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ORIGINAL PAPER

Heteronuclear complexes of phosphocreatine with copper(II) and magnesium(II) ions

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Abstract It has been found that in the binary systems Mg(II)/phosphocreatine and Cu(II)/phosphocreatine at low pH, metal ions mainly coordinate via the phosphate group. At higher pH for copper(II) system, the efficiency of $-PO_3^{2-}$ group is low and coordination takes place via amine and carboxyl groups (Mg^{2+} is still coordinated to phosphate group). Differences in the coordination mode for copper and magnesium ions in binary systems at high pH suggested that it would be interesting to determine the mode of coordination in heteronuclear complexes and the coordination competitiveness of metal ions towards phosphate group at low and at physiological pH. The formation of heteronuclear complexes of phosphocreatine with Mg^{2+} and Cu^{2+} ions was investigated. The overall stability constants of these species were determined by the potentiometric studies and the coordination mode was concluded on the basis of several spectral investigations (EPR, Visible Spectroscopy, 2D ^{1}H-^{15}N NMR, ^{31}P NMR and FT-IR).

Keywords Heteronuclear complexes · Phosphocreatine · Copper and magnesium complexes · Stability constants · Coordination mode

Introduction

Phosphocreatine is a well-known naturally occurring substance. It is essentially distributed within the skeleton muscle of vertebrates. Phosphocreatine is formed in the body by phosphorylation of creatine. Both biomolecules, phosphocreatine and creatine, play very important roles in the energetical processes. Phosphocreatine is accumulated in muscles and used up in periods of physical effort. The concentration of PCr in muscles depends on the ATP and total creatine concentration [1]. Phosphocreatine molecules regenerate ATP cellular reserves during ischemia. It is known that alkali metal salts of phosphocreatine have found pharmacological and therapeutical use in pathologic conditions of striated musculature (muscular atrophy and dystrophy), and also of heart musculature (myocardial sclerosis, degenerative myocardiopathies and anoxies in which the myocardial contractility must be restored as quickly as possible) [2]. On the other hand, phosphocreatine promoted recovery of ischemic heart tissue in vitro and reduced arrhythmias in patients with acute myocardial infarctions [3–6]. Magnesium complexes of creatine as well as phosphocreatine increase the latency to population spike disappearance and significantly reduce neuronal hyperexcitability during anoxia. Exogenous phosphocreatine and N-amidino piperidine are not useful for brain protection, while chelates of both creatine and phosphocreatine do replicate some of the known protective effects of creatine. The amount of brain phosphocreatine is substantially increased by treatment with creatine [7, 8], and it has been repeatedly shown that creatine pretreatment protects against anoxic damage in vivo [9–14]. Elevating cytosolic PCr with exogenous supplementation could provide the extra energy needed to restore ionic homeostasis following injury [5].

Copper is a trace metal in human organism which definitely plays an important role (for example as electron transport and antioxidant defence). Copper is essential to sustain life but toxic accumulation of copper can be deleterious to human health [15, 16]. The majority of Cu^{2+} ions in ceruloplasmin are in the form of mixed complexes with

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amino acids, peptides, and other biomolecules [17]. Understanding of the complexation processes of biomolecules is important for explanation of the role of metals in living organisms [18–21].

Magnesium is one of four metals belonging to macroelements in the body. The main function of Mg^{2+} ion was traditionally assumed to coordinate reagents, to keep them along the reaction pathway, and perhaps to slightly modify their chemical reactivity by complexation accompanied by redistribution of charges in a complex. The Mg^{2+} ion has always been considered as an assistant, and it has never been assumed that the ion participates directly in the phosphorylation reaction as a reagent [22–24]. To understand the role of phosphorylation in biological processes, it is essential to characterise the site at which it occurs.

This paper presents results of potentiometric and spectral studies of phosphocreatine complexes formation in the heteronuclear system. The phosphorylated creatine has three potential coordination sites (carboxyl group, phosphate group and guanidine group). Identification of the role of magnesium as an agent interfering in the double system copper/phosphocreatine seems an important problem which solution will allow explanation of the competitiveness of metal ions in living organisms at the molecular level.

