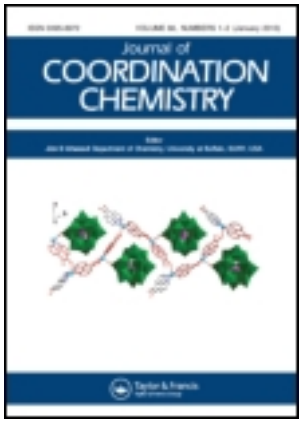

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Potentiometric and spectral studies of complex formation in the Cu(II), 3′,5′-cyclic adenosine monophosphate, and tetramine systems

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Potentiometric and spectral studies of complex formation in the Cu(II), 3′,5′-cyclic adenosine monophosphate, and tetramine systems

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Reactions in the systems composed of copper(II), 3′,5′-cyclic adenosine monophosphate (cAMP), and tetramines (PA) were studied. On the basis of potentiometric and spectroscopic data in metal-free systems, formation of molecular complexes (cAMP)H_x(PA), $x = 2$–4, was found. Stabilities of the complexes were determined and their centers of interactions were identified. In Cu(II)/cAMP, formation of Cu(cAMP) and Cu(cAMP)(OH) was observed, with the phosphate as the main site of metallation, while in ternary systems, formation of Cu(cAMP)H_4(Spm) and Cu(cAMP)(3,3,3-tet) was established. Characteristic differences in the coordination character of tetramines were found. In the Cu(II)/cAMP/Spm system, oxygens from the nucleotide phosphate are involved in metallation and protonated amines are engaged in noncovalent interaction with endocyclic nitrogens of nucleoside. In the Cu(II)/cAMP/3,3,3-tet system, a MLL′ complex is formed in which the inner coordination sphere includes polyamine nitrogens as well as the nucleotide phosphate.

Keywords: Copper(II); cAMP; Tetramine; Mixed complexes; Molecular complexes

1. Introduction

3′,5′-Cyclic adenosine monophosphate (cAMP) is an important intracellular mediator in both prokaryotes and eukaryotes. In vertebrate cyclic nucleotides, it serves as a second messenger by transducing the action of various hormones, controlling multiple cellular processes in the brain, neurotransmitters, and light, and thus, plays a key role in regulation of physiological functions such as visual response or immune response [1, 2]. The cAMP inhibits inflammatory cell proliferation and release of proinflammatory cytokines [3–5] or stimulates some enzymes, e.g. L-arabinose isomerase [6]. Moreover, similar to cGMP, cAMP also directly activates ion channels by binding to a site on the channel protein [7]. cAMP receptor protein (prokaryotic transcription factor) is the global regulator controlling transcription of about 200 genes; for example, the genes for carbon source utilization in the absence of glucose [8] and it is a dual regulator, acting as an activator

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or repressor [9–11]. The protein, allosterically activated by cAMP binding, is bonded to DNA and interacts with RNA polymerase [12, 13]. cAMP-activated cation current is widely distributed among central molluscan neurons [14–17]. Taking into account complex-forming properties of donors in cAMP, the interactions with metal ions present in a cell or with organic cations (polyamines) should be taken into consideration while discussing the role of nucleotides in a living organism, particularly in bioprocesses including interactions as a second messenger.

Polyamines are low molecular weight aliphatic amines which are fully protonated at physiological pH. Spermine is the most active and putrescine the least active in control of various biological processes [18, 19]. Polyamines stabilize DNA [20] as well as human erythrocyte membranes [21]; they are necessary for activation of DNA synthesis and cell replication [22–27] and stimulate gene transcription and translation [28]. Spermine and spermidine are activity regulators for membrane ion channels and have a blocking effect on certain potassium channels [29]. PAs modulate the activity of receptors [30] and play the major role in cell proliferation and wound healing [31]. Copper is used as a chemical control agent for micro-organisms and is required in trace amounts for growth and functioning of micro-organisms since it is a cofactor for numerous enzymes [32]. Copper(II) ions are borderline hard acids, thus their coordination ability is dominated by N-type as well as O-type donors. There are no data in the literature on the interaction of Cu(II) in the systems containing cAMP and polyamines.

This paper presents results of a study on complexation reactions in binary and ternary systems of Cu(II)/cAMP/PA.

