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Influence of Copper(II) on Proton Nuclear Relaxation Rates of Poly-L-histidine

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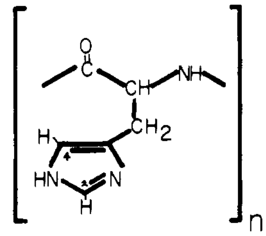
Abstract: Proton NMR chemical shifts and nuclear relaxation rates have been measured for the C_2 and C_4 protons of poly-L-histidine (PLH) as a function of pH. The NMR titration curves of chemical shift indicate a pK_a value of 6.1 for the imidazole groups. The C_4 proton is relaxed more efficiently than the C_2 proton at all values of pH due to its proximity to the C_β protons. The relaxation rates are not affected appreciably by changes in pH. In the presence of Cu(II) the relaxation rates show a pronounced maximum at ca. pH 3.5. Above this pH value, temperature dependence studies indicate that slow exchange is occurring between Cu(II) and PLH. On the basis of this and other evidence, we conclude that the maximum in the relaxation rates appears to represent the pH of most rapid exchange of Cu(II) ions between the ligand sites.



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Poly-L-histidine (PLH) (I) forms complexes with Cu(II),[2a] the nature of which have been examined by a variety of physical techniques.[2b] Maximum binding occurs at pH 5 and the binding constant at this pH is 10^{19} M^{-1}.[2b] It has been suggested that three of the four ligands which bind Cu(II) at this pH are imidazole nitrogens while the fourth ligand may be a peptide nitrogen.[2b] Part of the interest in the Cu(II)-PLH complex arises from the observation that it is effective in catalyzing the oxidation of ascorbic acid, *p*-hydroquinone, and several other organic substrates.[3,4]

In the present study we have measured both transverse ($1/T_2$) and longitudinal ($1/T_1$) proton NMR relaxation rates to study the nature of the Cu(II)-PLH complex as a function of pH. Transverse relaxation rates have previously been determined from resonance line widths by several workers to investigate Cu(II)-amino acid and Cu(II)-peptide interactions.[5,6] The danger of measuring only transverse relaxations rates, to determine metal-ligand distances, has been discussed previously.[7-11]

At low pH, PLH is an extended chain or at least possesses segments of extended chains; however, at pH values above the imidazolium pK_a value the polymer undergoes a conformational transition to a more ordered state.[12-16] PLH is quite insoluble in aqueous solutions when in this relatively ordered conformation (pH 6.3 to 13). Potentiometric titration data[2a] indicate a pK_a of 6.15 for the imidazolium groups of the polymer, essentially the same value observed for histidine.[17,18] In this study we have also used proton chemical shifts to estimate the pK_a of the imidazolium groups in PLH.

Experimental Section

PLH was obtained from Schwartz-Mann (degree of polymerization, dp, = 45), lot U3821, and from Miles-Yeda, mol wt $\simeq$ 11 100 (dp = 81), lot HS36. A stock solution of 0.075 M copper sulfate in D_2O was prepared and analyzed for Cu(II) by atomic absorption spectroscopy.

All PLH solutions were 10 mg/ml (7.3×10^{-2} M in histidine residues) in 0.1 M NaCl-D_2O. The amount of the standard copper sulfate solution added gave ratios of imidazole groups to Cu(II) ion of 100:1 and 50:1, except that for the variable temperature work a ratio of 775:1 was used.

Measurements of pH were made on a Radiometer pH meter with an Ingold long thin combination electrode inserted directly into the NMR tube. Adjustments of pH were made directly in the NMR tubes with standard solutions of NaOD (0.5 M) and DCl (1 M) in hand-held burets. Values reported are direct pH meter readings in D_2O.

NMR spectra were recorded at 220 MHz on a Varian Associates HR220 spectrometer equipped with an accessory for pulse Fourier transform spectroscopy. Measurements were carried out at 20 ± 1°, except where temperature was adjusted to higher values with a Varian Variable Temperature Controller, and the temperature was determined with a standard ethylene glycol sample.

