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The Infra-Red Absorption Spectra of Some Amide- and Dipeptide-Metal Chelates

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Changes in the infra-red absorption spectra of amino acid amides and dipeptides after chelate formation with Cu(II) are interpreted as a result of the shift in the resonance equilibrium of the peptide group. The magnitude of these changes for chelates of Cu(II), Ni(II) and Zn(II) has been used as a measure for the interaction of metal ions with the peptide group.

The behaviour of the infra-red (IR) absorption frequencies of $-NH_2$ and -COOH groups in amino acid-metal chelates was examined in a previous publication.[1] In the present investigation the behaviour of the amide group, as represented by its IR absorption spectra, is studied in some amide- and dipeptide-metal chelates. The chelation of peptides and amides by metal ions has mainly been studied in solutions, by methods aimed at the determination of complex constants.[2-4] A number of crystalline chelates has been prepared and analyzed and the ratio of metal to ligand thus determined.[5,6] The need for a better elucidation of metal-peptide bond interaction has arisen from studies of metal-activated enzymes. Smith and Spackman [7], for example, explain the role of metal ions in proteolytic enzyme systems by assuming a metal chelate bridge between the enzyme and the substrate molecule. The interaction of the peptide group with the metal ion is viewed as resulting in an electron displacement rendering the bond susceptible to nucleophilic attack by hydroxyl ions. If this were the case, the IR frequencies of the amide linkage should be considerably influenced by the metal ion. An IR study of chelate formation between metals and peptides has been published by Chouteau [8]. Since Chouteau's main interest was the investigation of the biuret reaction, the solid chelate compounds were precipitated or evaporated at extremely high pH values. Working at the pH values of 12—13, Chouteau found that the frequencies ascribed to the amide group did change considerably. The behaviour of the amide group at such extreme pH values has probably no direct bearing on the biological role of the metal ions. The solid chelates of peptides used in the present examination are all of neutral character, isolated from aqueous solutions where the pH value never exceeded 9.

PREPARATIONS

Bis-glycinamido-Cu(II)monohydrate, $(GA)_2Cu,H_2O$, Bis-L-leucinamido-Cu(II), $(La)_2Cu$, and Bis-L-leucinmethylamido-Cu(II) trihydrate, $(LMA)_2Cu,3H_2O$, were prepared from the corresponding amides with Cu(II) acetate as described by Rising and Yang [9] with the modification that the volume of the reaction mixture was increased to prevent an instantaneous precipitation. The solution was then slowly evaporated until the reddish-blue crystals appeared. The crystals were dried over P_2O_5 and analyzed . The $(LMA)_2$-Cu(II) showed a tendency to crystallize with varying numbers of water molecules. These differences were not essential for the IR investigation, as the different species showed only changes in the diffuse background absorption.

Glycylglycino-Cu(II) trihydrate, GG-$Cu,3H_2O$, DL-alanylglycino-Cu(II) dihydrate, AG-$Cu,2H_2O$, DL-leucylglycino-Cu(II) hydrate, LG-Cu,H_2O and Glycyl-L-leucino-Cu(II) hydrate GL-Cu, H_2O were prepared from commercial dipeptides and $CuCO_3$ according to Tomita *et al.*[5]. The crystalline chelates were recrystallized ırom water and washed with alcohol. When dried over P_2O_5, the dark blue trihydrate of glycylglycine is converted to a blue monohydrate. The monohydrate of GG-Cu(II) can be obtained directly when the chelate is precipitated from aqueous solution by ethanol.

The Bis-glycylglycino-Ni(II) didhydrate, $(GG)_2Ni,2H_2O$, was obtained as pale blue crystals. An aqueous solution of GG was vigorously shaken with an excess of freshly precipitated Ni(II) hydroxide. The pH of the suspension was adjusted to approx. 8. The residual Ni(II) hydroxide was removed by filtration. The volume of the blue solution was reduced and ethanol added. The chelate crystallized even from a solution of GG and $NiCl_2$ in proportions 2 to 1 when the pH of the solution was adjusted to 8 and ethanol added.

Bis-glycylglycino-Zn(II) dihydrate, $(GG)_2Zn,2H_2O$. was prepared from $Zn(OH)_2$ and GG. An excess of freshly precipitated and washed $Zn(OH)_2$ was added to an aqueous solution of GG. The pH of the suspension was adjusted approx. to 8 and the suspension shaken vigorously before filtration. The chelate crystallized as white needles upon addition of ethanol. Bis DL-alanylglycino-Zn(II) dihydrate was obtained by the same method.

Table 1. Analyses.*

Chelate	% N		% Metal	
	Calc.	Found	Calc.	Found
$(GA)_2Cu,H_2O$	24.61	24.41	27.91	27.79
$(LA)_2Cu$	17.40	17.26	19.74	19.53
$(LMA)_2Cu,3H_2O$	13.87	14.20	15.73	15.58
GG-$Cu,3H_2O$	11.31	11.53	25.65	25.71
GG-Cu,H_2O	13.23	12.98	29.90	30.02
AG-$Cu,2H_2O$	11.49	11.56	26.07	26.01
LG-Cu,H_2O	10.46	10.47	23.72	23.68
GL-Cu,H_2O	10.46	10.54	23.72	23.83
$(GG)_2Ni,2H_2O$	15.69	15.52	16.44	16.36
$(GG)_2Zn,2_2HO$	15.40	15.38	17.97	18.01
$(AG)_2Zn,2H_2O$	14.31	14.26	16.69	16.74

* N was determined according to Dumas and the metals titrated with EDTA.

