The influence of copper(II) ions on noncovalent interactions in the systems including phosphoserine and biogenic amines†

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Ternary systems of copper(II) complexes with phosphoserine and biogenic amines (putrescine or spermidine or spermine) have been investigated. The studies were performed in aqueous solution using the potentiometric method with computer analysis of the data, ^{13}C and ^{31}P nuclear magnetic resonance, visible and electron paramagnetic resonance spectroscopies. The composition and overall stability constants of the complexes were determined by the pH-metric study and the coordination sites were identified by spectroscopic methods. The reaction centres are phosphate, carboxyl and amine groups from phosphorylated serine as well as amine groups from polyamine. These centres are also the potential sites of the noncovalent interactions (in the systems without metal). In the systems studied only protonated complexes formed. Besides the heteroligand complexes and heteroligand complexes with intermolecular interactions, molecular complexes with the protonated bioamine in the outer coordination sphere appeared. In these molecular species polyamine was involved in noncovalent interactions with the anchoring phosphoserine complex.

Introduction

The presence of metal ions in living organisms modifies the character of bioprocesses as metal has a significant influence on noncovalent interactions among biomolecules. The reactions between the amino acid and the metal ions are considered as models of the processes which take place at the molecular level in the metal–protein system. Copper is a trace metal in human organism in which it definitely plays an important role *e.g.* in lipoprotein metabolism it is becoming of increasing importance in understanding cardiovascular problems such as atherosclerosis.[1] The majority of Cu(II) ions in ceruloplasmin are in the form of mixed complexes with amino acids, peptides, and other biomolecules[2] *e.g.* complexation with a component of human serum (immobilised *O*-phosphoserine).[3] Copper in trace amounts is essential to sustain life but toxic accumulation of copper can be deleterious to human health (*e.g.* Wilson and Menkes disease genes[4] and copper binding to β-amyloid protein is considered as inducing Alzheimer's disease[5]).

Phosphoserine is one of the naturally occurring phosphorylated amino acids. Two of the most important mechanisms regulating many processes in living organisms are phosphorylation and dephosphorylation.[6] To understand the role of phosphorylation in biological processes, it is essential to characterise the site at which it occurs and the mode of interaction of phosphorylated amino acids with other species (*e.g.* metal ions and small organic ligands like polyamine).[7]

Biogenic polyamines: putrescine, spermidine and spermine, play an essential role in the living processes of eukaryotic cells;[8] they stabilise membranes, activate some enzymes and, together with their metabolites, catalyse and control biosynthesis of nucleic acids and proteins.[9] At the physiological pH biogenic amines occur in the protonated form and are involved in noncovalent interactions with the negatively charged groups of other biomolecules, *e.g.* negatively charged phosphate groups of nucleic acids and phosphorylated proteins.[10] The character of interactions is affected by the length of the amine chain and the number of amine groups.[11] Understanding of the complexation processes of amino acids and their natural derivatives and biogenic amines is important for the explanation of the role of metals in living organisms.[12]

This paper presents results of a study on complexation process of phosphoserine and aliphatic biogenic amines to copper(II) ions. The question is how a metal introduced into a binary system (phosphoserine/polyamine) can change the mode of interaction. Recognition of the interactions of metal ions in biological metal free systems is the initial step for evaluation of the nature of their role in the living system.

Results and discussion

On the basis of the computer analysis data of potentiometric measurements (HYPERQUAD program), the composition of particular complexes forming in the systems studied and their overall stability constants were determined (log β), Table 1. The structural formulae of the ligands studied are presented in Fig. 1.

The stability constants of the complexes forming in the Cu/Ser-P/PA system, Table 3, were calculated using the earlier determined protonation constants of the ligands and overall stability constants (log β) of complexes forming in binary systems: Cu/Ser-P, Cu/Put, Cu/Spd and Cu/Spm, Table in ESI.†[13] The hydrolysis constants for the copper ion were also taken into account.[14] In all studied systems, the potential

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Table 1 The overall stability constants of the ternary complexes as well as statistic parameters (Σ, χ^2)

Overall stability constants log β of the ternary complexes			
	Put Σ = 1.13, χ^2 = 15.33	Spd Σ = 1.79, χ^2 = 18.70	Spm Σ = 4.66, χ^2 = 26.62
Cu(Ser-P)H_6L	—	—	68.28 (3)
Cu(Ser-P)H_5L	—	53.45 (8)	61.97 (5)
Cu(Ser-P)H_4L	44.80 (4)	49.50 (7)	55.41 (3)
Cu(Ser-P)H_3L	40.61 (4)	43.83 (7)	47.85 (2)
Cu(Ser-P)H_2L	34.76 (4)	36.61 (6)	38.96 (2)
Cu(Ser-P)HL	26.28 (5)	28.34 (5)	—

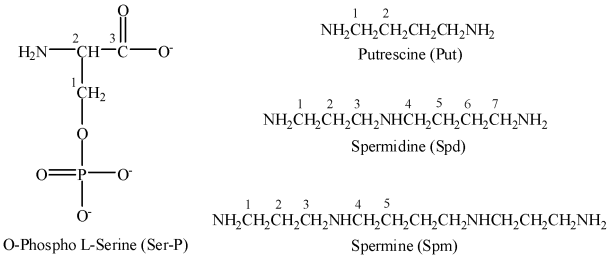

Fig. 1 The ligands studied in the totally deprotonated forms.

coordination centres are the amine groups from PA and carboxyl, phosphate and amine groups from phosphoserine.