2. Experimental

3′,5′-Cyclic adenosine monophosphate (cAMP), 1,12-diamino-4,8-diazadodecane (Spm), and 1,11-diamino-4,8-diazaundecane (3,3,3-tet) were purchased from Sigma and used without purification. Spm $4HNO_3$ and 3,3,3-tet $4HNO_3$ were obtained from the reaction of HNO_3 solution with Spm or 3,3,3-tet in methanol [33]. The results of elemental analyzes (% C, %N, and %H) performed on an Elemental Analyzer CHN 2400, Perkin-Elmer, were consistent with the calculated ones (±0.5%). $Cu(NO_3)_2$ purchased from POCH Gliwice (Poland) was twice recrystallized from water before use. The method for determination of Cu(II) concentration in a solution of about $1.5 \cdot 10^{-2}$ mol dm^{-3} was described earlier [34, 35]. Potentiometric titration was performed using the titration set Methrom: 713 pH-meter, 725 dosimat, 728 stirrer, and a combined glass electrode 6.233.100. Electrodes were calibrated in terms of the hydrogen ion concentration [36]. The concentration of ligands in the metal-free systems cAMP/Spm and cAMP/3,3,3-tet was 0.01 mol dm^{-3} (molar ratio 1 : 1). In the binary system, Cu(II)/cAMP (molar ratio 1 : 2, 1 : 4 and 1 : 5) concentration of the ligand was $2 \cdot 10^{-3}$ mol dm^{-3} and in the ternary Cu(II)/cAMP/Spm and Cu(II)/cAMP/3,3,3-tet systems, concentration of ligands varied from $1 \cdot 10^{-3}$ to $2 \cdot 10^{-3}$ mol dm^{-3} (molar ratio from 1 : 1.5 : 1.5 to 1 : 2.5 : 2.5). The measurements were performed in neutral gas atmosphere at ionic strength of $\mu = 1 \cdot 10^{-1}$ mol dm^{-3} (KNO_3) at $T = 20 \pm 1$ °C using CO_2-free solution of NaOH as titrant. The data obtained from the potentiometric titration (150–350 points in each titration) were subjected to computer analysis using SUPERQUAD for model selection and determination of the stability constants [37] and HALTAFALL for determination of distribution of particular species [38]. The criteria of model verification have been described earlier [39].

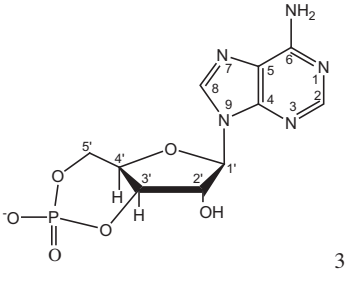
The samples for NMR study were prepared by dissolving cAMP, Spm, 3,3,3-tet, and Cu $(NO_3)_2$ in D_2O and adjusting pH with addition of NaOD or DCl. The pH values were corrected using the formula pD = pH-meter readings +0.4 [40]. The concentration of ligands in the systems was 0.01 mol dm^{-3}. In the binary system, the ratio of Cu(II) to ligand was 1 : 100. In the ternary systems, the ratio Cu(II)/ligand1/ligand2 varied from 1 : 50 : 50 to 1 : 100 : 100. The ^{13}C NMR spectra were recorded on a Gemini 300 VT Varian spectrometer using dioxane as internal standard. The signal positions in ^{13}C NMR spectra were given on the TMS (tetramethylsilane) scale. ^{31}P NMR spectra were measured on an NMR Unity-300 Varian spectrometer (H_3PO_4 as a standard). UV-vis spectra were recorded on a UV-vis JASCO V-500 spectrometer for the same ligand concentrations as in the samples for potentiometric titrations at metal : ligand ratio 1 : 2 in the binary system and metal : ligand1 : ligand2 ratio 1 : 1.5 : 1.5 in the ternary systems. EPR spectra were recorded at 77 K in a water–glycol solution (3 : 1, v/v) on a Radiopan SE/X 2547 spectrometer (C_{Cu2+} = 1 × 10^{-3} mol dm^{-3} or 5 × 10^{-3} mol dm^{-3}).

3. Results and discussion

The structures of the compounds studied are given in scheme 1.

3.1. *Protonation constants of cAMP*

The protonation constant of cAMP$^+$ was determined by the potentiometric method on the basis of computer analysis of potentiometric data collected in the pH range 2.5–10.5. The protonation constant log K_2 = 3.81(8) corresponding to attachment of a proton to the endocyclic nitrogen N1 from the adenine residue of the nucleotide is in agreement with literature data on cyclic and noncyclic nucleotides [41–46]. The protonation constant of



3',5'-cyclic adenosine monophosphate (cAMP)

1 2 3 4 5 5 4 3 2 1
NH_2-CH_2-CH_2-CH_2-NH-CH_2-CH_2-CH_2-CH_2-NH-CH_2-CH_2-CH_2-NH_2
1,12-diamino-4,8-diazadodecane (Spm)

1 2 3 4 5 4 3 2 1
NH_2-CH_2-CH_2-CH_2-NH-CH_2-CH_2-CH_2-NH-CH_2-CH_2-CH_2-NH_2
1,11-diamino-4,8-diazaundecane (3,3,3-tet)

Scheme 1. Chemical formulae of the bioligands studied.

the phosphate from cAMP is close to 1.4 and is much higher than that for AMP protonation (log $K_1 = 0.4$), which is explained by a different number of oxygens engaged in the ester bond [41–48].

3.2. *Studies of metal-free systems: cAMP/Spm and cAMP/3,3,3-tet*

As a result of noncovalent interactions between cAMP and spermine, the following molecular complexes are formed: (cAMP)H_4(Spm), (cAMP)H_3(Spm), and (cAMP)H_2(Spm), concluded on the basis of computer analysis of potentiometric titration data (stability constants, see table 1). The adduct formation is confirmed by the spectroscopic data discussed below.

