The Interaction of Cu(II) with Phe–Phe–Ser–Asp–Lys and other Peptides Containing the Phe–Phe Sub-unit

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(Received April 12, 1989; revised June 2, 1989)

Abstract

The synthesis of Phe–Phe–Ser–Asp–Lys is reported together with the results of a potentiometric and spectroscopic (ESR, CD and UV–Vis) study of its complexes with H^+ and Cu^{2+}, together with complexes of Phe–Phe and Phe–Phe–Phe. Evidence is presented for axial coordination of the β-carboxyl group of the Asp residue.

Introduction

Phenylalanine, Phe, coordinates to metal ions able to form square planar complexes (e.g. Cu(II) and Pd(II)) through the expected amino-N and carboxylate-O donors. The initial complex formed with peptides in which the N-terminal is Phe is similar with N,O coordination to the terminal-N and carbonyl-O of the neighbouring peptide bond. In addition to the expected coordination, however, there appears to be significant interaction between the aromatic ring of Phe and the metal ions, which affects a number of properties. For example, the ratio of the stepwise stability constants for the bis and mono phenylalanato–Cu(II) complexes is larger than expected [1] and ternary amino acid–Cu(II) complexes are stabilized when one ligand is aromatic and the other aliphatic [2]. In addition there is significant stereoselectivity in the formation of ternary complexes of L-His and D/L Phe [3]. Evidence for metal ion–aromatic ring interaction also comes from ESR and spectroscopic studies of Cu(II) complexes [4] and NMR studies of Pd(II) complexes [5] and from detailed studies of Cu(II) complexes of peptides using potentiometric and spectroscopic techniques [6, 7] and calorimetry [8]. There have been no detailed studies of the coordination of peptides containing the residues Phe–Phe as neighbouring amino acid sub-units, partly as a result of the low solubility of Phe–Phe at intermediate pH, but it is present in a number of biologically important peptides such as Substance P (Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2). The $-CO_2^-$ group of Asp bonds strongly to Cu(II) when Asp is one of the first three residues of a peptide chain [9–11]. In the fourth position it would not have an opportunity to coordinate effectively until the Cu(II) ion had deprotonated the 3 peptide-N atoms between the N-terminal and the Asp side chain to form a 4N complex. Under these circumstances the carboxyl group could only coordinate axially, when the bonding would be expected to be less significant. We wish to report the synthesis of Phe–Phe–Ser–Asp–Lys (H_2L) and the results of a study of its Cu(II) complexes, together with those formed with Phe–Phe and Phe–Phe–Phe. The pentapeptide is of interest because it is the 17–21 fragment of γ-bovine casein. It also contains Asp in the fourth position and Lys in the fifth. The –OH group of Ser would not be expected to coordinate and the ϵ-amino group of Lys is generally too far removed from the peptide backbone to coordinate, even when the neighbouring peptide-N atoms are deprotonated (as in Arg–Lys–Asp–Val–Tyr [11] and Gly–His–Lys [12]) so bonding through this centre, particularly when the Lys residue is removed from other coordination centres, is not to be expected unless encouraged by the conformation of the backbone [13].

Experimental

Synthesis

Phe–Phe–Ser–Asp–Lys was synthesized by solid phase peptide synthesis techniques. The α-amino groups were protected using the t-butoxycarbonyl group while the ϵ-amino group of lysine was protected with the benzyloxycarbonyl group. The lateral carboxyl group Asp and the hydroxy group Ser were protected with the benzyl group. Cleavage from the resin was carried out using HF gas.

0020-1693/89/$3.50

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The peptide was purified by gel filtration (Sephadex G10) and subsequently by HPLC on bondapak C_{18} with an eluent gradient of water (with 0.05% trifluoracetic acid)–methanol. Analysis of amino acids gave the following results: Phe 1.97; Ser 0.72; Asp 0.98; Lys 1.05 (results for Ser are always low as a result of oxidation). Purity was further confirmed by the pH-metric titrations.

Samples of Phe–Phe and Phe–Phe–Phe were purchased from the Sigma Chemical Co. and the purity checked by titration.

Potentiometric Studies

Stability constants for H^+ and Cu^{2+} complexes were calculated from titration curves carried out at 25 °C using total volumes of 1.5 cm^3. Alkali was added from a 0.1 cm^3 micrometer syringe which had been calibrated by both weight titration and the titration of standardized materials. Changes in pH were followed using a glass electrode calibrated in H^+ concentrations with $HClO_4$. All solutions were of ionic strength 0.10 mol dm^{-3} (KNO_3) and peptide concentrations of 0.003 mol dm^{-3}. Calculations were made with the aid of the SUPERQUAD computer program [14]. 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.