Glycinamide-hydrochloride, GA, HCl, was prepared according to Bergell and Wülfing [10]. Mp. for hydrochloride: 185—187° and for free glycinamide 64—65°.

L-Leucinamide, LA, was prepared from L-leucine ethyl ester according to Yang and Rising [11]. The L-leucine ester used was obtained according to Fischer [12] M.p. found for L-leucinamide hydrochloride 235°. L-Leucine methylamide, LMA, was prepared from

L-leucine ethyl ester. The ester was cooled with dry CO_2 in ethanol and an equal volume of liquid methyl amine was added. The glass tube containing the mixture was sealed and kept at 50° for 3 days. The amide was then distilled *in vacuo*; bp/9 mm Hg 139°C. (Found: N 19.17. Calc. 19.46).

Glycylglycine ethyl ester, GGet, was obtained by the same method as L-leucine ester. (Found: N 14.30. Calc. 14.25).

A Perkin-Elmer model 21 spectrophotometer equipped with CaF_2 and NaCl prisms was used for the IR-absorption measurements. The chelates were pressed into transparent KBr discs as described by Schiedt and Reinwein [13]. In order to achieve the best resolution possible, the IR absorption of the chelates was generally measured at low temperature. The low-temperature cell and the effects of low temperature have been described previously [1]. The values obtained at normal temperatures are directly compared with those obtained at low temperatures only when the temperature change has no effect on the position of the absorption bands.

The deuterium exchange was obtained by recrystallizing the substance under investigation from 99.7 % D_2O.

RESULTS OF THE ABSORPTION MEASUREMENTS

The first chelates investigated were the Cu(II)-amide chelates. Fig. 1—3 show the IR-absorption in the 3 μ region for 3 pairs of substances. GA and $(GA)_2$ Cu, H_2O, LA and $(LA)_2$ Cu, LMA and $(LMA)_2$ Cu, $3H_2O$. All the absorption curves except that for the pure LMA were obtained at low temperatures. The spectra of LMA can still be compared with the spectra of its Cu(II) chelate as the positions of the chelate frequencies were not influenced by a change of temperature.

The positions of the absorption bands due to N-H or O-H stretching frequencies are compiled in Table 2.

Table 2.

	Stretching frequencies in the 3 μ region		
GA	3 347	3 279	3 137, 3 084
$(GA)_2$,Cu,H_2O	3 329	3 218	3 106
LA	3 349	3 279	3 096
$(LA)_2$Cu	3 323	3 202	3 112
LMA	3 312		3 086
$(LMA)_2$Cu,$3H_2O$	3 232		3 117

Figs. 4—6 show the 6 μ region of the same chelates.

The amides themselves show, in this region, two well-known bands produced by the amide group frequencies. In the following these bands are referred to as the amide I and amide II bands.

The dashed curves in Figs. 4—6 represent the absorption of the deuterated compounds.

Deuterization removes from this region the possible amide II band and all the bands due to N-H bending movements. The frequencies of the free amides and amide hydrochlorides are presented in Table 3 together with the corresponding values for Cu-amide chelates.

Table 3.

	Solid		Solution	
	Amide I	Amide II	Amide I	Amide II
GA	1 695	1 608	1 686	1 553
GA, HCl	1 709	1 597		
$(GA)_2Cu,H_2O$	1 570			
LA	{1 664 1 681	1 600	1 678	1 548
LA, HCl	{1 678 1 686	1 618		
$(LA)_2Cu$	{1 588 1 554			
LMA	1 658	1 541	1 678	1 520
LMA, HCl	1 675	1 572		
$(LMA)_2\,Cu,3H_2O$	1 567			

The next group of substances examined, were the metal chelates of simple dipeptides. Fig. 7 shows the 3 μ region of GG and GG-Cu(II) trihydrate.

Fig. 8 shows the same spectral region of three further dipeptide-Cu(II) chelates. The AG-Cu(II) has two molecules of water attached to the chelate molecule, the other two LG-Cu(II) and GL-Cu(II) posess only one molecule of water each. The next two figures (9 and 10) show the IR absorption of the chelates in the 6 μ region. Fig. 9 represents the absorption of GG-Cu, H_2O.

