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AN NMR INVESTIGATION OF THE INTERACTIONS OF TRANSITION-METAL IONS WITH PHOSPHORUS-CONTAINING ESTERS AND AMINO ACIDS

by

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A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

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OF TRANSITION-METAL IONS WITH
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Leslie T. Gelbaum

Adviser: Professor Robert Engel

The investigations of metal ion-ligand coordination described herein, are based on the changes in nuclear relaxation times due to the presence of paramagnetic transition metal ions. The first investigation is a study of the decoupling of $^{31}P - {}^{1}H$ interactions caused by the direct bonding metal ions Fe^{III} and Co^{II}, and the indirectly bonding $Fe(acac)_3$. It is found that the phosphorus-hydrogen decoupling is observed at a lower concentration ratio than in the proton spectrum. This fact is an indication that line broadening due to the paramagnetic species is obscuring the true chemical decoupling. Investigations with $Fe(acac)_3$ suggest that direct chemical bonding is needed for decoupling, since $^{31}P - {}^{1}H$ decoupling is not observed with this reagent.

The second investigation is a study of the line broadening in dilute solutions of $NiCl_2$ with amino acids

in D_2O solutions at different pD values. It is shown that the line broadening is a linear function of the metal ion concentration. It is also found that the functional groups incorporated in the amino acid has a marked effect on the complexes with Ni^{II}. The effect of the functional group varies as the pD of the solution is changed and protonation or deprotonation of the functional group takes place.

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TABLE OF CONTENTS

LIST OF TABLES

TO MY PARENTS

CHAPTER I

INTRODUCTION

The effect of paramagnetic metal ions on the relaxation times of nuclei in solution has been known since the inception of NMR spectrometry. Block, Hansen, and Packard[1] observed the decrease of the spin-lattice relaxation time of H_2O in aqueous solutions of $Fe(NO_3)_3$. Subsequently, Bloembergen, Purcell, and Pound[2] conducted a theoretical and experimental investigation of nuclear relaxation in the presence of metal ions. Using the saturation technique for the spin-lattice relaxation time, T_1, and the line width for the spin-spin relaxation time, T_2, they determined that both T_1 and T_2 decrease in the presence of paramagnetic metal ions.

However, the theoretical considerations of Bloembergen et al. did not include the situation of chemical exchange. McConnell[3] modified the Bloch equations to include chemical exchange. These equations made it possible to determine the rates of fast chemical reactions from the nuclear magnetic resonance line shapes. The realization by chemists that it was now possible to investigate fast exchange reactions opened a new era of research in this field.

The following is a brief compilation of the investigations which have used the line shape technique.

Swift and Connick[4] were able to use the McConnell equation to determine the rate constants for exchange

in aqueous solutions of Mn^{+2}, Fe^{+2}, Co^{+2}, Ni^{+2} and Cu^{+2}. These experiments were performed using the line broadening of the ^{17}O resonance signal. Luz and Meiboom[5] used proton NMR to determine the solvation of Ni^{+2} and Co^{+2} in methanolic solution. Glaeser[6] et al. investigated the exchange of $Ni(NH_3)_6^{+2}$ in liquid ammonia solution with the aid of ^{14}N NMR line broadening. While investigating the exchange of nickel and chromium hexa-aquo cations in aqueous solution, Swift and Stephenson[7] found that the hydronium ion concentration was involved in the exchange equation. Babiec et al.[8] were able to determine that Co^{+2} reacts faster than Ni^{+2} in solutions of N,N-dimethyl formamide using ^{17}O line broadening.

Biochemical investigations were also aided by use of the line broadening technique. Sternlicht et al.[9] studied the binding of Mn^{+2}, Co^{+2} and Ni^{+2} with adenosine triphosphate; not only were they able to determine the exchange rate for the binding but also the site of binding. Cohn and Hughes[10] use ^{31}P line broadening to determine which of the phosphorus atoms in adenosine di- and triphosphate was binding to Mn^{+2} and Cu^{+2}. The preceding list, while showing but a small fraction of the studies which have used the line broadening technique, illustrates its versatility.

Zumdahl and Drago[11] while studying the exchange reaction of hexamethylphosphoric triamide with dihalo bis

(hexamethylphosphoric triamide) cobalt(II), noticed that the protons were decoupled from the phosphorus. It was concluded that this was caused by the rapid spin-inversions of the phosphorus nuclei caused by the presence of the paramagnetic Co^{+2}. These rapid spin-inversions caused the protons to be subjected to only an average value of the two spin states and was therefore "decoupled". Frankel[12] in a series of papers termed this effect "chemical exchange spin decoupling".