Spectroscopic Studies

Complexes with Phe–Phe–Ser–Asp–Lys were also studied spectroscopically. Solutions were of the same concentrations as those used in the potentiometric studies. Absorption spectra were recorded on a Cary 19 spectrometer and circular dichroism (CD) spectra were measured on a Mark III Jobin-Yvon dichrographe in the 200–800 nm region. All CD spectra are expressed in terms of $\Delta\epsilon(\epsilon_l - \epsilon_r)$. Electron spin resonance (ESR) spectra were obtained on a Varian E102 spectrometer at liquid nitrogen temperatures, diphenylpicrylhydrazyl being used as a standard.

Results and Discussion

The completely deprotonated free ligand anion of Phe–Phe–Ser–Asp–Lys, L^{2-}, can coordinate

TABLE 1. Stability constants for H^+ and Cu^{2+} complexes at 25 °C and I = 0.10 mol dm^{-3} (KNO_3)

	log β values				
	011	012	013	014	
H^+ complexes					
Phe–Phe	7.27(1)	10.53(1)			
Phe–Phe–Phe	7.13(1)	10.65(2)			
Phe–Phe–Ser–Asp–Lys	10.05(1)	17.23(1)	21.20(2)	23.98(2)	
Stepwise constants	Lys-ϵ-NH_2	α-NH_2	carboxyl-O-	carboxyl-O-	
Phe–Phe		7.27	3.26		
Phe–Phe–Phe		7.13	3.52		
Phe–Phe–Ser–Asp–Lys	10.05	7.18	3.97	2.78	
	110	11-1	11-2	11-3	11-4
Cu^{2+} complexes					
Phe–Phe[a]	5.31(7)	1.75(1)	−7.49(1)		
Phe–Phe–Phe		−0.31(1)	−6.17(1)	−16.79(1)	
Phe–Phe–Ser–Asp–Lys[b]	5.08(2)	−0.19(1)	−6.65(1)	−14.35(1)	−24.16(2)
Gly–Gly[c]	5.55	1.56	−7.74		
Gly–Gly–Gly[c]	5.24	0.02	−6.58	−18.5	
Gly–Gly–Gly–Gly[c]	5.08	−0.42	−7.31	−16.6	
Stepwise constants					
Phe–Phe	5.31	3.56	9.2		
Phe–Phe–Phe			5.86	10.6	
Phe–Phe–Ser–Asp–Lys	5.08	5.27	6.46	7.70	9.8
Gly–Gly	5.55	3.99	9.3		
Gly–Gly–Gly	5.24	5.22	6.60	11.9	
Gly–Gly–Gly–Gly	5.08	5.50	6.89	9.3	

[a]log $\beta_{12\text{-}1}$ = 4.48(8). [b]In Cu(II) complexes the free ligand (L^-) for Phe–Phe–Ser–Asp–Lys is defined as the ligand with the ϵ-amino-N of the Lys residue protonated. [c]Ref. 15.

four hydrogen ions, two on nitrogen atoms (the terminal α-NH_2 and the lateral ϵ-NH_2 of the Lys side chain) and one on each of the two carboxylate groups. Hydrogen ion complex stability constants for the three peptides studied are given in Table 1. The stepwise constants of Phe–Phe–Ser–Asp–Lys represented by $\log K_1$ and $\log K_2$ will correspond to protonation of the ϵ-NH_2 and α-NH_2 nitrogens respectively while $\log K_3$ and $\log K_4$ will be the macro constants for protonation of the carboxylate groups since the micro constants would be expected to be very close in value.

Stability constants of Cu(II) complexes are also given in Table 1 and species distribution curves for 1:1 mixtures of Cu(II) with Phe–Phe–Ser–Asp–Lys are shown in Fig. 1. Spectroscopic data for complexes with Phe–Phe–Ser–Asp–Lys are presented in Table 2. The lateral amino group of the Lys residue is generally protonated up to pH 10 (see Table 1) and, assuming no interaction between this ϵ-amino-N and Cu(II), its protonation constant should be neglected when making direct comparison with results for tetraglycine. This can be best done by redefining the 'free ligand' (L^-) as the ligand with the Lys ϵ-amino group protonated. This has been done when compiling Table 1 so that the CuL complex would now be coordinated through the N-terminal nitrogen and neighbouring carbonyl oxygen (a 1N species directly comparable to the 1N species of tetraglycine), $CuH_{-1}L$ would be a 2N species (terminal-N and first peptide-N coordination) $CuH_{-2}L$ a 3N species and $CuH_{-3}L$ a 4N species. Eventual deprotonation of the Lys amino-N would give a $CuH_{-4}L$ complex. The spectroscopic data given in Table 2 show that absorption, CD and ESR spectra all support these assignments although the absorption maximum for the 4N complex is a little higher than normally expected (see below).