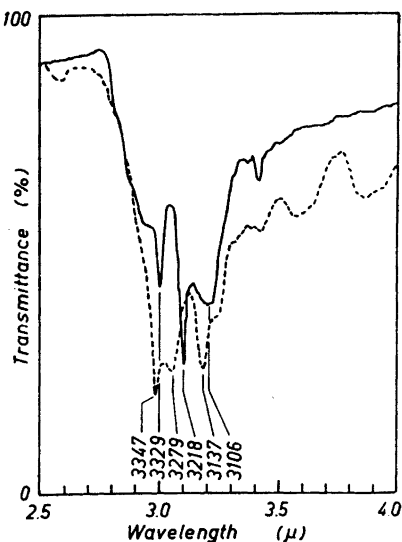

Fig. 1. IR absorption of glycinamide, GA, ----------- and of $(GA)_2Cu,H_2O$ ———

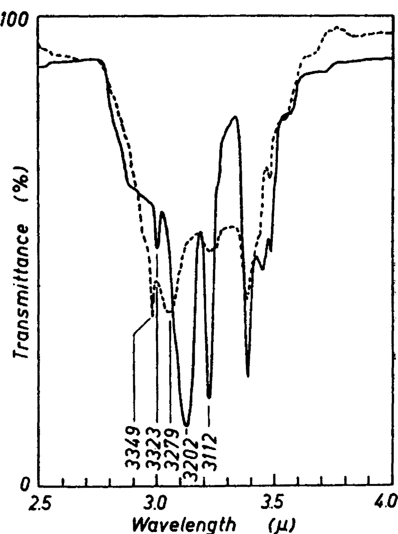

Fig. 2. IR absorption of L-leucinamide, ----------- LA, and of $(La)_2Cu$ ———

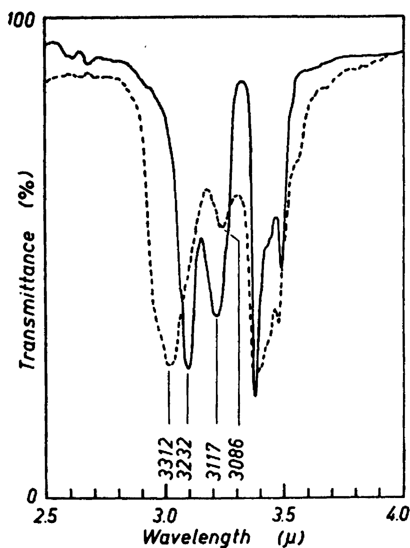

Fig. 3. IR absorption of L-leucinmethylamide, LMA, ------------ and of $(LMA)_2Cu,3H_2O$ ————

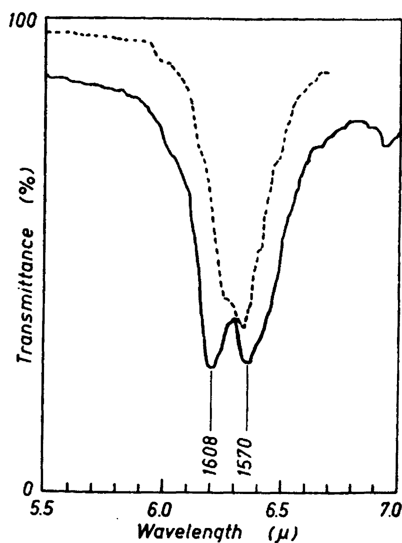

Fig. 4. IR absorption of $(GA)_2Cu,H_2O$ ———— and of the deuterated compound ------------

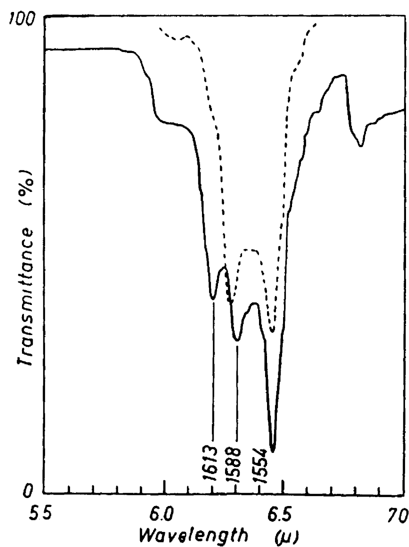

Fig. 5. IR absorption of $(LA)_2Cu$ ———— and of the deuterated compound ------------

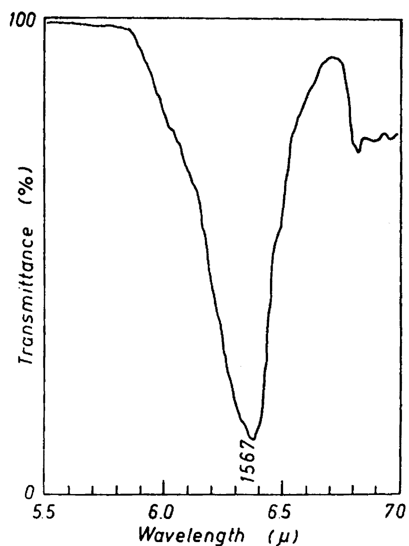

Fig. 6. IR absorption of $(LMA)_2Cu,3H_2O$

The spectrum of the trihydrate shown in Fig. 11 is complicated due to the three water molecules. In order to simplify the picture the chelate was recrystallized from D_2O. The IR absorption of the deuterated compound is represented by the broken curve in Fig. 9. The absorption spectra of the deuterated products of GG-Cu, H_2O and GG-Cu, $3H_2O$ were identical in this wavelength region.

Fig. 10 shows the 6 μ region for three further dipeptide chelates. The values of the two main bands, ν (COO^-) and ν $\left(-C\begin{smallmatrix}O\\ N-\end{smallmatrix}\right)$, are collected in Table 4. The extra bands visible in the figure are due to N-H or O-H bendings as they all vanish after deuterization.