The type of chromophore was proposed on the basis of the spectroscopic investigation ($g_\parallel$ as well as $A_\parallel$ values obtained from the EPR and energy of d–d transitions obtained from the Vis spectra) in the pH range in which particular complexes dominate, as established on the basis of the equilibrium study.[15] Direct relation between the d–d transition energy and EPR spectral parameters permits determination of the composition of the inner coordination sphere of copper. In the complexes with N or O donating ligands, with increasing number of donor atoms coordinated with Cu^{2+}, the values of λ_{max} and $A_\parallel$ increase, while that of $g_\parallel$ decreases.[15] When oxygen atoms (not originating from water molecules) are replaced by nitrogen ones in the tetragonal geometry in cupric ion, the maximum absorption wavelength shifts to smaller values, indicating the appearance of a stronger ligand field, and the molar absorptivity usually increases (λ_{max} for {N3} coordination is 600 nm, for {N4} 550 nm and for {N3,O1} λ_{max} is 584 nm).[16] The conclusions from the spectroscopic studies were supported by the chemical shifts in the ^{13}C and ^{31}P NMR spectra of the ligand in the complex with respect to those of the free ligand and by analysis of the equilibrium studies. Taking into account the limitations of this method following from the presence of paramagnetic ions (such as Cu^{2+}) in the systems studied, the NMR spectra of the species were recorded by the decoupling technique at low concentrations of metal ions.[17] As shown in the earlier works,[18] a considerable decrease in the copper ion concentration has no significant effect on the pH ranges of domination of a given species, relative to those in the 1 : 1 : 1 system. Significant changes in the chemical shifts were found to occur only in the pH ranges of complex formation (the ranges had been determined from potentiometric studies). Good agreement of the results obtained by different methods permits description of the ligands arrangement in the complex with high probability.

Cu/Ser-P/Put system

In the Cu/Ser-P/Put system Cu(Ser-P)H_x(Put) complexes was found, x = 1–4, Fig. 2. The values of the overall stability constants of the species are in

The results of the Vis and EPR studies (λ_{max} = 760 nm, $g_\parallel$ = 2.392 and $A_\parallel$ = 133 × 10^{-4} cm^{-1}, Table 2) for Cu(Ser-P)H_4(Put) indicate the formation of a species with the oxygen chromophore {O_x}. Changes in the chemical shift of C(1) and P atoms from phosphoserine in the complex Cu(Ser-P)H_4(Put) indicate that the main site of the copper ion coordination is the phosphate group Ser-P.

Relatively smaller changes in the chemical shift of the carbon atoms C(3) from Ser-P and C(1′) and C(2′) from putrescine evidence the noncovalent interaction of these bioligands and formation of the molecular complex Cu(H_2Ser-P)|||||H_2Put, in which putrescine is engaged in noncovalent interaction with Cu(H_2Ser-P). A similar mode of interactions was found in Cu/Asp/Put, in which the fully protonated amine is left outside the inner coordination sphere and interacts with the anchoring complex Cu(Asp).[18] The presence of putrescine outside the inner coordination sphere was confirmed by the Vis spectral study. To the binary system Cu/Ser-P at the pH of domination of Cu(Ser-P)H_4(Put) complex, putrescine was added in the equimolar ratio or in fivefold excess, which did not change the position of the maximum absorption (Fig. 3), so Put does not bind copper ions.

On the other hand, addition of phosphoserine to the binary system Cu/Put at the same pH leads to changes in the Vis spectrum, which proves that phosphoserine is included in the inner coordination sphere of copper (Fig. 4).

The mode of interaction of putrescine with complexed phosphoserine proposed is analogous to that in the system without metal, Ser-P/Put, at high pH at which the amine group from PA and the carboxyl group from phosphoserine are engaged in noncovalent interaction.[19] The mode of interaction that is realised in the adduct (Ser-P)H_4(Put), dominating in the system without metal at the same pH as

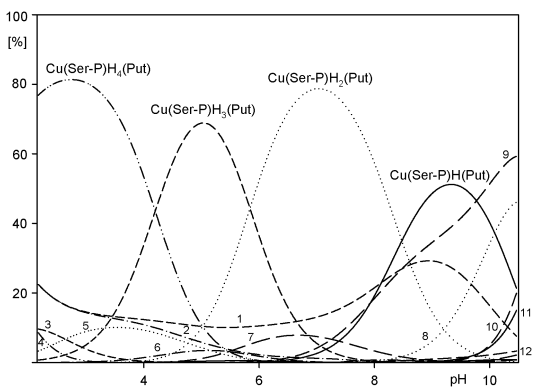

Fig. 2 Distribution diagram for the Cu/Ser-P/Put system: 1—H_2Put; 2—Cu^{2+}; 3—H_3PSer; 4—H_4PSer; 5—H_2PSer; 6—Cu(HPSer); 7—Cu(PSer); 8—HPut; 9—Cu(PSer)(OH); 10—Put; 11—Cu(PSer)$(OH)_2$; 12—Cu(Put)(OH). The percent of the species refers to the relative concentration of the metal c_{Cu} = 0.002 M.

