Binding Ability of Cu^{2+} Ions by Opiate-Like Fragments of Bovine Casein

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Abstract

The coordination modes of Cu(II) to α-casein (90–95) and α-casein (90–96) peptides with opioid activity isolated from pepsin hydrolisates of α-casein were investigated by means of electron paramagnetic resonance, absorption, and circular dichroism spectroscopy and potentiometry. The results allow the identification of the complex species involved and the attribution of the spectral data set to the various complex structures. According to the spectroscopic data, a phenolate side-chain of Tyr residue belonging to the Gly–Tyr–Leu or Gly–Tyr–Leu–Gln fragment of the peptides is involved in the metal coordination in a complex which is a minor species at neutral pH range. *Journal of Inorganic Biochemistry 66, 19–22 (1997)* ***© 1997 Elsevier Science Inc.***

Introduction

A number of peptide hormone-like substances have been isolated from food [1]. It has been suggested that these "food hormones" (formones) may act on gut luminal receptors as exogenous regulators of gastrointestinal motility and hormone release [2]. It has been shown that peptic digestion of some dietary proteins such as casein and wheat gluten results in the production of substances that have opiate-like activity in both receptor and bioassays tests. Peptides with opioid activity have been isolated from pepsin hydrolysates of α-casein [1]. Analysis of these peptides has shown that they correspond to the α-casein sequences of 90–96, Arg–Tyr–Leu–Gly–Tyr–Leu–Glu and 90–95, Arg–Tyr–Leu–Gly–Tyr–Leu. Both peptides have a unique sequence of amino acid residues, mainly due to the presence of two Tyr residues at the 2 and 5 positions in the peptide sequence.

Earlier work [3] has shown that binding abilities of the other opiate-like peptide fragment of β-casein, β-casomorphin and its fragments, containing Tyr and Pro residues are very specific in coordination to Cu^{2+} ions. The stabilization of the complex species coordinated through either amide nitrogens of residues beyond the inserted prolyl residue or through Tyr side-chain phenolic oxygen results in the formation of either large chelate rings or dimeric complexes [3–5].

This study was performed in order to examine the binding ability, especially the effect of two Tyr residues on the formation of complexes with Cu^{2+} ions. The study was carried out with two model peptides Arg–Tyr–Leu–Gly–Tyr–Leu (RYLGYL) and Arg–Tyr–Leu–Gly–Tyr–Leu–Gln (RYLGYLQ).

Experimental

The peptides were synthesized by standard liquid-phase methods according to the procedure described earlier in [6].

Potentiometric Studies

The stability constants of H^+ and Cu^{2+} complexes of RYLGYL and RYLGYLQ in 0.1 mol·dm^{-3} KNO_3 were determined at 25°C using pH-metric titration. The pH changes were monitored using a glass-calomel electrode (Russell TR-CMAW711) calibrated in hydrogen concentrations using HNO_3 with the MOLSPIN automatic titrator, 1.5 cm^3 solution, 1×10^{-3} mol·dm^{-3} RYLGYL or RYLGYLQ with a copper (II) to ligand molar ratio of 1:1.05. The data were analyzed by the SUPERQUAD program [7]. The standard deviations reported were calculated by assuming error randomness.

Spectroscopic Measurements

Absorption spectra were recorded with a Perkin-Elmer Lambda 11 spectrophotometer. Electron paramagnetic resonance (EPR) measurements were carried out with a Bruker ESP 300E in the X-band (9.3 GHz) at 120 K; 1:2 ethanediol:water was used as a solvent. Circular dichroism (CD) spectra were obtained with a JASCO J-600 spectropolarimeter. Concentrations used in the spectro-

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scopic measurements were similar to those given for pH-metric titrations.

Results and Discussion

Protonation constants for RYLGYL and RYLGYLQ are presented in Table 1. The protonation constants of RYLGYLQ and RYLGYL peptides differ only slightly, and they are close to those expected for comparable peptides [3–5].

Potentiometry detects a range of Cu^{2+} complexes with the formation constants reported in Table 1. Spectroscopic properties of major complexes are given in Table 2. Analysis of log β and log K values related to the formation of Cu^{2+} complexes (Table 1) reveals a very close similarity of both peptides.