Experimental

Phosphocreatine disodium salt hydrate enzymatic (PCr) was purchased from Sigma-Aldrich. Copper(II) nitrate and magnesium(II) nitrate from Merck were used without purification. The concentration of copper and magnesium ions was determined by the method of inductively coupled plasma optical emission spectrometry (ICP OES). The potentiometric measurements were carried out using Titrino 702 Metrohm (calibrated daily on the two buffers pH = 4.00 and pH = 9.22) equipped with an autoburette with a glass electrode (Metrohm 6.233.100). The electrode was calibrated daily according to a literature method before each series of study [25]. All potentiometric titrations were made under helium atmosphere, at the constant ionic strength $\mu = 0.1$ M (KNO_3), temperature (20 ± 1) °C, using as a titrant CO_2-free NaOH at a concentration of 0.183 M. The concentration of phosphocreatine was 0.002 M, and the metal to ligand ratios used were 2:1, 1:1 and 1:2 in the binary systems, and 1:1:1 in the ternary system. All solutions were prepared using fresh deionised (conductivity below 0.055 μS/cm) carbonate-free water. The protonation constants of the ligands and stability constants of the complexes were determined using the HYPERQUAD program [26] (using a value $pK_w = 13.83$). The stability constants were evaluated for the equilibria:

$$pM + qH + r(PCr) \rightleftarrows M_pH_q(PCr)_r$$

where M = Cu or Mg (charges were omitted for simplicity)

and calculated in the following equation:

$$\log\beta = \left[M_pH_qPCr_r\right]/[M]^p[H]^q[PCr]^r.$$

The calculations were performed using 150–350 points for each job taking into consideration only those parts of titration curves in which there was no precipitate in solutions. The correctness of the model was confirmed by verification of the results obtained [27]. The distribution of particular forms was performed by the HYSS (Hyperquad Simulation and Speciation) program [28].

The Visible and EPR Spectroscopy measurements were made for the systems containing copper ions. The samples for visible spectroscopic studies were performed in H_2O at the Cu:PCr ratio 1:1 and 1:2 for the binary system and 1:1:1 for the ternary system (Cu(II) concentration was 0.05 M) (Table 1). The spectra were recorded at 20 °C in PLASTIBRAND PMMA cell with 1 cm path length on an 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). EPR spectra were recorded on an SE/X 2547 Radiopan instrument at −196.15 °C, using glass capillary tubes. The concentration of Cu(II) was 0.005 M in water:glycol (3:1) mixture, and the Cu:PCr ratio was 1:1 and 1:2 and for Cu:Mg:PCr ratio was 1:1:1.

Measurements of ^{31}P, 2D ^{1}H-^{15}N NMR and FT-IR were performed in D_2O, and the pD of the solution was adjusted using NaOD or DCl, taking into account that $pD = pH_{readings} + 0.40$ [29]. The concentration of the ligands and magnesium ions used for NMR measurements was 0.1 M, while their concentration for the system including Cu^{2+} was 75 times smaller. Nuclear Magnetic Resonance spectra were recorded on Gemini 300 Varian and Bruker Advance 600 MHz spectrometers using orthophosphoric acid as an internal standard for ^{31}P NMR spectra. The 2D ^{1}H-^{15}N NMR spectra were recorded by the HETCOR—long range method. The ligand concentration for the IR studies was 0.5 or 0.25 M and the ratio of M:L concentrations varied from 2:1, 1:1 and 1:2 and 1:1:1 for ternary system. The infrared spectra were taken on an IFS 113v FT-IR Bruker spectrophotometer equipped with a DTGS detector; resolution 2 cm^{-1}. A cell with Si windows (thickness 100 μm) was used.

Results and discussion

Phosphocreatine (Fig. 1) has four sites of protonation assigned to dissociation of the first proton from $–POH_2$

Table 1 Concentration of each species for methods used in the paper

	C_{PCr}	c_{Cu}	c_{Mg}	pH
Cu/PCr				
Potentiometry	0.002 M	0.001–0.004 M	–	2.5–11.0
UV–vis	0.05–0.1 M	0.05 M	–	5.0; 6.5; 9.5
EPR	0.005–0.01 M	0.005 M	–	5.0; 6.5; 9.5; 10.0
IR	0.25–0.5 M	0.25–0.5 M	–	4.0
NMR	0.1 M	0.0013 M	–	4.0; 6.5
Mg/PCr				
Potentiometry	0.002 M	–	0.001–0.004 M	2.5–11.0
IR	0.25–0.5 M	–	0.25–0.5 M	4.0; 7.0
NMR	0.1 M	–	0.1 M	7.0
Cu/Mg/PCr				
Potentiometry	0.002 M	0.002 M	0.002 M	2.5–11.0
UV–vis	0.05 M	0.05 M	0.05 M	5.0; 7.0
EPR	0.005 M	0.005 M	0.005 M	5.0; 7.0
IR	0.5 M	0.5 M	0.5 M	4.0
NMR	0.1 M	0.0013 M	0.1 M	7.0

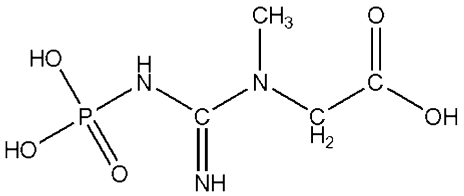

Fig. 1 The scheme of phosphocreatine in the totally protonated form

group, carboxyl group, the second proton from the phosphate group and guanidine. Four protonation constants were determined for phosphocreatine only by NMR methods [30, 31] and as yet two of them have been determined by potentiometric methods (deprotonation takes place at relatively low pH).

