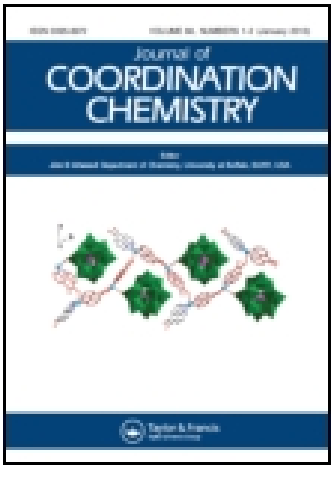

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Interactions of histone amino acid: lysine with copper(II) ions and adenosine 5′-triphosphate as well as in a metal-free system

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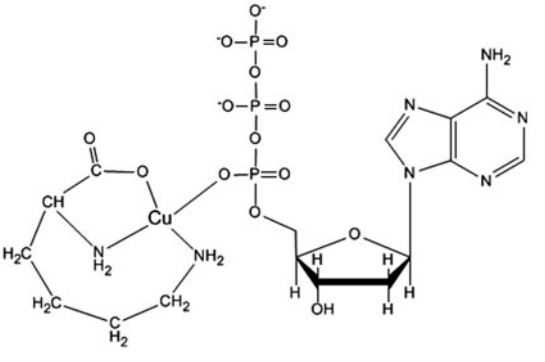

Thermodynamic stability and mode of coordination were studied in the binary system of copper(II)/lysine, ternary system of copper(II)/ATP/lysine, and compared to the intermolecular interaction in the metal-free ATP/lysine system. In the system ATP/Lys, groups P_β and P_γ from ATP, participate in the interaction with the amino acid, while group P_α from ATP is involved as reaction center only at high pH. The endocyclic nitrogens from the nucleotide N(1) and N(7) take part in the interactions only below physiological pH. In the binary complexes, oxygens from carboxylic group and nitrogen from the α-NH_2 amino group are involved in coordination. However, introduction of the second ligand, ATP, into the system Cu(II)/Lys changes the amino acid coordination in alkaline medium. In protonated mixed-ligand complexes, the α, β, and γ oxygens of the phosphate from ATP coordinate, but at higher pH, only α oxygens coordinate in Cu(ATP)(Lys). Although Cu(II) ions do not coordinate nitrogen from ATP in the ternary system, the presence of metal ions excludes interaction of these atoms over the whole pH range studied.

Keywords: Copper(II); Lysine; ATP; Complexes

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1. Introduction

Lysine belongs to the group of amino acids essential for living organisms which cannot be synthesized in these organisms and have to be supplied through diet. This amino acid has been used for the treatment of respiratory system diseases and can also be used in nutritional intervention for decreasing diarrhea morbidity and for improvement of the nutritional status of populations in some developing countries [1]. Treatment with lysine has been found to suppress the clinical symptoms of infection with herpes virus [2] and studies have shown that lysine benefits go beyond protein quality and growth [3]. Lysine is a strong inhibitor of collagen digestive enzymes spreading tumor cells in the tissues of living organisms [4–7]. This compound has two amine groups of positive charge at physiological pH and is incorporated into proteins strongly binding the negatively charged DNA helix fragments such as histones (components of nucleoprotein complex of chromatin). The histones bind to the polyanionic DNA helix to form electrically neutral nucleoproteins, which permit formation of specific third-order structures and gene packing. Particularly rich in lysine is the most alkaline and the largest of histones – H1, which is a linker histone stabilizing nucleosomes and responsible for condensation of chromatin. The histones incorporated in chromatin are subjected to post-translational modifications resulting in loosening of chromatin structure needed for DNA replication or transcription. Histone lysine methylation is of particular interest given the diverse set of methylation-associated biological processes, including transcriptional activation and repression, heterochromatin-mediated transcriptional silencing, the DNA damage response, and X chromosome inactivation [8–10]. Acetylation of lysine is essential in post-translational modification of histones as a direct regulator of structure and function of chromatin, stabilizer of proteins, or multifunctional factor acting not only on transcription but also on a variety of other cellular processes [11]. Reversible acetylation of lysines on the amino-terminal tails of nucleosomal histones is correlated in the chromatin structure and transcription [12]. Taking into account that the potential sites of lysine interaction with ATP are also the sites of coordination with biologically active metal ions, e.g. Cu(II), it is relevant to study such interactions. In the lysine molecule, the potential sites of interactions are oxygens from the carboxyl group and nitrogens from the α and ω amine groups, while in the ATP molecule such sites are oxygens from the α, β, and γ phosphates and endocyclic N(1) and N(7) from the purine ring of the nucleotide. For lysine, the mode of coordination with Cu(II) has not been fully resolved, in particular, the participation of the nitrogen from the ω-NH_2 [13–18]. Brubaker and Busch as well as Wilson *et al.* [19, 20] suggested that lysine coordination is glycine-like, while Conato *et al.* [13] suggested that lysine should be treated as a three-functional ligand, which means that the ω-NH_2 group is involved in coordination. Significant differences have also been found in descriptions of the system character, e.g. in the pH range at which a precipitate is formed which prevents analysis of results [18, 21], and this information is vital for the possibility of using potentiometric methods. Controversial also are the conclusions about participation of the endocyclic nitrogens N(1) and N(7) from the ATP purine ring in coordination. According to our results, the main donor centers in ATP are oxygens from phosphate, although N(7) has also been indicated as taking part in coordination [22, 23]. The question is if these two ligands compete for the copper(II) ions or if they cooperate in coordinating this ion. The aim of this study is an attempt to answer this question on the basis of a study of the complexation reaction in the binary system Cu(II)/Lys and in the ternary systems Cu(II)/ATP/Lys.