In these reports by Frankel it is stated that the requirement for the McConnell equation is the existence of two distinct magnetic environments. In the previous investigation only single absorbances were used and the exchange observed was between the bound solvent state and bulk solvent molecules. In Frankel's studies the two unique environments are caused by the two spin states of a phosphorus nucleus.

An excellent system where one could use this decoupling effect is the complex of phosphoryl compounds with transition metal compounds. In these compounds the metal ion binds to the phosphoryl oxygen, decoupling the phosphorus from the protons. One can then observe the effect of the metal ion on the phosphorus and proton nuclei independently. Frankel, using a number of these transition metal complexes, was able to determine the relaxation times for the phosphorus and the activation parameters for the exchange reactions of

the ligand with the bulk solvent. The bulk solvent in each case was the same as the ligand. Engel[13] in a similar study used trialkyl phosphites in phosphite solution. He was able to determine that the order of decoupling strength for a number of paramagnetic ions was: Fe^{+3} greater than Ni^{+2} which was greater than Co^{+2}. Engel[14] also studied the complexes of Co^{+2}, Ni^{+2} and Fe^{+3} with phosphorus esters in solutions of acetonitrile and methylene chloride.

In subsequent investigations Engel[15] studied the decoupling of proton-proton interactions caused by complexation at oxygen in alcohols and ketones using Fe^{+3}, Co^{+3} and $Fe(acac)_3$; studies were also performed by Engel[16] involving metal complexation at nitrogen in amines and nitriles. These investigations used $FeCl_3$, $CoCl_2$ and $Fe(acac)_3$, the last of which acts by a second coordination mechanism.

The investigations which are herein described were performed in order to better understand this effect of the transition metal ions. The first is a ^{31}P decoupling study of trialkyl phosphites, phosphates and phosphonates with $CoCl_2$, $FeCl_3$ and $Fe(acac)_3$ in acetonitrile. The second is a detailed investigation of the interactions of six amino acids with $NiCl_2$ in aqueous solution using the line broadening technique.

CHAPTER II[17]

^{31}P NUCLEAR MAGNETIC RESONANCE SPECTRA OF PHOSPHORUS-CONTAINING ESTERS IN THE PRESENCE OF TRANSITION-METAL IONS

There have previously been reported[13,14] investigations of paramagnetic decoupling of ^{31}P-^{1}H and ^{1}H-^{1}H interactions in phosphorus-containing esters. It is of practical interest to consider the same decoupling process as it would be observed in phosphorus NMR spectra.

Observations of ^{31}P-^{1}H chemical decoupling in systems similar to those mentioned here have been reported by several other groups[12,18,19]. In all of these cases the proton spectra were measured and directly co-ordinating paramagnetic reagents were used. The most intensive of these studies is that by Frankel,[12] who has given a detailed theoretical evaluation of the spectra produced.

It has also been noted previously[15,16] that chemical decoupling of ^{1}H-^{1}H interactions occurs by a second co-ordination spheremechanism, not involving direct chemical interaction between paramagnetic reagent and the molecule under consideration. It is predicted[12] that decoupling of ^{31}P-^{1}H interactions by such a mechanism would not be oberved in proton spectra but might be observed in phosphorus spectra. Results obtained by Zumdahl and Drago[11] using non-labile

chromium(III) reagents agree with the first part of this prediction, no decoupling having been observed in proton spectra with dimethyl methylphosphonate and dimethyl phosphite.

Investigation of paramagnetic metal ion interaction with phosphorus systems by observation of ^{31}P spectra has not been extensive; the technique has been used with several systems of biological interest to confirm earlier data concerning metal ion binding sites.[9,10,20] The primary concern has been with simple line broadening and paramagnetic shifts. The interest of the present work is to provide a basis for more detailed investigations of this type.

EXPERIMENTAL SECTION

All ^{31}P NMR spectra were measured with a Varian HA-100 spectrometer at 40.5 MHz; proton spectra were measured with a Varian A-60A instrument. All spectra were measured at ambient temperature with operating parameters optimised by use of a standard sample.

Phosphorus chemical shifts were measured relative to external phosphoric acid (85%) by use of a Varian V-4315 frequency counter. Proton chemical shifts were measured relative to the acetonitrile solvent signal.

The anhydrous cobalt(II) chloride, iron(III) chloride, and iron(III) acetylacetonate [$Fe(acac)_3$] were commercial

materials and were used without further purification. The phosphites and phosphates were commercial materials and were repurified by distillation. Dimethyl methylphosphonate was prepared by a standard procedure.[21]

The acetonitrile solvent was commercial material of spectrometric quality and was used without further purification.