Binary systems

Although the protonation constants as well as stability constants of copper and magnesium complexes can be found in literature, they were refined in this paper in the same experimental condition and are consistent with the literature data [30–33]. The values of successive protonation constants: $\log K_1 = 10.14$, $\log K_2 = 4.94$ determined for PCr from the computer analysis of titration data correspond to the protonation of the guanidine group and the second site at the phosphate group, respectively. The overall and successive protonation constants of phosphocreatine are given in Table 2.

Computer analysis of the potentiometric titration data for the system M/PCr ($M = Cu^{2+}$ or Mg^{2+}) of the metal–ligand ratio 2:1, 1:1 and 1:2 revealed the formation of M(HPCr) as well as M(PCr) complexes. Formation of Cu(PCr)(OH) and $Cu(PCr)_2$ complexes in the system Cu/PCr system was also found (only at the copper: phosphocreatine 2:1 ratio) (Table 2). Computer analysis of the potentiometric titration data was performed taking into regard the protonation constants of PCr and the constants for Cu(II) and Mg(II) hydrolysis ($\log\beta = -14.15$ for $Cu(OH)_2$ and $\log\beta = -10.93$ (2) for Mg(OH)) [34].

The coordination mode in the complexes formed in the binary copper systems was concluded on the basis of analysis of d–d energy transitions in the Vis spectra (characteristic of the d^9 configuration of Cu^{2+}), $g_{\|}$ as well as $A_{\|}$ values in EPR study (taking into account the relation of these values to the type and number of coordinated donor atoms [35–37]). Replacement of oxygen atoms from water molecules by the electron donor atoms of oxygen or nitrogen from the coordinated ligand in tetragonal geometry of a copper ion results in a shift of the d–d band towards lower energies (for nitrogen atoms the changes are more pronounced—λ_{max} for Cu^{2+} is about 815 nm, for 1O 800 nm, for 2O 750–770 nm, for 4O 735 nm and for 1 N coordination is 700 nm; for O,N the d–d maximum is between 720 and 700 nm). The conclusions were supported by the analysis of changes in the chemical shifts in the ^{31}P and 2D ^{1}H-^{15}N NMR spectra of the free ligand and the ligand in the complexes taking into regard our experience and critical analysis of the results for paramagnetic ions as well as IR spectra [38–41].

The protonated complexes Cu(HPCr) and Mg(HPCr) start forming in the solution at about pH 2.0 and 3.0, respectively. The protonated forms of the first complex dominate at pH close to 5.00 and bind 45 % of copper ion in the solution, while those of the second one dominate at

Table 2 Overall and successive stability as well as equilibrium constants of binary and ternary complexes and protonation constants of phosphocreatine (standard deviation are given in parenthesis)

Species*	Overall protonation constants log β	Reactions	Successive protonation constants log $K_{1\text{-}3}$
H(PCr)	10.14 (2) 11.04 [Ref. 28]	$PCr + H^+ \leftrightarrows H(PCr)$	10.14
H_2(PCr)	15.08 (3) 15.29 [Ref. 28]	$H(PCr) + H^+ \leftrightarrows H_2(PCr)$	4.94

Species	Overall stability constants log β	Reactions	Equilibrium constants log K_e
Cu(HPCr)	13.24 (3) 14.41 [Ref. 28]	$Cu^{2+} + HPCr \leftrightarrows Cu(HPCr)$	3.10
Cu(PCr)	7.92 (4) 7.89 [Ref. 28]	$Cu^{2+} + PCr \leftrightarrows Cu(PCr)$	7.92
Cu(PCr)(OH)	0.71 (3) 1.97 [Ref. 28]	$Cu^{2+} + PCr + H_2O \leftrightarrows Cu(PCr)(OH) + H^+$	14.54
$Cu(PCr)_2$	11.94 (5) not detected	$Cu^{2+} + 2PCr \leftrightarrows Cu(PCr)_2$	11.94
Mg(HPCr)	13.83 (2)	$Mg^{2+} + HPCr \leftrightarrows Mg(HPCr)$	3.69
Mg(PCr)	4.98 (3)	$Mg^{2+} + PCr \leftrightarrows Mg(PCr)$	4.98
CuMg(HPCr)	18.17 (3)	$Cu^{2+} + Mg(HPCr) \leftrightarrows CuMg(HPCr)$	4.34
CuMg(PCr)	13.11 (2)	$Cu^{2+} + Mg(PCr) \leftrightarrows CuMg(PCr)$	8.13

