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Interaction Centers of Purine and Pyrimidine Nucleotides in Their Reactions with Cu(II), Ni(II), Co(II), Cd(II) and Hg(II) Ions

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It has been established that in ATP complexes with Cu(II), Co(II) and Cd(II) ions, the metallation centres are the oxygen atoms of the phosphate group and the nitrogen atom N(7) from the purine ring. The spectral data suggest some involvement of N(1) atom in these interactions. In the ATP complexes with Ni(II) the main centres of coordination were found to be N(7) and N(1), while the contribution of the oxygen atoms from the phosphate group is of secondary importance. In the ATP complexes with Hg(II) ions, above pH 7, the metallation involves only the oxygen atoms from the phosphate group, while the N(1) and N(7) atoms are outside the inner sphere of coordination. In the complexes of CTP with the metal ions studied the interaction centres are the oxygen atoms from the phosphate group and N(3) from the pyrimidine ring. However, in the case of complexes with Ni(II), the main centre of interaction is N(3), while the involvement of the oxygen atoms from the phosphate group is of minor importance.

Key words: complexes, Cu(II), Ni(II), Co(II), Cd(II), Hg(II), nucleotides

Nucleotides and their derivatives take part in many fundamental processes in living organisms. ATP is one of the most important metabolic substances, used and resynthesized by the living organisms within 24 hours in an amount almost equal to their mass [1]. In the reactions taking place in living organisms, nucleotides occur at different concentrations, sometimes relatively high, *e.g.* in chromaffin granules, in which catecholamines are stored, the concentration of ATP is close to 0.1 M [2–4]. Practically all enzymes react with nucleotides but, according to many data, the substrates in a lot of reactions are not free nucleotides but their complexes with metals [5,6]. Despite intense investigation of the interaction of nucleotides with metal ions, the role of metals in these processes has not been fully recognized and more, there are ambiguities concerning the coordination mode of metal ions with the bioligands. According to literature, some authors suggest that the reactions of divalent metal ions with ATP as well as CTP take place only with the involvement of oxygen atoms from the phosphate group, but others indicate that also the nitrogen atom N(7) of ATP or N(3) of CTP are involved. Moreover, the presence of complexes with H_2O bridges between the metal ions and the metallation centres, that is the phosphate group or a donor nitrogen atom, has also been suggested. These ambiguous data concern the

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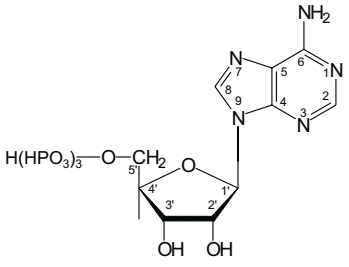
systems with metal ions present in living organisms, such as Cu(II), Ni(II), Co(II) as well as toxic metal ions such as Cd(II) or Hg(II) [7–15]. As follows from our studies, the metallation of bioligands is significantly affected by polyamines in living cells [16–21]. The first stage of our study of the reactions in the ternary systems including metal ions, polyamines and nucleic acid fragments has been devoted to the interactions between metal ions and nucleotides, in order to resolve some controversial questions on the involvement of particular functional groups in the metallation of the ATP or CTP. Without full recognition of the nature of these interactions, it is not possible to assess the role of biogenic amines, essential in the process of genetic information transfer.