RESULTS

Concentration data for ^{31}P-^{1}H decoupling as noted by observation of phosphorus signals are summarized in Table I; those as noted by observation of proton signals, are in Table II. Paramagnetic shifts of the phosphorus signals at decoupling concentrations are also listed in Table I.

The ^{31}P NMR spectra of trimethyl phosphite, trimethyl phosphate, triethyl phosphite, tributyl phosphite, and dimethyl methylphosphonate were observed upon association with $CoCl_2$ and $FeCl_3$. In all cases coalescence of the (multiplet) signals to broadened singlets were observed as previously noted for the proton spectra[14]. A higher concentration ratio (of metal to ligand) was required for $FeCl_3$ decoupling than for $CoCl_2$.

At the concentration ratio for decoupling required of the ^{31}P-^{1}H interactions, downfield paramagnetic shifts were noted for the ^{31}P signals in all cases except tributyl phos-

phite, for which the shift was zero within experimental error, and triethyl phosphite with $CoCl_2$, in which case downfield shifts were noted with increased concentration of the paramagnetic species.

In addition to the foregoing reagents, iron(III) acetylacetonate was used with triethyl phosphate and tri-isopropyl phosphite. With this reagent, in neither case was decoupling noted in the proton spectra and only incomplete decoupling was observed in the phosphorus spectra at the highest concentration ratios used (10^{-2}).

DISCUSSION

The results are qualitatively similar to those previously reported for proton spectra.[14] There are several noteworthy variations which can be rationalized.

Decoupling of the ^{31}P-^{1}H interaction is observable when paramagnetic species which co-ordinate directly with ligand molecules are used. In the absence of direct chemical co-ordination of the phosphorus ligand no decoupling is observed in the proton spectra and only partial, incomplete decoupling in the phosphorus spectra (vide infra).

Paramagnetic shifts of the phosphorus signals, when present, were in a downfield direction; proton shifts for the same molecules in the same solvent systems were either upfield or downfield. Presumably only positive electron spin

density is transferred to the region of the phosphorus upon chemical interaction whereas positive or negative electron spin density may be induced at the proton site. Conformational dependence of the direction of contact shifts has been noted previously in rigid and semi-rigid systems.[22,23] With (relatively) free conformational interconversion within the ester linkages of the present systems, a priori predictions of the directions of contact shifts are hardly possible.

The relative values of the concentration ratios for decoupling are of interest. First, decoupling of the phosphorus-hydrogen interaction is always observed at a lower concentration ratio in the phosphorus spectrum than in the proton spectrum. In fact, what is observed in the phosphorus spectra is probably not the true chemical decoupling, but rather an initial paramagnetic line-broadening effect which partially obscures the signal resolution and occurs prior to true chemical decoupling. This has been discussed by Frankel[12] for the proton spectrum of nickel(II)-dimethyl methylphosphonate systems. This effect, noted to be of little significance in the proton spectrum, should be of considerably more importance with the phosphorus spectrum owing to the relative closeness of the phosphorus nucleus to the paramagnetic species.

It had been noted earlier that in the series $NiCl_2$, $CoCl_2$, $FeCl_3$, the more paramagnetic $FeCl_3$ was most effective

in decoupling the proton spectra of phosphites[13] and nitrogen-bound ligands;[16] these observations were made for solutions in benzene and dioxane. In the present work, with acetonitrile as the solvent it is observed that $CoCl_2$ is the most effective in producing decoupling in both the proton and the phosphorus spectra. This is presumed to be due to the nature of the solvent system; the competition of the ligand with solvent (acetonitrile) for the metal ion is significantly less efficient for $FeCl_3$ than for $CoCl_2$.

It has been noted previously[14,15] that $Fe(acac)_3$ can be used in the decoupling of proton-proton interactions, presumably acting by a second co-ordination sphere mechanism rather than by a direct co-ordination process. It is predicted that phosphorus decoupling in the proton spectrum will probably not occur through a second co-ordination sphere mechanism, and thus far the systems investigated have behaved in accord with this prediction.[8] Attempts in the present work, using the non-labile $Fe(acac)_3$, agree with these prior results, no change in the proton spectrum being observed.

However, studies of the phosphorus spectra are inconclusive; only partial, incomplete decoupling of the phosphorus signals was observed. Concentrations of $Fe(acac)_3$ sufficiently high for complete decoupling could not be used with reliability as severe disturbance of the homogeneity of the magnetic field resulted.

