An NMR Study of a Poly(glutamic acid) Metal Complex

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ABSTRACT: The interactions of poly(glutamic acid) with cobaltous ions (Co(II)) in aqueous solution were investigated by means of NMR in the temperature range from 0 to 70°C. It was found that as Co(II) is added the α -CH and the NH peaks shift to higher fields and the β -CH₂ and γ -CH₂ peaks shift to lower fields. It is considered that these shifts are paramagnetic shifts due to the paramagnetism of Co(II). Large shifts of the γ -CH₂ peak indicate that Co(II) is coordinated with the carboxyl group. High-field shifts of the peak suggest that Co(II) is coordinated with the peptide NH. Paramagnetic shifts of the β -CH₂ and γ -CH₂ increased and those of the α -CH and NH decreased with increasing temperature. These results are interpreted in terms of changes in a chemical equilibrium between the Co(II)-free residues of PGA and the bonding ones. It is considered that the amount of Co(II) bonded to the carboxyl groups of PGA increases with increasing temperature. The temperature dependence of the shifts of β -CH₂ and γ -CH₂ suggests that the pseudocontact mechanism is also involved as well as the contact one.

KEY WORDS Nuclear Magnetic Resonance / Poly(glutamic acid) / Cobaltous Ion / Paramagnetic Shift /

So far the interactions of poly(glutamic acid) (PGA) with metal ions have been studied by various methods, such as potentiometric, spectroscopic and optical rotatory dispersion measurements.^{1,2} Most of these investigations are concerned with changes in physicochemical properties accompanied with the helix-coil transition. On the basis of ESR measurements, Yamazaki, *et al.*,³ reported that Cu(II) is bonded not only with the carboxyl group, but also with the peptide NH. Except for these reports, there have been few reports on the interaction between PGA and transition metal ions in aqueous solution.

The purpose of this study is to use nuclear magnetic resonance (NMR) spectroscopy to clarify the mechanism of the interaction between PGA and Co(II). It is known that Co(II) has a short electron relaxation time,⁴⁻⁶ so that, in spite of the paramagnetism of Co(II), proton NMR spectra of solutions containing Co(II) are not

broad compared to those of solutions containing other transition metal ions, such as Cu(II) and Mn(II). Luz and Meiboom⁵ observed NMR spectra of methanolic solutions of Co(II) and Ni(II) over a wide temperature range and they estimated the exchange rate of the methanol coordinated to Co(II) and Ni(II). McDonald and Phillips⁶ showed the proton signals of an aqueous solution of histidine is shifted by the presence of Co(II). They proposed possible structures for a histidine-Co(II) complex.

In this report, we present results of studies on the Co(II) induced shifts in the NMR spectra of PGA.

EXPERIMENTAL

A sample of the sodium salt of poly(D-glutamic acid) (NaPDGA) was kindly offered by Ajinomoto Co., Ltd. The molecular weight of this NaPDGA was about 80000. Any trace of

impurity was not detected in the NMR spectra. $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, and $\text{BaCl}_2 \cdot 6\text{H}_2\text{O}$ were purchased from Koso Chemical Co. and used without further purification.

NaPDGA was dissolved in D_2O to give concentrations of 2, 4 and 6 g/100 cc. The Co(II) solutions were made by dissolving $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ into D_2O . The molar mixing ratio, R , of cobaltous ion to a glutamic acid residue was varied from 0 to 0.23, by varying the Co(II) concentration and keeping the polymer concentration constant.

The NMR spectrometer used was a JNM-C-60HL (JEOL. Co.). A 100-MHz spectrometer (JNM-4H-100) was also used, if needed. Both instruments were equipped with variable temperature probes. The sample temperature was varied between 0 and 70°C . Spectra were observed in the mode of NMR controlled field sweep. A few drops of *t*-butanol were added to each solution, and its methyl resonance line was taken as an internal reference standard. All values for chemical shifts are given in ppm from this signal and down field shifts are assigned positive values.

RESULTS AND DISCUSSION

The 60-MHz proton NMR spectrum of 2-% NaPDGA in D_2O solution at 28°C is presented in Figure 1. Peak E is the signal of *t*-butanol. Peak B, appearing at *ca.* 1 ppm, is asymmetric in shape and in the 100-MHz spectrum was found to be separated into two lines, a broader one, B_1 , at higher field and a sharper one, B_2 , at lower field. When H_2O was used as the solvent, a signal D appeared at *ca.* 7.3 ppm.