Taking into account a different number of protons in particular species, the overall stability constants logβ cannot be used directly for determination of the character of the interactions. Therefore, the results were analyzed on the basis of the calculated equilibrium constants log K_e. The value of $\log K_e = \log\beta_{(cAMP)H(m+n)(PA)} - \log\beta_{Hm(cAMP)} - \log\beta_{Hn(PA)}$ corresponds to the effectiveness of interaction of ligands in the molecular adducts. Analogously, for interactions in the systems including metal ions: Cu(cAMP) + H_n(PA) ⇄ Cu(cAMP)H_n(PA), $\log K_e = \log\beta_{Cu(cAMP)Hn(PA)} - \log\beta_{Cu(cAMP)} - \log\beta_{Hn(PA)}$ (PA = polyamine).

In the system cAMP/Spm, the molecular complex (cAMP)H_4(Spm) dominates (figure 1). As follows from analysis of the protonation constants of bioligands and the number of labile hydrogens (table 1), this species is formed with involvement of fully protonated spermine (high protonation constants) interacting with deprotonated cAMP. This conclusion is confirmed by spectroscopic study discussed below.

With increasing pH and deprotonation of the endocyclic nitrogen of the nucleotide, the concentration of the (cAMP)H_4(Spm) adduct increases (Spm is still fully protonated). This observation suggests participation of the endocyclic nitrogen of cAMP in the interaction, as confirmed by spectroscopic data. Starting from pH close to 7, (cAMP)H_3(Spm) forms and at pH 9, it binds a maximum of about 40% of the bioligands (total concentration of cAMP = Spm = 0.01 M). With further increase in pH, formation of (cAMP)H_2(Spm) is observed to bind a maximum of about 50% of the bioligands at pH close to 10.

Table 1. Overall stability constants (log β) and equilibrium constants (log K_e) for the adducts and complexes formed in cAMP–Spm or cAMP-3,3,3-tet systems and in Cu(II)-cAMP, Cu(II)-cAMP-3,3,3-tet or Cu(II)-cAMP–Spm systems.

Species	Equilibrium	log β	log K_e
(cAMP)H_4(Spm)	cAMP + $4H^+$ + Spm ⇄ (cAMP)H_4(Spm)	42.05(4)	3.38
(cAMP)H_3(Spm)	cAMP + $3H^+$ + Spm ⇄ (cAMP)H_3(Spm)	33.39(6)	3.00
(cAMP)H_2(Spm)	cAMP + $2H^+$ + Spm ⇄ (cAMP)H_2(Spm)	23.99(7)	2.71
(cAMP)H_4(3,3,3-tet)	cAMP + $4H^+$ + 3,3,3-tet ⇄ (cAMP)H_4(3,3,3-tet)	39.69(5)	3.30
(cAMP)H_2(3,3,3-tet)	cAMP + $2H^+$ + 3,3,3-tet ⇄ (cAMP)H_2(3,3,3-tet)	23.21(7)	2.83
Cu(cAMP)	Cu + cAMP ⇄ Cu(cAMP)	4.66(2)	4.66
Cu(cAMP)OH	Cu + cAMP + H_2O ⇄ Cu(cAMP)(OH) + H^+	−0.68(9)	
Cu(cAMP)(3,3,3-tet)	Cu + cAMP + 3,3,3-tet ⇄ Cu(cAMP)(3,3,3-tet)	21.29(6)	16.63
Cu(cAMP)H_4(Spm)	Cu + $4H^+$ + cAMP + Spm ⇄ Cu(cAMP)H_4(Spm)	47.37(15)	4.04

Notes: Protonation constants: HcAMP 3.81(8) (this work); H_4Spm 38.67(2); H_3Spm 30.39(2); H_2Spm 21.28(3); HSpm 10.91(1); H_4(3,3,3-tet) 36.39(2); H_3(3,3,3-tet) 29.01(3); H_2(3,3,3-tet) 20.38(3); H(3,3,3-tet) 10.36(2); CuH_2(Spm) 27.63(26); $CuH(Spm)_2$ 29.32(14); Cu(Spm) 14.66(3); CuH_2(3,3,3-tet) 27.49(7); $CuH_2(3,3,3\text{-tet})_2$ 42.03(7); and Cu(3,3,3-tet) 16.36(3) [49].

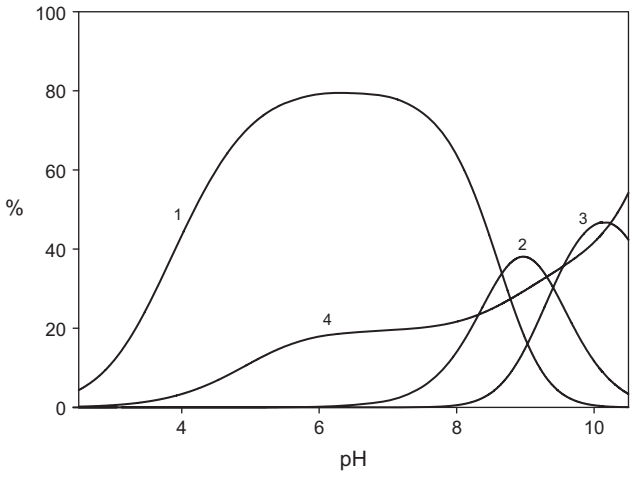


Figure 1. Distribution diagram for cAMP/Spm (L : L′ = 1 : 1); the percentage of the species refers to total cAMP; 1 – (cAMP)H_4Spm, 2 – (cAMP)H_3Spm, 3 – (cAMP)H_2Spm, and 4 – cAMP; $c_{cAMP} = 1 \times 10^{-2}$ mol dm^{-3}; and $c_{Spm} = 1 \times 10^{-2}$ mol dm^{-3}.