2. Materials and methods

L-lysine dihydrochloride (Lys) and adenosine 5′-triphosphate disodium salt (ATP) were purchased from Sigma and used without purification. Copper(II) nitrate purchased from POCH Gliwice (Poland) was twice recrystallized from water before use. The method of determination of the Cu(II) concentration in a parent solution of concentration about 0.17 M was described earlier [24, 25]. Potentiometric measurements were performed on a Methrom 702 SM Titrino with an auto-burette. A glass electrode Methrom 6.0233.100 was calibrated for hydrogen ion concentration [26] with a preliminary use of borax (pH 9.225) and phthalate (pH 4.002) standard buffers. The concentrations of ATP and Lys in the titrated systems were from 1×10^{-2} to 2.5×10^{-3} M. The concentration ratio of L : L′ (L = ATP and L′ = Lys) in the samples studied was 1 : 1 and the ratio of Cu : L : L′ was 1 : 1 : 1 to 1 : 2.5 : 2.5 in the ternary systems. The measurements were performed under helium at constant ionic strength of $\mu = 0.1$ M (KNO_3), at $T = 20 \pm 1$ °C, using CO_2 free solution of NaOH as a titrant. Addition of a NaOH solution did not change the ionic strength, because the measurements were performed, starting from fully protonated polyamines, such that $-NH_x^+$ cations were replaced by equivalent amounts of Na^+. Selection of the models and determination of the stability constants of the complexes were done using the HYPERQUAD computer program [27]. The calculations were done using 100–350 points for each evaluation. The titrations were performed up to a pH of 10.5. Species distribution curves were determined by the HALTAFALL computer program [28]. The criteria of model verification were described earlier [29]. The samples for ^{13}C and ^{31}P NMR studies were prepared by dissolving appropriate amounts of the ligands and $Cu(NO_3)_2$ in D_2O. DNO_3 and NaOD were used to adjust the pD of the solutions, correcting pH-readings (a pH meter CP-501 made by ELMETRON and electrode HAMILTON SLIMTRODE) according to the formula pD = pH-meter readings + 0.40 [30]. The concentration ratio of Cu(II) to Lys and Cu(II) to ATP and Lys was 1 : 100 and 1 : 100 : 100, respectively. The ^{13}C NMR spectra were recorded on an NMR Gemini 300VT Varian spectrometer using dioxane as an internal standard. Positions of the ^{13}C NMR signals were converted to the tetramethylsilane scale. ^{31}P NMR spectra were measured on an NMR Unity-300 Varian Spectrometer (H_3PO_4 as a standard). In order to observe a clear NMR spectrum, the ligand concentration was higher than for the potentiometric measurements. The UV–Vis spectra were measured in 1 cm^3 cells using a JASCO V-500 UV–Vis spectrophotometer and UV–Vis Thermo Fisher Scientific Evolution 300 (the concentrations of metal and ligands were the same as in the samples for potentiometric titrations). EPR spectra were recorded using an SE/X 2547 Radiopan spectrophotometer at 77 K in glass capillary tubes of 130-μL capacity. Samples were made using a water : glycol mixture (3 : 1). The concentration of Cu(II) ions and the ligands was 5×10^{-3} M.

The hydrolysis constants for Cu(II) were taken from the literature [31] and were employed in the calculations.

The ligands studied are presented in scheme 1.

3. Results and discussion

3.1. *ATP-Lys system*

On the basis of potentiometric and spectroscopic studies, it has been concluded that as a result of noncovalent interactions, in the system ATP-lysine, the molecular complexes

formed are of the type (ATP)H_x(Lys). At pH < 3 the species (ATP)H_5(Lys) is formed, while starting from pH of about 3.5, (ATP)H_4(Lys), figure 1, binds about 80% of the total number of ligands. The species (ATP)H_3(Lys) binds almost 80% of the reagents at pH close to 6. Starting from a pH of about 6.5, the formation of the adduct (ATP)H_2(Lys) begins and at pH close to 9, it binds about 40% of the ligands.