Table 2 Spectral data and modes of interactions in the studied complexes

Species	pH	λ_{max}/nm	ε/M^{-1} cm^{-1}	$g_{\parallel}$	$A_{\parallel} \times 10^{-4}$/cm^{-1}	Chromophore
Cu(Ser-P)H$_4$(Put)	3.0	750	17.5	2.392	133.0	{O$_x$}
Cu(Ser-P)H$_3$(Put)	5.0	725	14.4	2.300	161.5	{O$_x$}
Cu(Ser-P)H$_2$(Put)	7.0	705	33.3	2.290	156.0	{N1,O$_x$}
Cu(Ser-P)H(Put)	9.5	606	76.6	2.243	185.5	{N2,O$_x$}
Cu(Ser-P)H$_5$(Spd)	3.0	751	17.2	2.382	130.0	{O$_x$}
Cu(Ser-P)H$_4$(Spd)	4.75	725	28.1	2.299	164.0	{O$_x$}
Cu(Ser-P)H$_3$(Spd)	6.5	642	85.9	2.238	169.5	{N1,O$_x$}
Cu(Ser-P)H$_2$(Spd)	7.75	603	81.5	2.207	195.0	{N2,O$_x$}
Cu(Ser-P)H(Spd)	10.0	598	86.7	2.209	193.0	{N2,O$_x$}
Cu(Ser-P)H$_6$(Spm)	4.0	715	30.2	2.319	165.5	{O$_x$}
Cu(Ser-P)H$_4$(Spm)	7.0	620	57.0	2.225	199.5	{N1,O$_x$}
Cu(Ser-P)H$_3$(Spm)	8.25	599	78.7	2.219	197.5	{N2,O$_x$}
Cu(Ser-P)H$_2$(Spm)	10.0	591	89.6	2.218	199.0	{N2,O$_x$}

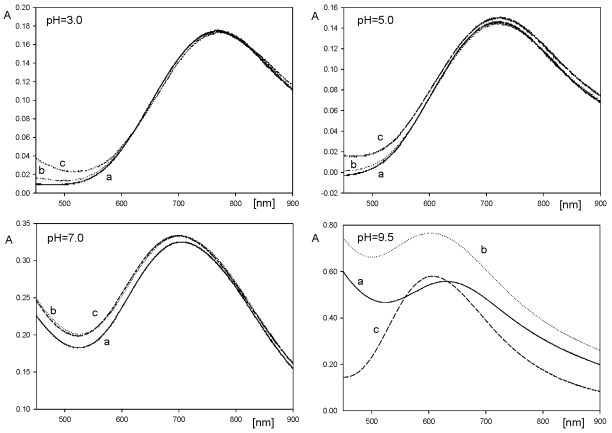

Fig. 3 Vis spectra taken in pH range of complexes domination; $C_{Cu(II)}$ = 0.01 M: (a) Cu(II)/Ser-P 1 : 1 system, (b) Cu(II)/Ser-P/Put 1 : 1 : 1 system, (c) Cu(II)/Ser-P/Put 1 : 1 : 5 system.

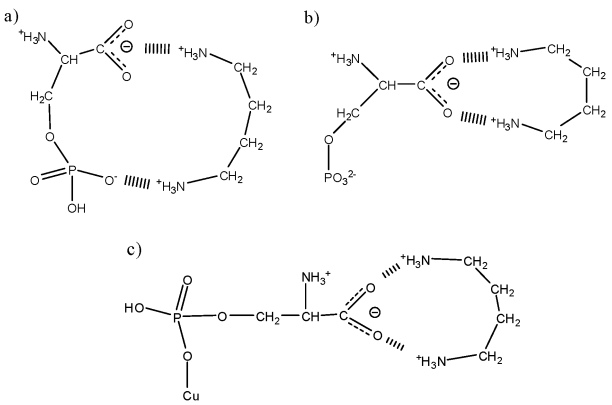

Fig. 4 Tentative scheme of interaction in binary system Ser-P/Put in: (a) (Ser-P)H$_4$(Put), (b) (Ser-P)H$_3$(Put) adducts and ternary system Cu/Ser-P/Put, (c) Cu(Ser-P)H$_4$(Put) molecular complex (|||||| indicates non-covalent interaction).

the species Cu(Ser-P)H$_4$(Put) in the system with metal, is impossible as the partly protonated phosphate group makes stronger bonds with Cu^{2+}. In this system the copper ion is an interfering agent changing the mode of noncovalent interactions, Fig. 4. The character of changes in the chemical shifts, Table 3, and the spectral parameters, Table 2, for Cu(Ser-P)H$_3$(Put) suggest the mode of interactions analogous to that in Cu(Ser-P)H$_4$(Put), *i.e.* putrescine outside the inner coordination sphere interacts with the carboxyl group of Cu(HSer-P) where the coordination is *via* the phosphate group. The equilibrium constant of formation of Cu(HSer-P) + H$_2$(Put) ⇆ Cu(Ser-P)H$_3$(Put), log K_e = 5.08 very close to that for the species with aspartic acid log K_e = 4.95, confirms that in both complexes the protonated polyamine interacts with the anchoring Cu(HSer-P) or Cu(HAsp) complex.[18]