Equilibrium constants log K_e determined for (cAMP)H_4(Spm), (cAMP)H_3(Spm), and (cAMP)H_2(Spm) are 3.38, 3.00, and 2.71, respectively. The values decrease with increasing deprotonation of the polyamine, a consequence of a reduced number of interacting centers (disappearance of positive $-NH_x^+$). The log K_e values determined for molecular complexes of noncyclic nucleotide with spermine are much smaller, 2.18, 1.81, and 1.83 for (AMP)H_4(Spm), (AMP)H_3(Spm), and (AMP)H_2(Spm), respectively. This difference is due to difference in the mode of interaction. Phosphate groups from noncyclic nucleotides do not participate in these interplays [50], which is not in agreement with the differences in the protonation constants, suggesting that phosphate from AMP (in contrast to cAMP) should show a greater tendency to form molecular complexes. It is probably related to the effect of the bioligand geometry.

In the system (cAMP)/3,3,3-tet, similar to the system with spermine, the four-proton molecular complex dominates, binding a maximum of about 80% of the polyamine and nucleotide at pH 6 (figure 2). Starting from pH close to 7, formation of (cAMP)H_2(3,3,3-tet) begins, which at pH near 9 binds 80% of the bioligands. Similar values of log K_e determined for (cAMP)H_4(3,3,3-tet) and (cAMP)H_4(Spm) species, 3.30 and 3.38 as well as for (cAMP)H_2(3,3,3-tet) and the (cAMP)H_2(Spm) species, 2.83 and 2.71, respectively (table 1), suggest a similar number of active centers taking part in the interaction. In cAMP/3,3,3-tet, no detectable amount of the three-protonated adduct was found. As a result of decomposition of the four-protonated adduct at pH 8, the concentration of unbound nucleotide considerably increases. Formation of an adduct (cAMP)H_x(3,3,3-tet) involves the fully deprotonated cAMP, so the concentration of free cAMP depends only on the adduct formation and is not a function of nucleotide protonation.

Analysis of ^{13}C NMR and ^{31}P NMR spectra of both systems shows that the phosphate from the nucleotide is involved in formation of molecular complexes. ^{31}P NMR signals assigned to phosphorus from cAMP at pH of adduct domination are shifted by 0.169, 0.127, and 0.097 ppm for (cAMP)H_4(Spm), (cAMP)H_3(Spm), and (cAMP)H_2(Spm) as well as by 0.137 and 0.105 ppm for (cAMP)H_4(3,3,3-tet) and (cAMP)H_2(3,3,3-tet), respectively (table 2).

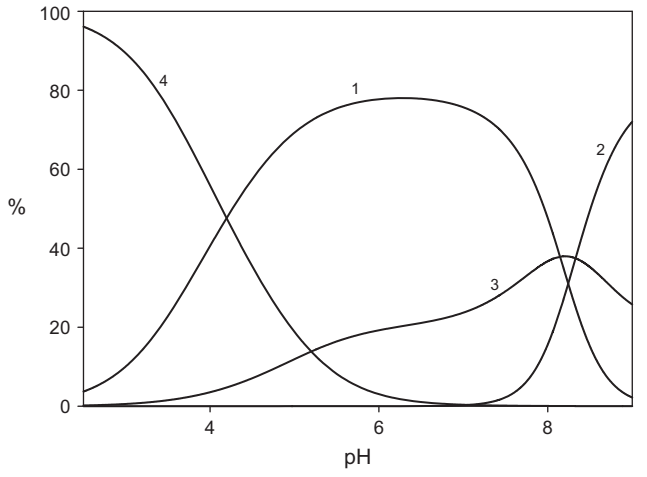


Figure 2. Distribution diagram for cAMP/3,3,3-tet (L : L′ = 1 : 1); the percentage of the species refers to total cAMP; 1 – (cAMP)H_4(3,3,3-tet), 2 – (cAMP)H_2(3,3,3-tet), 3 – cAMP, and 4 – HcAMP; and $c_{cAMP} = 1 \times 10^{-2}$ mol dm^{-3}, $c_{3,3,3\text{-tet}} = 1 \times 10^{-2}$ mol dm^{-3}.

In both systems studied, at pH 2, at which the complexes bind less than 5% of the biomolecules, the shift of the signal assigned to phosphorus from cAMP reaches only 0.003 ppm and 0.004 ppm for cAMP/Spm and cAMP/3,3,3-tet, respectively.

