the Raman frequencies obtained for polycrystalline samples. The infrared peaks in the solid phase, however, generally cannot be resolved sufficiently to allow a direct correspondence of VCD and IR signals. This is apparently due to inherently broader bands in the infrared and consequently Raman spectra are required to obtain a complete and detailed picture of the vibrational frequencies. Thus, in the solid phase, there is a direct correspondence between ordinary vibrational features and VCD peaks. In solution, however, the dominant VCD peaks in alanine and serine occur at frequencies different from the peak frequencies of vibrational bands. The transitions responsible for VCD intensity often do not produce distinct peaks in the vibrational spectra, thus making the correlation between the vibrational and VCD features rather difficult. On the other hand, vibrational optical activity may prove very helpful from the standpoint of the vibrational spectroscopist by locating transitions which are unobserved in conventional vibrational techniques.

V. Conclusion

We have reported the first detailed VCD observations of molecules in a polycrystalline, solid phase. We have found that VCD can be obtained from transparent mulls, and that the spectra show very detailed features in both N-H and C-H stretching regions. Solid-phase spectra have aided in the assignment of solution VCD, although a direct correlation between solid- and solution-phase VCD is not generally observed. We have also found that computer control of the spectrometer can produce a significant increase in the sensitivity of the experiment, especially under the adverse conditions encountered in the solution spectra of serine.

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Contribution of Minor Species to Paramagnetic Line Broadening of ^{1}H Nuclear Magnetic Resonance Spectra in Peptide–Copper(II) System

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Abstract: pH dependence of the paramagnetic line broadening of ^{1}H NMR spectra has been observed for histidine (Gly·Gly·His) and glycine (Gly, Gly·Gly, Gly·Gly·Gly, and Gly·Gly·Gly·Gly) peptide-copper(II) systems. The effective line width at half-height, $(\pi T_{2P})^{-1}$, of the ^{1}H NMR signals showed a bell-shaped curve with a maximum for each system. The cause of the bell-shaped curve was examined in the Gly·Gly·His-copper(II) system as the most typical and dramatic case. It was found that the line broadening was caused by the small amount of incomplete complexes present in the solution and that an extreme decrease of the incomplete complexes and exclusive formation of a complete complex having a large τ_M result in narrowing of the line width. This finding indicates that the ^{1}H NMR line broadening does not always reflect the dominant complex species present in solution, so care is required in the use of line-broadening data in the study of metal binding sites.

Selective broadening of ^{1}H NMR spectra by a paramagnetic ion has often been used to investigate metal ion binding sites.[2] Among others, the amino acid and/or peptide-copper(II) systems are the ones most often studied. However, recently a question has been raised as to whether the broadening truly means or represents selective binding of the metal ion to the ligand.[3,4] This is because there are two successive restrictions when we combine the line broadening data with the

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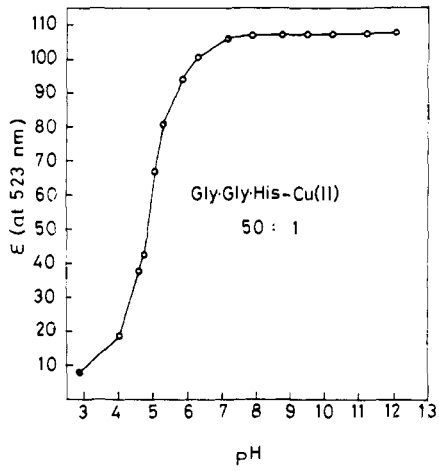


Figure 1. Dependence of the molar extinction coefficient ϵ (cm^{-1} M^{-1}) at 523 nm upon pH in the Gly·Gly·His-copper(II) 50:1 system.

binding sites. Firstly, the so-called "fast exchange" condition must be satisfied for the observed line width to be controlled by relaxation and not by chemical exchange, and secondly the "dipolar term" must be dominant to the transverse relaxation time of the bound ligand, T_{2M}, in order for the inverse form T_{2M}^{-1} to depend on the distance between the paramagnetic ion and the measured nucleus as the r^{-6} form.