Cu^{2+}–RYLGYL Complexes

The coordination of metal ion starts at pH around 4.5. The minor species, (CuH_2L), with {NH_2, CO} coordination detected around pH 5 is usually found by potentiometric data calculations in metal–peptide systems, and cannot be supported by spectroscopic data because of its very low concentration. The next species, CuHL, is detected at pH 6 as a major complex. The EPR parameters $g_{\|} = 2.250$ and $A_{\|} = 179\ cm^{-1} \times 10^{-4}$ and the d–d transition energy of 648 nm are consistent with {NH_2, N^-, CO} coordination (Table 2) [8, 9]. With increasing pH above 6 in the absorption spectra, a new band appears around 400 nm (Fig. 1). This band corresponds to the phenolic oxygen Cu^{2+} charge transfer transition [9], and strongly indicates that the phenolic group of the tyrosine residue is deprotonated and bound to metal ion to form monomeric or dimeric species [3, 4]. The absorption maximum at about 400 nm (Table 2) is, however, relatively weak, indicating a rather low concentration of this species. The species distribution shown in Figure 2 assumes that the phenolate-bound complex is a dimeric species. This assumption improves the fitting of the potentiometric data calculations, but its low concentration does not allow us to prove its existence with, e.g., the EPR spectra. It should be mentioned here that earlier studies have suggested the formation of dimeric species

Table 1. Stability Constants of H^+ and Cu(II) with RYLGYL and RYLGYLQ at 25°C and $I = 0.1\ mol \cdot dm^{-3}$ (KNO_3)

	Species	RYLGYL	RYLGYLQ
log β	HL	10.60(1)	10.40(2)
	H_2L	20.09(1)	19.78(3)
	H_3L	27.08(1)	26.64(3)
	H_4L	30.92(2)	30.48(3)
log K	Tyr-OH	10.60	10.40
	Tyr-OH	9.49	9.38
	NH_2 (Term)	6.99	6.86
	COOH (Term)	3.84	3.84
log β	CuH_2L	23.73(3)	
	CuHL	19.11(03)	18.77(05)
	CuL	12.60(04)	11.92(1)
	$Cu_2H_{-1}L_2$	19.18(9)	
	Cu_2L_2		26.29(11)
	$CuH_{-1}L$	4.56(1)	4.45(1)
	$CuH_{-2}L$	−5.36(1)	−5.15(2)
	$CuH_{-3}L$	−16.26(2)	−15.72(3)
log K	CuHL	4.62	
	CuL	6.51	6.85
	$CuH_{-1}L$	8.04	7.48
	$CuH_{-2}L$	9.92	9.60
	$CuH_{-3}L$	10.90	10.57

Table 2. Spectroscopic Parameters for Cu(II) Complexes of RYLGYL and RYLGYLQ

		UV–Vis		CD		epr	
Ligand	Species (Donor Set)	λ [nm]	(ε) [$dm^3 \cdot mol^{-1} \cdot cm^{-1}$]	λ [nm]	(Δε) [$dm^3 \cdot mol^{-1} \cdot cm^{-1}$]	$g_{\|}$	$A_{\|}$ [$cm^{-1} \times 10^{-4}$]
RYLGYL	CuHL	648	(48)	542	(−0.321)	2.250	179.6
	(NH_2,N^-,CO)			323	(+0.446)		
	CuL	569	(52)	535	(−0.722)	2.220	199.3
	(NH_2,N^-,CO)(NH_2,N^-,N^-)	390	(36)	319	(+0.747)		
	$CuH_{-1}L$	498	(81)			2.165	225.4
	(NH_2,N^-,N^-,N^-)						
	$CuH_{-2}L$	498	(85)	517	(−1.680)	2.166	215.4
	(NH_2,N^-,N^-,N^-)			312	(+0.229)		
	$CuH_{-3}L$	499	(80)			2.170	214.8
	(NH_2,N^-,N^-,N^-)						
RYLGYLQ	CuHL	648	(32)	580	(+0.18)	2.254	182.6
	(NH_2,N^-,CO)			330	(+0.05)		
	CuL	592	(47)	524	(−0.36)	2.214	202.6
	(NH_2,N^-,CO)(O^-)	389	(45)	325	(+0.53)		
	$CuH_{-1}L$	502	(68)	517	(−1.16)	2.175	211.2
	(NH_2,N^-,N^-,N^-)	390	(14)	315	(+0.44)		
	$CuH_{-2}L$	496	(97)	521	(−1.28)	2.170	215.8
	(NH_2,N^-,N^-,N^-)			305	(+0.24)		
	$CuH_{-3}L$	496	(92)	521	(−1.44)	2.166	220.5
	(NH_2,N^-,N^-,N^-)			304	(+0.11)		