TABLE Ia

Compound	Paramagnetic Reagent	$M/L \times 10^3$ [a] for Phosphorus-Hydrogen Decoupling
Trimethyl phosphite	$CoCl_2$	0.549
	$FeCl_3$	16.7
Trimethyl phosphate	$CoCl_2$	1.33
	$FeCl_3$	2.71
Triethyl phosphite	$CoCl_2$	0.500
	$FeCl_3$	5.63
Triethyl phosphate	$CoCl_2$	0.229
	$FeCl_3$	0.342
	$Fe(acac)_3$	10
Tri-isopropyl phosphite	$CoCl_2$	0.0913
	$FeCl_3$	0.128
	$Fe(acac)_3$	10
Tributyl phosphite	$CoCl_2$	0.358
	$FeCl_3$	3.22
Dimethyl methylphosphonate	$CoCl_2$	0.190
	$FeCl_3$	0.129

a) Molar concentration ratio of paramagnetic metal ion species to ligand.

TABLE Ib

Compound	Paramagnetic Reagent	Phosphorus Paramagnetic Shift At Decoupling M/L[b]
Trimethyl phosphite	$CoCl_2$	25
	$FeCl_3$	16
Trimethyl phosphate	$CoCl_2$	5
	$FeCl_3$	1
Triethyl phosphite	$CoCl_2$	0
	$FeCl_3$	10
Triethyl phosphate	$CoCl_2$	24
	$FeCl_3$	7
	$Fe(acac)_3$	0
Tri-isopropyl phosphite	$CoCl_2$	3
	$FeCl_3$	7
	$Fe(acac)_3$	0
Tributyl phosphite	$CoCl_2$	0
	$FeCl_3$	0
Dimethyl methylphosphonate	$CoCl_2$	42
	$FeCl_3$	0

b) All shifts are downfield and given in Hz.

TABLE Ic

Compound	Paramagnetic Reagent	Concentration Range of Compound (mol/l)	Maximum Concentration (mol/l) Of Paramagnetic Reagent
Trimethyl phosphite	$CoCl_2$	0.259-2.51	1.94×10^{-3}
	$FeCl_3$	0.259-2.51	4.33×10^{-3}
Trimethyl phosphate	$CoCl_2$	0.951-1.61	3.05×10^{-3}
	$FeCl_3$	0.951-1.61	4.96×10^{-3}
Triethyl phosphite	$CoCl_2$	0.611-2.04	1.94×10^{-3}
	$FeCl_3$	0.611-2.04	3.44×10^{-3}
Triethyl phosphate	$CoCl_2$	0.246-1.43	1.66×10^{-3}
	$FeCl_3$	0.246-1.43	4.51×10^{-3}
	$Fe(acac)_3$	0.246-1.43	2.76×10^{-3}
Tri-isopropyl phosphite	$CoCl_2$	0.170-1.87	1.16×10^{-2}
	$FeCl_3$	0.170-1.87	2.41×10^{-3}
	$Fe(acac)_3$	0.170-1.87	3.01×10^{-3}
Tributyl phosphite	$CoCl_2$	0.747-1.46	1.95×10^{-3}
	$FeCl_3$	0.747-1.46	2.41×10^{-3}
Dimethyl methylphosphonate	$CoCl_2$	1.75 -1.88	1.66×10^{-3}
	$FeCl_3$	1.75 -1.88	4.51×10^{-4}

TABLE II

Compound	Paramagnetic Reagent	M/L X 10^3 For $^{31}P-\{^1H\}$ Decoupling
Triethyl phosphate	$Fe(acac)_3$	Not observed
Tri-isopropyl phosphite	$CoCl_2$	0.685
	$FeCl_3$	0.770
	$Fe(acac)_3$	Not observed

CHAPTER III[24]

AN NMR INVESTIGATION OF THE INTERACTION OF $NiCl_2$ WITH AMINO ACIDS

INTRODUCTION

The study of metal ion complexes of amino acids has been of interest for a considerable period of time. These studies, however, have been concerned mainly with a limited number of amino acids which contain imidazole[25] or sulfhydryl[26] functional groups. The hydroxyl[27] and simple alkyl functional groups have received little or no attention. Furthermore, relatively little work has been done to understand changes in complexation with changing acidity.[25,28]

The majority of this prior work has involved the analysis of formation constants which were determined by titrimetric or polarographic techniques.[29] Not until recently has proton magnetic resonance (PMR) been used to investigate these complexes.[25,27] The PMR method has the great advantage that one can study competitive or concerted binding at different sites in the molecule. Perrin and Sharma have shown that these complexes do not involve a single species but rather that there exists an equilibrium involving varying numbers of amino acids and solvent ligands.[30] The PMR method is very useful in the investigation of systems of

this type; if the exchange rate is very low (on the NMR time scale), separate absorptions will be observed for each species present. However, if the exchange rate is high, only an "average" set of absorptions will be observed for the complexes in solution. This single set of absorptions allows one to study the "average" complex state.