The purpose of the present study is to show another restriction and its examples, which was also implied by Espersen and Martin,[4] and Beattie et al.[3] This is a problem arising from unavoidable experimental restrictions. That is, NMR experiments on the ligand-copper(II) system must be performed with a large excess of ligand, there may be various kinds of species, all of the structures of which we cannot imagine in a stoichiometric sense, and they may contribute more or less to the observed line width, $(\pi T_{2P})^{-1}$, of the NMR signal even if those species are minor components in the solution. Estimation of the contribution to line broadening of each type of complex species is difficult; this is a serious problem which is more fundamental than the preceding two restrictions. In the following experiment, by analyzing line widths of some histidine and glycine peptide-copper(II) systems, this third problem is discussed especially for the Gly·Gly·His-copper(II) system. To elucidate the complex formation, visible absorption spectra at 50:1 to 200:1 and electron spin resonance (ESR) spectra at 50:1 ligand to metal ion ratios were also studied, besides the stoichiometric conditions.

Experimental Section

Materials. Glycylglycylhistidine was synthesized by the method reported previously[5] or purchased from the Protein Research Foundation, Osaka. Tetraglycine was purchased from the Tokyo Kasei Kogyo Co., Ltd., Tokyo; glycine, diglycine, triglycine, KOH, HCl, and $CuCl_2 \cdot 2H_2O$ were obtained from the Wako Pure Chemical Industries Co., Ltd., Kyoto; and D_2O, NaOD, DCl, and TSP (sodium 3-trimethylsilyltetradeuteriopropionate) were from E. Merck AG, Darmstadt.

NMR Spectra. The ^1H NMR spectra were measured at 26 °C on a Varian HA-100 D NMR spectrometer operating at 100 MHz. TSP was used for an internal lock signal and as an internal reference. The temperature variation was made with a Varian variable-temperature accessory unit (V-6040) calibrated by ethylene glycol. The proton signals of the methylene group adjacent to the terminal amino group of Gly, Gly·Gly, Gly·Gly·Gly, and Gly·Gly·Gly·Gly and the methine signals of the imidazole group of Gly·Gly·His were chosen as monitor lines of ^1H NMR line broadening. The D_2O solutions (0.15–0.20 M) of the following ligand to metal ion ratios were employed for measurements, i.e., Gly, 8000:1; Gly·Gly, 10 000:1; Gly·Gly·Gly, 10 000:1; Gly·Gly·Gly·Gly, 2000:1; Gly·Gly·His, 500:1 and 30:1. The pH (pD) value was varied by adding a suitable amount of the 5 M D_2O solution of DCl or NaOD and was measured with a Hitachi-Horiba M-7E pH meter with a combination electrode CE-150 A of Toko Chemical Laboratory Co., Ltd., Tokyo. The direct pH meter readings were used without any further correction between pH and pD.

Visible Absorption Spectra. Ligand-copper(II) solutions of various ligand to metal ratios (1:1 to 200:1) were prepared. For pH variation, 4 M KOH and 2 M HCl were used. The pH value was determined with a CE-150 A electrode and a Hitachi-Horiba F-7DE pH meter. The spectra at various pH values were recorded on a Shimadzu UV-300 spectrophotometer. The copper(II) concentration varied from 0.004 to 0.0027 M during pH variations in the high ratio of ligand to copper(II) systems. The spectra of stoichiometric systems were measured at 0.01 M copper(II) concentration. The observed visible absorption spectral characteristics are summarized in Table I. The molar extinction coefficient ϵ (cm^{-1} M^{-1}) was calculated on the basis of the molar concentration of copper(II).

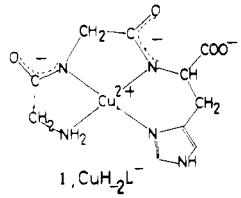
ESR Spectra. X-band ESR spectra were measured at 77 and 293 K on a JEOL ME-3X spectrometer operating with 100-kHz magnetic field modulation (modulation amplitude, 6.3 G). The magnetic fields were calibrated by the splitting of Mn(II) in MgO ($\Delta H_{3,4}$ = 86.9 G). An aqueous solution containing 50:1 mol ratio of Gly·Gly·His and copper(II) chloride ($1.0 \times 10^{-2} \sim 5.0 \times 10^{-3}$ M) was employed for these measurements. The pH value was varied by adding 5 M NaOH and/or 1 M HCl and was measured with a Hitachi-Horiba M-7E pH meter.