Table 4.

	ν (COO^-)	$\nu\left(-C\begin{smallmatrix}O\\ N-\end{smallmatrix}\right)$
GG-Cu,H_2O	1 592	1 541
AG-Cu,$2H_2O$	1 587	1 548
LG-Cu, H_2O	1 597	1 534
GL-Cu,H_2O	1 595	1 524

Fig. 11 represents the IR absorption of Cu(II), Ni (II) and Zn(II) chelates of glycylglycine (GG). The absorption in wavelength regions between 2.5-4 μ and 5.5-7.0 μ was measured with a CaF_2 prism, for the region from 7 to 14 μ a NaCl prism was utilized. Low temperature has been used for all of the measurements. As the absorption from water molecules conceals most of the absorption bands of GG-Cu, $3H_2O$ in the 6 μ region, the absorption of the monohydrate, represented by the broken curve, was added to the original spectrum.

The frequencies of the peptide and carboxyl groups for the different forms of GG are collected in Table 5.

Table 5.

	Amide I	Amide II	COO^-		Amide I	Amide II	COO^-
GG	1 678 1 657	d	1 608	GG-Cu,$3H_2O$	1 541		1 613[c]
GG, HCl	1 686	1 562	1 748[a]	$(GG)_2Ni,2H_2O$	1 653 1 631	1 583	1 600
GG-et	1 664	1 536	1 745[b]				
GG-et $CHCl_3$ sol.	1 672	1 536	1 748[b]	$(GG)_2Zn,2H_2O$	1 661 1 642	1 577	1 600

a Frequency of the COOH group.
b Frequency of the COOet group; et = ethylgroup.
c Deuterated compound.
d assignment uncertain.

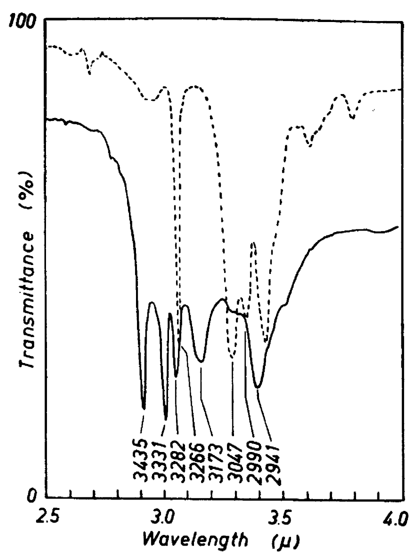

Fig. 7. IR absorption of glycylglycine, GG, ------------ and of GG-Cu,$3H_2O$ ————

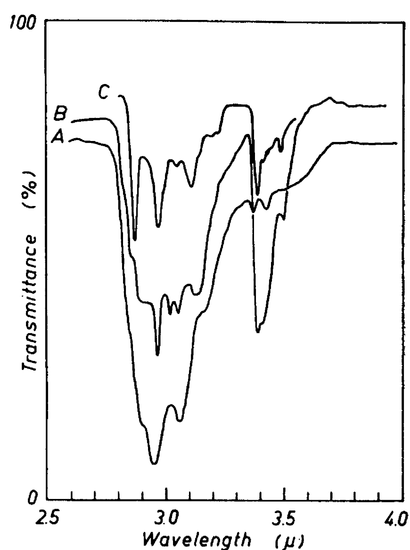

Fig. 8. IR absorption of AG-Cu,$2H_2O$ curve A, of GL-Cu,H_2O curve B, and of LG-Cu,H_2O curve C.

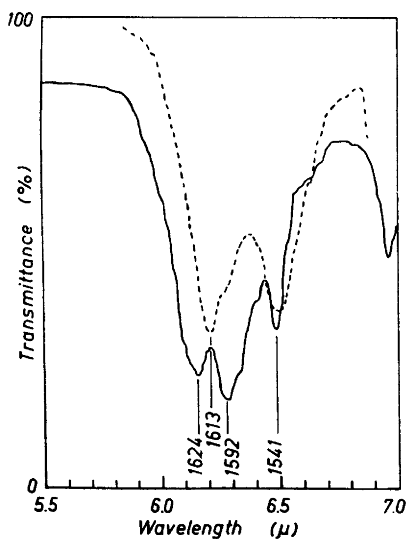

Fig. 9. IR absorption of GG-CuH_2O ———— and of the deuterated compound ------------

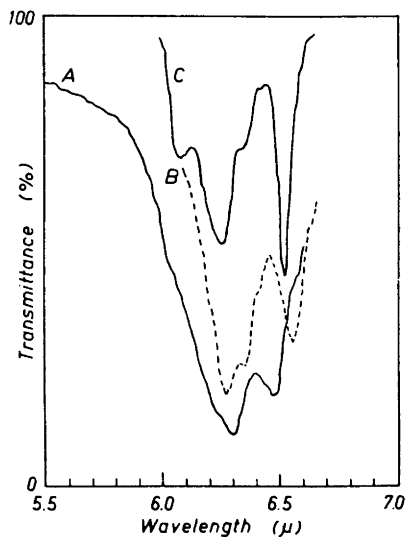

Fig. 10. IR absorption of AG-Cu,$2H_2O$ curve A, of GL-Cu,H_2O curve B and of LG-Cu,H_2O curve C.

