$$n_3 = kK_3/(1 + kK_3) \quad (3)$$

$$n_6 = kK_6/(1 + kK_6) \quad (4)$$

where k is a constant denoting the substituting power of the reaction medium, and K_2, K_3, and K_6 designated respectively the equilibrium constants for the substitution reactions of the hydroxyls at positions 2, 3, and 6.

The fraction of the completely substituted AHG units, f_3, is

$$f_3 = (2,3,6) = n_2n_3n_6 = k^3K_2K_3K_6/[(1 + kK_2) \times (1 + kK_3)(1 + kK_6)] = k^3K_2K_3K_6/d \quad (5)$$

$$(2,3) = n_2n_3 - (2,3,6) = k^2K_2K_3/d \quad (6)$$

$$(2,6) = k^2K_2K_6/d \quad (7)$$

$$(3,6) = k^2K_3K_6/d \quad (8)$$

$$(2) = n_2 - (2,3) - (2,6) - (2,3,6) = kK_2/d \quad (9)$$

$$(3) = kK_3/d \quad (10)$$

$$(6) = kK_6/d \quad (11)$$

where (2), (3), and (6) are the fractions of AHG units with substituents only in the positions named. Similarly, (2,3) designates the fraction of AHG units containing substituents only in the 2 and 3 positions.

The equations for the fractions of unsubstituted, monosubstituted, and disubstituted AHG units are

$$f_0 = 1/d \quad (12)$$

$$f_1 = k(K_2 + K_3 + K_6)/d \quad (13)$$

$$f_2 = k^2(K_2K_3 + K_2K_6 + K_3K_6)/d \quad (14)$$

The average degree of substitution of the cellulose derivative is

$$DS = n_2 + n_3 + n_6 = 3 - 3f_0 - 2f_1 - f_2 \quad (15)$$

An additional set of correlations can also be derived as follows:

$$(2)/f_1 = K_2/(K_2 + K_3 + K_6) \quad (16)$$

$$(3)/f_1 = K_3/(K_2 + K_3 + K_6) \quad (17)$$

$$(6)/f_1 = K_6/(K_2 + K_3 + K_6) \quad (18)$$

$$(2,3)/f_2 = K_2K_3/(K_2K_3 + K_2K_6 + K_3K_6) \quad (19)$$

$$(2,6)/f_2 = K_2K_6/(K_2K_3 + K_2K_6 + K_3K_6) \quad (20)$$

$$(3,6)/f_3 = K_3K_6/(K_2K_3 + K_2K_6 + K_3K_6) \quad (21)$$

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A Nitrogen-15 Nuclear Magnetic Resonance Study on the Copper(II) Complex of Poly(L-lysine)

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ABSTRACT: Nitrogen-15 nuclear magnetic resonance spectra of 46% ^{15}N-enriched poly(L-lysine) in aqueous solutions at different pHs were obtained. On the addition of a small amount of the copper(II) ion, the ^{15}N signal of the side chain amino group almost disappeared in the alkaline pH region, while the signal of the amide nitrogen remained. This fact indicates that the side chain amino groups have much more tendency to bind to the copper(II) ion than to the amide groups even in alkaline solutions.

The copper(II) complex of poly(L-lysine·HBr) (PLL) has a stereospecific catalytic activity for the oxidation of L-3,4-dihydroxyphenylalanine (DOPA) and is regarded as a metallo enzyme model of DOPA oxidase.[2] Several workers have investigated the copper(II) complex of PLL by means of potentiometric, circular dichroism and other physicochemical techniques. They discussed the conformations and properties of the PLL–Cu(II) complex in aqueous solution, especially the mode of interaction with copper ion.[3–6] Nozawa and Hatano proposed a model in which the side-chain amino groups coordinate to the copper(II) ion in a planar tetragonal form below pH 8, but

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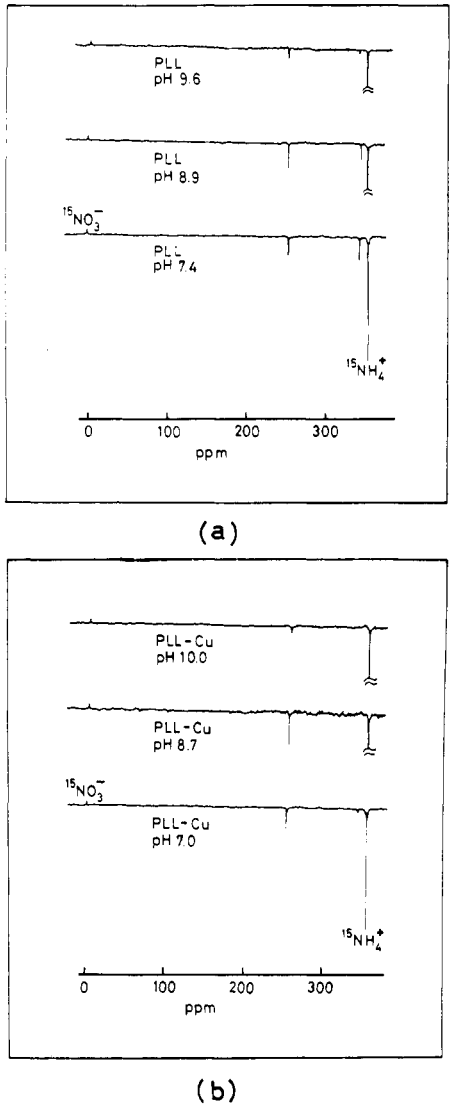