Analysis of ^{13}C NMR results points to participation of the endocyclic nitrogens from the nucleotide in interactions in molecular complexes. Changes in the positions of the signals assigned to carbons C(2), C(6) and C(5), C(8) neighboring to the nitrogens N(1) and N(7) in cAMP are 0.176, 0.236, 0.112, and 0.137 ppm for (cAMP)H_4(Spm). Similar changes were observed for analogous complexes with 3,3,3-tet, table 2). Moreover, changes in the signal positions of Spm and 3,3,3-tet in ^{13}C NMR spectra prove the involvement of all amine groups (table 2), except those from (cAMP)H_2(3,3,3-tet), in interaction with the nucleotide. In conditions of domination of the latter species, the shift of C_1 signal from 3,3,3-tet is only 0.020 ppm (table 2), while the shifts of the signals assigned to C_3 and C_4 are 0.063 and 0.081 ppm, respectively, which suggest that contribution of protonated amine groups located at C(1) is of little effectiveness. As found earlier [51, 52], at the first stage of deprotonation, the two terminal nitrogens from 3,3,3-tet are abstracted. With increasing pH, after dissociation of the two polyamine terminal protons from (cAMP)H_4(3,3,3-tet), (cAMP)H_2(3,3,3-tet) is formed. The pattern of deprotonation of the asymmetric spermine is different and implies that this spermine behaves as two independent groups $NH_3^+CH_2CH_2CH_2NH_2^{+-}$ (as discussed earlier [50]), which explains the differences in the character of interaction of both tetramines with cAMP.

3.3. *Investigation of Cu(II)/cAMP system*

In systems studied at metal : ligand molar ratios of 1 : 2, 1 : 4, and 1 : 5, titration to pH is close to 5. Above this pH, a precipitate appeared which suggests low effectiveness of the metal–ligand bond. Computer analysis of the potentiometric titration data indicated formation of Cu(cAMP) and Cu(cAMP)(OH). Figure 3 presents the distribution curves of particular species. The stability constants and equilibrium constants of complex formation

Table 2. Differences between ^{13}C NMR and ^{31}P NMR chemical shifts for the ligands in the cyclic 3′,5′-AMP/Spm (or 3,3,3-tet), Cu(II)/3′,5′-AMP, and Cu(II)/3′,5′-AMP/Spm (or 3,3,3-tet) systems in relation to the free isolated ligands (ppm).

		cAMP								Spm or 3,3,3-tet				
Complex	pH	C(6)	C(2)	C(4)	C(8)	C(5)	C(5′)	C(3′)	P	C_4	C_3	C_1	C_2	C_5
cAMP/Spm	2	0.003	0.004	0.003	0.002	0.003	0.001	0.000	0.003	0.004	0.001	0.004	0.003	0.002
(cAMP)H_4(Spm)	6	0.236	0.176	0.007	0.221	0.135	0.112	0.137	0.169	0.098	0.082	0.077	0.052	0.045
(cAMP)H_3(Spm)	9	0.353	0.280	0.011	0.303	0.279	0.241	0.197	0.127	0.097	0.083	0.083	0.066	0.032
(cAMP)H_2(Spm)	10	0.211	0.229	0.008	0.182	0.217	0.123	0.009	0.097	0.081	0.079	0.038	0.050	0.047
cAMP/3,3,3-tet	2	0.002	0.004	0.002	0.004	0.003	0.003	0.002	0.004	0.001	0.003	0.003	0.002	0.001
(cAMP)H_4(3,3,3-tet)	6	0.250	0.213	0.009	0.215	0.213	0.208	0.262	0.137	0.089	0.078	0.079	0.047	0.054
(cAMP)H_2(3,3,3-tet)	9	0.128	0.122	0.010	0.144	0.228	0.149	0.162	0.105	0.081	0.063	0.020	0.045	0.049
Cu(cAMP)	4.5	0.011	0.009	0.013	0.009	0.007	0.115	0.088	0.128					
Cu(cAMP)H_4(Spm)	4.0	0.153	0.197	0.007	0.108	0.098	0.107	0.079	0.221	0.127	0.108	0.078	0.059	0.097
Cu(cAMP)(3,3,3-tet)	6.9	0.017	0.009	0.018	0.021	0.010	0.156	0.092	0.307	0.107	0.123	0.105	0.092	0.089

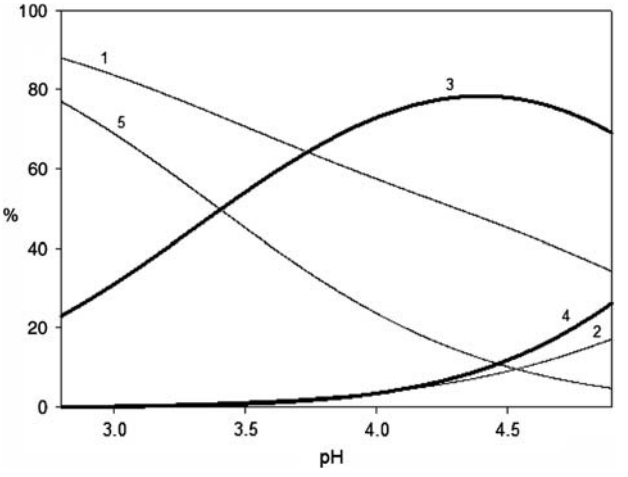


Figure 3. Distribution diagram for Cu(II)/cAMP (M : L = 1 : 2); the percentage of the species refers to total metal; 1 – HcAMP, 2 – cAMP, 3 – Cu(cAMP), 4 – Cu(cAMP)(OH), and 5 – Cu^{2+}; and $c_{Cu2+} = 1 \times 10^{-3}$ mol dm^{-3}, $c_{cAMP} = 2 \times 10^{-3}$ mol dm^{-3}.

are given in table 1. In this study, potential sites of metal coordination in the cAMP molecules are phosphates of the nucleotide and endocyclic nitrogens from adenine [53].