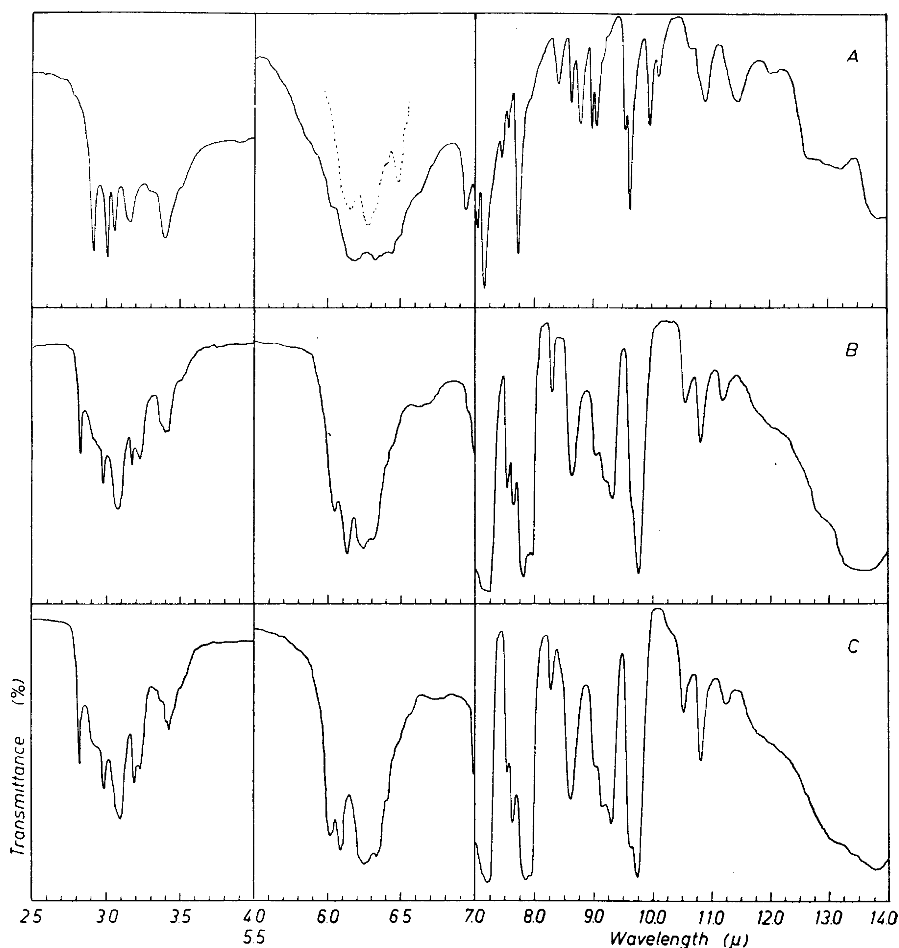

Fig. 11. IR absorption of GG-Cu,$3H_2O$ curve A, of $(GG)_2Ni,2H_2O$ curve B and of $(GG)_2Zn,2H_2O$ curve C.

In order to check the assignments of absorption frequencies in the Zn(II) chelates, the Zn(II) chelates of DL-alanylglycine and glycyl-L-leucine were prepared. $(AG)_2Zn,2H_2O$ crystallized beautifully and showed an IR spectrum in the 6 μ region that was almost identical with the spectrum of $(GG)_2Zn,2H_2O$. The amide I doublet was found at 1 658 and 1 639 cm^{-1}, the absorption of the carboxyl group appearing at 1 600 cm^{-1}. The Zn(II) chelate with glycyl-L-leucine is not included in the experimental part because a chelate with a definite number of water molecules could not be isolated. The product with an undefined number of water molecules exhibited broad absorption bands in the same region as $(GG)_2Zn,2H_2O$ and $(AG)_2Zn,2H_2O$, no absorption being visible below 1 580 cm^{-1}.

DISCUSSION

Amide-Cu(II) chelates. The interpretation of the observed changes in the IR absorption spectra of amide molecules after chelate formation is comparatively simple. The red Cu(II) chelates of DL-leucinamide and glycinamide have previously been isolated and analyzed by Rising and Yang [9]. They found that two amide molecules combine with one Cu(II) ion to form a neutral chelate. $(GA)_2Cu(II)$ crystallizes with one molecule of water whereas $(LA)_2$ Cu(II) lacks water of crystallization. This eliminates the possibility that the neutral molecule is formed by dissociation of hydrogen ions from coordinatively bound water molecules. The results of analyses of the amide chelates prepared for this investigation are in accordance with the values given by Rising and Yang. Potentiometric measurements, recently described by Datta and Rabin [16], confirm the assumption that, in each of the two amide molecules, one hydrogen is ionized from the amide nitrogen so that the chelate formed carries no net charge. The structure of the Cu(II) chelates with amino acid amides can be represented by Fig. 12.