Transverse relaxation times (T_2) were determined by measurement of line width at half-height ($W_{1/2}$) with an expanded recorder scale, using the relationship $T_2 = (1/\pi W_{1/2})$. Longitudinal relaxation times (T_1) were determined by the inversion recovery method,[19] with delay times always $>5 \times T_1$. A 180° pulse was 90 μs and a 90° pulse was 45 μs.

Theoretical Section

Before presenting and discussing our experimental results it will be useful to briefly present the theory of NMR relaxation rates which is pertinent to this study.

In the absence of paramagnetic metal ions the ^{1}H nuclear relaxation rates of protons in a molecule in dilute solutions of deuterated solvents are generally dominated by intramolecular dipole–dipole relaxation.[20,21]

The increase in nuclear relaxation rates of ligand nuclei due to the presence of paramagnetic metal ions can be calculated using the following equations:

$$\frac{1}{T_{1,\mathrm{P}}} = \frac{1}{T_{1,\mathrm{obsd}}} - \frac{1}{T_{1,0}} \qquad (1)$$

and

$$\frac{1}{T_{2,\mathrm{P}}} = \frac{1}{T_{2,\mathrm{obsd}}} - \frac{1}{T_{2,0}} \qquad (2)$$

where $1/T_{1,\mathrm{obsd}}$ and $1/T_{2,\mathrm{obsd}}$ are the observed relaxation rates in the presence of the paramagnetic metal ions and $1/T_{1,0}$ and $1/T_{2,0}$ are the rates of relaxation for the same ligand nuclei in the absence of paramagnetic metal ions. $1/T_{1,\mathrm{P}}$ and $1/T_{2,\mathrm{P}}$ will depend upon the fraction of ligands bound to metal, f, the lifetime of the metal in the bound site, τ_M, and on the rate of relaxation for the ligand nuclei in the bound state, $1/T_{1,\mathrm{M}}$ and $1/T_{2,\mathrm{M}}$,[22-29]

$$\frac{1}{T_{1,\mathrm{P}}} = \frac{f}{T_{1,\mathrm{M}} + \tau_\mathrm{M}} \qquad (3)$$

A similar relationship holds between $1/T_{2,\mathrm{P}}$ and $1/T_{2,\mathrm{M}}$, the transverse relaxation rate for the bound state.[22-29] However, there is a difference in the contributions to $1/T_{1,\mathrm{M}}$ and $1/T_{2,\mathrm{M}}$; specifically there is a scalar contribution to $1/T_{2,\mathrm{M}}$ which is negligible for $1/T_{1,\mathrm{M}}$. Therefore, if one observes $1/T_{2,\mathrm{P}}$ to be significantly greater than $1/T_{1,\mathrm{P}}$, this is generally considered to arise from the scalar mechanism for $1/T_{2,\mathrm{M}}$.

The rate of exchange of paramagnetic metal ion between ligand sites may appreciably affect the observed relaxation according to eq 3. The extreme cases of fast and slow exchange can be delineated.

Slow Exchange ($T_{1,\mathrm{M}}$, $T_{2,\mathrm{M}} \ll \tau_\mathrm{M}$). In this case eq 3 reduces to

$$1/T_{1,\mathrm{P}} = 1/T_{2,\mathrm{P}} = f/\tau_\mathrm{M} \qquad (4)$$

That is, the influence of the paramagnetic ion on the nuclear relaxation rates is directly proportional to the metal–ligand exchange rate. Thus, increasing the temperature would lead to a decrease in τ_M and hence an increase in $1/T_{1,\mathrm{P}}$ and $1/T_{2,\mathrm{P}}$.

