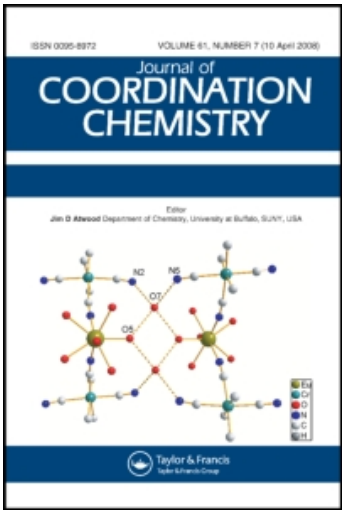

Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

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To cite this Article Kowalik-Jankowska, Teresa , Kozlowski, Henryk , Stasiak, Marcin and Leplawy, Miroslaw T.(1996) 'IMPACT OF α-HYDROXYMETHYLSERINE RESIDUES ON BINDING ABILITY OF DIPEPTIDES TOWARDS CU^{II} IONS', Journal of Coordination Chemistry, 40: 1, 113 — 120
To link to this Article: DOI: 10.1080/00958979608022850
URL: http://dx.doi.org/10.1080/00958979608022850

J. Coord. Chem., 1996, Vol. 40, pp. 113–120
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IMPACT OF α-HYDROXYMETHYLSERINE RESIDUES ON BINDING ABILITY OF DIPEPTIDES TOWARDS Cu^{II} IONS

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(Received 5 February 1996)

Potentiometric and spectroscopic studies performed on the binding ability of dipeptides containing α-hydroxymethylserine have shown that presence of the hydroxylmethyl substituent on the α-carbon changes considerably stability constants and binding modes. The indirect and direct involvement of the additional hydroxyl group is likely and this protects metal-peptide complexes against hydrolysis in very basic solutions.

Keywords: copper(II); complexes; α-hydroxymethylserine; dipeptides; stability constants; esr

INTRODUCTION

α-Hydroxymethylserine (hmSer) has been identified as the *N*-terminal amino acid residue in several antibiotics (cirratiomicines and antrimicins).[1–5] HmSer was also found in a plant *Vicia pseudoorobus*.[6] Not much is known about the role of α, α-disubstituted amino acids but potentially they should have a distinct impact on conformational preferences of peptide in which they are inserted and as a consequence they can change (most likely increase) peptide resistance towards proteinases.

HmSer has four potential binding sites including glycine-like $\{NH_2,COO^-\}$ chelation and two alcoholic groups. Its analogue *L*-serine (Ser), having three potential donor sites, *i.e.*, COO^-, NH_2 and one OH, has been shown to be quite an interesting ligand. The involvement of the protonated hydroxyl group may stabilise complexes at lower pH,[7–8] but direct binding of deprotonated hydroxyl oxygen occurs only at very high pH, making Ser an efficient chelator in basic solutions.[9–10]

Recent work on the coordination ability of α-hydroxymethylserine[7] has shown that this ligand is generally more efficient in Cu^{2+} and VO^{2+} binding than *L*-serine. Both of the deprotonated hydroxyl groups of hmSer can be involved in coordination of VO^{2+}, while Cu^{2+} ions are bound in the same way as with *L*-serine.

HmSer inserted into the peptide sequence besides providing alcoholic groups as potential donors, may also influence the peptide conformation, its rigidity and the basicity of potential nitrogen (NH_2 or N^-) and oxygen (COO^-) donors. In order to evaluate the impact of the hmSer residue on the binding ability of oligopeptides we have studied Cu^{2+} coordination by dipeptides containing this residue on *N*– and *C*–terminals. The effect of Ser residue on coordination ability of oligopeptides was recently discussed in detail.[11]

EXPERIMENTAL

Synthesis of ligands

The dipeptides (starting compounds mentioned below) were synthesized from protected aminoacids using TBTU[12] (*O*-benzotriazolyl tetramethyluronium tetrafluoroborate) as coupling reagent. The α-hydroxymethylserine (hmSer) derivatives used in these preparations will be described elsewhere.[13] Deprotection procedures were as follows: saponification was performed by means of 2M NaOH in dioxane, hydrogenation was carried out in a Parr apparatus at 50 psi with Pd on charcoal as catalyst, Boc (*t*-butoxycarbonyl) and Ipr (*O*, *O'*-isopropylidene) cleavage was accomplished using 90% aqueous TFA (trifluoroacetic acid). All transformations were monitored by RP HPLC (C_{18}) using various compositions of acetonitrile and water (both acidified with TFA). Resulting products were lyophilized. The deprotected compounds were checked by TLC (2-propanol: 25% aq. ammonia, 7:3) and showed a single spot (ninhydrin). The purity of final products was confirmed by spectroscopy (300 MHz ^1H NMR) and potentiometry.