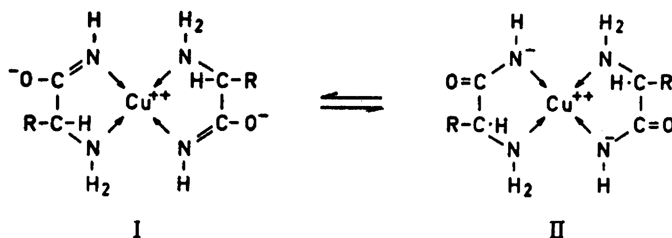

Fig. 12.

These changes in the amide molecule ought to be accompanied by changes in the IR absorption due to hydrogen stretching movements in amino and amide groups and in the intense vibration bands of the amide group in the neighbourhood of 6 μ.

The chelate formation results in a shift of the N-H bond stretching frequencies of amides towards lower wavenumbers. The shift is generally exhibited by amino groups when the free electron pair of the nitrogen is used for bond formation [1,17]. The behaviour of the N-H frequencies observed follows this rule. The spectrum of amides in the 3 μ region is rather complicated as the unsubstituted amides exhibit at least four N-H absorption bands in this region, some of these being certainly influenced by hydrogen bonds with the oxygen of the peptide group. The band structure of the Cu(II) chelates seems to be somewhat simpler. The amino group contributes with two frequencies and the remaining amide N-H with one frequency. It can be inferred from studies of the metal chelates of amino acids [1] that amino frequencies appear at slightly lower wavenumbers than the N-H frequencies in free amides. The Cu(II) chelate of leucine, for example, has its main amino frequencies at 3 308 and 3 240 cm^{-1}. The Cu(II) chelates of GA and LA show 3 frequencies in this region. The band, at 3 106 cm^{-1} for $(GA)_2Cu$ and at 3 112 cm^{-1} for $(LA)_2Cu$,

can thus tentatively be ascribed to the N-H stretching of the amide group. The Cu(II) chelate of LMA shows as expected only two absorption bands in this region. The interpretation of the absorption data of N-H stretchings is not entirely unambiguous as some of the bands are broad enough so that the possibility of two overlapping frequencies is not excluded. At the same time some of these frequencies might be split into two bands due to crystal effects. It must be stressed that, owing to above mentioned difficulties, the position or absence of the amide N-H frequency can scarcely be used as a general indication of the influence of the metal ion on the peptide linkage.

The region of 6 μ is far more suitable for this purpose as the intense amide I frequency can easily be identified. The amide I, at approx. 6 μ, is usually considered to be a stretching frequency of the amide carbonyl in resonance with the C-N bond. Amide I is only slightly influenced by deuterization. The amide II, at approx. 6.2—6.4 μ, is a combination band where the N-H bending movement participates. It is strongly influenced by deuterization. These assignments have recently been reviewed and discussed by Miyazawa *et al.*[14] in connection with the calculation of frequencies for monosubstituted amides. The frequencies of the chelates show a vastly changed picture. The region for amide I is devoid of any absorption, the band structure being markedly shifted towards lower wavenumbers. The picture is further completed by the deuterized compounds, where the absorption due to hydrogen bending movements is removed from this wavelength region. The band remaining must be the stretching frequency of the resonating structure corresponding to the amide I band $\left(—C\begin{smallmatrix}\nearrow O \\ \searrow N—\end{smallmatrix}\right)$. The only deviation from the simple picture expected is the split of this band for $(LA)_2$ Cu. When interpreting these changes, the influence of the hydrogen bonds on the amide I frequency ought to be considered. The positions of the frequencies of free amides in the solid state and in dilute $CHCl_3$ solutions can be compared in Table 3. The frequency changes do not exceed the value of approx. 20 cm^{-1}.

Further, the effect of metal ion-amino group interaction on the amide frequency ought to be considered.

A comparison between the free amide and its hydrochloride can be considered as relevant, the hydrogen ion taking the place of the metal ion. The observed shift of the amide I frequency is at its height approximately 20 cm^{-1} towards higher wavenumbers. The difference between the normal amide I frequency and the frequency observed in Cu(II) chelates is of the order 90—100 cm^{-1}. This can hardly be due to other effects than to the definite shift of the resonance equilibrium of the amide group towards the canonical form (I) in Fig. 12. From his IR studies of the biuret reaction of amides at high pH values, Chouteau [8] puts forth the same explanation.

Dipeptide-Cu(II) chelates. The next step is to compare these results with the IR-absorption of dipeptide-metal chelates. GG-Cu, $3H_2O$ and LG-Cu, H_2O have been crystallized and analyzed by Tomita *et al.*[5] and GG-Cu,H_2O by Manyak *et al.*[6] In all these chelates one dipeptide molecule combines with one Cu(II) ion, Manyak *et al.* proposed the structure III in Fig. 13 for the glycylglycine chelate.

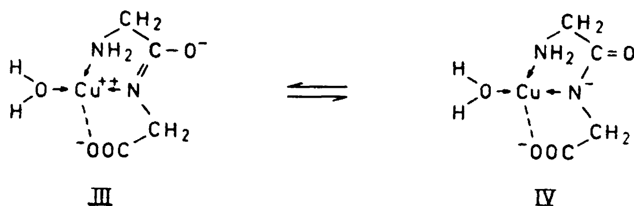

Fig. 13.