Analysis of the shifts of signals in the ^{13}C NMR and ^{31}P NMR spectra of (ATP)H_4(Lys), at pH 3 (table 1), appearing as a result of noncovalent interactions, provides information on which of the potential reaction centers in the ligands in the tetraprotonated complex are involved in the interaction. The changes were noted in the positions of the signals assigned to carbons of ATP in the vicinity of HN(1) : C(2) by 0.535 ppm and C(6) by 0.597 ppm, and in the vicinity of N(7) : C(5) by 0.187 ppm and C(8) by 0.237 ppm, and in the positions of the signals assigned to phosphorus from the two terminal phosphates of this nucleotide (P_β; 0.754 ppm and P_γ; 0.270 ppm). These changes indicate that the above atoms are involved in adduct formation. However, the changes in the positions of the signals of carbons near N(7) are significantly smaller than those of the carbons near N(1), which suggests a stronger involvement of the latter in the reaction (table 1). In the pH range of the adduct (ATP)H_4(Lys) domination, N(1) is protonated so it is a positive center of interaction (the overall protonation constants are given in table 2).

The insignificant shift of the signal assigned to P_α (0.008 ppm) means that this phosphate group is excluded from the interaction. The change in the position of the signals (with respect to their positions in the spectrum of free ligand) assigned to $C_{(6)}$ near the $-NH_3^+$ group and $C_{(1)}$ of the carboxyl group (table 1) from lysine suggests involvement of these two groups in the formation of the molecular complex (ATP)H_4(Lys). On the other hand, a change in the position of the signal assigned to $C_{(2)}$ by only 0.022 ppm excludes the participation of the α-amino group of the nucleotide from interaction. Analysis of the protonation constants shows that in the pH range of formation of a tetraprotonated adduct, the amino group is a positive reaction center and the carboxyl group is a negative one.

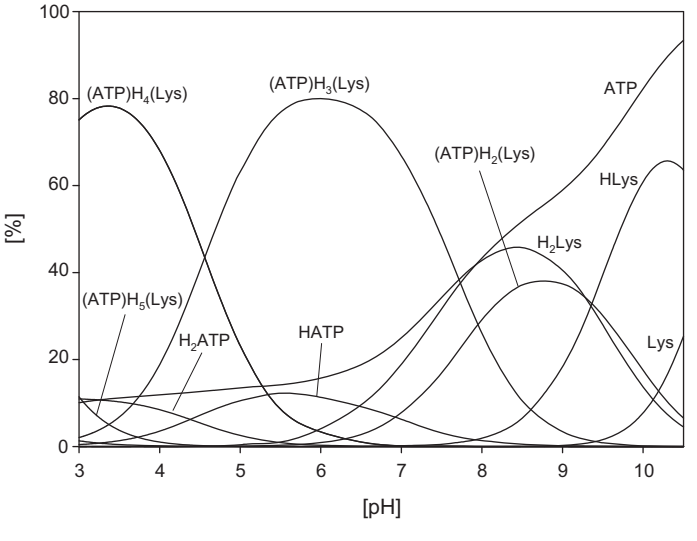


Figure 1. Distribution diagram for the ATP/Lys system; percentage of the species refers to total ATP. $C_{ATP} = 1 \times 10^{-2}$ M; $C_{Lys} = 1 \times 10^{-2}$ M.

Table 1. ^{13}C and ^{31}P NMR signal positions for ATP and lysine and differences between chemical shifts for the ligands in ATP/Lys system in relation to the free ligands (in parentheses); [ppm].

		ATP							Lys					
pH	Species	C(2)	C(5)	C(6)	C(8)	P_α	P_β	P_γ	$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	$C_{(4)}$	$C_{(5)}$	$C_{(6)}$
3	$(ATP)H_4(Lys)$	149.369	118.273	151.793	142.525	−11.164	−22.703	−10.596	175.153	55.183	27.183	22.282	30.628	39.941
		(0.535)	(0.187)	(0.597)	(0.237)	(0.008)	(0.754)	(0.270)	(0.185)	(0.022)	(0.000)	(0.005)	(0.004)	(0.089)
6	$(ATP)H_3(Lys)$	153.564	119.354	156.312	140.721	−11.115	−22.243	−8.554	175.400	55.343	27.196	22.282	30.688	39.948
		(0.256)	(0.173)	(0.271)	(0.219)	(1.994)	(2.140)	(2.208)	(0.178)	(0.100)	(0.008)	(0.009)	(0.009)	(0.069)
9	$(ATP)H_2(Lys)$	153.038	119.362	156.347	140.729	−10.790	−20.996	−5.324	176.569	55.496	27.290	22.363	31.255	39.988
		(0.005)	(0.020)	(0.043)	(0.026)	(0.080)	(0.038)	(0.115)	(0.014)	(0.161)	(0.007)	(0.044)	(0.366)	(0.116)

Table 2. Overall stability constants ($\log\beta$) and equilibrium constants ($\log K_e$) of adducts in ATP/Lys system.