Fast Exchange ($\tau_\mathrm{M} \ll T_{1,\mathrm{M}}$, $T_{2,\mathrm{M}}$). In this case,

$$1/T_{1,\mathrm{P}} = f/T_{1,\mathrm{M}} \qquad (5)$$

and

$$1/T_{2,\mathrm{P}} = f/T_{2,\mathrm{M}} \qquad (6)$$

Thus, an increase in temperature in the fast exchange region leads to a decrease in $1/T_{1,\mathrm{P}}$ and $1/T_{2,\mathrm{P}}$.

More detailed discussions of the theory pertinent to the results discussed below can be found in recent review articles.[27-29]

Results and Discussion

Chemical Shifts. By monitoring the chemical shifts of $C_2\underline{H}$ as a function of pH we have calculated a value of 6.11 ± 0.05 for the ionization constant of PLH (dp = 81). Since the material precipitated at pH >6, we assumed a change in chemical shift of 1.0 ppm, the same value as that found in histidine.[30]

Proton Nuclear Relaxation Rates. Relaxation rates for the $C_2\underline{H}$ and $C_4\underline{H}$ of PLH were measured as a function of pH and showed no significant pH dependence. The values of $1/T_1$ obtained with two samples of PLH (dp = 45, 81) were quite similar; for example at pH 4.4, $1/T_1 = 0.34\ \mathrm{s}^{-1}$ for the dp = 81 sample, while for the dp = 45 sample at pH 4.6 $1/T_1 = 0.39\ \mathrm{s}^{-1}$. Since these samples differ by a factor of almost 2 in their molecular weights this indicates that rotation of the side chains and segmental motion of the backbone are probably the relevant motions responsible for spin–lattice relaxation.[31,32]

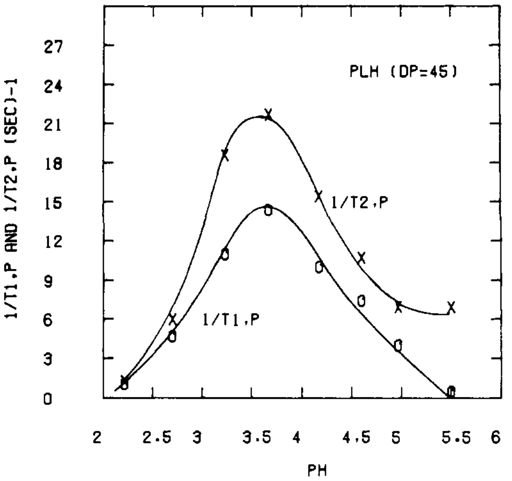

Figure 1. Plot of $1/T_{1,\mathrm{P}}$ and $1/T_{2,\mathrm{P}}$ for the $C_2\underline{H}$ imidazole protons of poly-L-histidine (dp = 45) vs. pH at 20 ± 1 °C. The ratio of imidazole residues to Cu(II) ion is 100:1.

In general the $C_4\underline{H}$ are relaxed more efficiently than the $C_2\underline{H}$ in PLH, as found previously for histidine.[27,33] For example, for PLH of dp = 81 at pH 4.4 $1/T_1$ for $C_4\underline{H}$ was $0.55\ \mathrm{s}^{-1}$. This results from the closer proximity of the C_β protons to $C_4\underline{H}$.

The line widths at half-heights, from which $1/T_2$ was calculated, were essentially independent of pH and degree of polymerization for both the C_2 and $C_4\underline{H}$, with a mean value of 3.0 ± 0.4 Hz. This value includes the spin–spin coupling between $C_2\underline{H}$ and $C_4\underline{H}$ which was measured directly from the splitting (1.4 Hz) of the $C_2\underline{H}$ resonance.

Relaxation Rates in the Presence of Cu(II). In the presence of Cu(II) ion both relaxation rates of the $C_2\underline{H}$ show a sharp maximum between pH 3 and 4 (Figure 1). The same phenomemon was observed for the $C_4\underline{H}$ resonance and for the C_2 and $C_4\underline{H}$ of the higher dp (81) material.