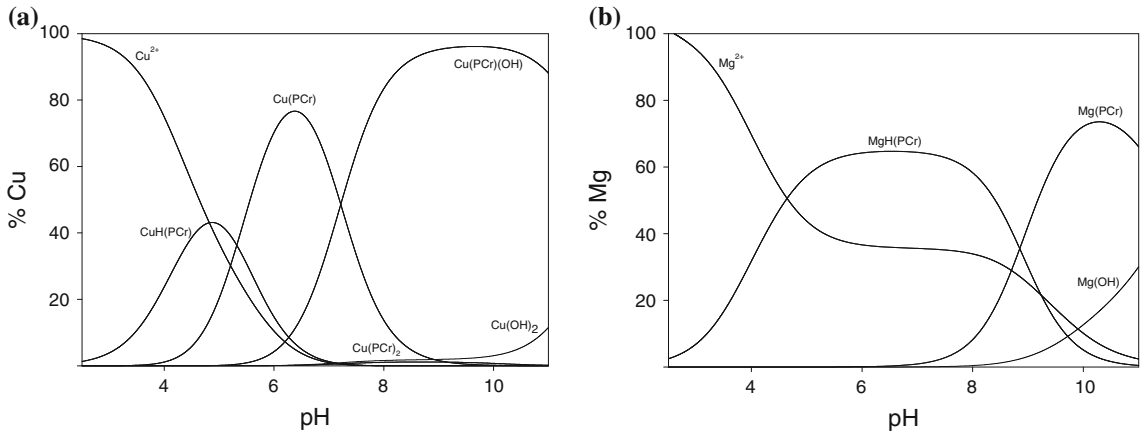

Fig. 2 HYSS distribution form created in M:PCr systems: **a** Cu:PCr; **b** Mg:PCr, as a function of pH

Table 3 Spectral parameters for binary and ternary complexes with Cu^{2+} ions

Complex	pH	λ_{max} [nm]	ε [$dm^3\ mol^{-1}\ cm^{-1}$]	$g_{\parallel}$	$A_{\parallel}$ [$10^{-4}\ cm^{-1}$]	Chromophore
Cu(HPCr)	5.0	787	17.6	2.357	154	{2O}
Cu(PCr)	6.5	715	54.8	2.307	164	{1 N,xO}
Cu(PCr)(OH)	9.5	685	55.0	2.273	163	{1 N,xO}
$Cu(PCr)_2$	10.0	–	–	2.265	171	–
MgCu(HPCr)	4.0	795	16.1	2.378	148	{1O}
MgCu(PCr)	7.0	689	28.3	2.251	175	{1 N,xO}

pH 5.5–8.0 and bind 60 % of magnesium ion (Fig. 2). The equilibrium constants of formation of M(HPCr) are 3.69 for Mg(II) and 3.10 for Cu(II). Close logK_e values for the two complexes indicate their similar stability and suggest a similar mode of coordination.

$$M^{2+} + HPCr \rightleftarrows M(HPCr) \qquad \log K_{eMg(HPCr)} = 3.69$$
$$\log K_{eCu(HPCr)} = 3.10$$

The Vis and EPR spectral data for Cu(HPCr) complex ($\lambda_{max} = 787$ nm, $g_{\parallel} = 2.357$ and $A_{\parallel} = 154 \cdot 10^{-4}\ cm^{-1}$, Table 3) indicate that only oxygen atoms are engaged in coordination [37].

Analysis of changes in the chemical shifts in the ^{31}P NMR spectra (taken in the pH range of complex domination) indicates that the main site of metalation in both