Species	Reactions	$\log\beta$	$\log K_e$
$(ATP)H_5(Lys)$	$H_2ATP + H_3Lys \leftrightarrows (ATP)H_5(Lys)$	38.15 (15)	4.90
$(ATP)H_4(Lys)$	$H_2ATP + H_2Lys \leftrightarrows (ATP)H_4(Lys)$	35.97 (7)	4.85
$(ATP)H_3(Lys)$	$HATP + H_2Lys \leftrightarrows (ATP)H_3(Lys)$	31.41 (6)	4.66
$(ATP)H_2(Lys)$	$ATP + H_2Lys \leftrightarrows (ATP)H_2(Lys)$	23.46 (6)	3.21

Notes: Overall protonation constants of the ligands: HATP, 6.50(1); H_2ATP, 10.88(2) [22], HLys, 10.90; H_2Lys, 20.25; H_3Lys, 22.37 – this work; $\log K_e$ values were calculated using protonation and overall stability constants of particular species: $\log K_e = \log\beta_{(ATP)H(m+n)(Lys)} - \log\beta_{HmATP} - \log\beta_{HnLys}$.

In the ^{31}P NMR spectrum of $(ATP)H_3(Lys)$, although the α-phosphate is protonated, a significant shift of the signal assigned to P_α (1.994 ppm) is observed, in contrast to the observations made for the tetraprotonated species, which means that this group is involved in interactions as a positive center. In the pH range of $(ATP)H_3(Lys)$ formation, HN(1) from ATP is deprotonated and the nitrogen loses its positive character, which is in agreement with the results obtained from the molecular electronic structure calculation (program GAUSSIAN 03 [32], Ground State method/DFT/B3LYP/LAND2DZ level, solvation model); the partial charges on HN(1) and N(7) in H_2ATP are +0.105 and −0.091, respectively, while in HATP, the partial charges on N(1) and N(7) are −0.069 and −0.089, respectively. The shifts of the signals assigned to C(2) and C(6) in the ^{13}C NMR spectra (table 1) are a consequence of the difference in electron density between the protonated and deprotonated N(1) (this nitrogen becomes a negative reaction center). In $(ATP)H_3(Lys)$ species, the character of noncovalent interaction changes and $-COO^-(Lys)\cdots\alpha\text{-}PO_3H(ATP)$ is formed. As follows from analysis of the NMR data, the shifts in positions of the signals assigned to P_α, P_β, and P_γ mean that all phosphate groups of ATP are involved in the interactions (although P_α is a positive center). The changes in positions of the signals assigned to $C_{(1)}$, $C_{(2)}$, and $C_{(6)}$ in the spectrum of Lys with respect to those in the spectrum of the free ligand indicate that also the α $-NH_3^+$ group of the amino acid is involved in the interactions, while in the tetraprotonated species only the ω-amino group was engaged in the interaction, see table 1. Surprisingly, the equilibrium constants of $(ATP)H_4(Lys)$ and $(ATP)H_3(Lys)$ adduct formation are 4.85 and 4.66 (table 2), despite a different number of interacting centers. Most probably this is a consequence of exceptionally strong dependence of the stability of weak interactions on spatial conditions, as discussed [33]. The equilibrium constant of $(ATP)H_2(Lys)$, $\log K_e = 3.21$, is much lower than those of the other complexes, which indicates a substantial change in the character of interactions in this diprotonated species.

Under the conditions of this complex domination, at pH close to 9, the nucleotide becomes fully deprotonated, while the amine groups of lysine remain protonated. The changes in position of the signal assigned to $C_{(1)}$ from the carboxyl group of lysine is only 0.014 ppm, which means that this group is excluded from the interactions, while the two $-NH_3^+$ groups are involved in the interaction (see table 1, the largest changes in the positions of the signals assigned to $C_{(2)}$, $C_{(5)}$, and $C_{(6)}$). Moreover, the shifts of the signals assigned to phosphorus from ATP point to engagement of the oxygens from phosphate groups in the interaction, while N(1) and N(7) do not interact with the amino acid (table 1).

3.2. *Spectral investigation of Cu(II)/Lysine system*

Literature data [13, 21, 34, 35] indicate formation of the complexes MHL, MH_2L_2, MHL_2, and ML_2 in the binary system Cu(II)/Lys. The agreement of the results of the equilibrium

studies for the above univocal model made us assume it for the study of the binary system Cu(II)/Lys and ternary system Cu(II)/ATP/Lys. Mean values of overall stability constants of the following complexes: CuH(Lys), 18.34; $Cu(HLys)_2$, 35.44; $CuH(Lys)_2$, 25.44; $Cu(Lys)_2$, 14.92 [13, 21, 35] were calculated and used for the determination of pH ranges of their domination (program HALTAFALL), in which the spectral investigation was done (table 3).