With increasing pH the dominant species becomes Cu(Ser-P)H$_2$(Put), Fig. 2. The spectral parameters of this species: λ_{max} = 720 nm, $g_{\parallel}$ = 2.300 and $A_{\parallel}$ = 161 × 10^{-4} cm^{-1}, Table 2, indicate that the inner coordination sphere contains a nitrogen atom apart from the oxygen. To find out the origin of this nitrogen atom, the Vis study was performed for Cu/Ser-P at pH = 7.0 to which putrescine was added, Fig. 3. No changes in the spectral parameters mean that putrescine is outside the inner coordination sphere and the coordinated nitrogen atom must come from phosphoserine. Changes in the chemical shift in the NMR spectrum, Table 3, show that the copper ion is coordinated with the amine and carboxyl groups from phosphoserine, while the relatively smaller changes in the neighbourhood of the phosphate group and C(1) and C(2) of putrescine point to the involvement of PO_4^{2-} from Ser-P and NH_3^+ from Put in a weak interaction. Moreover, the value of log K_e = 4.36, close to the value of the equilibrium constant of formation of the complex Cu(Ser-P)H$_3$(Put) (log K_e = 4.95) and close to that of the analogous species with aspartic acid formation (log $K_{e\ Cu(Asp)H_2(Put)}$ = 4.36), confirms that amine must be outside the inner coordination sphere.

The spectral parameters obtained for the Cu(Ser-P)H(Put) complex dominant at pH 9.5, Table 2 and Fig. 2, indicate the formation of {N2,O$_x$} chromophore, which means that in this complex putrescine is bonded to copper ions. Addition of putrescine to the system of Cu/Ser-P results in a shift of λ_{max} by about 50 nm, which indicates that only one nitrogen atom is involved in the coordination (the other nitrogen atom comes from phosphoserine), Fig. 3. The inclusion of the amine into the inner coordination sphere is also indicated by an increase in log K_e to 5.55, so to a value higher than those obtained for the species with putrescine not involved in the coordination; log $K_{e\ Cu(Ser-P)H_3(Put)}$ = 5.08 and log $K_{e\ Cu(Ser-P)H_2(Put)}$ = 4.35. Changes in the chemical shifts in the NMR spectra confirm the

Table 3 Equilibrium constants (log K_e) of Cu(Ser-P)H_x(PA) complexes formation and NMR data-differences between NMR chemical shifts for the ligands in the investigated systems in relation to the free ligands in the same pH [ppm]

			NMR										
			Phosphoserine						Polyamine				
Species	log K_e	Equilibrium of formation	C(1)	C(2)	C(3)	P	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C(6′)	C(7′)
Cu(Ser-P)H_4(Put)	—	Cu(H_2Ser-P) + H_2(Put) ⇆ Cu(Ser-P)H_4(Put)	0.42	0.10	0.36	0.22	0.13	0.12	—	—	—	—	—
Cu(Ser-P)H_3(Put)	5.08	Cu(HSer-P) + H_2(Put) ⇆ Cu(Ser-P)H_3(Put)	0.34	0.25	0.27	0.18	0.17	0.15	—	—	—	—	—
Cu(Ser-P)H_2(Put)	4.35	Cu(Ser-P) + H_2(Put) ⇆ Cu(Ser-P)H_2(Put)	0.26	0.22	0.39	0.20	0.15	0.16	—	—	—	—	—
Cu(Ser-P)H(Put)	5.55	Cu(Ser-P) + H(Put) ⇆ Cu(Ser-P)H(Put)	0.04	0.24	0.35	0.04	0.25	0.09	—	—	—	—	—
Cu(Ser-P)H_5(Spd)	—	Cu(H_2Ser-P) + H_3(Spd) ⇆ Cu(Ser-P)H_5(Spd)	0.38	0.12	0.40	0.28	0.08	0.09	0.07	0.12	0.13	0.06	0.11
Cu(Ser-P)H_4(Spd)	4.87	Cu(HSer-P) + H_3(Spd) ⇆ Cu(Ser-P)H_4(Spd)	0.30	0.12	0.36	0.27	0.12	0.07	0.08	0.15	0.14	0.05	0.10
Cu(Ser-P)H_3(Spd)	7.78	Cu(HSer-P) + H_2(Spd) ⇆ Cu(Ser-P)H_3(Spd)	0.36	0.15	0.28	0.26	0.45	0.29	0.30	0.19	0.20	0.14	0.18
Cu(Ser-P)H_2(Spd)	5.68	Cu(Ser-P) + H_2(Spd) ⇆ Cu(Ser-P)H_2(Spd)	0.08	0.21	0.23	0.04	0.42	0.21	0.22	0.15	0.16	0.10	0.06
Cu(Ser-P)H(Spd)	7.47	Cu(Ser-P) + H(Spd) ⇆ Cu(Ser-P)H(Spd)	0.27	0.78	1.63	0.20	1.25	0.75	0.96	1.59	1.71	0.58	0.60
Cu(Ser-P)H_6(Spm)	—	Cu(H_2Ser-P) + H_4(Spm) ⇆ Cu(Ser-P)H_6(Spm)	0.36	0.09	0.16	0.26	0.08	0.09	0.06	0.07	0.09	—	—
Cu(Ser-P)H_4(Spm)	10.0	Cu(HSer-P) + H_3(Spm) ⇆ Cu(Ser-P)H_4(Spm)	0.28	0.07	0.14	0.25	0.08	0.09	0.12	0.15	0.10	—	—
Cu(Ser-P)H_3(Spm)	7.56	Cu(Ser-P) + H_3(Spm) ⇆ Cu(Ser-P)H_3(Spm)	0.20	1.22	1.63	0.32	0.09	0.11	0.10	0.13	0.11	—	—
Cu(Ser-P)H_2(Spm)	7.78	Cu(Ser-P) + H_2(Spm) ⇆ Cu(Ser-P)H_2(Spm)	0.18	1.28	1.72	0.33	0.12	0.15	0.25	0.30	0.32	—	—

