A Potentiometric and Spectroscopic Study of the Interaction of the N Terminal Tetrapeptide Fragment of Fibrinopeptide A with Cu^{II} and Ni^{II}†

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The syntheses are reported of the amino-terminal tetrapeptide fragment of human fibrinopeptide A (Ala-Asp-Ser-Gly) and derivative tetrapeptides with the β-carboxylate of the aspartate residue and the hydroxy group of the serine residue blocked to prevent co-ordination to metal ions. Complexes with hydrogen ion and copper(II), and in the case of Ala-Asp-Ser-Gly with nickel(II) also, have been studied by a combined approach of potentiometric and spectroscopic [absorption, circular dichroism, e.s.r. and, with nickel(II), ^1H n.m.r.] techniques. Stability constants of copper(II) and nickel(II) complexes of 'tetra-alanine' (Ala-Ala-Ala-Ala) have also been measured. The β-carboxylate group has been shown to co-ordinate strongly to Cu^{II} over the pH range 4—9 to give an unusually stable $[CuH_{-1}L]$ species (overall charge omitted). Fibrinopeptide A, which is present in locally high concentrations near the sites of bodily injury, would similarly be expected to co-ordinate strongly to Cu^{II}.

Human fibrinopeptide A is a peptide containing 16 amino acid sub-units, Ala-Asp-Ser-Gly-Glu-Gly-Asp-Phe-Leu-Ala-Glu-Gly-Gly-Gly-Val-Arg. Fibrinogen is a dimer, the two halves being held by disulphide bridges, and fibrinopeptides A and B, together with fibrin, are produced by the cleavage of fibrinogen by thrombin when there is some injury to the body. Once the fibrinopeptides have been released the fibrin residues form a clot by side-to-side and end-to-end aggregation.[1] Local concentrations of fibrinopeptides near the site of injury will be high and they would be competitive with albumin in interaction with metal ions, particularly Cu^{II}. Of the two fibrinopeptides, peptide A would be expected to co-ordinate most strongly to metal ions, having Asp as the second residue, hence fibrinopeptide A has been selected for this study. In peptides, histidine or cysteine residues are generally the most effective donor centres to Cu^{II} and these are absent from the chain of firbrinopeptide A, however the lateral carboxylate group of the aspartic acid residue in the second position could be an effective donor centre. To investigate the role of this side chain in complex formation we have undertaken the synthesis of the $-NH_2$ terminal tetrapeptide, and of analogous tetrapeptides in which the possible side-chain donor centres are blocked, and studied their metal complexes with Cu^{II} using potentiometric and spectroscopic techniques. For comparison stability constants of the Cu^{II} and Ni^{II} complexes of 'tetra-alanine' (Ala-Ala-Ala-Ala) have also been measured potentiometrically. The Ni^{II} complexes of Ala-Asp-Ser-Gly have been studied potentiometrically and by ^1H n.m.r. The only comparable peptide for which data are available is thymopoietin, the active centre of which is probably Arg-Lys-Asp-Val-Tyr.[2] This differs from the tetrapeptides studied, however, by having the Asp residue in the third position.

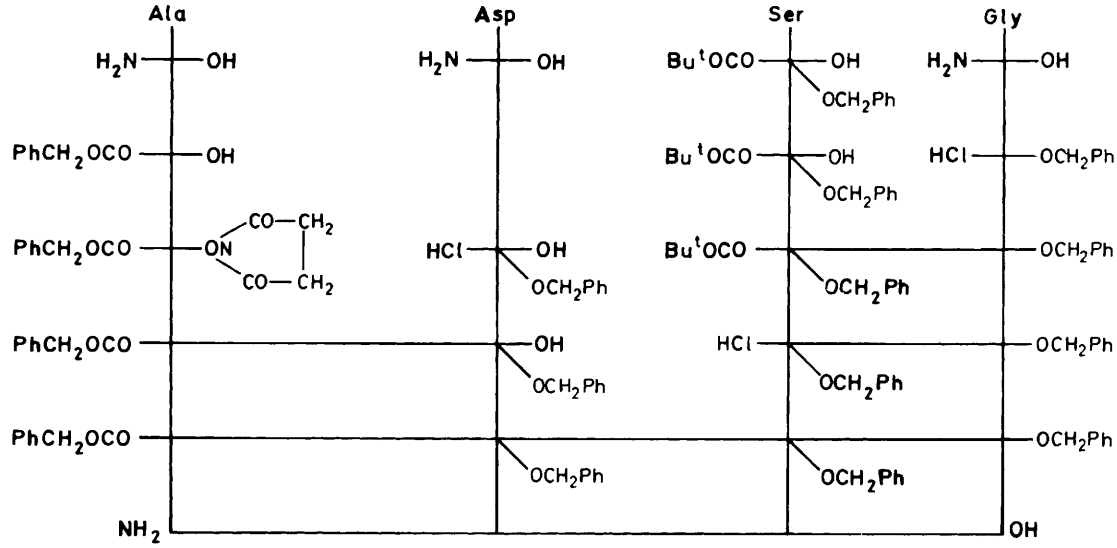
† Abbreviations used for the amino acid residues are those recommended by I.U.P.A.C. I.U.B. in 'Nomenclature and Symbolism for Amino Acids and Peptides,' *Pure Appl. Chem.*, 1984, **56**, 595.