Cu(cAMP) is dominant at pH close to 4.5. At basic pH, a precipitate appears and, as indicated by elemental analysis, the precipitate is a mixture of $Cu(OH)_x$ and $CuL(OH)_x$, which is in agreement with experimental data [41]. However, the complex stability constants differ significantly from the literature data.

At pH of 4.5, in the pH range of Cu(cAMP) domination, the UV-vis spectrum shows a band at 791 nm, corresponding to coordination with $\{Cu–O_x\}$ chromophore with a significant contribution of the phosphate only, established in study of analogous systems [50, 54–57]. The UV band at 270 nm does not change its position as a result of coordination, suggesting exclusion or very weak interaction of the endocyclic nitrogen in metallation. At pH 4.5, positions of the NMR signals assigned to phosphorus and C(5′) from the nucleotide change by 0.128 and 0.115 ppm, respectively. Positions of the signals assigned to C(2), C(6), C(5), and C(8) from the nucleotide in the neighborhood of the endocyclic nitrogens N(1) and N(7) change only by 0.009, 0.011, 0.007, and 0.009 ppm, respectively. In order to minimize NMR signal broadening caused by the paramagnetic Cu(II), the spectra were recorded at low concentrations of the ions. As follows from the calculation, pH ranges of dominance of species formed at low concentration of metal ions are practically the same, both for binary and ternary systems of higher metal ion concentration and metal/ligand(s) ratio.

Significant changes in the chemical shifts were only observed in the pH ranges in which occurrence of complexes was deduced on the basis of the potentiometric measurements. The NMR method has been earlier applied to study similar systems [50, 58–60].

The combined results of the Vis and EPR studies ($\lambda_{max} = 791$ nm, $g_{\|} = 2.365$ and $A_{\|} = 138 \cdot 10^{-4}$ cm^{-1}) for Cu(cAMP) complex clearly indicate formation of a species with the $\{Cu–O_x\}$ chromophore (maybe with an insignificant participation of endocyclic nitrogen), as earlier concluded on the basis of spectral parameters for Cu(II)/ADP/Spm and Cu(II)/AMP/O-Spm [54, 56, 61]. A similar coordination via oxygen donors has also been

observed in the copper complex with cyclophosphate (tetrametaphosphate); the spectral parameters of this Cu(tetrametP) were $\lambda_{max} = 769$ nm, $g_{||} = 2.378$, and $A_{||} = 139 \times 10^{-4}$ cm^{-1}, and the type of coordination was {2O} [61].

Although the phosphate groups in cAMP are different than that in AMP, we can say that the mode of copper(II) bonding proposed on the basis of our results, involving mainly oxygens from the cyclic phosphate, is in agreement with the conclusion that the main center of copper(II) coordination is phosphate from the nucleotide [61–66].

3.4. *Study of Cu(II)/cAMP/Spm and Cu(II)/cAMP/3,3,3-tet systems*

In contrast to Cu(II)/cAMP systems, in the whole pH range studied, from 2.5 to 10.5, in the Cu(II)/cAMP/Spm and Cu(II)/cAMP/3,3,3-tet systems (Cu(II)/cAMP/polyamine molar ratios of 1 : 2.5 : 2.5), no precipitate was observed. Computer analysis of the potentiometric titration data revealed formation (in detectable concentration) of the hetero ligand complexes Cu(cAMP)H_4(Spm) and Cu(cAMP)(3,3,3-tet) (stability constants given in table 1).

Cu(cAMP)H_4(Spm) is dominant at pH close to 4 (figure 4), while Cu(cAMP)(3,3,3-tet) at pH near 5 (figure 5). The stoichiometry of both complexes and pH ranges of their occurrence as well as the equilibrium constant values suggests different interactions. The equilibrium constant of Cu(cAMP)H_4(Spm) formation is 4.04 (table 1), much lower than the corresponding value of CuSpm (log $K_e = 14.66$). The presence of spermine in the fully protonated form (implied by the protonation constants) suggests that Cu(cAMP)H_4(Spm) is a molecular complex formed as a result of noncovalent interaction between the fully protonated polyamine with the anchoring Cu(cAMP): Cu(cAMP) + H_4Spm $\rightleftarrows$ Cu(cAMP)H_4(Spm). Spermine is located in the outer coordination sphere, not involved in direct metal binding, resulting in the low K_e. The equilibrium constant of Cu(cAMP)H_4(Spm) formation is close to that of Cu(AMP)H_4(Spm), log $K_e = 4.20$, in which only the phosphate is involved in metallation [49]. Similar to the Cu/polyamine system [48], in $CuH(Spm)_2$ present in the Cu/cAMP/Spm system at high pH, the first ligand binds metal through four