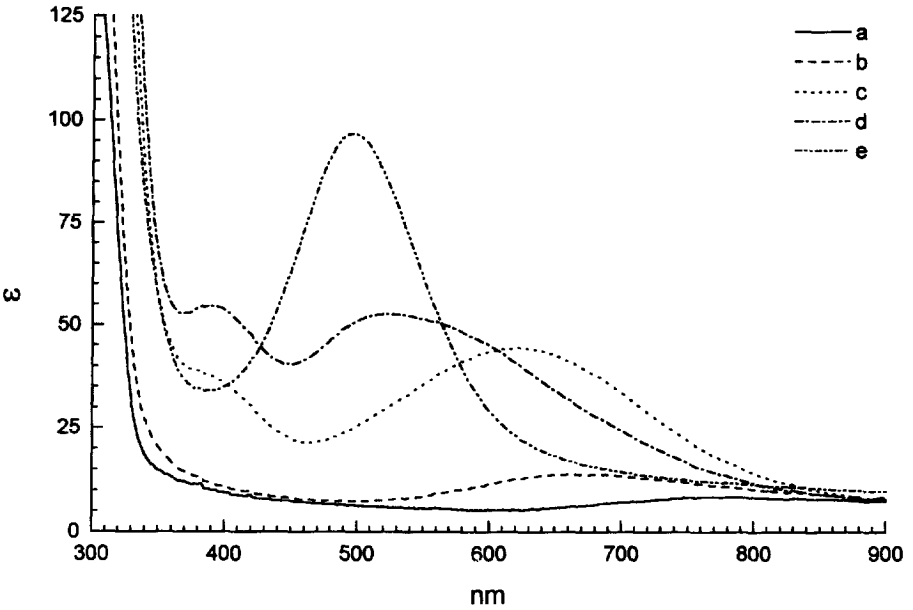

Figure 1. UV–Vis spectra for the Cu^{2+}–RYLGYLQ system at: (a) pH = 4.24, (b) pH = 5.43, (c) pH = 6.85, (d) pH = 7.46, (e) pH = 9.38; metal concentration 3 mmol·dm^{-3}, ligand concentration 3 mmol·dm^{-3}.

with phenolate–metal binding only when Tyr is situated at the N-terminal position [3–5] and monomeric complexes when Tyr is inserted several residues away from the N-terminal [4].

The CuL complex formed as a major species above pH 6 is clearly the 3N species. The EPR parameters $g_{\parallel} = 2.220$ and $A_{\parallel} = 199$ cm$^{-1} \times 10^{-4}$ and d–d energy around 550 nm (Table 2) are consistent with (NH_2, $2N^-$, CO) coordination [8, 9].

Above pH 8.5, the band diagnostic of phenolate binding is no longer observed (Fig. 1), and in the meantime, a $CuH_{-1}L$ complex with the (NH_2, $3N^-$) coordination mode is formed. This binding mode is easily seen in the EPR, absorption, and CD spectra (Table 2). The d–d energy close to 500 nm as well as $A_{\parallel} > 200$ cm$^{-1} \times 10^{-4}$ corresponds to four nitrogen binding modes [8, 9]. The further deprotonations leading to $CuH_{-2}L$ and $CuH_{-3}L$ complexes correspond to proton release from two Tyr residues. This does not change the binding mode around the Cu^{2+} ion, and the spectroscopic parameters remain almost unchanged (Table 2).

RYLGYLQ

The results obtained for the Cu^{2+}–RYLGYLQ system are very similar to those discussed above for RYLGYL (see Figs. 2 and 3). CuHL exhibits (NH_2, N^-, CO) coordination. A further increase of pH provides the formation of a CuL complex. Simultaneously, the phenolate–Cu^{2+} charge transfer band appears, similar to that observed for the Cu^{2+}–RYLGYL system (*vide supra*). The intensity of the charge transfer band is again very low, suggesting that phenolate binding occurs in the minor complex formed in