With increasing pH, protons are removed from lysine in the following sequence: the carboxyl group, α-amine group, and ω-amine group [18]. The protonated complex CuH(Lys) forms according to the reaction: Cu + HLys $\leftrightarrows$ CuH(Lys). The sequence of unblocking of the donors suggests a glycine-like coordination in CuH(Lys) species with participation of the oxygens from carboxyl and α-nitrogen from lysine in coordination. This hypothesis is supported by the change in spectral data. At pH 6.0, λ_{max} is 630 nm, while $g_{\|} = 2.265$ and $A_{\|} = 173$ in the EPR spectra (table 3), which suggests formation of a type $\{N,O_x\}$ chromophore. This mode of coordination is also indicated by analysis of the ^{13}C NMR spectra. The shifts in the positions of the signals assigned to $C_{(1)}$, $C_{(2)}$, and $C_{(6)}$ of lysine located close to donors from $-COO^-$, α-NH_2, and ω-NH_2 groups in the spectrum of the complex with respect to their positions in the spectrum of the free ligand are 0.140, 0.087, and 0.018 ppm, respectively, which confirms participation of oxygen from the carboxyl and nitrogen from α-NH_2 from the amino acid in coordination in CuH(Lys). Taking into account the limitations in the use of NMR in the investigation of paramagnetic ions, the NMR spectra of the species were recorded by the decoupling technique at low concentrations of metal ions. The pH ranges of the complex dominance in the distribution diagram of the species are practically the same as for systems of higher concentrations of metal ions and ligands. Significant changes in the chemical shifts were observed only in the pH ranges in which the complexes were present (determined on the basis of potentiometric measurements), as discussed earlier [36–38].

With increasing pH and formation of $Cu(HLys)_2$, a shift of the d–d bands in the UV–Vis spectrum (relative to their positions in the spectrum of CuH(Lys)) towards higher energies is observed (pH 9.0, $\lambda_{max} = 618$ nm), which corresponds to involvement of another nitrogen in metallation. EPR parameters for $Cu(HLys)_2$ determined as $g_{\|} = 2.224$ and $A_{\|} = 188$ (table 3) confirm formation of the $\{2N,O_x\}$ chromophore. Changes in the positions of the ^{13}C NMR signals assigned to carbons in the vicinity of the coordination centers in the lysine with respect to their positions in the spectrum of the free ligand are, $C_{(1)}$ 2.940 ppm, the $C_{(2)}$ signal is quenched, and $C_{(6)}$ 0.000 ppm. Thus, the carboxyl groups and α-NH_2 amine groups from both lysine molecules are involved in coordination.

When $CuH(Lys)_2$ is formed (pH > 8.0), however, no significant changes in spectroscopic parameters are observed (table 3), suggesting that both in $Cu(HLys)_2$ as well as $CuH(Lys)_2$,

Table 3. Vis and EPR spectral data for Cu(II)/Lys and Cu(II)/ATP/Lys systems.

			EPR		
Complex	pH	λ_{max} [nm]	$g_{\|}$	$A_{\|}$ (10^{-4} cm^{-1})	Chromophore
CuH(Lys)	6.0	630	2.265	173	$\{N,O_x\}$
$Cu(HLys)_2$	9.0	618	2.224	188	$\{2N,O_x\}$
$CuH(Lys)_2$	10.5	617	2.220	191	$\{2N,O_x\}$
$Cu(ATP)H_2(Lys)$	4.5	768	2.382	148	$\{O_x\}$
Cu(ATP)H(Lys)	6.5	706	2.323	162	$\{N,O_x\}$
Cu(ATP)(Lys)	9.0	622	2.282	184	$\{2N,O_x\}$
$Cu(ATP)(Lys)(OH)_2$	10.5	622	2.281	181	$\{2N,O_x\}$

a similar chromophore is formed {2N,O_x}. These results confirm the earlier conclusions of Conato *et al.* [13]. Moreover, in the ^{13}C NMR spectrum at pH 10.5 where this complex dominates, the signals assigned to $C_{(1)}$ and $C_{(2)}$ are quenched and the position of the signal of C(6) is shifted by 0.043 ppm, which indicates coordination of two nitrogens in both these species (besides the oxygens), i.e. with two glycine-like-coordinated ligands, while the terminal ω-NH_2 groups are outside the inner coordination sphere of the copper(II) ions. Gergely *et al.* [21] have also postulated that nitrogen from ω-NH_2 is excluded from interactions in $CuH(Lys)_2$ and $Cu(Lys)_2$, and both molecules of lysine are coordinated to the Cu(II) in a glycine-like manner.

3.3. *Cu(II)/ATP/Lys systems*

In the pH range studied (from 2.5 to 10.5), computer analysis of the potentiometric titration data indicates formation of the following complexes: $Cu(ATP)H_2(Lys)$, Cu(ATP)H(Lys), Cu(ATP)(Lys), and $Cu(ATP)(Lys)(OH)_2$ (table 4, figure 2).

From pH ~2.5 to ~7 formation of $Cu(ATP)H_2(Lys)$ species is observed, which dominates at pH close to 4.5. The values of the spectroscopic parameters obtained from UV–Vis and EPR spectra at a pH of 4.5, in the pH range of $Cu(ATP)H_2(Lys)$ complex domination, are $\lambda_{max} = 768$ nm, $g_{\|} = 2.382$ and $A_{\|} = 148$ (table 3), which suggests that only oxygens are involved in coordination [39–42]. Moreover, an experiment was performed in which increasing amounts of lysine or ATP were added to Cu(II)/ATP or Cu(II)/Lys, respectively. The shifts of the d–d band in spectra of the resulting solutions towards higher energy (figure 3) in both systems clearly show that in the ternary system also the oxygens from the second ligand are involved in coordination and the {O_x} chromophore is formed.