Table I. Visible Absorption Characteristics of Peptide-Copper(II) Systems

system	pH	λ_{max}, nm	ϵ_{max}, cm^{-1} M^{-1}
Gly·Gly·His-Cu(II) 50:1	4.84	540, 640	45.3, 47.2
	5.92	524	94.4
	6.19	523	102.4
	7.20	523	106.0
	10.28	523	106.8
	12.05	523	107.5
Gly·Gly·His-Cu(II) 1:1	8.34	523	101.2
Gly·Gly·Gly·Gly-Cu(II) 60:1	8.33	598	78.2
	11.02	523	106.2
	12.26	513	152.3
	13.20	513	160.2
Gly·Gly·Gly·Gly-Cu(II) 1:1	12.28	513	139.0
Gly·Gly-Cu(II) 200:1	5.67	628	78.5
	7.01	615	86.6
	10.57	615	87.3
	12.60	573	60.1
	13.60	550	59.8
Gly·Gly-Cu(II)1:1	8.69	638	80.4
Gly·Gly·Gly-Cu(II) 100:1	4.87	688	47
	7.98	603	56
	9.07	556	50
	13.30	516	119
Gly·Gly·Gly-Cu(II) 1:1	11.01	555	149

Results and Discussion

Glycylglycylhistidine is known to form a very stable complex with copper(II) ion under 1:1 stoichiometric conditions.[6,7] As shown in Table I and Figure 1, the λ_{max} and ϵ_{max} of the Gly·Gly·His-copper(II) 50:1 system show remarkable constancy above pH 6 and agree with those of the 1:1 system. These results indicate that the structure of the 1:1 complex, 1



1, $CuH_{-2}L^-$

($CuH_{-2}L^-$), is maintained at the high ligand concentrations in which the NMR experiments were performed. Figure 2

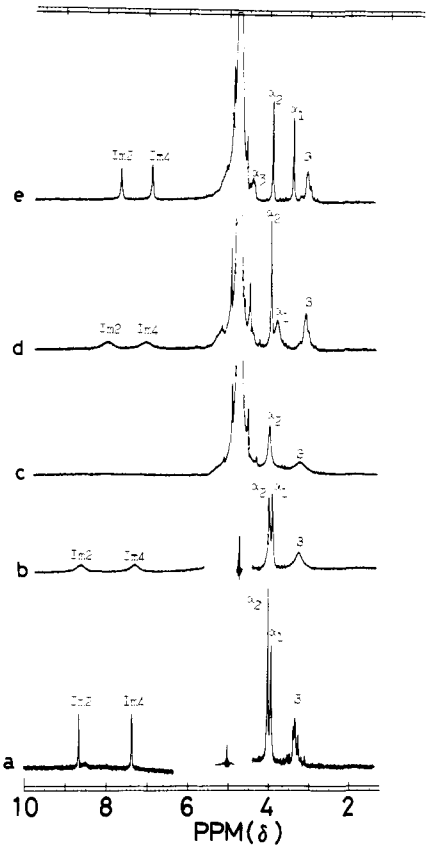


Figure 2. ^{1}H NMR spectra of Gly·Gly·His-copper(II) 30:1 system. (a) pH 0.5; (b) pH 2.0; (c) pH 5.5; (d) pH 7.0; (e) pH 9.5. Because of overlap with the residual HDO peak, the α_3 signal is not clear except in spectrum e.