The previous PMR investigations involved the use of high concentrations of paramagnetic or diamagnetic metal ion; with the paramagnetic systems this results in large changes in the chemical shifts of the protons close to the metal ion. Moreover, these high concentrations of metal ions do not approximate the biological systems for which the initial studies were begun. A more sensitive PMR method for investigation of the metal ion binding in these complexes involves solutions relatively dilute in metal ion. Here, line broadening phenomena rather than chemical shift observations are made.

The presence of paramagnetic ions in solution is known to have several effects on NMR signal shapes.[12,31] A primary source of line broadening is the change in nuclear relaxation time caused by the relatively large, localized magnetic field of a species bearing an unpaired electron in the proximity of the NMR active nucleus.[31] The relaxation of the nucleus in closest proximity (bound?) to the paramagnetic species should be affected to a greater extent than those which are at greater distances, either within the li-

gand molecules or in the bulk solvent.[32]

The situation of chemical exchange between a localized paramagnetic environment and its diamagnetic surroundings (solvent) can be described by equation 1 where M is the

$$ML_x + L' \rightleftharpoons ML_{x-1}L' + L \qquad 1$$

paramagnetic metal ion and L is the exchanging ligand. (L' may be identical to L or a molecule of solvent). The perturbation of NMR signals of the uncomplexed ligand will be determined by the times the ligand spends in a diamagnetic environment (τ_A) and in the paramagnetic environment (τ_B) together with the nuclear relaxation times in the respective environments (T_{2A} and T_{2B}). The Bloch equations have been solved for systems involving chemical exchange[3,33] yielding equation 2 where P_B is the fraction of the NMR active nuclei in the coordination sphere of the paramagnetic ion. These

$$\frac{1}{T_2} = \frac{1}{T_{2A}} + \frac{P_B}{T_{2B} + \tau_B} \qquad 2$$

functions are related to the observable line width ($\Delta\nu$) by the simple expression 3.[33]

$$\frac{1}{T_2} = \pi\Delta\nu \qquad 3$$

From this it is readily seen that the observable line

width is a linear function of P_B (4). Moreover, the fraction of NMR active nuclei bound to the paramagnetic species

$$P_B = \Delta\nu[\pi(T_{2B} + \tau_B)] - \frac{T_{2B} + \tau_B}{T_{2A}} \qquad 4$$

is directly proportional to the concentration of metal ion, if the concentration ratio of ligand to metal (L/M) is large. Under these conditions the observable line width should be linearly related to the metal ion concentration; significant deviations would be expected at smaller values of (L/M), i.e. relatively high concentrations of metal ion.

In the present work with α-amino acids, a linear dependence of proton relaxation times with metal ion concentration has always been observed.

Equation 2 contains both T_{2B} and τ_B thereby relating T_2 to the nuclear relaxation and the rate of ligand exchange. If relaxation is dominated by the rate of ligand exchange ($\tau_B >> T_{2B}$), equation 2 reduces to 5. However, if the nuclear relaxation dominates the rate of ligand exchange

$$\frac{1}{T_2} = \frac{1}{T_{2A}} + \frac{P_B}{\tau_B} \qquad 5$$

($T_{2B} >> \tau_B$) then equation 2 is reduced to 6.

$$\frac{1}{T_2} = \frac{1}{T_{2A}} + \frac{P_B}{T_{2B}} \qquad 6$$

The problem which now arises for consideration is a determination of which of these two situations actually exists in the systems being studied. (A third possibility exists wherein nuclear relaxation and ligand exchange are "competitive" leaving only equation 2 valid.)

This problem may be resolved in one of two ways. The first of these involves a realization that the τ_B is simply the rate of a chemical reaction and therefore would be expected to exhibit a significant temperature dependence. While T_{2B} is also temperature sensitive, its dependence is expected to be much less significant. An experimental determination showing that the relaxation time is reasonably sensitive to temperature variation would indicate that τ_B was the dominant factor in the relaxation. A second method involves only the secondary observation of some non-equivalent proton of the ligand; if all NMR active nuclei (protons) within the ligand exhibit the same degree of line broadening, then the relaxation is dependent on T_{2B}. The absence of this phenomenon, each non-equivalent nucleus exhibiting a different degree of line broadening, indicates that T_{2B} is the dominant factor in the observed relaxation. For this last situation equation 6 would be viable. This latter method has been used in the present investigation and obviates difficulties in variable temperature experiments.[34]

In all of the experiments described in this report each non-equivalent proton set of a particular ligand exhibited

a unique relaxation time indicating that nuclear relaxation is the dominant process and equation 6 represents a useful approximation.