The neutral chelate is formed either by the dissociation of the amide hydrogen or by dissociation from the coordinatively bound water molecule. From titrimetric and spectrometric data Dobbie and Kermack [2], and later Datta and Rabin [4,16], arrive at the conclusion that the dissociation takes place from the peptide group.

The IR absorption of Cu dipeptides in the 3 μ region is exemplified by Figs. 7 and 8. The absorption of amide chelates, and especially that of the secondary amide $(LMA)_2Cu$, indicates that GG-Cu is liable to exhibit two N-H frequencies in the neighbourhood of 3 200—3 300 cm^{-1}. Of the 4 possible absorption bands in Fig. 7, those at 3 331 and 3 282 cm^{-1} are most likely to belong to the N-H stretching frequencies. When the two other peaks present are considered it must be borne in mind that OH frequencies can appear in this wavelength region originating from the three molecules of water attached to the chelate. It is evident from a comparision with the N-H bands of the amide-Cu(II) chelates that the band at 3 435 cm^{-1} in GG-Cu(II),$3H_2O$ appears at a wavenumber too high for the N-H stretching frequency; it must be assigned to an OH stretching frequency. The band at 3 173 cm^{-1} is possibly the other OH frequency of the coordinated water molecule. It is obvious from Fig. 8 that the 4 main bands found in GG-Cu(II),$3H_2O$ dominate also the spectra of Cu dipeptide chelates with only one water of crystallization as LG-Cu (II),H_2O. This indicates that the OH bands originate from the water molecule bound to the metal ion as a fourth ligand (Fig. 13). The other two water molecules in GG-Cu(II),$3H_2O$ contribute presumably only with broad and undefined absorption. The special position of one water molecule is even emphasized by the fact that whereas it is easy to remove two molecules of water from GG-Cu (II),$3H_2O$ the third molecule is retained even at high temperatures.

The OH absorption and the fact that some of the absorption bands are not sharp enough to exclude an overlapping of frequencies, are the reasons why the IR-absorption in the 3 μ region does not give a conclusive answer to the question of metal ion influence on the peptide linkage. We have, as in the case of amide-Cu(II) chelates, to rely mainly on the results obtained in the double bond region. The IR absorption of dipeptide-Cu (II) chelates in the 6 μ region is characterized by two strong bands unaffected by deuterization. The frequency due to the stretching of the carboxyl group is generally found [15] in the neighbourhood of 1 590 cm^{-1}. When the carboxyl group participates in some kind of covalent bound formation with the metal ion, the stretching frequency shifts to higher wavenumbers as observed for Pt(II) chelates of glycine and, to a certain extent, for the water-free Cu(II) chelates

of some amino acids [1]. Table 4 summarizes the behaviour of the carboxyl frequency for Cu-dipeptide chelates. The COO^- frequencies show no appreciable trend towards higher wavenumbers so that the interaction between the carboxyl group and the metal ion ought to be of the same kind as in alkali salts of carboxylic acids. The only change in the position of the carboxyl frequency is displayed by the deuterization product of GG-Cu(II) as shown by Fig. 9. The position of similar bands in other dipeptide-Cu chelates, uninfluenced by deuterization, indicates that of the two bands remaining after deuterization, the carboxyl frequency at 1 613 cm^{-1} is represented in the monohydrate by the band at 1 592 cm^{-1} and not by the band at 1 624 cm^{-1}.

The band at 1 541 cm^{-1} in GG-Cu(II),H_2O must be ascribed to the amide I frequency, representing the same mode of vibration of the peptide group as observed in the Cu(II) chelates of amino acid amides. The position of this band in different Cu(II) dipeptides is fairly constant. The wavenumbers of these bands are gathered in Table 4. The observed shift of the amide I band, due to chelate formation, is for the peptides examined, approx. 100 cm^{-1}, which is in close agreement with the values observed for amide-Cu(II) chelates. The frequency is thus found at a considerably lower wavenumber than the normal peptide group vibration or a conjugated $C = N$ bond frequency, indicating that the resonance structure of the peptide group is preserved but the equilibrium shifted towards the canonical structure III in Fig. 13.

Dipeptide chelates with Ni(II) and Zn(II). Manyak *et al.*[6] presume that the chelates formed by dipeptides with Ni(II) and Zn(II) are of the same type as the corresponding Cu(II) chelates. The Ni(II) and Zn(II) chelates isolated for this investigation are, as the analysis results show, neutral compounds consisting of two dipeptide molecules and one metal ion. These proportions and the absence of alkali ions rule out the possibility of further hydrogen ionisation in addition to the carboxyl group dissociation. The structure can be represented by *V* in Fig. 14.

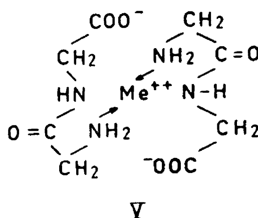

Fig. 14.