Experimental

Peptide Synthesis.—The tetrapeptide Ala-Asp-Ser-Gly was synthesised by standard liquid-phase methods as outlined in the Scheme. The coupling reagents were dicyclohexylcarbodi-imide (Merck) and 1-hydroxybenzotriazole (Aldrich). The Bu^tOCO groups were cleaved by 4 mol dm^{-3} HCl in dioxane. The benzyloxycarbonyl and benzyl groups were removed by hydrogenolysis using 10% Pd/C in an acetic acid–water (90:10) mixture. The peptide was purified by gel filtration (Sephadex G15, eluant water). Sample purity was checked by paper chromatography [Whatman no. 1, eluant (% v/v): water (30), pyridine (35), and butanol (35)] and by high-performance liquid chromatography (h.p.l.c.) on bondapak C_{18} with an eluant gradient of water (with 0.05% trifluoroacetic acid)–methanol. Amino acid analysis gave the following ratios: aspartic acid (1.07), serine (0.91), glycine (1.02), alanine (1.01). The tetrapeptide Ala-Asp-SerOCH_2Ph-Gly was synthesised in the same way with the exception of the hydrogenolysis which was performed in methanol. Under these conditions the benzyl ether group of the serine residue was not cleaved. In the case of the tetrapeptide Ala-AspOBu^t-Ser-Gly, the β-carboxyl group of the aspartic acid residue was protected by the t-butyl group which is not cleaved by hydrogenolysis on Pd.

Spectroscopic Studies.—The metal-ion sources were $Cu(ClO_4)_2 \cdot 6H_2O$ and $Ni(ClO_4)_2 \cdot 6H_2O$ (Fluka) and solutions with molar ratios of metal:peptide of 1:1 were used with concentrations of 0.002—0.003 mol dm^{-3}. Absorption spectra were measured on a Cary 219 spectrometer and circular dichroism (c.d.) spectra on a Mark III Jobin-Yvon dichrographe in the 200—800 nm region. All c.d. results are expressed in terms of $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$. Electron spin resonance (e.s.r.) spectra were recorded on a Varian E102 spectrometer at liquid-nitrogen temperature, diphenylpicrylhydrazyl being used as a standard.

^1H N.m.r. spectra were recorded on a 400-MHz Bruker spectrometer with a peptide concentration of 0.01 mol dm^{-3} at



Scheme.

300 ± 2 K. Peptide:Ni^{II} ratios of 1:1.25 and 1:5 were studied. Analysis and simulation of the proton ABC spectra were carried out on a Hewlett-Packard 9826 computer.

Potentiometric Studies.—Stability constants for complexes with H^+ and Cu^{II} were calculated from titration curves carried out at 25 °C using total volumes of 1.5—2 cm^3. Alkali was added from a 0.1- or 0.25-cm^3 micrometer syringe which had been calibrated by both weight titration and by titration of standardized materials. Changes in pH were followed using a glass electrode calibrated in H^+ concentration with $HClO_4$.[3] All solutions were of ionic strength 0.10 mol dm^{-3} (KNO_3) and had peptide concentrations of 0.003 mol dm^{-3}. Calculations were made with the aid of the SUPERQUAD computer program.[4] This allows for the refinement of total ligand concentrations so that it is possible to confirm agreement between calculated values and those found from Gran plots.[5] It also confirmed the absence of acetate, a frequent impurity in peptide samples, or other co-ordinating ions. In all cases duplicate or triplicate titrations were carried out at Cu:ligand ratios of 1:1 and 1:2. The standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They give, however, a good indication of the importance of the particular species in the equilibrium.

Results and Discussion

(*i*) *Hydrogen Ion Complexes.*—Ala-Asp-Ser-Gly (H_2L) and Ala-Asp-SerOCH_2Ph-Gly (H_2L) have three protonation centres, the amino nitrogen and two carboxylate groups, while Ala-AspOBu^t-Ser-Gly (HL) and tetra-alanine (HL) have one carboxylate less.