RESULTS

In Table III are summarized the data obtained for the systems of α-amino acid - $NiCl_2$ in D_2O at several pD values. The slopes were calculated using a least squares technique[35] on a minimum of seven determinations. The standard deviations for these slopes are given below for each system investigated (Table IV).

Alanine: As is shown in Table III both the α- and β-protons of alanine exhibit a very significant pD dependence in their interaction with $NiCl_2$. The slopes of $\Delta\nu$ vs. metal ion concentration show a large increase upon changing the acidity from pD=7 to pD=5 but then decrease upon further increase of the acidity to pD=3. The concentration ratio, (L/M), in all cases was maintained large enough so that no significant changes in chemical shifts of α- or β- protons (relative to HOD) were noted.

For these measurements the alanine concentration range was 0.7990 M to 0.3060 M and the $NiCl_2$ concentration range was 0.02616 M to 0.01682 M.

Valine: The α- and γ- protons of valine exhibit only a slight variation of the slope of $\Delta\nu$ vs. metal ion concen-

tration over the pD range investigated. Interestingly enough, however, the β-protons exhibit a very significant dependence in the same direction but of greater magnitude than for the β-protons of alanine. Again, no significant changes in chemical shifts (relative to HOD) were noted within the concentration ratios used.

For these measurements the valine concentration range was 0.2804 M to 0.1284 M and the $NiCl_2$ concentration range was 0.01682 M to 0.004806 M.

Threonine: Threonine provides an interesting system of some difficulty in analysis. Only slight changes were noted for the α-proton in the slope of Δν vs. metal ion concentration over the pD range investigated; moreover, a minimum is reached at pD=5 in contrast to the systems previously mentioned. Reliable measurements for the β-proton were not possible due to the low intensity of the very complex signal. The γ-protons exhibit a measureable change of slope with changing pD, reaching a maximum at pD=5. No change in chemical shifts was noted over the concentration ranges used.

The threonine concentration range for the experiments was 0.4906 M to 0.1792 M and the $NiCl_2$ concentration range was 0.2523 M to 0.01346 M.

Methionine: The signal for the α-proton of methionine exhibits a pD dependence opposite that of the α-proton of alanine and the β-protons of valine; the slope of Δν vs. metal ion concentration decreases upon increasing the acidity

from pD=7 to pD=5, increasing again as the acidity is increased to pD=3. As with the systems previously mentioned, no significant changes in chemical shifts were noted over the concentration ranges used.

The methionine concentration range for these measurements was 1.059 M to 0.5994 M and the $NiCl_2$ concentration range was 0.01529 M to 0.002103 M.

Histidine: The signal for the α-proton of histidine exhibits only a relatively small dependence on added paramagnetic metal ion; the slope of $\Delta\nu$ vs. metal ion concentration is smaller than that for any of the systems previously mentioned. At pD=3 reliable data could not be obtained due to overlap with the HOD signal.

The signal for the β-protons, on the other hand, exhibits a very significant dependence both on the concentration of paramagnetic metal ion and the acidity of the solution. At pD=7 a negative slope of $\Delta\nu$ vs. metal ion concentration is observed which upon increasing the acidity becomes positive. Opposite behavior is noted for both the high field and low field imidazole absorptions; at pD=7 large, positive slopes for $\Delta\nu$ vs. metal ion concentration were observed which upon increasing the acidity decreased to near zero and became negative. Unlike the systems discussed previously, histidine exhibits small (but significant) contact shifts. Ghanges in the chemical shifts of the imidazole protons are noted at pD=7; downfield shifts of

0.2 ppm and 0.1 ppm are observed for the low and high field imidazole absorptions respectively at the lowest (L/M) ratio used. At higher acidities contact shifts are insignificant with the exception of the low field imidazole absorption which at pD=5 exhibits a downfield shift of 0.1 ppm at the smallest (L/M) ratio investigated.

The histidine concentration range for these measurements was 0.1349 M to 0.05653 M and the $NiCl_2$ concentration range was 0.01346 M to 0.002103 M.