The ^{13}C NMR signal assigned to $C_{(1)}$ from the carboxyl group of lysine (pH 4.5) in the complex changes position by 5.396 ppm with respect to that in the spectrum of the free ligand. The ^{31}P NMR spectrum at a pH of 4.5 reveals changes in the positions of the signals assigned to α, β, and γ phosphorus by 0.131, 0.159, and 0.087 ppm. These results confirm coordination of copper ions by oxygens of the phosphate of ATP and the carboxyl of Lys, which is in full agreement with the conclusions drawn from Vis and EPR results.

In the pH range from 6.0 to 8.0, Cu(ATP)H(Lys) dominates (figure 2). The value of λ_{max} at pH close to 6.5 is 706 nm, while the EPR parameters ($g_{\|} = 2.323$ and $A_{\|} = 162$) indicate formation of the {N,O_x} chromophore [39–42], in the Cu(ATP)H(Lys) complex. Shifts of the ^{13}C NMR signals assigned to $C_{(2)}$ and $C_{(3)}$ neighboring the α-amine group observed upon coordination are 0.700 and 0.510 ppm, respectively, which proves participation of the

Table 4. Overall stability constants ($\log\beta$), and equilibrium constants ($\log K_e$) of complex formation in Cu(II)/ATP/Lys systems ($\chi^2 = 17.65$, $\Sigma = 41.30$).

Species	Reactions	$\log\beta$	$\log K_e$
$Cu(ATP)H_2(Lys)$	$Cu(ATP) + H_2Lys \leftrightarrows Cu(ATP)H_2(Lys)$	29.35 (11)	2.47
Cu(ATP)H(Lys)	$Cu(ATP) + HLys \leftrightarrows Cu(ATP)H(Lys)$	24.44 (4)	6.91
Cu(ATP)(Lys)	$Cu(ATP) + Lys \leftrightarrows Cu(ATP)(Lys)$	16.14 (4)	9.51
$Cu(ATP)(Lys)(OH)_2$	$Cu(ATP)(Lys) + 2H_2O \leftrightarrows Cu(ATP)(Lys)(OH)_2 + 2H^+$	−4.52 (7)	–

Notes: Overall stability constants ($\log\beta$) for binary system Cu(II)/ATP: CuH(ATP) 10.53(3), Cu(ATP) 6.63(2), Cu(ATP)(OH) −1.25(2) [22]; Log K_e values were calculated using protonation and overall stability constants of particular species: $\log K_e = \log\beta_{Cu(ATP)H(m+n)(Lys)} - \log\beta_{CuHmATP} - \log\beta_{HnLys}$.

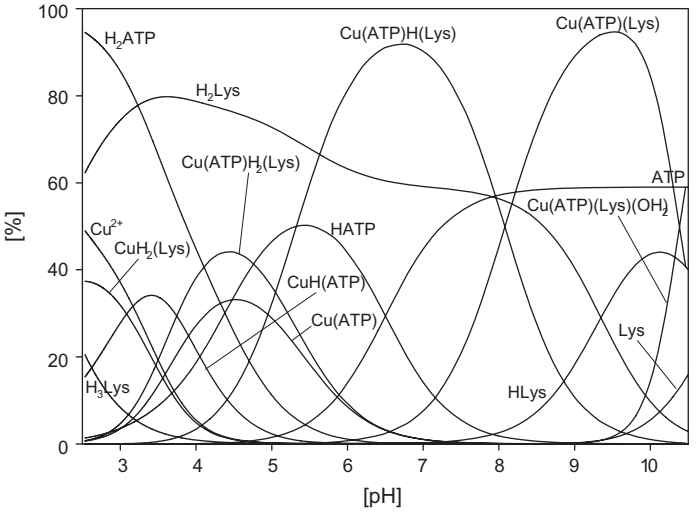


Figure 2. Distribution diagram for the Cu(II)/ATP/Lys system; the percentage of the metal species refers to total metal: $C_{Cu^{2+}} = 1 \times 10^{-3}$ M; $C_{ATP} = 2.5 \times 10^{-3}$ M; $C_{Lys} = 2.5 \times 10^{-3}$ M.