On the basis of the NMR spectra of glutamic acid monomer,⁷ and poly(γ -benzyl-L-glutamate),⁸ we assigned the peaks A, B_1 , B_2 , C, and D to $\alpha\text{-CH}$, $\beta\text{-CH}_2$, $\gamma\text{-CH}_2$, HDO, and peptide NH protons, respectively. When D_2O was used as the solvent, the peptide NH proton signal could not be observed. This is thought to be due to the exchange between the NH proton and the deuterium of D_2O .

It was found that, as Co(II) was added, the $\alpha\text{-CH}$ and the NH peaks shifted to higher fields, whereas the $\beta\text{-CH}_2$ and the $\gamma\text{-CH}_2$ peaks shifted to lower fields. At high Co(II) concentrations,

the $\gamma\text{-CH}_2$ peak was separated into two lines. The spectrum of 2-% NaPDGA solution containing Co(II) ($R=0.18$) observed at 28°C is shown in Figure 2. In Figure 3, the shifts of the $\alpha\text{-CH}$, $\beta\text{-CH}_2$, $\gamma\text{-CH}_2$, and NH lines are shown against the Co(II) concentration.

The shifts observed in the present work are more than 1 ppm, and are greater than that

Figure 1. The 60-MHz proton NMR spectrum of 2-% NaPDGA in D_2O solution at 28°C .

Figure 2. The 60-MHz proton NMR spectrum of 2-% NaPDGA in D_2O solution containing Co(II) ($R=0.18$) at 28°C .

Figure 3. The shifts of $\alpha\text{-CH}$, $\beta\text{-CH}_2$, $\gamma\text{-CH}_2$ and NH peaks vs. the ratio of the number of Co(II) ions to the number of glutamic acid residues, R . Data for NH were obtained at 24°C and other data at 28°C .

observed in the course of the helix—coil transition.⁹ It cannot be considered that the shifts are due to the helix-coil transition, which might be induced by adding the salt. It is reported that such large shifts could arise from an electrostatic effect or from the paramagnetism of Co(II).¹⁰

In order to estimate the contributions to the shifts from the electrostatic effects, we examined the NMR spectra of NaPDGA for the effects of divalent salts such as CaCl_2 , MgCl_2 , and BaCl_2 . The shifts induced by those ions are very small compared to those of Co(II). This fact indicates that the shifts induced by Co(II) are not due to the electrostatic effect, but the paramagnetism of Co(II).

There are two mechanisms for the paramagnetic shift, one is the contact shift and the other the pseudocontact shift. The contact shift is observed when an unpaired electron-spin density is finite at the resonance nucleus.¹¹ The pseudocontact shift is observed in complexes when a paramagnetic ion has an anisotropic g factor and occupies a particular position relative to the ligand molecule.¹² In general, Co(II) is known to have an anisotropic g factor.¹³

PGA has the carboxyl group COOH , the peptide NH and CO each of which is able to form a coordinate bond to Co(II). Large shifts of the $\gamma\text{-CH}_2$ and NH lines indicate that the Co(II) is coordinated with the γ -carboxyl and NH groups. In fact, it is reported that the protons bonding to nitrogen atoms show up-field shifts when the nitrogen atoms coordinate to Co(II).⁴ The fact that the $\beta\text{-CH}_2$ paramagnetic shifts are smaller than those of the $\gamma\text{-CH}_2$ is interpreted as follows. When a coordinate bond between Co(II) and the carboxyl group is formed, a little of the unpaired electron-spin density will be transmitted from the carboxyl group to the γ - and β -carbons of PGA. It is rapidly attenuated with going from the γ -carbon to the β -carbon. Therefore, the smaller unpaired electron density of the $\beta\text{-CH}_2$ results in a smaller paramagnetic shift for the $\beta\text{-CH}_2$.

Both areas under the two lines of the $\gamma\text{-CH}_2$ are equal to each other at all concentrations of Co(II). This indicates that the two lines are due to the respective protons of the $\gamma\text{-CH}_2$. The difference in shifts of the two protons of

$\gamma\text{-CH}_2$ shows a different magnetic environment at each proton, which is considered to be due to the particular orientation of the side chain of the PGA relative to the Co(II). The particular orientation of the side chain may be caused by a chelate ring, formed by the coordination of the γ -carboxyl and peptide NH . A similar result is reported for the lines of $\alpha\text{-CH}_2$ in the spectrum of the N -methyl glycine—Ni(II) complex.⁴ The line of $\alpha\text{-CH}_2$ splits into two lines, when a 5-membered ring is formed in the N -methylglycine complex with Ni(II).

