the temperature at three different pHs: (●), pH 7.0; (○), pH 8.7; (×), pH 10.4.

These values are in agreement with those reported by Saito and Smith.[30]

We obtained an activation energy of 4 kcal/mol for tumbling motions of the ϵ-C-ϵ-H bond from the temperature dependence of T_1^{-1} shown in Figure 8. This value is in agreement with that obtained from the results of the ϵ proton NMR in the preceding section.

Figure 9 shows $(fT_{1p})^{-1}$ for the ϵ carbon at pH 10.5 as a function of the reciprocal of the temperature. T_1^{-1} values are also plotted in the figure. $(fT_{1p})^{-1}$ for the ϵ carbon decreases with increasing temperature, suggesting that the exchange process is fast. The dotted line in the figure is parallel to the straight line of T_1^{-1}. The agreement with the experimental points is rather poor. If we make similar assumptions as in the preceding section, the distance between the Cu(II) and ϵ carbon can be evaluated to be 2.2-2.7 Å. Taking into account the fact that the amino

Table II
Correlation Times for Carbon Atoms of PLL at 297 K and pH 7.0

	α	β	γ	δ	ϵ
τ_R, s	6.7×10^{-10}	3.5×10^{-10}	1.6×10^{-10}	0.9×10^{-10}	0.5×10^{-10}

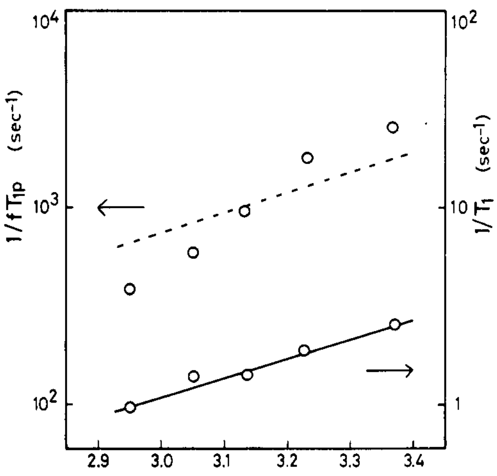

Figure 9. $(fT_{1p})^{-1}$ for the ϵ carbon of poly(L-lysine) at pH 10.5 as a function of the reciprocal of the temperature.

Table III
Ratio of NOE Values in the Presence and the Absence of Cu(II)[a]

	α	β	γ	δ	ϵ
ratio	1	1	1	0.5	0.4

[a] The concentrations of PLL residues and Cu(II) are 2.73×10^{-1} and 3.47×10^{-4} M, respectively (at 297 K and at pH 10.5).

group of the side chain is coordinated to Cu(II), this value seems reasonable.

It has been suggested that the NOE is quenched by the paramagnetic metal ions like Cu(II).[33-35] Table III lists the ratio of the NOE values in the absence and the presence of Cu(II) at a concentration of $f = 1.27 \times 10^{-3}$ for all protonated carbons at pH 10.5 and at 297 K. It is apparent that the NOEs for ϵ and γ carbons are quenched by the addition of Cu(II), but no remarkable effects on α, β, and γ carbons are observed. This is in accordance with the fact that the amino group is coordinated to Cu(II).

Enhancement of the Water Proton Relaxation Rate. The longitudinal relaxation rate of water proton increases with the addition of the paramagnetic ion like Cu(II) to water. If we further add the macromolecule to this aqueous solution of the paramagnetic metal ion, the longitudinal relaxation rate of the water proton may be further affected.[36] The enhancement factor of the water proton relaxation rate is defined as

$$\epsilon_1{}^* = \frac{1/T_{1p}{}^*(\mathrm{H_2O})}{1/T_{1p}(\mathrm{H_2O})} \qquad (17)$$

where the asterisk means the presence of the macromolecule.

If the metal ion does not bind to the macromolecule, we obtain $\epsilon_1{}^* = 1$. If the metal ion binds to the macromolecule, and if the metal ion binding to the macromolecule still has labile water molecules in the first coordination sphere, we get $\epsilon_1{}^* > 1$. On the other hand, if the metal ion binding to the macromolecule does not have a labile water molecule in the first coordination sphere, we have $\epsilon_1{}^* < 1$.

We measured the enhancement of the relaxation rate of the water proton of the Cu(II) aqueous solution by the addition of PLL. Figure 10 shows the enhancement factor $\epsilon_1{}^*$ as a function of pH at 300 K. The concentrations of PLL residues and Cu(II) are 2.08×10^{-2} and 1×10^{-3} M, respectively. It was found that the value of $\epsilon_1{}^*$ increases abruptly in the pH region 8–10 from unity at lower pH to a value of about 4 at higher pH. $\epsilon_1{}^* \approx 1$ at lower pH means that no binding of Cu(II) to PLL occurs at acidic pH. The increase in $\epsilon_1{}^*$ at alkaline pH is caused by the binding of Cu(II) to PLL. The results obtained here indicate that Cu(II), which is binding to the amino group of the side chain at alkaline pH, still accommodates the water molecule in the first coordination sphere.