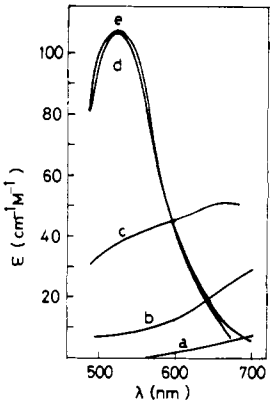


Figure 3. Absorption spectra in the range $480 \leq \lambda \leq 700$ nm for Gly·Gly·His-copper(II) 50:1 system. (a) pH 0.58; (b) pH 2.91; (c) pH 4.66; (d) pH 7.20; (e) pH 9.65. Absorbance is expressed as a molar extinction coefficient (ϵ, cm^{-1} M^{-1}).

shows ^{1}H NMR spectra of glycylglycylhistidine from pH 0.5 (a) to pH 9.5 (e) together with the assignments (abbreviations used are shown with structure 1). Visible absorption spectra at the corresponding pH are also shown in Figure 3. The ^{1}H NMR spectra show a rather curious behavior with pH changes; the signals of the imidazole protons (Im2, Im4) broaden and even disappear with increasing pH at first, but again appear and sharpen when the pH rises above 6. These phenomena cannot be simply explained by the degree of the coordination of the imidazole group to copper(II) ion since visible absorption spectra show a continuous formation of I which involves the binding of the imidazole nitrogen (see Figures 1 and 3). Although the same behavior was observed for the other protons,[8] the broadening is clearly significant with the imidazole and α_1 protons. It is unlikely that the sharpening of the signals of imidazole protons above pH 6 is due to the conversion of a complex involving imidazole nitrogen to one involving amino nitrogen or a carboxyl group.

An analytical expression about the paramagnetic line broadening of NMR spectra including chemical exchange is elegantly shown by the theory of Swift and Connick,[9] and, if we abbreviate their equation to our peptide-copper(II) system, the equation becomes

$$1/T_2 = 1/T_{2L} + \sum_i pq_i/(T_{2M_i} + \tau_{M_i}) + 1/T_{20} \quad (1)$$

Where all the symbols have their usual meaning,[9-11] the summation is carried out for all the complexed species, and the value $(\pi T_{2P})^{-1}$ is given by using the following relation

$$1/\pi T_{2P} = 1/\pi T_2 - 1/\pi T_{2L} \quad (2)$$

The $\Delta\omega_M^2$ term of the original equation is omitted in eq 1 since the electron spin relaxation time of copper(II) ion is relatively long ($\sim 10^{-8}$ s);[9] the relaxation in the bound sphere is so efficient that $1/T_{2M}^2 \gg \Delta\omega_M^2$. To complete the description of paramagnetic line broadening, the outer sphere contribution to the line width is included in the last term of eq 1 (i.e., $1/T_{20}$), which is not explicitly shown in the original equation. However, this term may be small compared with the second term of eq 1, since this term arises mainly from the dipole-dipole interaction between the paramagnetic ion and the bulk nuclei which decreases in magnitude according to the inverse sixth power of interacting distance. Therefore, its variation with pH may not cause such a remarkable change of line width as shown in Figure 2.

Now we have two possible interpretations for the pH profile of the line width, especially for the sharpening of signals in the pH region over 6. The first one is the idea that the main contributor to line broadening is the major species I, and the sharpening of the signals with increasing pH is due to the slowing down of the exchange rate of ligand. This is reasonable, because the dissociation rate and also, therefore, the association rate of I ($CuH_{-2}L^-$) should come down with increasing pH.[12] It is unlikely that the transverse relaxation time of the coordinated ligand T_{2M}, which is given by the Solomon-Bloembergen equation,[13] varies significantly with pH in the same complex, I.

The second interpretation is that the line broadening is not primarily based on the stable major complex which may have a large τ_M, but on incomplete minor species which may have a fast ligand exchange rate. For example, one can imagine a minor species which has a unidentate interaction between the imidazole group and copper(II) ion. While the amount of the major species is almost constant in the pH region above 6, the minor species should decrease extremely with increasing pH. That is, if the amount of the major species increases from 99.0 to 99.9% with pH, the amount of the minor species decreases from 1 to 0.1%. Such variation of the minor species should greatly affect the line width; therefore, one can easily expect the sharpening of signals with increasing pH.