participation of the amine and carboxyl groups from phosphoserine and the amine group from putrescine in the coordination. The changes in the chemical shift of the phosphate group are so small that its noncovalent interaction with the $-NH_3^+$ group of amine can be excluded. The lack of additional intramolecular interaction among the bioligands is also testified by the value of log K_e = 5.55. The presence of additional interactions, *e.g.* like in Cu(Asp)H(tn) complex, leads to increasing value of the equilibrium constant of formation to log $K_{e\ Cu(Asp)H(tn)}$ = 7.10.[18]

Cu/Ser-P/Spd system

As follows from the computer analysis of the potentiometric data, the species of Cu(Ser-P)H_x(Spd) type with x = 1–5 form in the system Cu/Ser-P/Spd; the overall stability constants are given in Table 1. At the lowest pH the complex Cu(Ser-P)H_5(Spd) is formed, Fig. 5. The Vis and EPR spectral parameters characteristic of this species, Table 2, indicate the presence of only oxygen atoms in the inner coordination sphere—{O_x} chromophore. Changes in the signal position of chemical shifts of the atoms located near the phosphate group of Ser-P in the NMR spectra indicate that the main coordination site is phosphate group. Relatively smaller changes in the chemical shifts of C(3) from Ser-P and the carbon atoms of spermidine, Table 3, imply the noncovalent interaction between these bioligands. The presence of spermidine outside the inner coordination sphere (similarly as the presence of putrescine in the Cu(Ser-P)H_{2-4}(Put) complexes) was confirmed by the Vis spectral results. Spermidine was added to the system Cu/Ser-P which causes no changes in the maximum absorption position (Fig. 6). NMR and Vis spectra indicated that molecular complex Cu(H_2Ser-P)|||||H_3Spd was formed in which the fully

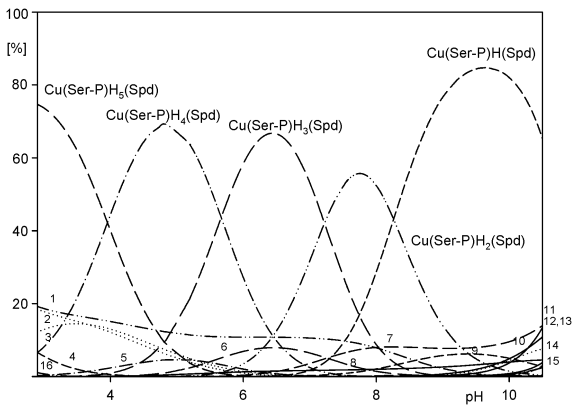

Fig. 5 Distribution diagram for the Cu/Ser-P/Spd system: 1—H_3Spd; 2—Cu^{2+}; 3—H_2Ser-P; 4—H_3Ser-P; 5—Cu(HSer-P); 6—Cu(Ser-P); 7—Cu(Ser-P)(OH); 8—HSer-P; 9—H_2Spd; 10—HSpd; 11—Cu(Spd)(OH); 12—Cu(Ser-P)$(OH)_2$; 13—Cu(Spd)$_2$(OH); 14—Spd; 15—Ser-P; 16—H_4Ser-P. The percent of the species refers to the relative concentration of the metal c_{Cu} = 0.002 M.

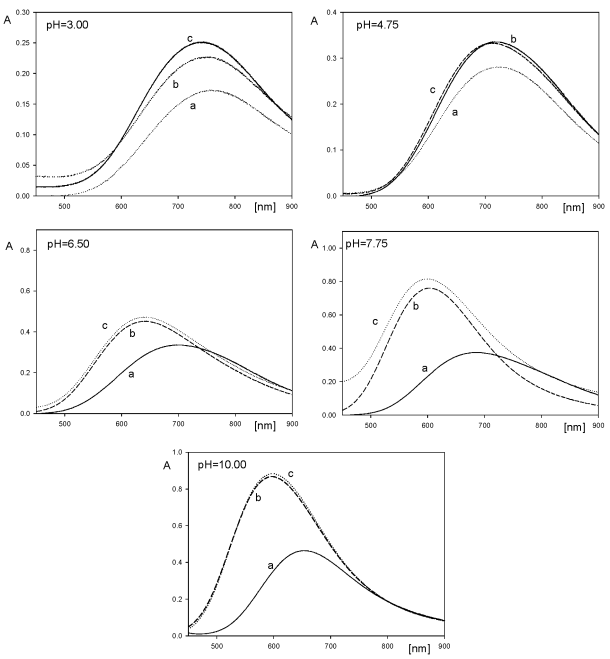

Fig. 6 Vis spectra taken in pH range of complexes domination; $C_{Cu(II)}$ = 0.01 M: (a) Cu(II)/Ser-P 1 : 1 system, (b) Cu(II)/Ser-P/Spd 1 : 1 : 1 system, (c) Cu(II)/Ser-P/Spd 1 : 1 : 5 system.

protonated amine is engaged in noncovalent interactions with the carboxyl group of the anchoring Cu(H_2Ser-P) complex.