HmSer-hmSer

Z-hmSer(Ipr)-hmSer(Ipr)-OH (*Z*-benzylocarbonyl) was reacted with 90% aqueous TFA (20 min) and the obtained product, (Z-hmSer-hmSer-OH), was hydrogenated (2 hours) in 50% aqueous methanol to give the dipeptide. TLC 0.41.

TFAxAib-hmSer-OH

Boc-Aib-hmSer(Ipr)-OMe (OMe-methyl ester) was saponified using 1.1 eq. of NaOH (21 hours at r.t.) and recrystallized acid was deprotected by means of 90% aqueous TFA (20 min) yielding the dipeptide (as trifluoroacetate). TLC 0.60.

HmSer-Aib-OH

Z-hmSer(Ipr)-Aib-OH was treated with 90% aqueous TFA (15 min) and the peptide (Z-hmSer-Aib-OH) was hydrogenated in 50% aqueous methanol (2 hours) to yield the dipeptide. TLC 0.57.

HmSer-Ala-OH

Z-hmSer(Ipr)-Ala-OBzl (OBzl-benzyl ester) was deprotected with 90% aqueous TFA (15 min) and (Z-hmSer-Ala-OBzl) was hydrogenated in 75% aqueous methanol for 4 hours to give the dipeptide. TLC 0.54.

Potentiometric Studies

All the ligands were sufficiently soluble in water. The ionic strength was 0.1 mol dm^{-3} (KNO_3), ligand concentration 2×10^{-3} mol dm^{-3}, Cu^{2+} to ligand ratios were 1:2 and 1:3. Stability constants for the H^+ and Cu^{2+} complexes were calculated from pH titrations carried out using total volumes of 2.0 cm^3. Alkali was added from a 0.100 cm^3 micrometer syringe which has been calibrated by weight and titrations of standard materials. The pH-metric titrations were performed at 25°C using a MOLSPIN automatic titration system with a micro-combined glass-calomel electrode calibrated in hydrogen ion concentration using HNO_3.[14] Titrations were performed in triplicate and the SUPERQUAD computer program was used for calculations of stability constant ($\beta = [M_pH_qL_r]/[M]^p[H]^q[L^r]$).[15] Standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They are, however, a good indication of the importance of a particular species in the equilibrium.

Spectroscopic Studies

EPR spectra were recorded on a Bruker ESP 300E spectrometer at X-band frequency (9.3 GHz) at 120K. Absorption spectra were recorded on a Beckman DU 650 spectrophotometer. Circular dichroism (CD) spectra were recorded on a Jasco J 600 spectropolarimeter in the 750–250 nm range. Metal concentration

in all spectroscopic measurements was adjusted to 5×10^{-3} mol dm^{-3} and metal to ligand ratio was1:2. ^{1}H–NMR spectra to check ligand purity were recorded on a Bruker AMX spectrometer at 300 MHz in D_2O. The ligand purity was also checked by potentiometric titrations.

RESULTS AND DISCUSSION

According to protonation constants collected in Table I for dipeptides the *N*-terminal hmSer residue has a distinct impact on the acidity of the amino group, while being on the *C*-terminal it does not affect the carboxylate pK much at all. The simple analogue of *L*-alanine, α-methylalanine (Aib), on the other hand, has a distinct influence on the acidity of the carboxylate when inserted at the *C*-terminal and almost no effect on amino group protonation constant in the *N*-terminal position (Table I).

The coordination of Cu^{2+} ions with dipeptides containing hmSer is almost the same as with simple Gly–Ala or Gly–Ser dipeptides (Table II). Spectroscopic data (Table III) show that the major complexes formed ($MH_{-1}L$ and $MH_{-2}L$) involve two nitrogens in metal ion coordination, $\{NH_2,N^-,COO^-\}$. The *d-d* transition around 610 – 630 nm clearly supports this binding mode.[16] It is interesting to note that in the Cu^{2+}–hmSer–Ala system deprotonation of $CuH_{-1}L$ leads to considerable changes of CD spectra in the *d–d* region. Three *d–d* transitions are observed for $CuH_{-2}L$ (Table III), while only one broad band for the $CuH_{-1}L$ species. This may indicate that deprotonation of metal-bound water (*vide infra*) in the latter complex induces a distinct change in the chelate ring conformation.