To examine the validity of both interpretations, we studied the temperature dependence of line broadening. If the first idea is true, the line width should increase with increasing temperature at a constant pH above 6, because in this case it is apparent that τ_M is larger than T_{2M}.[10] The temperature variation experiments are shown in Figure 4. Increasing temperature of the solution at pH 5.7 resulted in line width narrowing. This result is clearly in conflict with the first interpretation, but not with the second one. The minor species, in which copper(II) ion is not completely "captured" by Gly·Gly·His, may have a rather fast ligand exchange rate, so it is

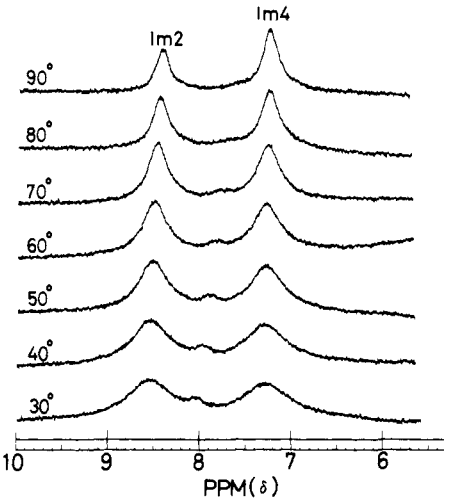


Figure 4. Temperature dependence of ^{1}H NMR spectra of imidazole methine protons of Gly·Gly·His-copper(II) 500:1 system from 30 to 90 °C, pH 5.7.

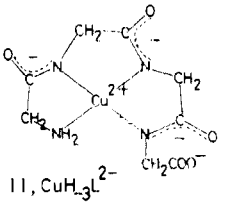
plausible that $\tau_M < T_{2M}$ with the minor species. In such a fast exchange region, the line width should decrease with increasing temperature.[10] That is the observation illustrated in Figure 4.[14]

The data in Figure 2 also support the same conclusion. It is noteworthy that the signal of the α_2 is almost intact at pH 7.0 (Figure 2d) where a significant broadening is still observed with the imidazole and α_1 protons. If the major complex has a predominant role for the line broadening, it is hard to expect the remarkable difference in the line broadening among the α_1, α_2, and imidazole protons. The results suggest that the broadening will be caused by minor species involving imidazole and/or amino bindings.

The presence of a minor species whose structure is not so rigid as I can also be supported from ESR spectra. Figure 5 shows the ESR spectra of the Gly·Gly·His-copper(II) 50:1 system at 77 and 293 K at varying pH. The spectrum at pH 10.6 in frozen solution at 77 K exhibits a typical copper hyperfine pattern with approximately axial symmetry, i.e., $g_x \simeq g_y$, and also shows nitrogen nuclear superhyperfine splittings. The ESR parameters are $g_\parallel = 2.174$, $g_\perp = 2.051$ and $A_\parallel = 233$ G. These spectral characteristics are compatible with those expected for structure I. On the other hand, the spectrum at pH 4.4 in frozen solution at 77 K shows an undoubtedly different spectral pattern from that at pH 10.6; namely, the value for $g_\parallel$ increases to 2.270 and the value for $A_\parallel$ decreases to 177 G and the ligand superhyperfine structure is not observed. The ESR spectrum at pH 6.1 is approximately made up of the superposition of the two types of spectra at pH 10.6 and 4.4. In addition, the ESR spectrum at room temperature and at pH 4.4 is fairly broad compared with the spectrum at pH 10.6. These observations clearly show the presence of minor species other than I in acid to neutral pH region and also imply that they are in a state of weak complexation; in other words, they should have a fairly fast ligand exchange rate in aqueous solution.

In conclusion, it is considered that the line broadening of the imidazole signals in the ^{1}H NMR spectra shown in Figure 2 is caused by the minor species having a fast ligand exchange rate and the sharpening of the signals with increasing pH is due to the extreme decrease of the minor species. The stable major complex, I, may have too large a τ_M to cause line broadening; in other words, copper(II) ion is "captured" by one Gly·Gly·His molecule in the major complex. This is quite probable since all the four coordination sites in the copper(II) plane are strongly occupied by four nitrogens of one Gly·Gly·His molecule.