With deprotonation of phosphoserine the Cu(Ser-P)H_4(Spd) complex is formed, Fig. 5. The spectral parameters of this species (λ_{max} = 725 nm, $g_{\|}$ = 2.299 and $A_{\|}$ = 164 × 10^{-4} cm^{-1}, Table 2) indicate the {O_x} chromophore. Addition of spermidine to the binary system Cu/Ser-P (λ_{max} = 730 nm) does not change the position of the d–d band which means that spermidine is not involved in coordination. The noncovalent interaction of the protonated amine with the anchoring Cu(HSer-P) complex is also confirmed by the equilibrium constant of formation of Cu(HSer-P) + H_3Spd ⇆ CuH_4(Ser-P)(Spd) log K_e = 4.87, whose value is comparable with those of putrescine complexes in which similar interaction was also observed (log K_e = 5.08 and log K_e = 4.35). The changes in the chemical shift of the carbon atoms of phosphoserine and spermidine in the NMR spectra, Table 3, testify the proposed mode of coordination.

The Cu(Ser-P)H_3(Spd) complex dominates in the system at pH close to 6.5, Fig. 5, its maximum absorption of d–d band is at λ_{max} = 642 nm and spectroscopic parameters are $g_{\|}$ = 2.238 and $A_{\|}$ = 170 × 10^{-4} cm^{-1}, Table 2. These values suggest that the inner coordination sphere contains oxygen atoms and one nitrogen atom, the {N1,O_x} chromophore. Addition of the polyamine to the binary system Cu/Ser-P at pH = 6.5 (λ_{max} = 700 nm), Fig. 6, resulted in a shift of λ_{max} by about 60 nm. The value of the shift towards higher energies indicates that one nitrogen atom from spermidine has been included into the inner coordination sphere. Higher log K_e = 7.78 of the reaction of this complex formation than that of formation of Cu(Ser-P)H_4(Spd), log K_e = 4.87, confirms the inclusion of spermidine into the inner coordination sphere. As follows from comparison of the equilibrium constant of formation of Cu(Ser-P)H_4(Spd) log K_e = 7.78 with that of formation of Cu(Ser-P)H(Put) log K_e = 6.10 (in which only one nitrogen atom from Put is involved in the coordination and no noncovalent interaction was detected) and that of Cu(Asp)H(tn) log K_e = 7.10 (in which there is a noncovalent interaction between the complexed ligands—tn coordinated *via* 1N atom), the deprotonated amine group of spermidine participates in the coordination, while the protonated fragments of spermidine are engaged in weak intramolecular interaction with phosphoserine. As established earlier, involvement of the second nitrogen atom from Spd in the coordination leads to a much greater increase in the equilibrium constant (log K_e = 9.68 for Cu(tn)[20]).

Above pH 6.0, the formation of Cu(Ser-P)H_2(Spd) and Cu(Ser-P)H(Spd) complexes takes place, Fig. 5. The spectral parameters of both species, Table 2, suggest the {N2,O_x} chromophore. The addition of the amine to the binary system Cu/Ser-P results in the shift of the absorption band towards lower energies by about 75 nm, which means that one nitrogen atom of Spd is involved in the coordination. The log K_e = 7.47 value for CuH(Ser-P)(Spd) confirms the Spd coordination and its involvement in noncovalent interaction, like in CuH_4(Ser-P)(Spd). For CuH_2(Ser-P)(Spd) the value of log K_e is 5.68, so it is lower, which suggests the lack of weak interactions between the molecules of this species (similarly as observed in Cu(Ser-P)H(Put) log K_e = 5.55). Changes in the chemical shifts in the NMR spectra, Table 3, confirm the conclusions drawn from the spectral and equilibrium study on the mode of coordination and thus confirm the presence of weak interactions between the protonated NH_x^+ groups from the amine and the phosphate group from phosphoserine.

Cu/Ser-P/Spm system

According to the potentiometric measurements analysed by a computer program, in the system with the longest polyamine studied, spermine, the complexes Cu(Ser-P)H_x(Spm) with x = 2–6 are formed; the values of the overall stability constants of the complexes are in Table 1.

The six-proton complex Cu(Ser-P)H_6(Spm) is present in the system at the start of the potentiometric measurements, Fig. 7, and binds all copper ions introduced into the system.

The Vis and EPR results obtained for this complex (λ_{max} = 715 nm, $g_{\|}$ = 2.319 and $A_{\|}$ = 165 × 10^{-4} cm^{-1}, Table 2) indicate the formation of a species with the oxygen chromophore {O_x}. Changes in the chemical shift of the signals positions in the ^{13}C and ^{31}P NMR spectra show that the main coordination site of copper ion is the phosphate group from phosphoserine. Relatively smaller changes in the chemical shift of carbon atom C(3) of Ser-P and carbon atoms of spermine, Table 3, prove the noncovalent interaction of spermine with the carboxyl group of the phosphoserine complex, so the formation of molecular complex Cu(H_2Ser-P)|||||H_4Spm. Similarly as for putrescine and spermidine at low pH, addition of spermidine to the system Cu/Ser-P does not change the position of the absorption band λ_{max}, so the amine is outside the inner coordination sphere, Fig. 8.