The minor CuL species with $\{NH_2,C=O\}$ binding mode is stabilised by the hmSer residue when compared to other dipeptides listed in Table II. The CuL

TABLE I Protonation constants for the dipeptides containing α-hydroxymethylserine (hmSer) and α-methylalanine (Aib) at 298 K and I = 0.1 mol dm^{-3} (KNO_3)

ligand	$log\beta(HL)$	$log\beta(H_2L)$	$logK_{COOH}$	$logK_{NH2}$
Gly-Ala 1	8.20	11.37	3.17	8.20
Gly-Ser 2	8.09	11.03	2.94	8.09
Ala-Ser 2	8.16	11.22	3.06	8.16
Aib-hmSer	8.07 ± 0.01	11.08 ± 0.01	3.01	8.07
Ala-Ala 1	8.26	11.34	3.08	8.26
Ser-Ala 3	7.40	10.57	3.17	7.40
hmSer-Ala	6.76 ± 0.01	9.86 ± 0.01	3.10	6.76
hmSer-Aib	6.86 ± 0.01	10.42 ± 0.01	3.56	6.86
hmSer-hmSer	6.50 ± 0.01	9.48 ± 0.01	2.98	6.50

[1] Reference 21
[2] Reference 22
[3] Reference 11

TABLE II Equilibrium data (logβ) for copper(II) complexes of dipeptides containing α-hydroxymethylserine (hmSer) and α-methylalanine (Aib) at 298 K and I = 0.1 mol $dm^{-3}(KNO_3)$

	Species							
ligand	*ML*	$MH_{-1}L$	$MH_{-2}L$	$MH_{-3}L$	$logK_{HL}^{ML}$	$logK_{ML}^{MH_{-1}L}$	$logK_{MH_{-1}L}^{MH_{-2}L}$	$logK_{MH_{-2}L}^{MH_{-3}L}$
Gly-Ala[1]	5.76	1.55	−7.94		−2.44	−4.21	−9.49	
Gly-Ser[2]	5.46	1.68	−7.67		−2.63	−3.78	−9.35	
Ala-Ser[2]	5.22	1.76	−7.53		−2.94	−3.46	−9.29	
Aib-hmSer	6.28 ± 0.05	3.04 ± 0.01	−6.42 ± 0.01	−17.93 ± 0.07	−1.79	−3.24	−9.46	−11.51
Ala-Ala[1]	5.33	1.43	−8.01		−2.93	−3.90	−9.44	
Ser-Ala[3]	5.22	1.59	−7.69		−2.18	−3.63	−9.28	
hmSer-AIa	4.98 ± 0.04	1.70 ± 0.01	−7.54 ± 0.01	−18.68 ± 0.02	−1.78	−3.28	−9.24	−11.14
hmSer-Aib	5.69 ± 0.02	2.16 ± 0.01	−7.24 ± 0.01		−1.17	−3.53	−9.40	
hmSer–hmSer	6.04 ± 0.03	2.72 ± 0.01	−6.10 ± 0.02	−16.31 ± 0.02	−0.46	−3.32	−8.82	−10.21

[1] Reference 21

[2] Reference 22

[3] Reference 11

TABLE III Spectroscopic data for copper(II) complexes with dipeptides containing α-hydroxymethylserine(hmSer) and α-methylalanine (Aib)

Species	***UV-VIS***		***CD***		***EPR***	
	λ(nm)	*ε*	*λ(nm)*	*Δε*	$A_{//}$	$g_{//}$
Aib-hmSer						
$MH_{-1}L$	612[a]	93			190	2.235
$MH_{-2}L$	610[a]	89			167	2.234
$MH_{-3}L$	607[a]	89			170	2.232
hmSer-Ala						
$MH_{-1}L$	627[a]	103	671[a]	–0.559	177	2. 243
			326[b]	+0.053		
			270[c]	–1.360		
$MH_{-2}L$	629[a]	99	674[a]	–0.467	169	2.235
			571[a]	+0.027		
			503[a]	–0.108		
			322[b]	+0.072		
			263[c]	–1.880		
$MH_{-3}L$	630[a]	98	684[a]	–0.513	168	2.242
			573[a]	+0.075		
			500[a]	–0.154		
			315[b]	+0.063		
			256[c]	–2.270		
hmSer-Aib						
$MH_{-1}L$	612[a]	103			190	2.241
$MH_{-2}L$	613[a]	102			173	2.231
hmSer-hmSer						
$MH_{-1}L$	616[a]	109			186	2.237
$MH_{-2}L$	616[a]	110			169	2.230
$MH_{-3}L$	607[a]	111			174	2.237