The same situation is expected in the glycine peptide-copper(II) systems. This is especially so for a Gly·Gly·Gly·Gly-copper(II) system since, as can be seen from the spectral characteristics of the Gly·Gly·Gly·Gly-copper(II) 60:1 system (see Table I), the blue shift of λ_{max} with increasing pH ultimately resulted in a constant λ_{max}, i.e., 513 nm, which agrees well with that of the 1:1 system, and therefore the structure of the complex is thought to be II ($CuH_{-3}L^{2-}$).[15] The structure



II, $CuH_{-3}L^{2-}$

resembles that of the Gly·Gly·His-copper(II) system in view of the fact that all the four coordination sites in the copper(II) plane are occupied by four nitrogens of one molecule. The formation of the complex species II with pH is shown in Figure 6, where ϵ at 513 nm has been plotted against pH. These observations of the Gly·Gly·Gly·Gly-copper(II) system suggest the same sort of pH dependence of line broadening as in the Gly·Gly·His-copper(II) system. This is indeed the case, and the results are shown in Figure 7 as to the signal of the methylene group adjacent to the terminal amino group together with the other systems studied in this work. Here, in order to demonstrate the changes in line broadening quantitatively and

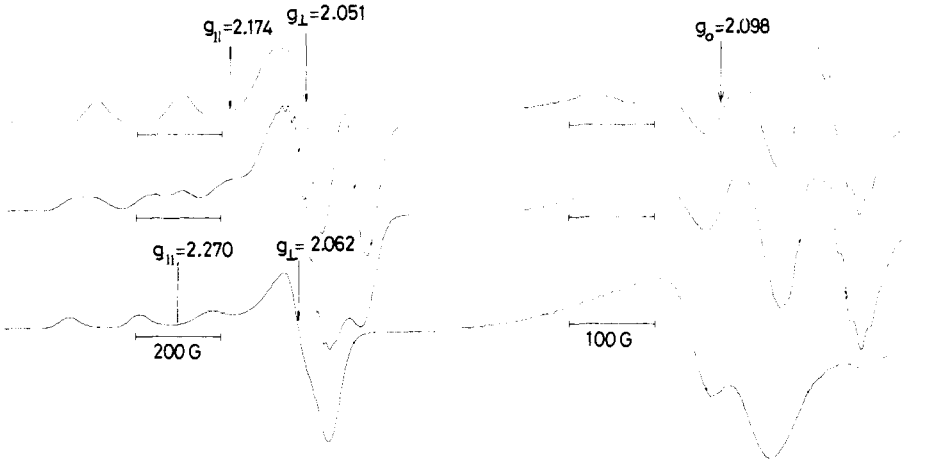


Figure 5. ESR spectra of solutions containing Gly·Gly·His and copper(II) chloride (ligand:metal ratio 50:1) at 77 (left) and 293 K (right) and pH (from top to bottom) 10.6, 6.1, and 4.4.

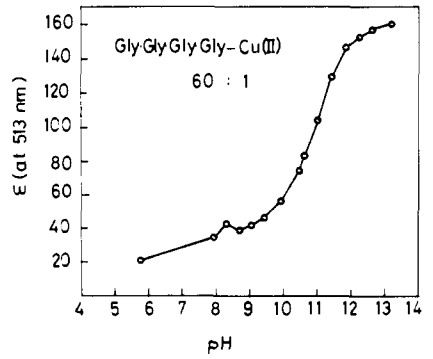
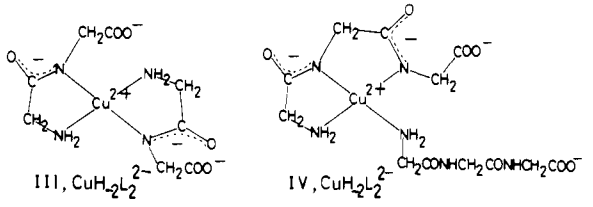


Figure 6. Dependence of the molar extinction coefficient ϵ (cm^{-1} M^{-1}) at 513 nm upon pH in the Gly·Gly·Gly·Gly-copper(II) 60:1 system.