Calculated protonation constants are given in Table 1. They are close to those expected from the structures of the tetrapeptides, with amine protonation in the region of pH 8 and carboxylate protonation in the pH region of 2.5—4.5. Amine protonation of Ala-Asp-Ser-Gly is unaffected by protection of the Ser hydroxyl group since this is far removed from the protonation centre. The terminal amino nitrogen (log β = 8.33) is, however, more basic than with 'tetraglycine' (Gly-Gly-Gly-Gly) (7.97) since it is stabilized by formation of an intramolecular hydrogen-bonded chelate ring between the terminal NH_3^+ group and the β-carboxylate group of the aspartate residue. In the ligand Ala-AspOBu^t-Ser-Gly this carboxylate is blocked so removing any possibility of internal hydrogen bonding. As expected, the protonation constant (log β = 7.90) is now very close to that for tetraglycine. Carboxylate protonation constants are as expected since replacement of the -OH proton of the serine residue by a benzyl group decreases the basicity of both carboxylate groups. As expected, the amino-nitrogen of tetra-alanine is a little more basic than with tetraglycine as a result of the inductive effect of the methyl group. This effect is even more marked in the carboxyl protonation constants, and is presumably influenced by the conformation of the tetrapeptide containing three chiral amino acid residues.

(*ii*) *Metal Ion Complexes.*—All three fibrinopeptide-based tetrapeptides form series of complexes with Cu^{II} comparable in stoicheiometry but very different in stability to those formed by tetraglycine[6] or tetra-alanine. Metal complex formation constants calculated from the potentiometric studies are given in Table 1, together with those for tetraglycine and for Arg-Lys-Asp-Val-Tyr.[2] The spectroscopic data are given in Table 2.

Tetra-alanine behaves very similarly to tetraglycine in its complexation reactions with Cu^{II} and Ni^{II}. With Cu^{II} the species distribution curves show reasonable agreement although the complexes $[CuH_{-1}L]$ and $[CuH_{-2}L]$ (overall charges omitted throughout for clarity) with tetra-alanine are of comparable importance while with tetraglycine the latter is significantly more important. This is probably a result of steric interference between the methyl side-chains of neighbouring Ala residues of the same chirality in the $[CuH_{-2}L]$ complexes with tetra-alanine. Such interference would be absent in the $[CuH_{-1}L]$ species and obviously cannot be present with tetraglycine.

With the three tetrapeptides based on fibrinopeptide A the first complex to form with Cu^{II} at low pH is the species [CuL]. As the pH is raised successive deprotonations take place giving the species $[CuH_{-1}L]$, $[CuH_{-2}L]$, and $[CuH_{-3}L]$ respectively in common with other tetrapeptides. However these species differ significantly in stability, and hence in pH ranges of existence, depending on the substituents present in the ligand molecule. In all cases there was good agreement between the ranges of existence predicted from the potentiometric results and those detected spectroscopically. This is shown clearly in Figure 1(*a*) and (*b*) in which the energies of absorption maxima for the *d*–*d* transitions are plotted, together with the species distribution

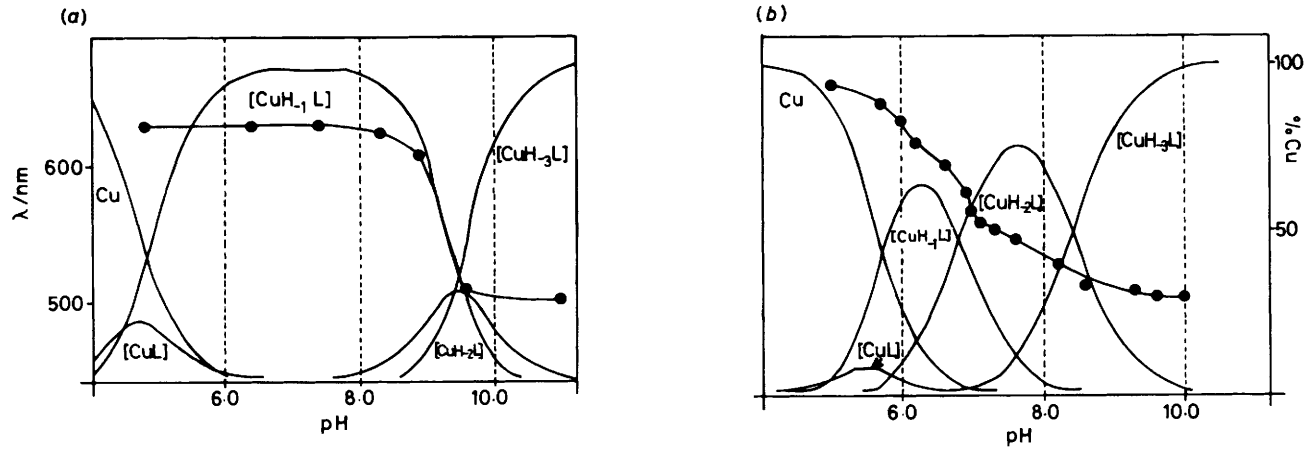


Figure 1. Species distribution curves and absorption maxima (*d*—*d* transition) for 1:1 mixtures of Cu^{2+} with (*a*) Ala-Asp-Ser-Gly and (*b*) Ala-AspOBu^t-Ser-Gly (0.001 mol dm^{-3})