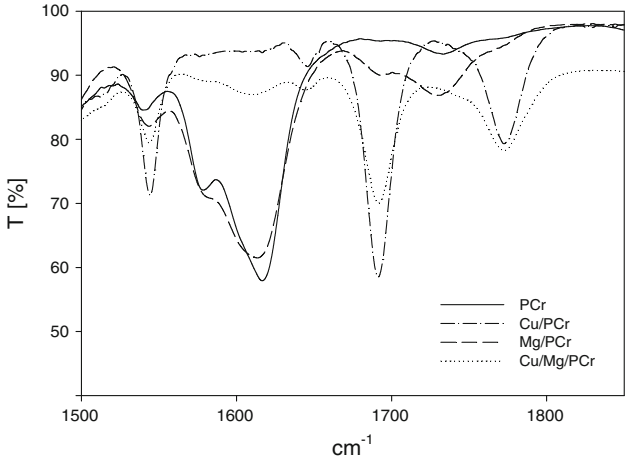

Fig. 3 IR spectra of PCr, M/PCr and Cu/Mg/PCr systems at pH = 4.0

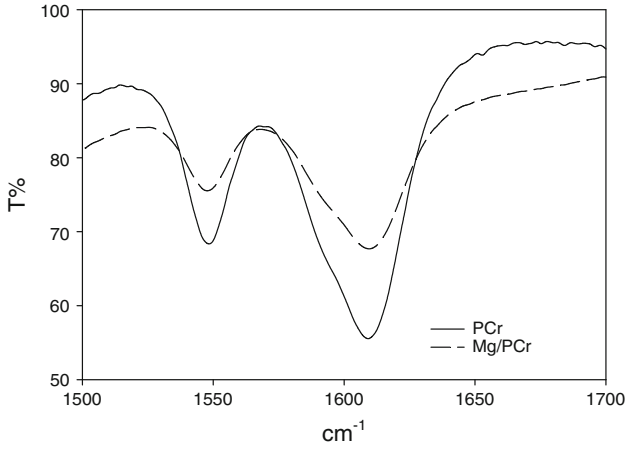

Fig. 4 IR spectra of PCr and Mg/PCr systems at pH = 7.0

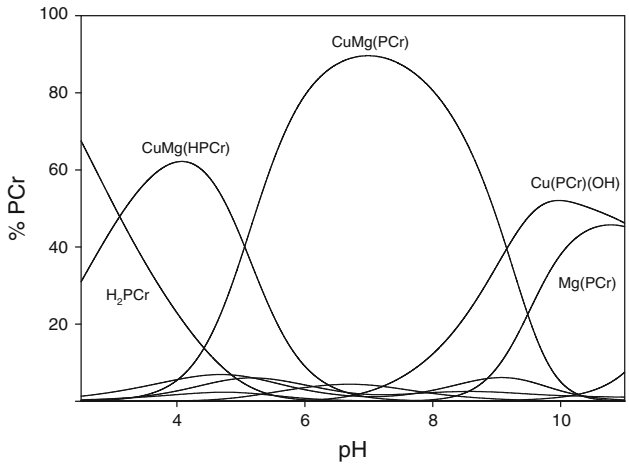

Fig. 5 HYSS distribution form created in Cu/Mg/PCr system as a function of pH

protonated complexes is the phosphate group (+0.51 ppm and −0.39 ppm for Cu(HPCr) and Mg(HPCr) respectively). In addition, the participation of the carboxyl group in metalation was checked by the IR measurements. For Cu(HPCr) complex, the asymmetric stretching band $\nu_{asC\text{-}O}$ assigned to $-COO^-$ group was shifted from 1,625 to 1,682 cm^{-1} and for Mg(HPCr), the asymmetric stretching bands were not shifted (position of the band 1,624 cm^{-1}), which means that this group in Mg(HPCr) is not involved in coordination (Fig. 3). Observation of changes in the bands in the far infrared assigned to the M–O and M–N bonds was impossible because of the conditions of the IR experiments. For M(HPCr) complexes, the participation of guanidine group was excluded on the grounds of comparative analysis of 2D ^1H-^{15}N NMR spectra of the free ligand and the complex, which for Cu(HPCr) confirmed the conclusions drawn from Visible and EPR Spectroscopy measurements (no nitrogen atoms in the inner coordination sphere of copper). A slightly higher $\log K_e$ for Mg(HPCr), 3.69, than for Cu(HPCr), 3.10, confirms the fact that magnesium shows higher affinity to oxygen and the fact that the macrochelate forming in copper complex slightly decreases the stability.

In parallel with the total deprotonation of phosphocreatine (deprotonation of the nitrogen from guanidine), simple ML type complexes start to form (Fig. 2). A comparison of $\log K_e = 4.98$ of Mg(PCr) with that of the protonated complex $\log K_{Mg(HPCr)} = 3.69$ shows that the nitrogen atom deprotonation enhances the complex stability. Changes in the chemical shifts in the ^{31}P NMR spectra of Mg(PCr) relative to the spectrum of the free phosphocreatine at the same pH values −0.35 ppm indicate that Mg^{2+} ion is linked to the phosphate group. Similarly as in Mg(HPCr) complex, the involvement of carboxyl group was excluded on the basis of IR study (characteristic band of carboxyl group was not shift, Fig. 4). In addition, the 2D ^1H-^{15}N NMR spectra excluded the participation of nitrogen from guanidine group in Mg(PCr) as no changes in the spectra of the free phosphocreatine and the complexes were noted. The Vis and EPR results obtained for Cu(PCr) ($\lambda_{max} = 715$ nm, $g_{\|} = 2.307$, $A_{\|} = 164 \quad 10^{-4}$ cm^{-1}) indicate that the inner coordination sphere besides oxygen atoms includes the nitrogen atom. This observation for copper complex was confirmed by comparison of 2D ^1H-^{15}N NMR spectra (no changes). Deprotonation of the nitrogen atom of guanidine leads to its unblocking and its incorporation into the inner coordination sphere of Cu^{2+}, which increases the complex stability (the equilibrium constant of formation of Cu(PCr) ($\log K_e = 7.92$) much higher than that of Cu(HCrP) ($\log K_e = 3.10$)). Moreover, the changes in chemical shift observed in the ^{31}P NMR

spectrum (+0.17 ppm) confirm the participation of the phosphate group in the coordination. For Cu(PCr) complex, the precipitate appearing at the concentration required for IR study excluded this method for verification of participation of the carboxyl group in coordination. Possible formation of tridentate coordination was identified in the solid-state complex Cu(PCr)(H_2O) by Almeida et al.; however, the same authors have excluded this mode of coordination in solution on the basis of pK_e values analysis [32, 42].