Cysteine: The α-proton of cysteine exhibits a very slight dependence on pD in the slope of $\Delta\nu$ vs. metal ion concentration. The signal for the β-protons appears as two overlapping doublets which could not be resolved sufficiently for analysis and was therefore considered as one single absorption. This absorption also exhibits only a slight variation with changing pD. The magnitudes of the slopes measured are only slightly smaller than those for the α-proton. No change in chemical shifts was observed over the concentration range studied.

For these measurements the cysteine concentration range was 0.3141 M to 0.1228 M and the $NiCl_2$ concentration range was 0.02018 M to 0.003946 M.

DISCUSSION

Fundamentally similar behavior is found for alanine and valine. This is not unexpected as both are simple alkyl de-

rivatives of glycine and exhibit similar values of I_pH.[36] Marked increases in the slopes of $\Delta\nu$ vs. metal ion concentration are noted for both upon increasing the acidity from pD=7 to pD=5, decreasing with further increase of acidity; with I_pH values of approximately 6, this would indicate that ligand association with the metal ion occurs predominantly in the zwitterionic form. This is in contrast, however, with the results of Cassatt and Wilkins[37] where it was concluded on the basis of spectrophotometric data that the zwitterionic form is very unreactive.

The greatly decreased effect noted for the γ-protons of valine is in accord with the concept of Pearson, et al.[32] relating to distance of the nucleus from the paramagnetic site. This is also in accord with the previous reports on chemical decoupling by paramagnetic ions.[13-16]

With threonine a significant structural departure from the simple alkyl substituted glycine is found; it is thus surprising that little work has been done using this amino acid. The hydroxyl group substituted at the β-position can provide not only a perturbation of the electronic aspects at the primary site of association but also a possible secondary site of association. The signal change (with metal ion concentration) for the α-proton remains almost constant (a slight decrease with increasing acidity is noted) over the pD range investigated; this is indicative that there is little change in the degree of coordination at the α-amino acid

function over this range.

Moreover, an interesting variation of signal change (with metal ion concentration) over the pD range investigated isobserved for the γ-protons. The sensitivity of the γ-proton signal is quite high, very much greater than that of the γ-proton signal value of valine, exhibiting a maximum at pD=5. This may be interpreted in terms of the hydroxyl and amino acid functions competing for coordination at pD=7; as the acidity is increased the amino function is first protonated leaving hydroxyl and carboxyl functions for coordination and finally the hydroxyl group becomes protonated leaving the carboxyl function as the sole coordination site. A similar result that the hydroxyl group participates in binding has previously been reported by Bowles, Szarek, and Baird.[27] However, Li, et al.[38] concluded that in serine the hydroxyl does not participate in binding.

Methionine presents another structural variation on the simple alkyl substituted glycine which allows an additional possible site of coordination aside from the α-amino acid function. This structural variation appears to have introduced some interesting deviations in the nature of the ligand coordination compared to the previously discussed systems.

At pD=7 an extremely large value of the slope of Δν vs. metal ion concentration for the α-proton indicates efficient coordination at the α-amino acid function; however, a rel-

atively great sensitivity to the paramagnetic ion is observed for the γ-protons as well, indicating that a significant degree of coordination with the sulfur is also present. Upon increasing the acidity to pD=5 the α-amino function is highly protonated and coordination involves primarily the carboxyl function and the sulfur atom, although there is significant protonation at the sulfur. Further increase of the acidity to pD=3 results in protonation of the sulfur leaving the carboxyl function as the sole site available for coordination.

The increasing protonation of the sulfur with increasing acidity is also shown by the effect of the paramagnetic ion on the signal of the S-methyl group. As the acidity increases, the slope of $\Delta\nu$ vs. metal ion concentration constantly decreases, paralleling the effect for the γ-protons, indicating decreasing coordination in that region of the molecule.

Histidine, unlike the previously mentioned amino acids, has been studied extensively due to the presence of the imidazole ring. These investigations involved the use of high concentrations of metal ion which tended to obscure a very subtle interaction between the amino acid function and the imidazole ring. This interaction can be seen in the ordinary PMR spectrum at pD=7 where an ABCXY spectrum is observed rather than the expected pseudo-first order spectrum. This indicates that there is ordinarily (in the absence of complexing ion) some degree of interaction between the α-amino acid function and the imidazole ring which rigidifies the

conformation. At greater acidities (pD=5 and pD=3) the normal pseudo-first order spectrum is observed. Here the imidazole ring is protonated removing the ability to coordinate with the α-amino acid function.