Table 1. Formation constants for H^+, Cu^{2+}, and Ni^{2+} complexes at 25 °C and I = 0.10 mol dm^{-3} (KNO_3), with standard deviations on the last figure given in parentheses

(*a*) H^+ complexes

Ligand	log values β_{HL}	β_{H_2L}	β_{H_3L}	K_{H_2L}	K_{H_3L}
Ala-Asp-Ser-Gly	8.33(1)	12.35(2)	15.56(2)	4.02	3.21
Ala-AspOBu^t-Ser-Gly	7.90(1)	11.28(1)		3.38	
Ala-Asp-SerOCH$_2$Ph-Gly	8.33(1)	12.03(1)	14.53(3)	3.70	2.51
Arg-Lys-Asp-Val-Tyr[a]	7.20	10.45	12.37	3.25	1.92
Tetra-alanine	8.13(1)	11.65(1)		3.52	
Tetraglycine[b]	7.97	11.15		3.18	
SerOCH$_2$Ph	9.03(1)	10.96(1)		1.93	
Ser[c]	9.22	11.46		2.24	

(*b*) Cu^{2+} complexes

Ligand	$\beta_{[CuL]}$	$\beta_{[CuH_{-1}L]}$	$\beta_{[CuH_{-2}L]}$	$\beta_{[CuH_{-3}L]}$	$\beta_{[CuL_2]}$
Ala-Asp-Ser-Gly	6.63(2)	2.16(1)	−7.41(2)	−16.77(1)	
Stepwise protonation constants		4.47	9.57	9.36	
Ala-AspOBu^t-Ser-Gly	4.78(9)	−0.19(1)	−6.99(2)	−15.40(2)	
Stepwise protonation constants		4.97	6.80	8.41	
Ala-Asp-SerOCH$_2$Ph-Gly	6.18(3)	2.28(2)	−6.39(4)	−16.2(1)	
Stepwise protonation constants		3.90	8.67	9.8	
Arg-Lys-Asp-Val-Tyr[a]	7.68	−3.42	−11.10	−21.13	
Tetra-alanine	4.77(5)	−0.45(1)	−8.09(1)	−17.33(2)	
Stepwise protonation constants		5.22	7.64	9.24	
Tetraglycine[b]	5.08	−0.42	−7.31	−16.60	
SerOCH$_2$Ph	7.47(4)				14.45(3)
Ser[c]	7.86				14.43

(*c*) Ni^{2+} complexes

Ligand	$\beta_{[NiL]}$	$\beta_{[NiH_{-1}L]}$	$\beta_{[NiH_{-3}L]}$
Ala-Asp-Ser-Gly	3.66(9)	−3.92(4)	−20.24(4)
Stepwise protonation constants		7.78	16.32
Tetra-alanine	3.06(2)	−4.97(2)	−21.29(2)
Stepwise protonation constants		8.03	16.32
Tetraglycine[d]	3.65	−4.45	−20.90

[a] Ref. 2; the ligand (assumed H_2L) contains two additional ionizable protons from the terminal NH_3^+- of Lys and the phenolic -OH of Tyr. For the sake of comparison, these have been ignored when calculating the above constants. [b] Ref. 6. [c] Ref. 11. [d] R. B. Martin, M. Chamberlin, and J. T. Edsall, *J. Am. Chem. Soc.*, 1960, **82**, 495.

curves, as a function of pH. Hence in the following discussion evidence from both experimental approaches is combined to ascertain the structures of the various complexed species, and explain their stabilities.

A comparison of the species distribution curves for 1:1 mixtures of Ala-Asp-Ser-Gly and Ala-AspOBu^t-Ser-Gly with Cu^{II} is shown in Figure 2. The species [CuL] are never major components of the equilibrium mixture, existing in the pH range