compare the degrees of broadening, the effective line width at half-height, $(\pi T_{2P})^{-1}$, normalized to the system at a 1000:1 ligand to metal ion ratio, is shown as common logarithms of $(\pi T_{2P})^{-1}$ against pH. The very similar profile of the Gly·Gly·His and the Gly·Gly·Gly·Gly-copper(II) systems as shown in Figure 7 clearly justifies the above prediction. Moreover, it is interesting to note that triglycine, diglycine, and glycine also show the same profile, that is, a "bell shaped curve" with pH variation. The pH which shows the maximum broadening (hereafter we call this the pH_{max}) varies among the ligands. The order of this pH_{max} is Gly·Gly·His (~5.5), Gly·Gly·Gly·Gly (~7.5), Gly·Gly·Gly (~8), Gly·Gly (~8.5), and Gly (~12) in the order of increasing pH. The magnitude of the $(\pi T_{2P})^{-1}$ also increases following this order. The sharpening of the Gly-copper(II) system at very high pH values may be caused by the formation of hydroxide of copper(II) ion, but this may not be so in the other systems. The orders of the pH_{max} and the magnitude of $(\pi T_{2P})^{-1}$ suggest that the stability of the complex which may be related to the ligand exchange rate between the bound and unbound form plays an important role for affecting the line width of ^1H NMR signals as was discussed above. The structures of the predominant complexes for the Gly·Gly and Gly·Gly·Gly-copper(II) systems are not so straightforward as those of the Gly·Gly·His and Gly·Gly·Gly·Gly systems from the visible absorption spectra at high ligand to metal ion ratios, since the λ_{max} for each system shows a continuous blue shift with increasing pH and the λ_{max} for each system does not agree with that of the stoichiometric conditions, suggesting the presence of a new species not found in the stoichiometric conditions. However, by taking into account the continuous blue shift and the structures under stoichiometric conditions, a complex like III ($CuH_{-2}L_2^{2-}$) for



Gly·Gly and like IV ($CuH_{-2}L_2^{2-}$) for Gly·Gly·Gly may be present in the solutions. The τ_M of these complexes may not be so large as in the case of Gly·Gly·His since two molecules are required to form complexes III and IV. However, the formation of III or IV may have resulted in line-width narrowing of the observed signals with increasing pH as in the Gly·Gly·His-copper(II) system.

The broadening of the signal by the minor components may be due mainly to unidentate interaction of the amino and imidazole groups. The formation of a more complete chelate with increasing pH in which the exchange rate is slow should cause narrowing of line width. The sharpening of signals at pH values

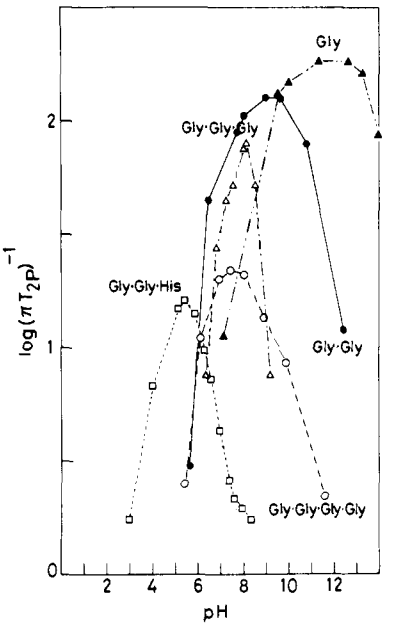


Figure 7. pH dependence of effective line width, $(\pi T_{2P})^{-1}$, for the Im2 proton of Gly·Gly·His and for the amino terminal methylene protons of Gly, Gly·Gly, Gly·Gly·Gly, and Gly·Gly·Gly·Gly. (— - - —) Gly; (— ●) Gly·Gly; (— - —△) Gly·Gly·Gly; (— — — ○) Gly·Gly·Gly·Gly; (- - - □) Gly·Gly·His.

lower than pH_{max} can, of course, be explained by a decreased interaction of imidazole or amino group with copper(II) ion. The concept of the interaction in a unidentate mode through imidazole or through amino nitrogens may be justified, since the order of pH_{max} agrees well with the following pK value of the interacting groups, that is: Gly·Gly·His, pK(imidazole), 6.99[5]; Gly·Gly·Gly·Gly, p$K(NH_3)$, 7.89[15]; Gly·Gly·Gly, p$K(NH_3)$, 7.90[15]; Gly·Gly, p$K(NH_3)$, 8.10[16] (and for reference Gly, p$K(NH_3)$, 9.62[17]).

To summarize our interpretation of the results, we can explain that, in some cases where relatively stable complex formation is expected, the line broadening is not due to the predominant complex species in the solution. Studies to determine ligand binding sites from selective broadening data should be undertaken with caution.