The line width data given in Table III for histidine are quite interesting. At pD=7 the imidazole proton signals are seen to be rather sensitive to the paramagnetic effect of the metal ion whereas the α-proton exhibits only a weak sensitivity. From this it appears that at this acidity the primary site of metal ion coordination is the imidazole ring. This is supported by the negative slope of $\Delta\nu$ vs. metal ion concentration for the β-protons. As the metal ion coordination with the imidazole ring increases the locking of the β-protons into a fixed conformation decreases yielding an increase in the mobility of the β-carbon. This greater mobility of the β-carbon is reflected in the sharpening of the absorption due to the β-protons.

As the acidity is increased to pD=5 and pD=3 the imidazole ring is protonated pre-empting complexation with the metal ion at that position. This leaves the α-amino acid function as the primary site of complexation. The great change in the efficiency of the paramagnetic effect on the β-protons illustrates this very well, along with the decrease in efficiency of the paramagnetic effect on the imidazole proton signals.

Cysteine, like histidine, has been extensively investi-

gated;[26,29,39] however, methionine, which is structurally similar to cysteine has received relatively little attention[40]. The present work indicates that they tend to interact somewhat differently with $NiCl_2$. The slopes of $\Delta\nu$ vs. metal ion concentration decrease more rapidly with increasing distance from the amino acid end of the molecule for methionine than for cysteine. This effect may be attributed to the greater ability of the S-H (compared with $S-CH_3$) to compete for the metal ion. The slight pD dependence indicates that protonation of the sulfhydryl function does not affect the metal interaction.

EXPERIMENTAL

DL-Alanine (Aldrich), L-valine (Aldrich), DL-histidine (Pfaltz and Bauer), DL-methionine (Pfaltz and Bauer), and L-threonine (Sigma) were purchased from the indicated suppliers and used without further purification. DL-Cysteine (Sigma) was dried (110°) for 12 hr prior to use. Anhydrous $NiCl_2$ was prepared as described previously.[41] D_2O of 99.8 atom% D (Stohler) was used without further purification. The pD of the solutions was adjusted using 1 M HCl and 1 M NaOH with the aid of a Corning Model 112 pH meter.

All experiments were performed using a Varian HA-100 NMR spectrometer in frequency sweep mode. Chemical shifts were measured relative to the internal lock signal (HOD) using a Varian V-4315 frequency counter.

CONCLUSION

From the discussion above it may be noted that the presence of a substituent other than an alkyl group on the fundamental glycine structure allows effective competition for metal ion association. This competition appears to be quite dependent on the acidity of the medium as protonation of the substituent becomes significant.

The present work provides a useful foundation for the investigation of more complex interactions of paramagnetic metal ions with peptide systems; such investigations should prove of significant interest in the elucidation and understanding of metal ion complexation and function in enzymatic systems.

TABLE III

Proton Signal	Slope of Δν vs. Metal Ion Concentration		
	pD=7	pD=5	pD=3
α-proton of:			
Alanine	307	637	351
Valine	298	374	350
Threonine	223	191	195
Methionine	364	133	226
Histidine	14.7	26.9	---
Cysteine	227	252	198
β-protons of:			
Alanine	349	511	309
Valine	87.8	265	152
Histidine	-88.2	119	120
Cysteine	152	170	165
γ-protons of:			
Valine	40.4	31.7	47.9
Threonine	104	196	4.34
Methionine	142	98.0	74.8
$S-CH_3$:			
Methionine	35.2	24.6	16.4
Imidazole protons of Histidine:			
Low Field	254	-3.7	-16.7
High Field	250	4.4	-21.1

TABLE IV

Proton Signal	Standard Deviation in Slopes of Δν vs. Metal Ion Concentration		
	pD=7	pD=5	pD=3
α-proton of:			
Alanine	0.3384	0.2064	0.3054
Valine	0.3422	0.2149	0.1614
Threonine	0.1967	0.1164	0.1648
Methionine	0.4052	0.1984	0.2758
Histidine	0.1212	0.1639	------
Cysteine	0.1085	0.0938	0.3095
β-protons of:			
Alanine	0.2011	0.8917	0.0989
Valine	0.1073	0.6279	0.2509
Histidine	0.1118	0.3267	0.2499
Cysteine	0.1814	0.1679	0.2189
γ-protons of:			
Valine	0.0418	0.1071	0.1136
Threonine	0.2180	0.1369	0.4548
Methionine	0.2084	0.2724	0.0778
$S-CH_3$:			
Methionine	0.2261	0.1022	0.0821
Imidazole protons of Histidine:			
Low Field	0.2493	0.1784	0.1397
High Field	0.3007	0.2878	0.2335

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