Table 2. Spectroscopic data (G = 10 ^{4}T) for Cu^{2+} complexes

Ligand	Species	Visible λ/nm[a]	C.d. λ/nm (Δε)	E.s.r. $g_{\parallel}$	E.s.r. $A_{\parallel}$ G
Ala-Asp-Ser-Gly (H_2L)	$[CuH_{1}L]$	630 (83)	615 (+0.07)[b]	2.26	185
			300 (−0.75)[c]		
			262 (+0.60)[d]		
	$[CuH_{3}L]$	506 (150)	522 (−0.9)[b]	2.18	210
			298 (+0.75)[c]		
			270 (−0.50)[d]		
Ala-AspOBu^t-Ser-Gly (HL)	$[CuH_{1}L]$	648 (52)	642 (−0.19)[b]	2.23	175
			310 (+0.08)[c]		
			268 (−0.23)[d]		
	$[CuH_{2}L]$	580 (104)	566 (−0.44)[b]	2.19	195
			310 (+0.47)[c]		
			274 (−0.34)[d]		
	$[CuH_{3}L]$	510 (147)	522 (−0.85)[b]	2.16	210
			302 (+0.39)[c]		
Ala-Asp-SerOCH$_2$Ph-Gly (H_2L)			268 (−1.88)[d]		
	$[CuH_{-1}L]$	635 (84)	618 (+0.09)[b]	2.245	192
			298 (−0.65)[c]		
			264 (+0.58)[d]		
	$[CuH_{-2}L]$	625 (130)	590 (−0.22)[b]		
			298 (−0.4)[c]		
			259 (+1.6)[d]		
	$[CuH_{-3}L]$	510 (180)	520 (−1.1)[b]	2.179	210
			300 (+0.70)[c]		
			272 (−0.34)[d]		

[a] Approximate absorption coefficients in parentheses (ε/dm^3 mol^{-1} cm^{-1}). [b] $B + E$ (d–d transition). [c] N^-–Cu (charge-transfer transition). [d] NH_2–Cu (charge-transfer transition).

4—6 with total concentrations always less than 20%, hence they could not be detected reliably in the spectroscopic studies. In common with other peptide complexes, they would be expected to bond through the terminal NH_2– group and the neighbouring carbonyl oxygen of the first peptide linkage. Although only a minor component in the solution equilibrium, the [CuL] complex with Ala-Asp-Ser-Gly is significantly more stable than with tetra-alanine (Δ log β = 1.9 log units) or the blocked Ala-AspOBu^t-Ser-Gly (1.9). These results suggest significant co-ordination through the Asp carboxylate group.

With Ala-Asp-Ser-Gly $[CuH_{-1}L]$ is the dominant species over the pH range 5—9 as shown in Figure 1(*a*). The complex species is also even more stable than with tetra-alanine (Δ log β = 2.6) or the Bu^t-blocked analogue (2.4). However it is of comparable stability to the Ser-blocked analogue, Ala-Asp-Ser OCH$_2$Ph-Gly (Δ log β = −0.1). When the species $[CuH_{-1}L]$ does eventually ionize to give $[CuH_{-2}L]$, this has a stability much closer to that of tetra-alanine (Δ log β = 0.7). The same is true of the $[CuH_{-3}L]$ species also (Δ log β = 0.6). These trends are shown clearly in the species distribution curves in Figure 2.

It is seen from Figure 1(*a*) that, over the pH range 5—8.5, the *d*–*d* transition has an absorption maximum of 630 nm, marginally lower than that normally expected for a 2N species (around 650 nm).[7] Over the pH range 8.5—9.5 the energy of the *d*–*d* band increases rapidly to reach a value of 506 nm which is characteristic of a 4N species.[8] The low importance of the 3N species with Ala-Asp-Ser-Gly ($[CuH_{-2}L]$) is confirmed by the species distribution curves in Figure 2. In the c.d. spectra, a positive Cotton effect in the *d*–*d* transition is observed at 615 nm over the pH range 5.8. Above pH 9 this becomes negative with a maximum at 580 nm and a shoulder at *ca.* 520 nm, characteristic of the simultaneous formation of 3N and 4N species. The bands in the charge-transfer region at 300 and 270 nm correspond to N^-–Cu and NH_2–Cu transitions respectively.[6] The e.s.r. spectrum at low pH corresponds to a 2N species and, as the pH is raised above 8.5, the spectrum changes first to a mixture of 3N and 4N species and finally gives values characteristic of 4N species alone.

Combination of the potentiometric and spectroscopic results demonstrates clearly that the $[CuH_{1}L]$ complex of Ala-Asp-Ser-Gly is much more stable than with tetra-alanine, is present over a wide range of pH, and is a 2N species. This can be completely explained by assuming co-ordination of the β-carboxylate group of the Asp residue to Cu^{II}. The relatively high value of the *d*–*d* transition energy for the 2N species and the change in sign of the Cotton effect between 2N and 3N species suggests that the carboxylate is bound in an equatorial position as shown in Figure 3, and that this bond is broken on formation of $[CuH_{-2}L]$. Stabilization of the $[CuH_{-1}L]$ species of Ala-Asp-Ser-Gly as compared to that of tetra-alanine (Δ log β = 2.6) would be expected to be greater than with the [CuL] species (1.9) since the chelate ring is two atoms larger in the latter complexes (see Figure 2).