In the system with Cu^{2+} at pH above 8.0 the Cu(PCr)OH complexes are dominated, while in the system with a twofold excess of phosphocreatine the $Cu(PCr)_2$ complex, so far not described in literature, is formed. According to the spectral analysis of these complexes, the inner coordination spheres of copper in both species contain O and N atoms and the mode of coordination is the same as in Cu(PCr) (Table 3). It should be emphasised that because of the pH range of formation, these complexes have not much significance in formation of heteronuclear species.

Heteronuclear system

Identification of the different mode of PCr coordination with copper and magnesium ions, especially at physiological pH, it seemed a natural consequence to perform potentiometric studies in the heteronuclear systems in order to estimate the competitiveness of metal ions towards the PCr donor group, especially the phosphate group. Computer analysis of the potentiometric measurements evidenced the formation of CuMg(HPCr) and CuMg(PCr) complexes in the system Cu/Mg/PCr. The reaction of heteronuclear complexes formation starts above pH of 2.5 with formation of the protonated complex (Fig. 5).

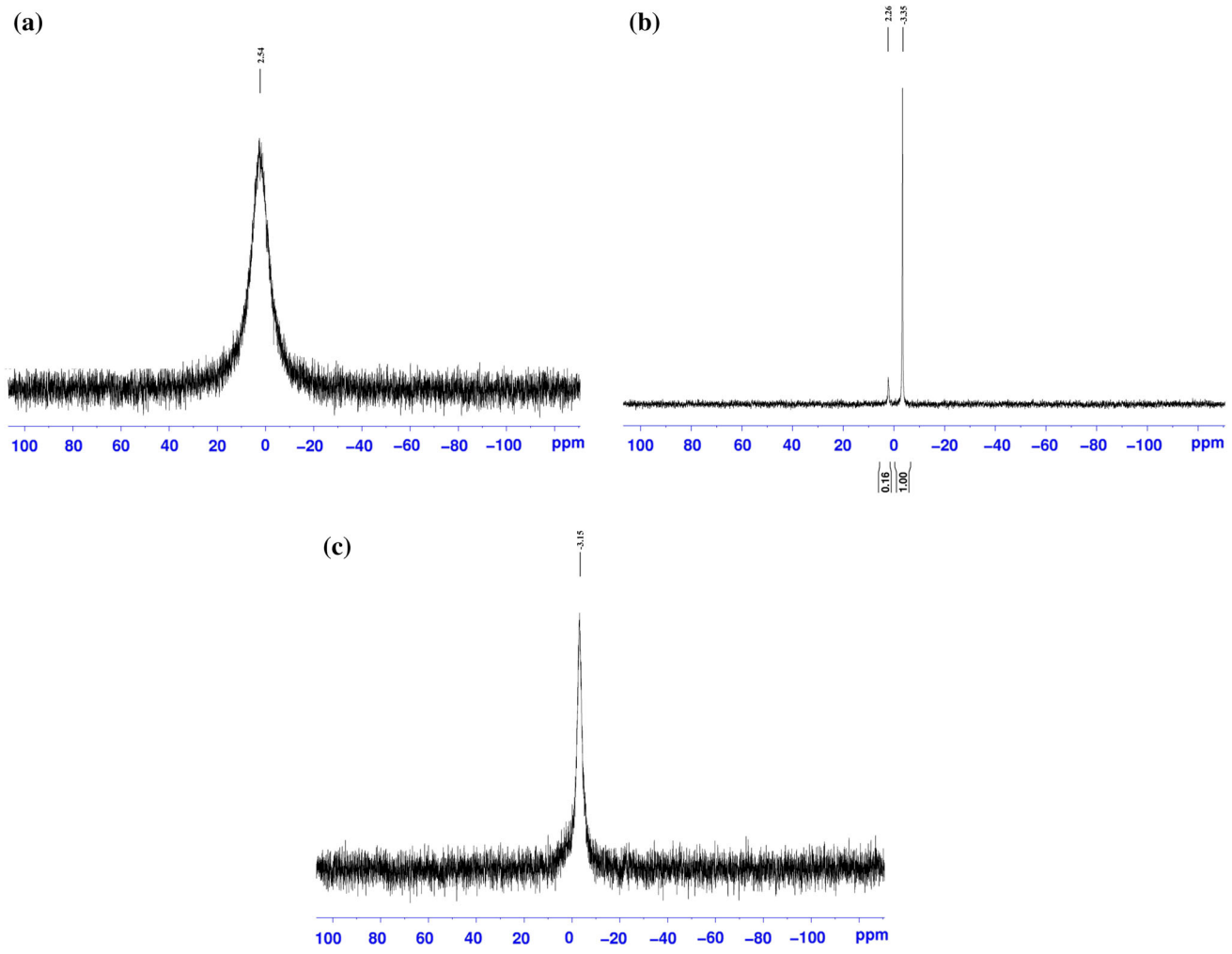

Fig. 6 ^{31}P NMR spectra of **a** Cu(HPCr); **b** Mg(HPCr); **c** CuMg(HPCr) complexes

Taking into regard that in the binary systems the Cu(HPCr) as well as Mg(HPCr) complexes formed in the same pH, and two reactions of CuMg(HPCr) forming are possible:

$$\mathrm{Cu(HPCr)} + \mathrm{Mg} \rightleftarrows \mathrm{CuMg(HPCr)} \quad \log K_e = 4.93$$
$$\mathrm{Mg(HPCr)} + \mathrm{Cu} \rightleftarrows \mathrm{CuMg(HPCr)} \quad \log K_e = 4.34$$