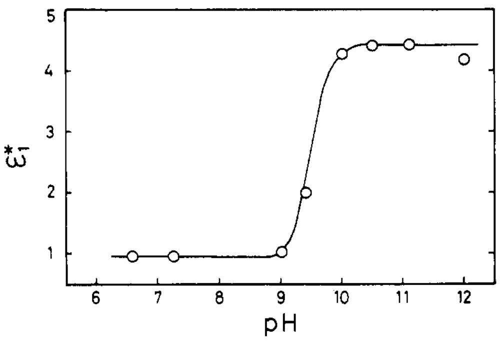

Figure 10. Enhancement factor of the water proton relaxation rate $\epsilon_1{}^*$ as a function of pH at 300 K. Concentrations of PLL residues and Cu(II) are 2.08×10^{-2} and 1×10^{-3} M, respectively.

Comparison with Models Previously Proposed. The results obtained in the present study suggest a structural model in which side-chain amino groups and water molecules are coordinated to Cu(II) at alkaline pH. This is consistent with the first type of complex reported by Peggion et al.[11,12] and Tosi et al.[9] at pH <8. They have reported that at higher pH, peptide nitrogens participate to form the second type of complex. The present studies which are carried out at a molar ratio of Cu(II) to PLL residue ranging from 4.6×10^{-4} to 1.3×10^{-3} show no evidence for peptide nitrogen to coordinate to Cu(II). This was confirmed by ^{15}N NMR experiments.[37] Peggion et al.[38] have stated that the stability of the second type of complex of poly(L-histidine)–Cu(II) depends on the Cu(II)/peptide molar ratio as well as pH and the conformation of the polypeptide. In this respect, it should be noted that the present NMR studies were done at lower Cu(II) concentrations and at higher PLL concentrations than earlier optical studies. It would be desired that both optical and NMR studies should be done at the same conditions, but at the present time it is difficult to do NMR measurements at PLL concentrations as low and at Cu(II) concentrations as high as the optical studies.

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^{15}N NMR Spectroscopy. 21.[1] Copolymerization of Glycine *N*-Carboxyanhydride with γ-Methyl Glutamate *N*-Carboxyanhydride, *S*-Benzylcysteine *N*-Carboxyanhydride, Leucine *N*-Carboxyanhydride, and Valine *N*-Carboxyanhydride

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ABSTRACT: The copolymerization of Gly-NCA with γ-MeGlu-NCA, *S*-BzlCys-NCA, Leu-NCA, and Val-NCA was investigated in various solvents but mostly in dioxane. Primary, secondary, and tertiary amines were used as initiators, and in some cases an electrophilic cocatalyst, e.g., 4-chlorophenyl isocyanate, was added. The 36.48 MHz natural abundance ^{15}N NMR spectra of the resulting copolypeptides, measured in trifluoroacetic acid, display four signals which represent the four different types of peptide groups which can exist in binary copolypeptides. A combined, quantitative evaluation of the ^{1}H NMR and ^{15}N NMR spectra allows one to calculate the average length of the homogeneous blocks and the copolymerization parameters. This evaluation demonstrates that the nature of the sequences depends mainly on the reactivity of the monomers, which decreases in the following order: Gly-NCA > γ-MeGlu-NCA > *S*-BzlCys-NCA > Leu-NCA > Val-NCA. While in most cases random sequences are predominantly formed, the copolymerization of Gly-NCA with Leu-NCA and Val-NCA shows a tendency to yield block copolymers.

The copolymerization of α-amino acid NCA's allows the synthesis of copolypeptides of all common α-amino acids. Since the ratio of the monomer units and the molecular weights can be varied over a wide range, these synthetic copolypeptides are useful model compounds for proteins. Chemical, physicochemical, and physical studies on the properties of such copolypeptides require that the primary structure is known, because most properties depend on the sequence. However, the enzymatic, chemical, and physical methods widely used for the sequence analysis of proteins cannot be applied to synthetic copolypeptides. Thus, spectroscopic methods useful for the characterization of peptide sequences are desirable, in as much as they are nondestructive, time saving, and routine. Since ^{1}H NMR spectra of copolypeptides do not contain sequential information, we have studied the ^{13}C NMR spectra of various copolypeptides; however, only in a few special cases of alanine containing binary copolypeptides is the ^{13}C NMR method successful, even if a magnetic field of 84 kG (8.4 T) is used. Since the ^{17}O spectra of oligopeptides display only extremely broad signals without fine structure,[2] only ^{15}N NMR spectroscopy is possibly useful for a spectroscopic sequence analysis of synthetic copolypeptides. A first success in this direction was achieved when copolymers obtained from Gly-NCA and β-Ala-NCA were spectroscopically analyzed.[3a] The present work was undertaken to test whether copolypeptides exclusively built up from α-aminoacyl units are also accessible to ^{15}N NMR

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