With Ni^{II} the complexes [NiL], $[NiH_{-1}L]$, and $[NiH_{-3}L]$ are formed, with no significant concentration of $[NiH_{-2}L]$. As expected, results for tetra-alanine give comparable species distribution curves to those for tetraglycine. With Ala-Asp-Ser-Gly absorption and c.d. spectra confirmed that below pH 8 octahedral complexes are formed ([NiL] and $[NiH_{-1}L]$) while above pH 8 a planar complex ($[NiH_{-3}L]$) is present with a characteristic *d*–*d* transition at 410 nm. In the c.d. spectrum there is a negative Cotton effect at 460 nm (Δε = −1.75) with a shoulder at 410 nm. The c.d. spectrum also shows a strong charge-transfer transition (N^-–Ni) at 265 nm (Δε = +4.8) confirming amide deprotonation. Stability constants are a little larger than those found for tetra-alanine and gave the species

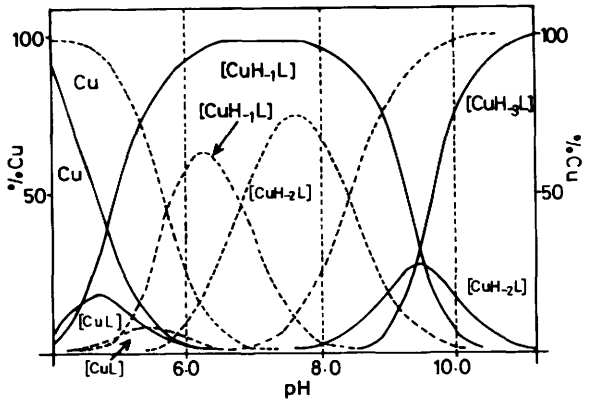


Figure 2. Comparison of the species distribution curves for 1 : 1 mixtures of Cu^{2+} with Ala-Asp-Ser-Gly (——) and Ala-AspOBu^t-Ser-Gly (– – –) (0.001 mol dm^{-3})

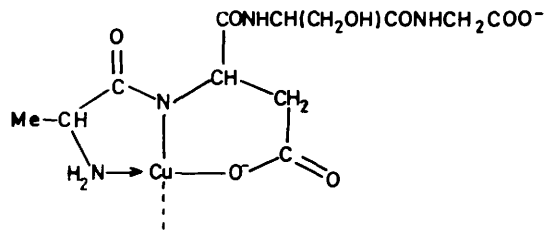


Figure 3. Bonding centres in the [CuH$_{-1}$L] complex of Ala-Asp-Ser-Gly

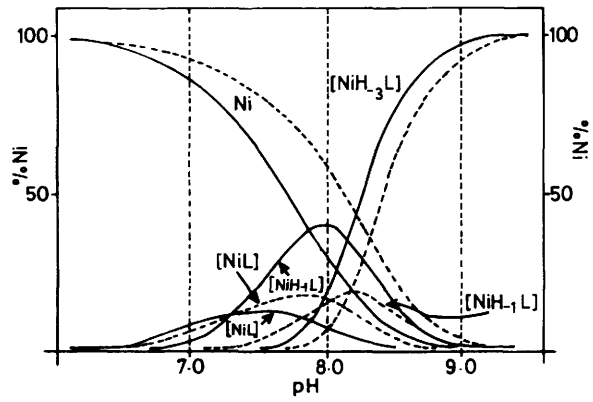


Figure 4. Comparison of the species distribution curves for 1 : 1 mixtures of Ni^{2+} with Ala-Asp-Ser-Gly (——) and tetra-alanine (– – –) (0.001 mol dm^{-3})

distribution curves shown in Figure 4. The major difference is the greater importance of the complex [NiH$_{-1}$L] with Ala-Asp-Ser-Gly, an effect comparable to the difference in analogous complexes with Cu^{II}, although rather smaller in magnitude. This suggests the presence of chelation to Ni^{II} through the β-carboxylate side chain, as has been found with Cu^{II}. The range of existence of this species (possibly six-co-ordinate) is limited by the formation of the stable, diamagnetic [NiH$_{-3}$L] complex around pH 8.