Because the Cu(Ser-P)H_5(Spm) complex occurs in the pH range of domination of Cu(Ser-P)H_6(Spm) and Cu(Ser-P)H_4(Spm), Fig. 7, determination of the mode of interaction in this species is impossible.

The Cu(Ser-P)H_4(Spm) complex dominates at pH close to 6.5, Fig. 7, and spectral parameters for this species, Table 2, suggest that besides oxygen atoms there is one nitrogen atom in the inner coordination sphere—{N1,O_x} chromophore.

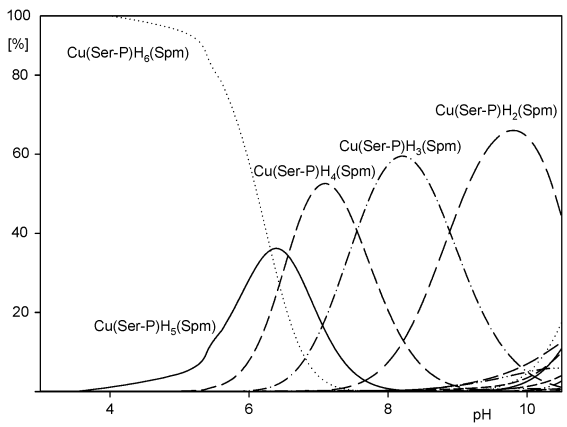

Fig. 7 Distribution diagram for the Cu/Ser-P/Spm system (for the sake of simplicity description of the binary complexes was omitted). The percent of the species refers to the relative concentration of the metal c_{Cu} = 0.002 M.

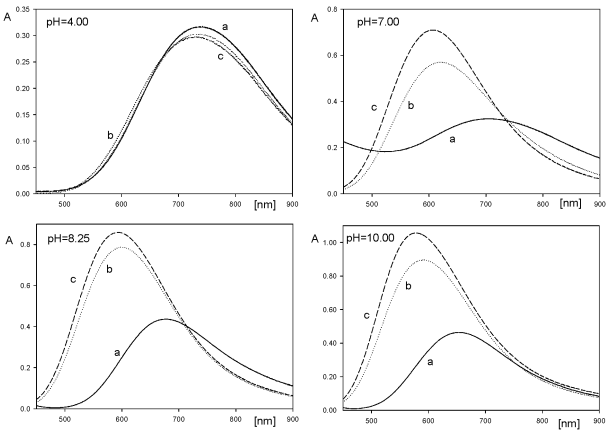

Fig. 8 Vis spectra taken in pH range of complexes domination; $C_{Cu(II)}$ = 0.01 M: (a) Cu(II)/Ser-P 1 : 1 system, (b) Cu(II)/Ser-P/Spm 1 : 1 : 1 system, (c) Cu(II)/Ser-P/Spm 1 : 1 : 5 system.

Addition of spermine to Cu/Ser-P at pH = 6.5, λ_{max} = 700 nm, shifted the maximum absorption wavelength by about 60 nm, Fig. 8, which means that the amine is coordinated by one nitrogen atom. Relatively high log K_e of formation of Cu(Ser-P)H_4(Spm) indicates that spermine is involved in the inner coordination sphere and that there is weak interaction between the protonated amine groups of spermidine and the complexed phosphoserine Cu(HSer-P) + H_3Spm ⇆ CuH_4(Ser-P)(Spm); log K_e = 10.0. Spectral results of the discussed CuH_4(Ser-P)(Spm) complex implied a chromophore {N1,O_x} and exclude the participation of two nitrogen atoms in the coordination. Therefore, this high log K_e can be explained by assuming that the complex is formed as a result of another process, *e.g.* Cu(H_2Ser-P) + H_2Spm ⇆ CuH_4(Ser-P)(Spm). Calculating the value of log K_e for this reaction is impossible.

Above pH 7.0 the formation of Cu(Ser-P)H_3(Spm) and Cu(Ser-P)H_2(Spm) was detected. The spectral parameters, Table 2, obtained for both complexes imply the presence of {N2,O} chromophore. Addition of spermidine to Cu/Ser-P causes a shift of the maximum absorption by about 65 nm towards lower energies, which indicates that one nitrogen atom of Spm is involved in the coordination. The log K_e values for the species 7.56 for Cu(Ser-P)H_3(Spm) and 7.78 for Cu(Ser-P)H_2(Spm) formed point to that besides the involvement in the coordination, spermine also participates in the noncovalent interactions (similarly as in Cu(Ser-P)H_3(Spd), log K_e = 7.78). Changes in the chemical shifts observed in the NMR spectra confirm the conclusions drawn on the equilibrium and spectral studies. In those species amine and carboxyl groups of phosphoserine and one amine group of spermine are involved in the coordination, and protonated –NH_x^+ groups are engaged in noncovalent interactions with the phosphate group of Ser-P.