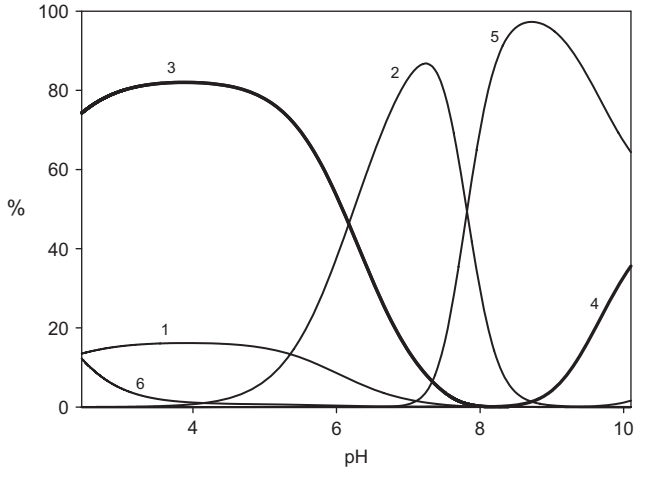


Figure 4. Distribution diagram for Cu(II)/cAMP/Spm; the percentage of the species refers to the total metal; 1 – Cu(cAMP), 2 – Cu(cAMP)(OH), 3 – Cu(cAMP)H_4(Spm), 4 – $CuH(Spm)_2$, 5 – Cu(Spm), and 6 – Cu^{2+}; and $c_{Cu2+} = 5 \times 10^{-4}$ mol dm^{-3}, $c_{cAMP} = 1 \times 10^{-3}$ mol dm^{-3}, $c_{Spm} = 1 \times 10^{-3}$ mol dm^{-3}.

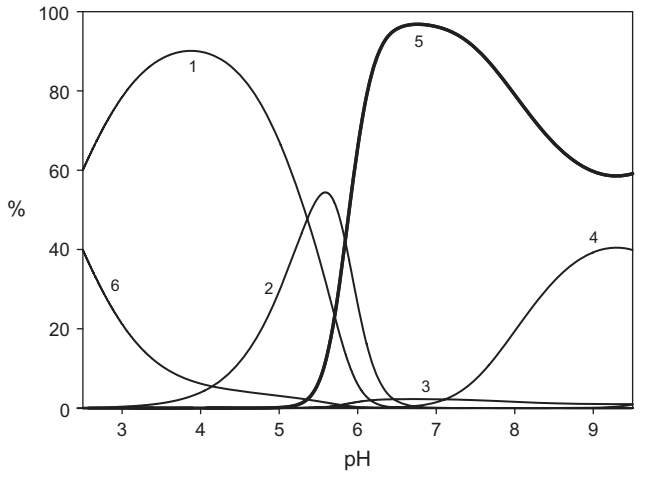


Figure 5. Distribution diagram for Cu(II)/cAMP/3,3,3-tet; the percentage of the species refers to the total metal; 1 – Cu(cAMP), 2 – Cu(cAMP)(OH), 3 – Cu(3,3,3-tet), 4 – $CuH_2(3,3,3\text{-tet})_2$, 5 – Cu(cAMP)(3,3,3-tet), and 6 – Cu^{2+}; and $c_{Cu2+} = 5 \times 10^{-4}$ mol dm^{-3}, $c_{cAMP} = 1 \times 10^{-3}$ mol dm^{-3}, $c_{3,3,3-tet} = 1 \times 10^{-3}$ mol dm^{-3}.

donor nitrogens from $-NH_x$ of the polyamine (which promotes deprotonation of the polyamine). The second ligand binds metal only through one nitrogen (the polyamine remains partly protonated), as indicated by the low log $K_e = 3.75$ of the reaction Cu(Spm) + HSpm $\rightleftarrows$ $CuH(Spm)_2$ (log K_e is 14.66 for reaction Cu + Spm $\rightleftarrows$ Cu(Spm), with four nitrogens involved in the coordination [48]). Analogous coordination has been established for $CuH_2(3,3,3\text{-tet})_2$.

Comparing to the system including Spm, the interaction in the Cu(cAMP)(3,3,3-tet) complex is much different. The equilibrium constant of reaction Cu(cAMP) + 3,3,3-tet $\rightleftarrows$ Cu(cAMP)(3,3,3-tet) is 16.63 (log $K_e = \log\beta_{Cu(cAMP)(3,3,3\text{-tet})} - \log\beta_{Cu(cAMP)}$ and is similar to that of Cu(3,3,3-tet) formation log $K_e = 16.36$ [48], which means that both ligands are located in the inner coordination sphere. This conclusion is confirmed by the fact that in contrast to the Cu/cAMP binary systems, in the ternary ones, no precipitate takes place.

Results of the spectroscopic studies confirm the proposed model of interactions in the mixed complexes $Cu(cAMP)H_4(Spm)$ and Cu(cAMP)(3,3,3-tet). The energy of d–d transition for $Cu(cAMP)H_4(Spm)$ at pH of maximum concentration is $\lambda_{max} = 789.5$ nm, which indicates formation of the $\{O_x\}$ chromophore with coordination only by the nucleotide phosphate [44, 57]. Thus, the fully protonated spermine does not bind the metal directly and is located in the outer coordination sphere. EPR parameters obtained exclude involvement of nitrogen in coordination of Cu(II). The parameters obtained for $Cu(cAMP)H_4(Spm)$ at pH of 4.0, $g_{||} = 2.398$, and $A_{||} = 124.3 \cdot 10^{-4}$ cm^{-1} correspond to participation in metal bonding only of oxygen.