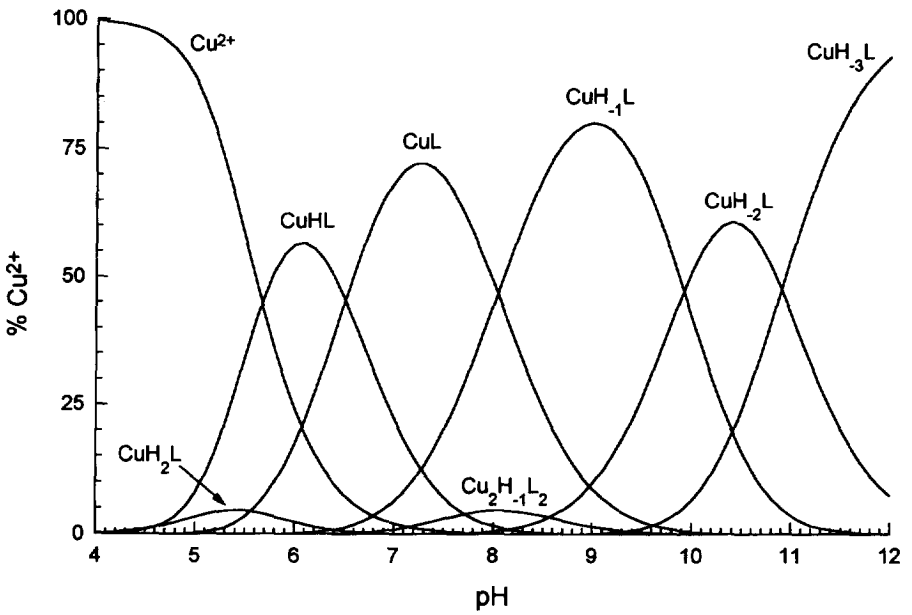

Figure 2. Species distribution curves for the Cu^{2+}–RYLGYL system; metal concentration 1 mmol·dm^{-3}, ligand concentration 1 mmol·dm^{-3}.

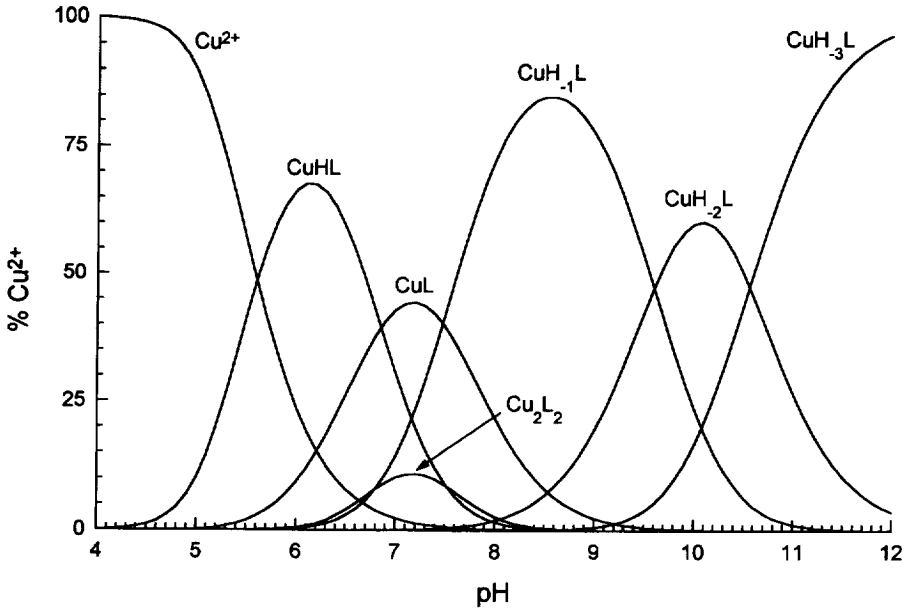

Figure 3. Species distribution curves for the Cu^{2+}–RYLGYLQ system; metal concentration 1 mmol·dm^{-3}, ligand concentration 1 mmol·dm^{-3}.

the solution studied. Above pH 8.5, the system exhibits spectroscopic parameters (Table 2) analogous to those of the RYLGYL characteristic for 4N species (Table 2).

Conclusions

Bovine casein fragments with opiate activity are efficient chelating agents for Cu^{2+} ions, and in the physiological pH, may form very effective 3N or 4N complexes. The side-chain donor groups like that of Tyr residues could be involved in metal ion coordination, but their biological implications could be of minor importance.

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