A comparison of the IR curves in Fig. 11 reveals that the IR spectra of Ni(II) and Zn(II) chelates are almost identical and of another type than that of the Cu(II) chelate. The difference between Ni(II) and Zn(II) chelates lies in the position of the amide I band. This suggests a difference in the influence of metal ions on the peptide link. A second more prominent feature, common for both Ni(II) and Zn(II) chelates, is the splitting of the amide I frequency. Such splitting is frequently observed in crystalline peptides. Glycylglycine

itself, for example, shows a similar double peak. It is presumably due to hydrogen bond effects between the peptide groups in the crystal lattice.

Table 5 summarizes the behaviour of the important frequencies of GG under different conditions. We choose the value of the amide I band of GG-ester at 1 672 cm^{-1} measured in dilute $CHCl_3$ solution, as a standard for the uninfluenced peptide group. When the solution of the GG-ester is evaporated to dryness, the remaining film of liquid ester shows a shift of the amide I band by 8 cm^{-1} towards lower wavenumbers. The effect is undoubtedly due to hydrogen bond formation. The same kind of shift is observed when the GG-ester is compared with free GG. The shift of the mean of two amide I frequencies is in this case 5 cm^{-1}. That the shift is so small is probably due to the fact that the GG molecule exists in the solid state as a "zwitterion" where the extra hydrogen at the amino group shifts the amide frequency in the opposite direction. This kind of influence was observed in the hydrochlorides of amides and is also exhibited by the hydrochloride of GG, where the shift of the amide I frequency is 14 cm^{-1} towards higher wavenumbers. The hydrochloride of the GG-ester, when compared to the free ester, shows a similar shift of 14 cm^{-1} in the same direction.

Thus the shifts of the amide I band due to changes in hydrogen bond structure and bond formation at the amino group do not exceed the value of 20 cm^{-1}.

The amide I band of the Cu(II) chelate of GG shows, as previously demonstrated, a shift of 131 cm^{-1} towards lower wavenumbers. The Ni(II) chelate displays a similar shift of 32 cm^{-1} and the Zn(II) chelate a shift of 20 cm^{-1} in the same direction. The mean value of the two amide I frequencies has been used for the Ni(II) and Zn(II) chelates. The shift in Ni(II) chelates is big enough to permit the conclusion that the metal ion has an influence on the resonance of the peptide bond. The influence of the Zn(II) ion is smaller and the shift of the amide I band exceeds only with 12 cm^{-1} the value shown by the free GG. The position of the amide I band in $(GG)_2Zn(II), 2H_2O$ at 1 653 cm^{-1} lies in a region where the normal amid I frequencies of secondary amides are due to appear [18]. For example the methylamide of L-leucine (Table 1) has its amide I band as near as at 1 658 cm^{-1}. The influence of the Ni(II) ion is not unexpected as Maynak *et al*[6]. have isolated, from a more alkaline solution, a chelate of the same proportions as the neutral compound investigated here, but with two Na ions attached. The presence of the Na ions indicates that the chelate formed has lost two protons in analogy to the GG-Cu(II) chelate. The observation that Ni(II) forms, at high pH values, a chelate of the same character as the Cu(II) ion can also be inferred from the titration curves [6,16]. The existence of such a chelate with Zn(II) ions has not been demostrated.

These differences observed for the chelates of different metal ions lead to the conclusion that, although Cu(II), Ni(II) and Zn(II) all form chelate structures with the amino group of the GG molecule, differences between the various ions are large enough, so that, for example, the neutral chelate of GG with Cu(II) isolated at pH 8, shows a major change in the peptide group resonance, whereas the neutral chelate with Zn(II) scarcely shows any change in the peptide group.

The question whether the neutral chelate with Cu(II) is formed by proton dissociation from the amide nitrogen or from the coordinatively bound water molecule cannot be solved directly from changes in hydrogen stretching frequencies due to the complexity of the absorption band structure in the 3 μ region. However, as the changes in the 6 μ region for GG-Cu(II)H_2O are of the same type as for $(LA)_2Cu(II)$, where the existence of two OH groups is excluded by the analysis results, the IR-investigation strongly supports the view that the proton dissociation takes place from the peptide group. The proton dissociation is also essential for the interpretation of the resonance shift in the peptide group, as in a chelate where the nitrogen binds both a proton and a metal ion, the peptide group resonance is blocked and the carbonyl frequency (amide 1) shifted towards higher wavenumbers. This kind of behaviour has been observed by Penland *et al.*[19] for different metal chelates of urea. The evidence that the metal ion-peptide group interaction in Cu(II) chelates is characterized by the substitution of the amide hydrogen with a metal ion does not imply that the observed interaction of Ni(II) with the peptide group does involve the nitrogen atom. On the contrary, the conclusions from urea chelates when applied to dipeptide chelates indicate that in case the amide hydrogen is retained after chelate formation and the amide I frequency still shows a shift towards lower wavenumbers, as in $(GG)_2$-Ni (II), the interaction between the metal ion and the peptide group probably takes place through the oxygen of the peptide link.

Although it is not quite correct to apply results obtained with solid chelates to aqueous solutions, it is interesting to note that the Zn(II) ion, which activates several proteolytic enzymes, shows practically no tendency to interact with the peptide group. This means that if chelate formation with the peptide group of the substrate molecule with a resulting electron displacement is a necessary step in the mechanism of metal activated enzymes, the role of the metal ion must be considerably influenced by the protein molecule [20].

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