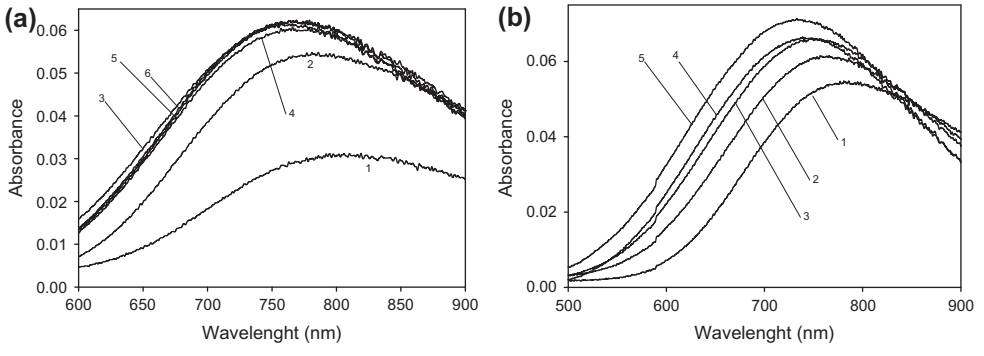


Figure 3. Vis spectra of Cu(II)/ATP/Lys and Cu(II)/Lys/ATP systems (pH 4.5); $C_{Cu(II)} = 1 \times 10^{-3}$ M. (a) Cu(II)/ATP/Lys: (1) 1 : 1 : 0, (2) 1 : 1 : 1, (3) 1 : 1 : 2, (4) 1 : 1 : 3, (5) 1 : 1 :4; (b) Cu(II)/Lys/ATP: (1) 1 : 1 :0, (2) 1 : 1 : 1, (3) 1 : 1 : 2, (4) 1 : 1 : 3, (5) 1 : 1 : 4.

α-nitrogen of lysine in coordination. Figure 4 presents the ^{13}C and ^{31}P NMR spectra of Cu(ATP)H(Lys).

Similarly as in the diprotonated complex, copper ion is also coordinated by oxygen from phosphate of ATP and the carboxyl from lysine. The changes in positions of the ^{31}P NMR signals assigned to α, β, and γ phosphorus from ATP are 0.121, 0.168, and 0.083 ppm, respectively, while the change in position of the ^{13}C NMR signal assigned to $C_{(1)}$ from the lysine carboxyl group is 0.386 ppm. Moreover, the equilibrium constant of Cu(ATP)H(Lys) formation, $\log K_e = 6.91$, corresponding to the energy of HLys attachment to the anchoring species Cu(ATP), is only slightly smaller than $\log K_e = 7.44$ for HLys attachment to copper ion ($\log K_e = \log \beta_{CuH(Lys)} - \log \beta_{HLys} = 18.34 - 10.90 = 7.44$), which confirms coordination with participation of the carboxyl and α amine from lysine.

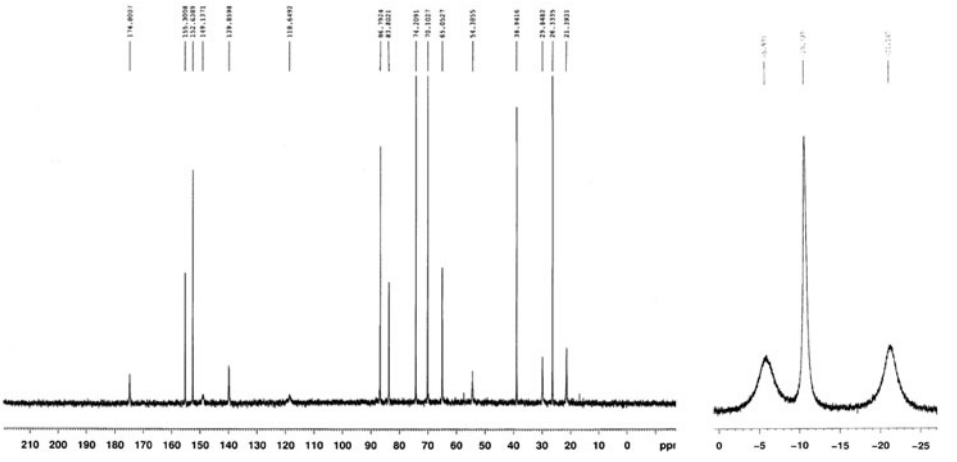
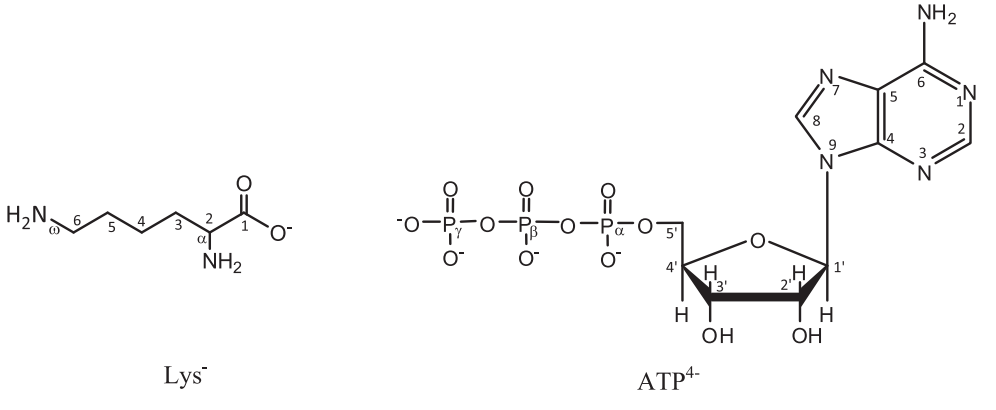


Figure 4. ^{13}C and ^{31}P NMR spectra for Cu(ATP)H(Lys) species (pH 7).