Comparison of constants for Phe–Phe–Ser–Asp–Lys with those for tetraglycine shows comparatively close agreement for the 1N and 2N species (CuL and $CuH_{-1}L$) but the 3N ($CuH_{-2}L$), and particularly the 4N ($CuH_{-3}L$), complexes of Phe–Phe–Ser–Asp–Lys are more stable by a factor of up to 2 log units. The similarities in stability of the 1N and 2N complexes suggests that the thermodynamic effects of aromatic ring interactions between the first two Phe residues is little affected by coordination. This is supported by the results for Phe–Phe and Phe–Phe–Phe, which again resemble their Gly analogues. Their complexes are a little less stable as would be expected from normal electronic effects. It can therefore be assumed that coordination has little effect on ring 'stacking' between neighbouring phenyl rings. As expected the results show no evidence for coordination of the Ser–OH group.

The relatively high stability of the 4N complex is the most marked difference with tetraglycine.

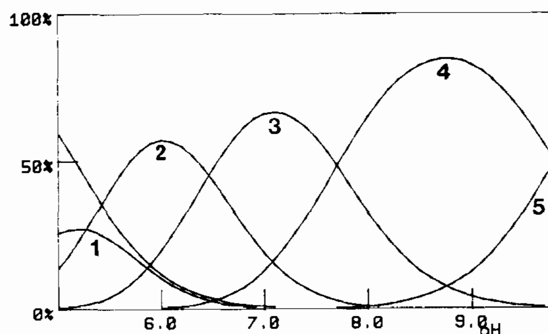

Fig. 1. Species distribution curves for 1:1 mixtures of Cu(II) with Phe–Phe–Ser–Asp–Lys (0.001 mol dm^{-3}): 1 = CuL, 2 = $CuH_{-1}L$, 3 = $CuH_{-2}L$, 4 = $CuH_{-3}L$, 5 = $CuH_{-4}L$.

TABLE 2. Spectroscopic data for complexes of Phe–Phe–Ser–Asp–Lys with Cu(II)

Species	Absorption spectra λ_{max} (nm)[a]	CD spectra λ_{max} (nm) ($\Delta\epsilon$)	ESR spectra $g_\parallel$	$A_\parallel$ (G)
CuL			2.31	150
$CuH_{-1}L$	670(80)	660(−0.32)[b] 328(+0.29)[c] 272(−1.26)[d] 220(+7.3)[e]		
$CuH_{-2}L$	610(125)	603(−0.35)[b] 322(+0.34)[c] 276(−1.56)[d] 220(9.6)[e]	2.22	170
$CuH_{-3}L$	572(160)	540(−0.95)[b] 316(+0.40)[c] 272(−2.30)[d] 218(+19.8)[e]	2.18	200

[a]Approx. absorption coefficients in parentheses (ϵ (dm^3 mol^{-1} cm^{-1})). [b]B + E (d–d transitions). [c]N^-–Cu(II) charge transfer transition. [d]NH_2–Cu(II) c.t. transition + ^1L_b aromatic ring transition. [e]Intraligand transition.

This could be a result of axial coordination from either the β-carboxyl group of the Asp residue or the Lys amino group. The amino group is far removed from other coordination centres and previous studies have shown no evidence of coordination [11, 12]. The Asp–carboxyl group, however, can coordinate effectively [9–11]. In the 4N complex of Phe–Phe–Ser–Asp–Lys this coordination would almost certainly be axial and axial coordination has been shown to produce a 'red shift' in the absorption maximum [15]. The calculated value for λ_{max} in a 4N tetrapeptide complex is 512 nm [15] and the typical range of values found is 500–525 nm. With Phe–Phe–Ser–Asp–Lys the value is 572 nm from pH 8 upwards, a 'red shift' of 60 nm, almost identical to that expected for apical

carboxylate coordination [16]. The $CuH_{-4}L$ complex would be of similar structure to the $CuH_{-3}L$ species but with the ϵ-amino group of the Lys residue deprotonated. The constant for protonation of the $CuH_{-4}L$ complex ($\log K = -4.30 - (-14.11) = 9.81$) is close to the protonation constant β_{HL}, supporting this conclusion. This similarity would not be found if ϵ-amino (Lys) bonding to Cu(II) was significant, so supporting the suggestion that the enhanced stability of the $CuH_{-4}L$ complex is a result of β-carboxyl (Asp) rather than ϵ-amino (Lys) coordination.

Acknowledgement

We would like to thank Mrs M. Baquet for purifying the sample of Phe–Phe–Ser–Asp–Lys by HPLC.

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