Spectra of PGA solutions containing Co(II) were obtained from 0 to 70°C . It was found that with increasing temperature the paramagnetic shifts of $\beta\text{-CH}_2$ and $\gamma\text{-CH}_2$ increased while those of NH and $\alpha\text{-CH}$ decreased. In Figure 4, the paramagnetic shifts measured from the values of chemical shifts in the absence of Co(II) are plotted against the reciprocal temperature. In general, paramagnetic shifts are reported to be proportional to the reciprocal temperature.^{11,12} The present results do not obey this rule. These results can be interpreted in terms of the change in a chemical equilibrium between the Co(II)-free residues of PGA and Co(II)-bonding ones. If a fast exchange between two species occurs, the position of the observed line is a weighted average of the two lines from the respective species. It is, therefore, expected that the observed shift varies if the relative concentration of the two species varies with temperature. The

Figure 4. Co(II)-induced paramagnetic shifts as a function of the reciprocal temperature for 4% NaPDGA solutions containing Co(II) ($R=0.06$ for NH and $R=0.23$ for others).

increase of the observed paramagnetic shifts of β -CH₂ and γ -CH₂ indicates an increase of the Co(II)-bonded carboxyl groups of PGA with increasing temperature.

The ratio of the observed paramagnetic shift of the β -CH₂ relative to that of the γ -CH₂ depends on temperature. If the shift is caused by the contact mechanism alone, the ratio will be independent of temperature, because the ratio is related simply to the hyperfine constant which is not considered to depend on temperature. On the other hand, if the shift is caused by the pseudocontact mechanism, the ratio may depend on temperature, since the ratio is related to the position of the metal ion relative to the protons of β -CH₂ and γ -CH₂, and this relative position may be varied with thermal motion.

Figure 5. The shifts of β -CH₂, γ -CH₂, and NH peaks vs. the ratio of the number of Ni(II) ions to the number of glutamic-acid residues, R . Data for NH were obtained at 36°C and other data at 28°C.

Figure 6. Ni(II) induced paramagnetic shifts as a function of reciprocal temperature for 4% NaPDGA solutions containing Ni(II) ($R=0.13$).

Ni(II) is known to have an isotropic g factor in aqueous solution,¹⁴ therefore, there is no contribution from pseudocontact shift. In order to clarify that the ratio in the case of contact mechanism is independent of temperature, the effect of Ni(II) upon the NMR spectra of PGA was examined with the same procedures as for Co(II). When Ni(II) was added to NaPDGA solutions, peaks of β -CH₂ and γ -CH₂ were found to shift to lower fields in the same way as those of Co(II). The peak of the α -CH was screened with HDO signal. In Figure 6, the paramagnetic shifts measured from the values of chemical shifts in the absence of Ni(II) are plotted against the reciprocal temperatures. The result shows that the ratio of the paramagnetic shifts of the β -CH₂ relative to that of the γ -CH₂ is independent of temperature. This fact supports the interpretation that the temperature dependence of the ratio in the case of Co(II) is due to the pseudocontact mechanism.

It may be concluded that Co(II) is coordinated with the carboxyl group and the peptide NH, and that a chelate ring is formed. The observed shifts are due to both contact and pseudocontact mechanisms. The amount of Co(II) bonded to the carboxyl group of PGA increases with increasing temperature.

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REFERENCES

1. A. L. Jacobson, *Biopolymers*, **1**, 269 (1963).
2. A. L. Jacobson, *ibid.*, **2**, 237 (1964).
3. H. Takesada and H. Yamazaki, *ibid.*, **4**, 713 (1965).
4. R. S. Milner and L. Pratt, *Disc. Faraday Soc.*, **34**, 88 (1962).
5. Z. Luz and S. Meiboom, *J. Chem. Phys.*, **40**, 2686 (1964).
6. C. C. McDonald and W. D. Phillips, *J. Amer. Chem. Soc.*, **85**, 3736 (1963).
7. Y. Kobayashi and H. Makino, *Biochim. Biophys. Acta*, **191**, 738 (1969).
8. E. M. Bradbury and C. Robinson, *Nature*, **220**, 1079 (1968).
9. J. L. Markly, D. H. Meadows, and O. Jardetzky, *J. Mol. Biol.*, **27**, 25 (1967).

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10. J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance." McGraw-Hill Book Company, New York, N.Y., 1959, p 449.
11. N. Bloembergen, *J. Chem. Phys.*, **27**, 595 (1957).
12. H. M. McConnell and E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).
13. B. Bleaney and D. J. E. Ingram, *Proc. Roy. Soc.*, **208**, 143 (1951).
14. G. N. La Mar, *J. Chem. Phys.*, **41**, 2992 (1964).