Figure 1. ^{15}N NMR spectra (10.1 MHz) of ^{15}N-PLL in H_2O, which were obtained with the proton-decoupled NOE mode after 10 000–30 000 pulses. The pulse interval was 2.0 s, and the pulse width was 15 μs (~30 °C). The signal intensity in each spectrum is normalized to that of the external standard, $^{15}NH_4{}^{15}NO_3$. (a) Spectra of ^{15}N-PLL without copper: bottom, at pH 7.4; middle, at pH 8.9; top, at pH 9.6. (b) Spectra of ^{15}N-PLL with copper: bottom, at pH 7.0; middle, at pH 8.7; top, at pH 10.0.

above pH 8.5 the main-chain amide group begins to participate in the coordination to the copper(II) ion at the apical position of the plane.[3] Palumbo et al.[4] and Garnier and Tosi[5] suggested another type of coordination in the higher pH region where at least one deprotonated main-chain amide participates in the coordination to the copper(II) ion at planar tetragonal positions.

Recently, the mode of interaction of copper(II) to PLL was examined by means of proton and carbon-13 magnetic resonances.[6] In the study neither the relaxation time nor the nuclear Overhauser enhancement (NOE) of the main-chain α-carbon was affected on the addition of copper(II) at any pH. On the other hand, the relaxation rates of the side-chain ε-proton and δ- and ε-carbons become drastically faster in the region of pH 8–11, and NOE of δ- and ε-carbons was also extremely quenched on the addition of the copper(II) ion at pH 10.4. These results indicate that the coordination of the side-chain amino groups to the copper(II) ion is plausible, and no evidence was obtained for the coordination of the main-chain amide group. In the present study we confirmed the results by means of ^{15}N NMR. ^{15}N spectra give direct information on the type of coordination to copper(II) ion, since the nitrogen atoms locate at the sites which directly participate in the binding with paramagnetic species.

Carbobenzoxy-L-lysine (46.4% ^{15}N enriched) was purchased from Hikari Kogyo Co. Ltd., Japan, and ^{15}N-PLL was prepared as described elsewhere.[7] Both amino and amide nitrogens were equally labeled. ^{15}N NMR spectra were taken on a JEOL-PFT-100 pulse Fourier transform NMR spectrometer operating at 10.1 MHz. Spectra was obtained with the proton decoupled NOE mode after 10 000–30 000 pulses repeated every 2 s. Chemical shifts were read from the external $^{15}NO_3^-$ signal of $^{15}NH_4{}^{15}NO_3$ in dimethyl-d_6 sulfoxide solution in a capillary. About 10 mg of PLL was dissolved in 2 mL of H_2O (2.1×10^{-2} M in lysine residue), and NaOH was used for the adjustment of pH. Inherited PLL was contaminated with paramagnetic ions in preparation, so that the pH adjusted PLL solutions were treated with Chelex-100 before NMR measurements, and pH was read again after the NMR measurements. To see the effect of the copper(II) ion, $CuCl_2$ was added until it was 10^{-4} M to the Chelex treated PLL solution.

^{15}N NMR spectra of the aqueous solutions of PLL (a) and PLL with copper(II) (b) are given in Figure 1. The strong inverted signal at 355 ppm upfield from external $^{15}NO_3^-$ is due to $^{15}NH_4^+$ of the reference. According to previous studies,[7,8] the lower field signal at 254 ppm and the upper field signal at 345 ppm at pH 7.4 (Figure 1a, bottom) are assignable to the main-chain amide nitrogen and the side-chain amino nitrogen, respectively. The backbone of PLL is known to take randomly coiled conformation in neutral solution. When the pH of the PLL solution was raised, both signals became broad and weak but remained to be observed. The phenomenon was attributed to dipolar broadening or to the partial quenching of NOE resulting from the slower segmental motion of PLL,[7,8] which is known to be either in α-helical or β forms in the alkaline region.[9]

For copper(II) containing PLL solutions, however, as are shown in Figure 1b, the signal of the amino nitrogen at 345 ppm almost disappeared at pH 7.0 and completely vanished at pH 8.7. It means that the coordination of the amino nitrogen to the copper(II) ion occurred around pH 7. On the other hand, the signal of the main-chain amide nitrogen still remained throughout the pH region examined. If the copper(II) ion directly binds to the amide nitrogen, NOE caused by the proton irradiation should be quenched and the signal would be hardly observed. Therefore, the present evidence excludes the possibility for the coordination of the amide nitrogen to the copper(II) ion at this condition.

Peggion et al. mentioned that the type of the copper(II) complex of PLL depends on the Cu(II)/peptide molar ratio as well as pH.[10] The present experiment was carried out at the ratio of 5×10^{-3}, and the other spectroscopic and physicochemical studies cited above were done at much higher Cu(II)/peptide ratios. Thus the present result does not directly deny the proposed models in the alkaline pH region, but it can be said at least that the side-chain amino groups have much more tendency to bind to the copper(II) ion even in alkaline solution compared with the main-chain amide groups.

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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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