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Computer Simulation of the Conformational Properties of Oligopeptides. Comparison of Theoretical Methods and Analysis of Experimental Results

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Abstract: A theoretical analysis of the conformational and statistical thermodynamic properties of series of oligopeptides containing alanine, methionine, valine, and glycine was carried out. A novel feature of these calculations is the determination of the relative vibrational *free energy* of different minimum-energy conformations. It was shown that in many of the peptides considered the entropic contribution to the relative stabilities of different conformations is comparable to the energy difference (~4-5 kcal/hexapeptide). In one case, Met_3-Gly-Met_2, the entropic contribution dominated, causing a reversal in stability of the energetically favored α-helical conformation to a more extended form. Monte Carlo simulations of several hexapeptides were carried out in order to better simulate the ensemble of conformations present in solution. Average energies, end-to-end distances, and probability maps of the conformational states of a given residue as a function of its position in the chain were generated. The effect of the insertions of a glycine in alanine host peptides was studied by this technique. It was found that, when glycine is present in the middle of a hexamer of alanine (Ac-Ala_3-Gly-Ala_2-NMe), a class of folded structures characterized by low entropy, low energy, and a small end-to-end distance dominates the conformations generated. This result permits an alternative interpretation of the CD spectra for Boc-Met_3-Gly-Met_2-OMe.

I. Introduction

A great deal of effort has been expended in recent years on the determination of the solution conformation of oligopeptides.[2,3] The impetus for these studies arises from the intrinsic interest in understanding the factors which influence molecular conformation in solution, from the fact that these compounds are composed of the same structural units as proteins and peptides, and with the firm conviction that biological activity is intimately related to the accessible, low-energy conformations of these molecules.[2a,4,5] The continuing discovery of short, biologically active peptides, perhaps the most well-known recent examples being the pentapeptide enkephalins,[6] serves to maintain interest and active research in this area.

Various techniques have been applied to the study of the conformation of peptides, including spectroscopic methods (IR, NMR, CD), X-ray crystallography, and theoretical conformational analysis.[2,3] Each of these methods has its limitations; spectroscopy, the indirect nature of the results from which only aspects of the structure can be deduced, and those only by implication; X-ray crystallography, the need for a crystal and the effect of crystal forces on the molecular conformation;[7] and finally theoretical analysis, where solvent effects, local minima, and the adequacy of the potential functions are the outstanding problems. It would seem clear that a combination of the three techniques, each contributing and providing feedback for the other, would be the desired approach to understanding conformational behavior at the molecular level.[8]

In this paper we treat a series of alanine and methionine oligopeptides as well as several host-guest peptides, all of which have been well characterized by experimental spectroscopic techniques, by a variety of theoretical methods. We have a twofold objective. The first is to investigate the effect of several common approximations and assumptions, such as rigid geometry and neglect of vibrational free energy, on the conclusions drawn from such calculations. The second objective is to determine to what extent the combination of various theoretical techniques such as energy minimization, Monte Carlo chain simulation, and the inclusion of the vibrational effects can help to extend the understanding of the conformational behavior of these molecules, as deduced from experiment, to the energetic and molecular level.

Secondary Structure. The study of the critical chain length for the onset of secondary structure in oligopeptides has been conducted using a number of physicochemical techniques including polarimetry, ultraviolet spectroscopy, circular dichroism (CD), and nuclear magnetic resonance spectroscopy.[2b,9-15] Peptides composed of amino acids such as γ-ethyl-L-glutamic acid and L-alanine were observed to begin forming helices in organic solvents at a chain length of six or seven residues. In additional studies, oligomers composed of hydrophobic amino acids were found to form β structures in pentamers and hexamers.[12-15] Although much information has been gained from these studies, there still remain many unanswered questions concerning the exact structure of these oligopeptides in solution. This is especially true in the case of peptides undergoing transition as they are undoubtedly in dynamic equilibrium between a number of energetically preferred conformations. Thus, for example, the CD patterns of hexamers, heptamers, and octamers of L-alanine or γ-ethyl-L-glutamic acid in organic solvents do not conform to

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