Scheme 1. Chemical formulas of the bioligands studied.

In the pH range above 8.5, the dominant species binding about 90% of copper ions is Cu(ATP)(Lys) (figure 2) and its maximum concentration is observed at pH close to 9.0. The UV–Vis result ($\lambda_{max} = 622$ nm) and EPR parameters ($g_{||} = 2.282$ and $A_{||} = 184$) imply a {2N, O_x} chromophore, while the shift of the ^{13}C NMR signals suggests involvement of both nitrogens from lysine in the coordination; the signals assigned to $C_{(2)}$ and $C_{(6)}$ are shifted by 0.104 and 0.108 ppm, respectively. It should be emphasized that signals assigned to $C_{(3)}$ and $C_{(4)}$ from lysine, which are not in the vicinity of the coordination sites, are shifted only by 0.042 and 0.007 ppm, respectively. The above results indicate that introduction of ATP into the binary system Cu(II)/Lys changes the character of the amino acid interaction, favoring the involvement of the terminal ω-NH_2 group from lysine in coordination of Cu^{2+} in Cu(ATP)(Lys). Although this unexpected involvement of the ω-NH_2 group in Cu(II) coordination arouses some doubts, such a possibility has been suggested [43, 44]. The ^{31}P NMR signals assigned to α, β, and γ phosphorus in ATP at pH 7.0 (for Cu(ATP)H(Lys)) shift by

0.121, 0.168, and 0.083 ppm, whereas at pH 9.5 by 0.074, 0.002, and 0.014 ppm, respectively, indicating participation of only the oxygen from the α-phosphate group of ATP in coordination in the Cu(ATP)(Lys) complex. An increase in the formation constant of Cu(ATP)(Lys), $\log K_e = 9.51$, corresponding to energy of Lys attachment to Cu(ATP), relative to $\log K_e = 6.91$ describing the formation of Cu(ATP)H(Lys) (HLys coordination to the anchoring Cu(ATP) in a glycine-like manner), confirms involvement of the second nitrogen from the amino acid molecule in the inner coordination sphere of copper ions in Cu(ATP)(Lys).

Starting from pH close to 9.5, formation of $Cu(AMP)(Lys)(OH)_2$ begins. The spectroscopic parameters measured at pH 10.5 ($\lambda_{max} = 622$, $g_{||} = 2.281$, $A_{||} = 181$) at which $Cu(AMP)(Lys)(OH)_2$ binds about 60% of the copper ions, take practically the same values as at pH 9.0 (table 2), at which Cu(AMP)(Lys) dominates, which suggests that the bioligands coordinate in the same mode in both species, and implies the presence of the $\{2N,O_x\}$ chromophore in both complexes.

4. Conclusion

In the metal-free ATP/Lys system, molecular complexes are formed. At low pH, N(1) as well as N(7) from ATP are engaged in interactions with lysine, but at high pH, these two nitrogens are not involved in the interaction, similarly as the oxygens from the carboxyl of lysine. At low pH, P_α of ATP does not take part in the interaction between the bioligands, but at higher pH, it is involved in this interaction. As follows from the spectroscopic results, in CuH(Lys), $Cu(HLys)_2$, $CuH(Lys)_2$, and $Cu(Lys)_2$ formed in the Cu(II)/Lys binary system, over the whole pH range studied, only oxygen from $-COO^-$ and nitrogen from the α-NH_2 group of lysine (glycine-like manner) are involved in coordination. In the hetero-ligand complexes $Cu(ATP)H_2(Lys)$ and Cu(ATP)H(Lys), the lysine interacts with Cu(II) with involvement of oxygens from the carboxyl and nitrogen from the α-NH_2 amine.

Introduction of ATP into the binary system Cu(II)/Lys changes the mode of lysine coordination in the alkaline pH range, where Cu(ATP)(Lys) and $Cu(ATP)(Lys)(OH)_2$ are formed. The spectroscopic studies and analysis of the equilibrium study unexpectedly indicate participation of the terminal ω-NH_2 in coordination. This conclusion confirmed the suggestions [43, 44] that nitrogen of the ω-NH_2 from lysine can be involved in coordination with Cu(II) in the alkali pH range. Near pH of 4 in $Cu(ATP)H_2(Lys)$, the α, β, and γ oxygens of the phosphate from ATP take part in metal coordination, but at higher pH only α oxygens from this phosphate take part in Cu(ATP)(Lys).

Introduction of Cu(II) ions into the system ATP/Lys changes the interaction between the bioligands in the metal-free system, as established for similar systems [45–48]. The endocyclic nitrogens from the nucleotide are unblocked in the entire pH range studied (however, they are not involved in coordination) and can be the potential centers of interactions with other biomolecules present in biological systems [49].

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