

SHORT COMMUNICATION

<sup>13</sup>C Paramagnetic Shift of Co(II)–Poly(D-glutamic acid) Complex

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Studies on the metal complexes of poly( $\alpha$ -amino acids) have been carried out extensively,<sup>1</sup> since these complexes may be considered as useful models for understanding the mode of action of metallo-proteins. The paramagnetic transition metal ions have great influence on the NMR of diamagnetic molecules in solution.<sup>2,3</sup> We have carried out NMR studies on the complexes of some paramagnetic metal ions with poly(D-glutamic acid) and poly(L-lysine),<sup>4–6</sup> and the structures and molecular dynamics of these complexes have been deduced from paramagnetic shifts and enhanced nuclear relaxation rates.

In this paper, the results of <sup>13</sup>C NMR studies on the Co(II)–poly(D-glutamic acid) (PGA) complex in aqueous solution (D<sub>2</sub>O) are reported. PGA was prepared as described previously<sup>7</sup> and CoCl<sub>2</sub>·6H<sub>2</sub>O was used. <sup>13</sup>C NMR spectra were obtained at 22.63 MHz using a Bruker SXP 4/100 FT spectrometer. Dioxane was used as an internal reference with chemical shifts converted to tetramethylsilane by adding 67.4 ppm to the measured shifts.

<sup>13</sup>C NMR spectra of PGA at pH 7.1 and 304 K are shown in Figure 1 at various Co(II) concentrations. The bottom spectrum is the one for PGA in the absence of Co(II). The addition of Co(II) to PGA progressively causes a shift and broadening of <sup>13</sup>C resonances of PGA, especially of C <sub>$\gamma$</sub>  and C <sub>$\delta$</sub>  resonances of the side chain. These effects are apparently caused by the unpaired electron spin of Co(II). Figure 2 shows the Co(II) concentration dependence of the observed paramagnetic shift. By increasing the molar ratio of Co(II) to the PGA residue,  $f$ , all the resonances shift downfield in the order of C <sub>$\gamma$</sub>  > C <sub>$\delta$</sub>  > C <sub>$\beta$</sub>  > C <sub>$\alpha$</sub> , C'. These paramagnetic shifts vary linearly with  $f$  at the Co(II) concen-

trations studied. The large shifts observed here show that Co(II) forms a complex with PGA; the carboxylate groups of the side chains participate to form the complex. Such large downfield shifts have also been observed for  $\beta$  and  $\gamma$  protons in the same complex.<sup>4</sup>

It is of interest to note that paramagnetic shifts are more effective at a neutral pH than at an acidic pH, at which PGA is in the helical form. This is attributed to the difference in conformation and/or the degree

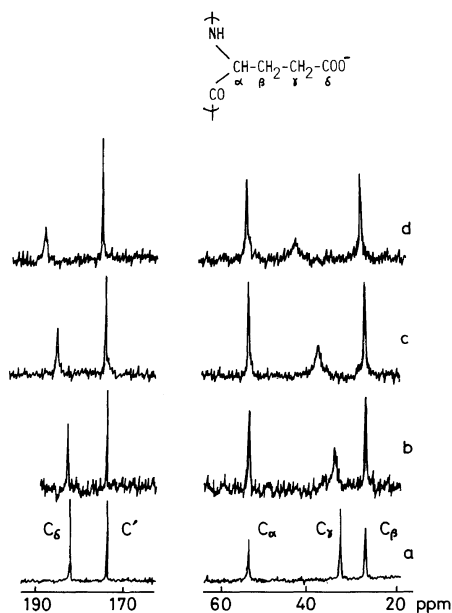
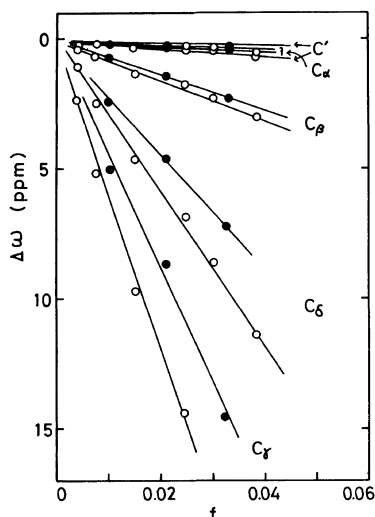
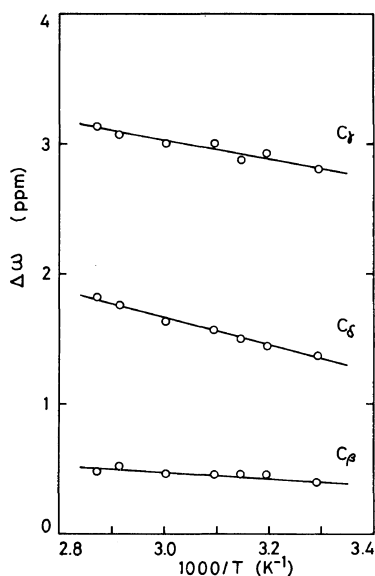