The n.m.r. parameters for the diamagnetic planar complex [NiH$_{-3}$L] of Ala-Asp-Ser-Gly are given in Table 3. Assignment of the protons for the free tetrapeptide was achieved by pH titration and for the complex by selective irradiation. The variation in chemical shift between the free peptide and the complex indicates clearly the involvement of the four residues in nickel co-ordination. The large variations in the chemical shifts

Table 3. ^{1}H N.m.r. chemical shifts* and coupling constants (Hz) for the free peptides and the [NiH$_{-3}$L] complex (pH 11.5) of Ala-Asp-Ser-Gly

	Free peptide	Complex	$-\Delta\delta$
Ala(1)			
$C_\alpha H$	3.544	3.278	0.266
$-CH_3$	1.277	1.237	0.040
J_{AB}	6.96	6.96	
Asp(2)			
H_A	2.656	2.512	0.144
H_B	2.754	2.690	0.064
H_C	4.658	3.777	0.881
J_{AB}	15.86	13.92	
J_{AC}	8.07	3.07	
J_{BC}	5.52	6.06	
Ser(3)			
H_A	3.877	3.587	0.290
H_B	3.938	3.668	0.270
H_C	4.478	4.136	0.342
J_{AB}	11.78	11.51	
J_{AC}	4.87	11.66	
J_{BC}	5.17	2.79	
Gly(4)			
H_A	3.766	2.931	0.835
H_B	3.821	3.513	0.308
J_{AB}	17.40	17.13	

* Chemical shifts (δ) are given from $SiMe_4$ with HDO at 4.8 p.p.m.

of the $C_\alpha H$ protons indicates that the N atoms are the binding sites. The two α-protons of the Gly residue, which give an AB spectrum in the free ligand, become significantly inequivalent in the planar complex becoming an AX system. This suggests that the unco-ordinated terminal carboxylate might interact in some way with another side chain, *e.g.* that of the Ser residue. The calculation of rotamer populations, defined in Figure 5 and given in Table 4, shows major changes in the side-chain conformations of the Asp and Ser residues on co-ordination to Ni^{II}. Upon co-ordination there is very large stabilization of rotamer III of the Asp residue while the population of rotamer III of the Ser side chain decreases considerably. The side-chains of both residues usually favour the pseudo-axial position (rotamer III) in their planar complexes.[9,10] In the complexes studied steric effects could prevent the same conformation of the Ser and Asp side-chains and, in addition, the –OH group of Ser could be involved in hydrogen bonding to an oxygen atom of the vicinal carbonyl or carboxyl groups.

The comparisons shown in Figures 1 and 2 demonstrate clearly the difference in behaviour between Ala-Asp-Ser-Gly and an analogous tetrapeptide with the Asp carboxylate blocked. In the absorption spectrum of the blocked peptide there is an almost smooth increase in energy of the *d–d* transition as the pH is raised, showing step-by-step deprotonation of the second and third peptide nitrogens to form 3N and 4N complexes. In the c.d. spectra the *d–d* transition energy for the 2N species has a value more characteristic of 2N complexes, and the sign of the Cotton effect is negative while it is positive for Ala-Asp-Ser-Gly itself. The e.s.r. spectra of the Asp blocked tetrapeptide also show values for $g_{\|}$ and $A_{\|}$ typical of those for 2N, 3N, and 4N complexes.[11]

In addition Ala-Asp-Ser-Gly has a potential donor centre in the Ser residue, although the study of amino acid complexes with Cu^{II} suggests that there is no interaction with the –OH group of serine.[12] A blocked analogue, Ala-Asp-SerOCH$_2$Ph-

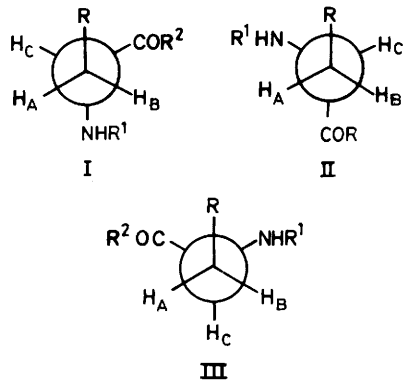


Figure 5. Notation for the rotamers of Asp (R = COO^-, R^1 = Ala, R^2 = Ser-Gly) and Ser (R = OH, R^1 = Asp-Ala, R^2 = Gly) side-chains of Ala-Asp-Ser-Gly