[a] Ad-d transition
[b] $N^- \Rightarrow$ Cu(II) charge transfer transition
[c] $NH_2 \Rightarrow$ Cu(II) charge transfer transition

complex in the case of hmSer-hmSer is about two orders of magnitude more stable than that of Gly-Ala or Gly-Ser (see $logK^{ML}_{HL}$ in Table II, Figure 1.) Also, the major $CuH_{-1}L$ complex with $\{NH_2,N^-,COO^-\}$ coordination is stabilised by hmSer, both on *N*– and *C*–terminals. The $CuH_{-1}L$ complex undergoes deprotonation with log K value around –8.8 to –9.5 which is characteristic for deprotonation of a water molecule bound to tri-coordinate Cu^{2+}.[17] It is interesting to note that in three of the studied systems, Cu^{2+}-Aib-hmSer, Cu^{2+}-hmSer-hmSer and Cu^{2+}-hmSer-Ala, the formation of $CuH_{-3}L$ could be recorded by potentiometry. Formation of this species is specially favoured for hmSer-hmSer. Log K of reaction $CuH_{-2}L \Leftrightarrow CuH_{-3}L + H^+$ for the latter ligand is relatively low (–10.21) suggesting that in $CuH_{-3}L$ the weakly bound carboxylate is substituted by a deprotonated alcoholic group of a *C*-terminal hmSer residue. The same reaction could be expected for the Cu^{2+} -Aib-hmSer system, while in the case of hmSer-Ala, hydrolysis of the metal-carboxylate bond is expected. The two types of CuH_{-3} L complexes formed, *i.e.*, terdentatde $\{NH_2,N^-,O^-\}$

with deprotonated water at the fourth position and bidentate $\{NH_2,N^-\}$ with two OH groups bound to the third and fourth positions of Cu^{2+} is seen also in absorption and EPR spectra. Substitution of carboxylate by a deprotonated hmSer alcoholic group (dipeptide remains terdendate) affects only slightly the EPR spectra and shifts the energy of the *d-d* transition towards higher energy (Table III). In the case of hmSer-Aib the *d-d* energy remains around 630 nm and in the EPR spectra the formation of soluble $Cu(OH)_4^{2-}$ species is seen (Figure II). This clearly indicate that in the case of *C*-terminal hmSer the $CuH_{-3}L$ complex is resistant towards hydrolysis, while lack of the alcoholic group in the second residue leads to complete complex hydrolysis which starts with hydrolysis of the metal-carboxylate bond.[10,18–20]

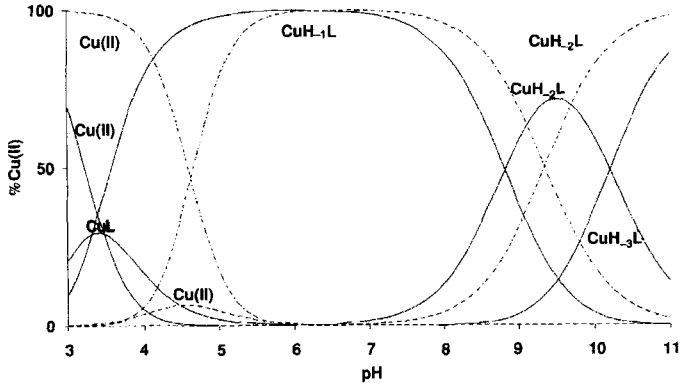

FIGURE 1 Species distribution curves for Cu(II)-Gly-Ser (.....) and Cu(II)-hmSer-hmSer (—); metal concentration 10^{-3} mol dm^{-3} and metal-to-ligand ratio 1:2.

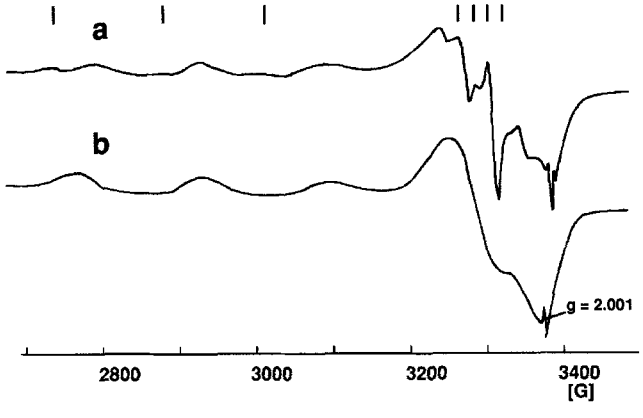

FIGURE 2 EPR spectrum of Cu(II)-hmSer-Ala (a) and Cu(II)-hmSer-hmSer (b) at pH 12.1 at 120 K; 1:2 metal to ligand molar ratio.

α-Hydroxymethyl-*L*-serine residue thus has a distinct influence on the binding ability of oligopeptides. It can stabilise considerably particular species or use its side chain acloholic group to bind Cu^{2+} ion. HmSer, having two alcoholic groups, is distinctly different to serine in its behaviour.

Acknowledgments

This work was supported by Polish State Committee for Scientific Research (KBN 3T09A06908).

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