The Visible Spectroscopy and EPR results obtained for CuMg(HPCr) show that the inner coordination sphere of copper contains only one oxygen atom. Moreover, addition to the binary system Cu/PCr of magnesium ions in the equimolar amount as well as in excess leads to insignificant changes in the position of d–d band, from 789 to 795 nm, which points to a change in the coordination sphere of copper from {2O} to {1O} (Table 3).

As follows from analysis of ^{31}P NMR data, the phosphate group which is a centre of coordination in the binary systems for both copper and magnesium at low pH, in the heteronuclear system, binds only Mg^{2+} (Fig. 6).

Moreover, it was found that the carboxyl group from phosphocreatine takes part in the complex formation and the shifts of the asymmetric stretching bands assigned to $-COO^-$ group are the same as in the spectrum of the Cu(HPCr) complex (Fig. 3). According to our analysis, the introduction of Mg^{2+} ion into the binary system Cu(PCr) excluded Cu^{2+} ion from the phosphate group and caused changes in the coordination sphere of copper ion which is coordinated only through the carboxyl group. The participation of the nitrogen atom from guanidine was also excluded on the basis of 2D ^{1}H-^{15}N NMR results (no significant changes) (Fig. 7).

The binuclear complex CuMg(PCr) forms in solution from pH of about 3.5, and dominants at pH close to 7.0 binding almost 90 % of the phosphocreatine in solution (Fig. 5). Relatively high equilibrium constant of CuMg(PCr) formation, determined on the basis of the reaction: $\mathrm{Mg(PCr)} + \mathrm{Cu}^{2+} \rightleftarrows \mathrm{CuMg(PCr)}$, $\log K_e = 8.13$, indicates that copper ion is coordinated to phosphocreatine similarly as in the binary complex Cu(PCr), so through the nitrogen atom, coordination through the phosphate group would give much lower $\log K_{Cu(HPCr)}$ of 3.10, similarly as in the Cu(HPCr) complex. EPR spectral parameters and the position of d–d absorption band evidence that the inner coordination sphere includes one nitrogen atom and one oxygen atom,