The models of interaction are clearly confirmed by simple spectroscopic experiments. Introduction of increasing amounts of cAMP to the Cu(II)/Spm system leads to changes in the d–d band to higher energy, from 820 nm to about 790 nm (pH 4.0), which proves that copper(II) is coordinated via phosphate from cAMP. Introduction of Spm to Cu(II)/cAMP does not lead to significant shifts in the position of the maximum absorption, indicating that the polyamine is not involved in direct metal bonding and CuL$\cdots$L' is formed (L = cAMP, L' = Spm) ($\cdots$ noncovalent interplay, tentative mode of interplay in figure 6(a)). Interaction

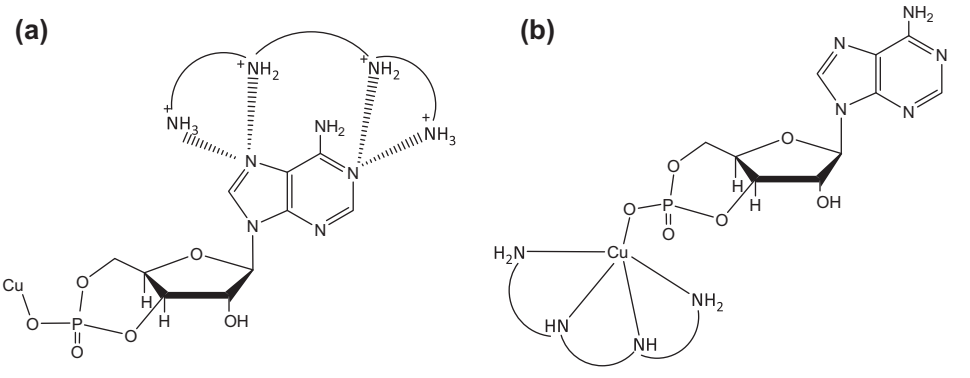


Figure 6. Tentative solution structure of the complexes studied: (a) Cu(cAMP)H_4(Spm) and (b) Cu(cAMP)(3,3,3-tet).

between the protonated polyamine and cAMP from the metal binary complex is confirmed by shifts of ^{13}C NMR signals in spectra of both ligands. The signals assigned to C(2), C(6), C(5), and C(8) from the nucleotide neighboring nitrogens N(1) and N(7) are shifted by 0.197, 0.153, 0.098, and 0.108 ppm, respectively (pH close to 4.0), while the signal assigned to C(4) which is not close to the potential centers of interaction is shifted only by 0.007 ppm. The ^{13}C NMR spectrum of spermine reveals changes in the positions of signals assigned to all carbons C_1, C_2, C_3, C_4, and C_5 of the polyamine equal to 0.078, 0.059, 0.108, 0.127, and 0.097 ppm, respectively. Binding of metal ions with phosphate prevents noncovalent interaction of this group with Spm. Interaction with both endocyclic nitrogen as well as phosphate is impossible due to steric reasons.

The Vis spectrum of Cu(cAMP)(3,3,3-tet), taken at pH = 7.5, $\lambda_{max} = 603$ nm, points to formation of $\{4\,N,O_x\}$ chromophore [50, 61], confirmed by EPR parameters $g_{\|} = 2.205$ and $A_{\|} = 180.1 \cdot 10^{-4}$ cm^{-1} at pH of 7.5 (tentative mode of coordination in figure 6(b)).

As a result of coordination, no significant changes are observed in the positions of signals assigned to carbons neighboring N(1) and N(7) in the ^{13}C NMR spectrum, which excludes participation of the endocyclic nitrogens from cAMP in the interaction. The ^{31}P NMR signal corresponding to phosphorus from the nucleotide at pH of 6.9 changes its position by 0.307 ppm, which points to participation of phosphate from the nucleotide in coordination. Signals assigned to C_1, C_2, C_3, C_4, and C_5 from 3,3,3-tet at pH 6.9 change their positions by 0.105, 0.092, 0.123, 0.107, and 0.089 ppm, respectively, which means that four nitrogens are involved in coordination.

4. Conclusions

In (cAMP)H_x(Spm) adducts formed in the metal-free cAMP/PA systems, the centers of interaction are phosphate of the nucleotides (in contrast to the systems with AMP), N(1) and N(7) from cAMP and protonated NH_3^+ from the polyamine. In the cAMP/3,3,3-tet complexes, the same centers of interaction are involved in adduct formation. The presence of metal ions in the system considerably changes the mode of interaction between both ligands.

The main site of coordination in Cu(cAMP) is the phosphate from the nucleotide. In the ternary system, Cu(II)/cAMP/Spm protonated complex is formed. Oxygens from the nucleotide phosphate are involved in metal bonding, while the protonated polyamine has noncovalent interaction with the anchoring Cu(cAMP). Significant differences in mode of coordination are observed in the system, including the shorter tetramine −3,3,3-tet. In this system, a hetero ligand complex of MLL′ type is formed in which Cu(II) binds the polyamine nitrogen and the nucleotide phosphate. Cyclic adenosine monophosphate activates protein kinases that catalyze phosphorylation of substrate proteins. Since copper(II) in the complexes with cAMP are bonded mainly by phosphate, the endocyclic nitrogens from this nucleotide are the potential centers of noncovalent interactions with other bioligands (as in the systems with spermine, but not in the system with 3,3,3-tet), which permits the nucleotide reaction on the signaling pathway of this second messenger.

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