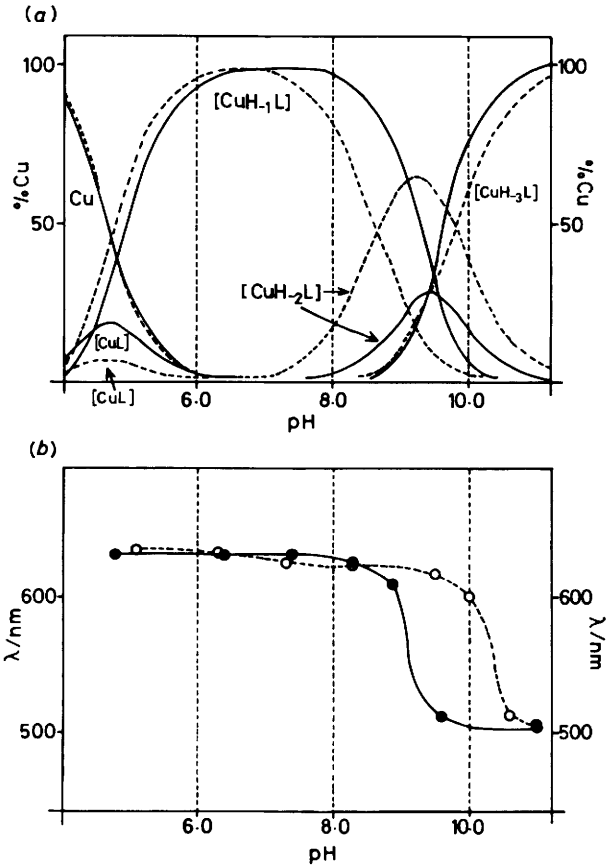


Figure 6. Comparison of (*a*) the species distribution curves and (*b*) the absorption maxima of 1:1 mixtures of Cu^{2+} and Ala-Asp-Ser-Gly (——) and Ala-Asp-SerOCH$_2$Ph-Gly (– – –) (0.001 mol dm^{-3})

Gly, was therefore studied to confirm the absence of interaction between Cu^{II} and the –OH side-chain of Ser. The species distribution curves calculated from the metal complex formation constants were almost completely superimposable up to pH 8, and the spectroscopic results showed a similar wide range of stability for the [CuH$_{-1}$L] species. Above pH 8 differences are noticed in both the species distribution curves and the spectroscopic results, the species [CuH$_{-2}$L] (a 3N species) having a wider range of existence as demonstrated in Figure 6. This is probably a result of hydrophobic interactions of the blocking benzyl group which stabilize the 3N complex. As an additional check on this point formation constants for Cu^{II} complexes of SerOCH$_2$Ph itself were measured for comparison with those found earlier for serine.[12] No significant difference was found.

Table 4. Rotamer population for Ala and Ser side-chains in the free peptide and Ni^{II} complexes of Ala-Asp-Ser-Gly

	P_I	P_{II}	P_{III}
(*a*) Asp residue			
Free peptide	0.27	0.50	0.23
Complex	0.32	0.04	0.64
(*b*) Ser residue			
Free peptide	0.24	0.21	0.55
Complex	0.02	0.82	0.16

From the above studies it is probable that fibrinopeptide A shows a significantly higher affinity for Cu^{II} than would most simpler peptides over the physiological pH range. Hence, since there is a high concentration of the peptide near the sites of bodily injury, it is possible that co-ordination to Cu^{II} could have biological implications. By analogy with Ala-Asp-Ser-Gly, the major complex at biological pH would be the species [CuH$_{-1}$L] (*cf.* Figure 3) where the peptide is locked in a partially coiled, and possibly biologically active, conformation. Since copper is known to have anti-inflammatory activity,[13] it is interesting to speculate whether a complex similar to that described is involved.

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References

1 B. Blomback and L. A. Hanson, 'Plasma Proteins,' Wiley, New York, 1979.
2 I. Sovago, T. Kiss, and A. Gergely, *Inorg. Chim. Acta*, 1984, **93**, L53.
3 H. M. Irving, M. G. Miles, and L. D. Pettit, *Anal. Chim. Acta*, 1967, **38**, 475.
4 P. Gans, A. Sabatini, and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1196.
5 G. Gran, *Analyst (London)*, 1952, **77**, 661.
6 H. Sigel and R. B. Martin, *Chem. Rev.*, 1982, **82**, 385.
7 E. J. Billo, *J. Inorg. Nucl. Chem. Lett.*, 1974, **10**, 613.
8 J. M. Tsangaris, J. W. Cheng, and R. B. Martin, *J. Am. Chem. Soc.*, 1969, **91**, 726.
9 H. Kozlowski and Z. Siatecki, *Chem. Phys. Lett.*, 1978, **54**, 498.
10 H. Kozlowski and B. Jezowska-Trzebiatowska, *Chem. Phys. Lett.*, 1976, **42**, 246.
11 G. Formicka-Kozlowska, H. Kozlowski, I. Z. Siemion, K. Sobczyk, and E. Nawrocka, *J. Inorg. Biochem.*, 1981, **15**, 201.
12 L. D. Pettit and J. L. M. Swash, *J. Chem. Soc., Dalton Trans.*, 1976, 2417.
13 J. R. J. Sorenson, *Chem. Br.*, 1984, **20**, 1110; 'Inflammatory Diseases and Copper,' ed. J. R. J. Sorenson, Humana Press, New Jersey, 1982.

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