The increase in the relaxation rates ($1/T_1$ and $1/T_2$) as the pH is increased to a value of 3.3 presumably arises from the onset of binding of Cu(II) to the imidazole moieties of PLH.[2b] However, the origin of the decrease of the relaxation rates above pH 3.6 was not immediately obvious, particularly since it has been demonstrated that Cu(II) binds to PLH above pH 3, with a maximum binding constant at pH 5.

Several reports have recently appeared indicating significant pH dependencies of relaxation rates of ligand nuclei in the presence of paramagnetic metal ions.[7,11,33-36] It has also been shown that slow exchange occurs for Cu(II) with glycine.[36] Therefore, a likely explanation for the decrease in the relaxation effect of Cu(II) on PLH above pH 3.3 could be slow exchange of the Cu(II) ions between imidazole groups. To test this hypothesis we carried out relaxation measurements at different temperatures.

It was found that while $1/T_{1,\mathrm{P}}$ showed no significant dependence on temperature at pH 1.9, the values increased significantly with temperature when the pH was raised to 3.7 and 4.8 (Table I). We therefore conclude that slow exchange is occurring at pH values above 3.7 and that this is responsible for the decrease in relaxation rates with increasing pH for the imidazole protons of PLH in the presence of Cu(II).

Further evidence for slow Cu(II) exchange comes from the observation that for PLH, $T_{1,\mathrm{P}}/T_{2,\mathrm{P}} < 2$ for both $C_2\underline{H}$ and

Table I. $1/T_{1,\mathrm{P}}$ Values for Imidazole Protons of Poly-L-histidine (dp = 81) as a Function of pH and Temperature[a]

pH		$1/T_{1,\mathrm{P}}$, s^{-1} 20 °C	37 °C	59 °C
1.9	C_2H	0.05	0.02	0.04
	C_4H	0.01	0.05	0.08
3.8	C_2H	0.54	1.55	2.45
	C_4H	0.45	1.21	2.11
4.8	C_2H	0.28	0.60	1.44
	C_4H	0.22	0.53	1.37

[a] The ratio of imidazole residues to Cu(II) ion is approximately 775:1.

$C_4\underline{H}$. Previous proton relaxation data on imidazole in the presence of Cu(II)[7,9,11] gave a value of $T_{1,\mathrm{P}}/T_{2,\mathrm{P}} > 20$ for $C_2\underline{H}$ and $C_{4,5}\underline{H}$ indicating a significant scalar contribution to $1/T_{2\mathrm{M}}$. Since the nature of the Cu(II) nitrogen bond in PLH and imidazole complexes is expected to be similar one would expect a substantial scalar contribution to $1/T_{2\mathrm{M}}$. The apparent absence of a scalar mechanism for PLH, therefore, also indicates that the rate of exchange of Cu(II) ions is slow in this case (see eq 5 and 6).

Using eq 3 and the data given in Figure 1 it is possible to estimate τ_{M} as a function of pH if one makes a number of assumptions. First, if we assume three imidazole side chains to be bound simultaneously to each Cu(II),[26] then the value of f in eq 3 is 3×10^{-2}. Using the results reported by Espersen and Martin[9] for $C_2\underline{H}$ of imidazole in the presence of Cu(II) and assuming fast exchange[9] and that on the average four imidazole molecules bind Cu(II) in an identical way, we calculate $T_{1,\mathrm{M}}^{-1} \simeq 10^3\ s^{-1}$. Assuming the same value for $T_{1,\mathrm{M}}^{-1}$ in PLH and using eq 3 we find τ_{M} equals approximately 10^{-3} and 10^{-2} s at pH values of 3.6 and 5.0, respectively.

It has been reported that the ratio of oxidation rates of PLH-Cu(II) compared to Cu(II) alone, for several substrates, shows a maximum in the pH range 3.5–4.5.[3,4] One may speculate that this maximum in the oxidation rate may be related to the fastest exchange of Cu(II) with PLH demonstrated here.

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