Conclusions

Analysis of the above presented results permits concluding that in the systems Cu/Ser-P/PA the centres of complexation are the oxygen atoms from the carboxyl and phosphate groups and the nitrogen atom from the amine group of phosphoserine as well as the donor nitrogen atoms from the amine groups of the amines. The reaction centres change depending on pH. Below the physiological pH, *Ser-P* coordinates only *via* the phosphate group and with increasing basicity the main reaction centres become the amine and carboxyl groups. In all systems in low pH amine is outside the inner coordination sphere and is engaged in noncovalent interactions with the negatively charged carboxyl group of phosphoserine. Additional non-covalent interactions have been found to occur between the ligands. This type of interaction is different than that in the system without metal. The presence of Cu(II) ions in the system was shown to influence the character of the noncovalent interactions of the bioligands. Copper ion coordinated to the phosphate group of Ser-P and blocked this part of Ser-P to interact with the amines. In the ternary systems with Cu(II) ions the formation of molecular complexes of the ML···L′ type was established (where L = Ser-P, L′ = PA). On the other hand, totally protonated PA with the active centres blocked for metallation was concluded to be in the outer coordination sphere and interacts noncovalently with the binary species Cu(HSer-P). Copper(II) was found to bind the phosphate group from the phosphorylated amino acid and block it as a centre of noncovalent interactions. In the Cu(Ser-P)H_x(PA) complex the presence of the ligand–ligand intramolecular interactions, additionally stabilising the complex, was established.

Experimental section

Materials

O-Phospho L-serine (Ser-P) as well as putrescine, spermidine and spermine were purchased from Sigma and were used without further purification. Polyamine (PA) nitrates were prepared by dissolving a proper amount of free amine and addition of an equimolar amount of HNO_3. The precipitate obtained was recrystallised, washed out with methanol and dried in desiccator over P_4O_{10}. The nitrate salts were checked by elemental analysis whose results (%C, %N, %H) were in agreement with the theoretically calculated values (±0.5%). The elemental analysis was performed on an Elemental Analyzer CHN 2400, Perkin–Elmer. Copper(II) nitrate(V) from Merck was purified by recrystallisation from water. The concentration of copper(II) ions was determined by the method of inductively coupled plasma optical emission spectrometry (ICP OES). All solutions and experiments were prepared using demineralised carbonate-free water.

Equilibrium study

The potentiometric titrations were carried out using Titrino 702 Metrohm equipped with an autoburette with a combined glass electrode—ROSS Ultra Orion calibrated in terms of H^+ concentration prior to each titration.[21] Prior to each series of measurements, the pH-meter indication was corrected with the help of two standard buffer solutions of pH 4.002 and pH 9.225. All potentiometric titrations were made in the atmosphere of neutral gas (helium), at the constant ionic strength of μ = 0.1 M (KNO_3), temperature of (20 ± 1) °C (titration dish placed in thermostatic bath set at this temperature), in the pH range from 2.5 to 10.5, using as a titrant CO_2-free

NaOH at a concentration of 0.171 M. The concentrations of phosphoserine and polyamines were 2×10^{-3} M, and the metal to ligands ratio was 1 : 1 : 1. The selection of the model as well as the determination of the stability constants of the complexes were made using the HYPERQUAD program which uses the nonlinear method of least squares[22] (determined ionic products for water was $pK_w = 13.89$). The calculations were performed using 150–350 points for each job, taking into account only that part of titration curves corresponding to the conditions in which no precipitate appeared in the solutions. The hydrolysis constants for copper(II) ion were taken from our previous publication.[14] In all cases the testing began with the simplest hypothesis and then in the following steps the models were expanded to include progressively more species, and the results were scrutinized to eliminate those species that were rejected by the refinement processes. The model assumed was verified by analysis of the standard deviations, the convergence of the experimental data with those obtained for the model evaluated by the Hamilton test and chi squared test.[23] The distribution of particular forms was obtained by the HALTFALL program.[24]

NMR measurements

Samples for ^{13}C and ^{31}P NMR studies were obtained by dissolving the relevant species (*Ser-P*, *PA* and copper salt) in D_2O. The pH values of the samples were corrected according to the formula $pD = pH_{meter\ readings} + 0.4$[25] adjusted by NaOD and DCl. The concentration of ligands for NMR study was in the range from 0.05 M to 0.1 M and molar ratio of M : L : L′ was 1 : 75 : 75. The NMR spectra were recorded on NMR Gemini 300VT VARIAN spectrometer, using dioxan as an internal standard for ^{13}C NMR and ortho-phosphoric acid as an external standard for ^{31}P NMR measurements. The positions of ^{13}C NMR signals were given in the TMS scale. The changes in the chemical shifts were calculated for the ligands in the investigated systems in relation to the free ligands in the same pH [ppm scale].

Visible (Vis) spectroscopy

Samples for visible studies were prepared in H_2O at the M : L : L′ ratio 1 : 1 : 1 and concentration of Cu(II) ion was 0.05 M. The spectra were recorded at 20 °C in PLASTIBRAND PMMA cell with 1 cm path length on Evolution 300 UV-VIS ThermoFisher Scientific spectrometer equipped with a xenon lamp (range 450–950 nm, accuracy 0.2 nm, sweep rate 120 nm min^{-1}).

EPR spectroscopy

EPR studies were carried out at −196 °C, using glass capillary tubes (volume 130 μm^3). The concentration of Cu(II) was 0.005 M in water : glycol mixture (3 : 1), and the M : L : L′ ratio was 1 : 1 : 1. The spectra were recorded on an SE/X 2547 Radiopan instrument.

Abbreviations

Ser-P	Phosphoserine
Put	Putrescine
Spd	Spermidine
Spm	Spermine
PA	Polyamine
TMS	Tetramethylsilane

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