Figure 1. Effects of Co(II) on <sup>13</sup>C NMR spectra of PGA [0.83 M in monomer units] at pH 7.1 and at 304 K: (a)  $f = [\text{Co(II)}]/[\text{PGA}] = 0$ ; (b)  $f = 3 \times 10^{-3}$ ; (c)  $f = 8 \times 10^{-3}$ ; (d)  $f = 1.5 \times 10^{-2}$ . A bandwidth of 6024 Hz was used with 8 K data points.



**Figure 2.** Co(II) concentration variation of paramagnetic shift  $\Delta\omega$  for Co(II)-PGA complex at 304 K: (○) pH 7.1; (●) pH 4.7–4.8. [PGA]=0.83 M. The positive value of  $\Delta\omega$  means lower field shift.



**Figure 3.** Temperature variation of paramagnetic shift of  $C_\beta$ ,  $C_\gamma$ , and  $C_\delta$  carbons at pH 7.1 and  $f=5.2 \times 10^{-3}$ .

of ionization of the carboxylate groups. It is clear from these results that Co(II) is bound to the carboxylate groups even in the helix state. It is also of interest to note that the observed shift is larger for the  $C_\gamma$  carbon than for the  $C_\delta$  carbon.

Figure 3 shows the temperature dependence of the observed paramagnetic shifts for the  $C_\beta$ ,  $C_\gamma$ , and  $C_\delta$  carbons and that the observed shifts increase with increasing temperature. These results are in good agreement with those of the previous  $^1\text{H}$  NMR.<sup>4</sup>

Since the observed shifts vary with  $f$  and the two separate resonances from the complexed and uncomplexed states are not observable for any resonances, the chemical exchange between these two sites occurs and the exchange rate is fast. Furthermore, it was found that the paramagnetic contribution to the line width of each resonance decreased with increasing temperature. Under these conditions, the observed paramagnetic shift,  $\Delta\omega$ , is given by,<sup>8</sup>

$$\begin{aligned} \Delta\omega &= P_M \Delta\omega_M \\ &= \Delta\omega_M [\text{Co(II)}] / (K_d + [\text{PGA}]) \end{aligned} \quad (1)$$

where  $P_M$  is the fraction of Co(II)-PGA complex,  $K_d$  is the dissociation constant of the complex, and  $\Delta\omega_M$  is the intrinsic paramagnetic shift of the complex. The temperature dependence of  $\Delta\omega$ , therefore, arises from those of  $\Delta\omega_M$  and  $K_d$ .

The intrinsic paramagnetic shift,  $\Delta\omega_M$ , consists of the contact and pseudo-contact shifts,<sup>9</sup> but it is impossible to separate both contributions from the observed shift. Both the contact and pseudo-contact shifts are proportional to the reciprocal of temperature (Curie's law).<sup>9</sup> The temperature dependence of the observed paramagnetic shift obtained here is opposite to that of Curie's law. It is more likely that the temperature dependence of  $\Delta\omega$  is due mainly to that of  $K_d$  rather than to that of  $\Delta\omega_M$  and  $K_d > [\text{PGA}]$ . These results indicate that  $K_d$  decreases with increasing temperature. The behavior of the observed paramagnetic shifts may be interpreted in terms of the chemical equilibrium between the complexed and uncomplexed states shifting in the direction of complex formation with increasing temperature, which is the same as the results of  $^1\text{H}$  NMR.<sup>4</sup>

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