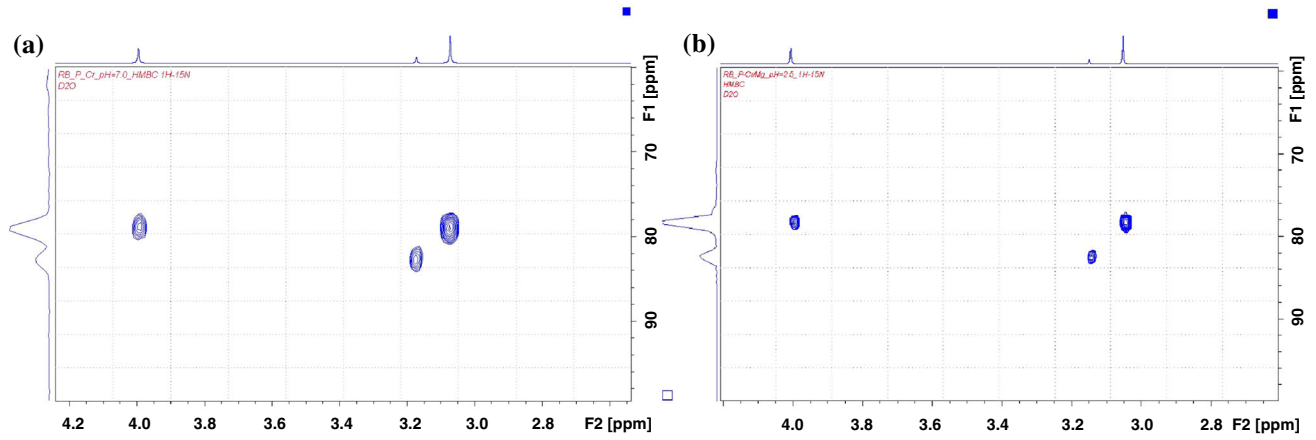

Fig. 7 2D ^{1}H-^{15}N NMR spectra of **a** PCr and **b** Cu/PCr systems at pH = 2.5

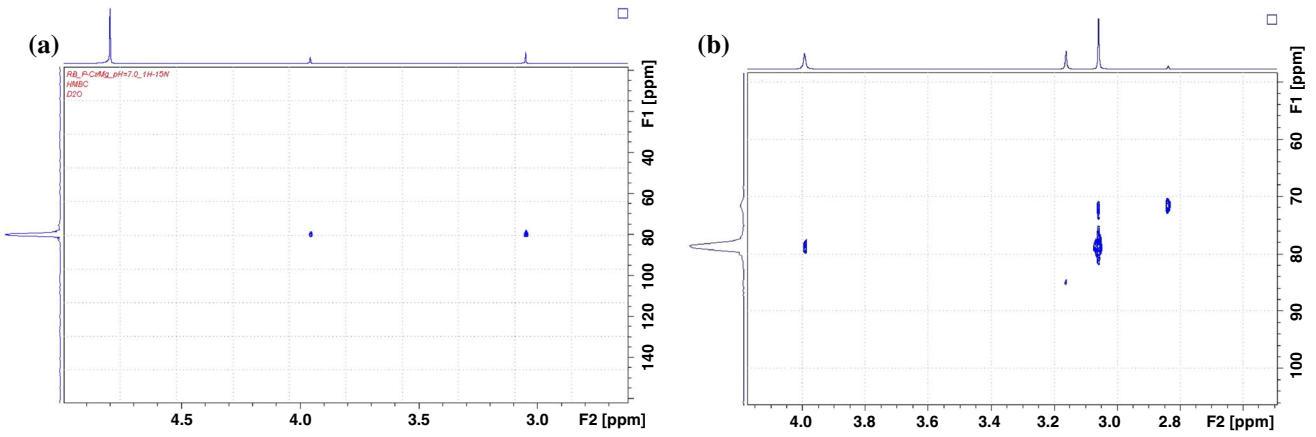

Fig. 8 2D ^{1}H-^{15}N NMR spectra of **a** PCr and **b** Cu/PCr systems at pH = 7.5

which means that as a result of Mg^{2+} ions introduction to Cu/PCr, one of the oxygen atoms is removed from the inner coordination sphere. The involvement of the phosphate group in the metalation is confirmed by the change in the signal position in the spectrum of ^{31}P NMR. The character of changes in the positions of signals assigned to the phosphate group suggests that this group is involved in the coordination with Mg^{2+} and not with the paramagnetic Cu^{2+} ion. In addition, changes in the 2D ^{1}H-^{15}N NMR spectrum indicate that the amino group is involved in the coordination (Fig. 8).

As the Visible Spectroscopy and EPR results confirmed the presence of oxygen in the inner coordination sphere of copper and NMR results excluded the involvement of oxygen atoms from the phosphate group from the coordination to Cu^{2+} ion, the conclusion is that the carboxyl group takes part in the complex formation. Similarly as in the protonated heteronuclear complex, the presence of Mg^{2+} ion in the system blocks the phosphate group and eliminates it from coordination of copper ion.

Conclusions

In all systems studied (binary and ternary ones), the formation of simple complexes and protonated ones was proved. As follows from analysis of the potentiometric and spectral results, in binary complexes with Mg^{2+} the main site of coordination in the entire pH range is only the phosphate group. In the binary system with copper ion, the Cu(HPCr) complex is a bidentate macrochelate in which the coordination involves the oxygen atoms from the phosphate and carboxyl groups. With deprotonation of the nitrogen atom from the guanidine group, this group becomes an active site of coordination of copper ion, which leads to formation of the macrochelate complex in which the copper ion is additionally coordinated through the phosphate and/or carboxylate group. High affinity of Mg^{2+} to the phosphate group is reflected in the heteronuclear complexes in which the magnesium ion drives out copper ion from the phosphate group. This tendency is observed in the whole pH range, which can be important, for example, during supplementation with magnesium preparations where excessive amounts of magnesium can disturb biochemical processes.

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