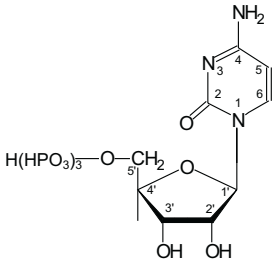
EXPERIMENTAL

Adenosine 5′-triphosphate sodium salt (ATP) and cytidine 5′-triphosphate disodium salt were purchased from Sigma and used without further purification. Cu(II), Ni(II) and Co(II) nitrates, purchased at POCH Gliwice, were used after twice repeated crystallization from H_2O. $Hg(NO_3)_2{\cdot}H_2O$ was purchased from Aldrich, and $Cd(NO_3)_2{\cdot}4H_2O$ from Merck, and they were used without further purification. The method of determination of the concentration of Cu(II), Ni(II) and Co(II) in the about $1.5{\cdot}10^{-2}$ M stock solutions was described earlier [15,22]. The concentration of Hg(II) ions ($\sim 1{\cdot}10^{-2}$ M) was determined by precipitation titration with NaCl solution in the presence of diphenylocarbazone, while the concentration of Cd(II) ions ($\sim 1{\cdot}10^{-2}$ M) was determined complexometrically using EDTA and pyrocatechol violet as indicator. Potentiometric titrations were performed using a titration set made by Methrom including a 713 pH-metr, 725 dosimat, and an electrode 6.233.100 for the ions Cu(II), Ni(II) and Co(II), and a DTS Radiometer 800 Multi-Titration System with a GK-2401C electrode for Cd(II) and Hg(II). The electrodes were calibrated in terms of hydrogen ions concentration [23]. The concentrations of ATP and CTP in the systems with Cu(II), Ni(II), Co(II) ions were $1{\cdot}10^{-3}$ M–$1.5{\cdot}10^{-3}$ M and with Cd(II), Hg(II) ions were $1.3{\cdot}10^{-3}$ M–$5.2{\cdot}10^{-3}$ M. The metal to ligand ratio for the systems with Cu(II) was from 1:3.3 to 1:4.6, with Ni(II) and Co(II) from 1:4 to 1:6.5 and with Cd(II) and Hg(II) from 1:1 to 1:4. The measurements were carried out in helium atmosphere at the ionic strength $\mu = 0.1$ (KNO_3), at T = (20±1)°C, using a CO_2 free solution of NaOH as a titrant. The titrations of the Cu(II), Ni(II) and Co(II) systems were carried out to pH about 9, and to pH ~9.5 for Cd(II) and Hg(II) systems, because at higher pH a precipitate appeared. 150 to 350 points for each titration were subjected to a computer analysis, using SUPERQUAD computer program for model selection and determination of stability constants [24]. HALTAFALL program was used to obtain species distribution [25]. The procedure of a model selection was described in [26]. The samples for NMR and IR study were prepared by dissolving of substrates in D_2O. The pD of the solution was adjusted by NaOD, DCl or DNO_3, taking into account that pD = pH meter readings +0.4 [27]. The concentration of the ligands in the samples was 0.05 M, and the metal to ligand ratio was 1:2 in the IR study and from 1:100 to 1:200 for Cu(II), 1:60 to 1:100 for Ni(II) and Co(II), 1:5 for Cd(II) and Hg(II) in the NMR measurements. The spectra ^{13}C NMR were taken on an NMR Gemini 300 VT Varian spectrometer with dioxane as internal standard. The positions of the signals in the ^{13}C NMR spectra were given in the TMS scale. The spectra ^{31}P NMR were recorded on an NMR Unity-300 Varian spectrometer with H_3PO_4 as internal standard. IR spectra were taken on a Bruker IFS-113v instrument with the use of a KRS5 cell. Vis spectra were taken on a UV 160 Shimadzu spectrometer at the ligand concentrations the same as in the samples used in potentiometric titration, and the metal to ligand ratio of 1:3.5. EPR spectra were recorded using a Radiopan SE/X 2547 spectrometer ($C^{2+}_{Cu} = 0.001$ M or 0.005 M) at the metal to ligand ratio of 1:2.5.

RESULTS AND DISCUSSION



ATP



CTP

Equilibrium studies of metal ion–nucleotide system: Table 1 presents stability constants of the complexes formed in the systems adenosine 5′-triphosphate (ATP) and cytidine 5′-triphosphate (CTP) with Cu(II), Ni(II), Co(II), Cd(II), Hg(II) and H^+ as well.

Table 1. Overall stability constants (logβ) and equilibrium constants ($\log K^{H}_{MHL}$) for the protonated complexes of ATP or CTP with H^+, Cu(II), Ni(II), Co(II), Cd(II) and Hg(II) ions.

	ATP			CTP		
	Species	logβ	$\log K^{H}_{MHL}$	Species	logβ	$\log K^{H}_{MHL}$
H^+	H(ATP)	6.50 (1)		H(CTP)	6.67 (2)	
	H_2(ATP)	10.88 (2)		H_2(CTP)	11.54 (2)	
Cu^{2+}	Cu(HATP)	10.53 (3)	3.90	Cu(HCTP)	10.14 (8)	3.95
	Cu(ATP)	6.63 (2)		Cu(CTP)	6.19 (3)	
	Cu(ATP)(OH)	–1.25 (2)		Cu(CTP)(OH)	–0.81 (5)	
Ni^{2+}	Ni(ATP)	4.69 (6)		Ni(HCTP)	9.42 (6)	5.07
				Ni(CTP)	4.35 (5)	
Co^{2+}	Co(ATP)	4.82 (4)		Co(CTP)	4.74 (7)	
				Co(CTP)(OH)	–3.69 (6)	
Cd^{2+}	Cd(HATP)	9.56 (7)	4.60	Cd(HCTP)	9.56 (7)	4.60
	Cd(ATP)	4.96 (4)		Cd(CTP)	4.96 (4)	
Hg^{2+}	Hg(HATP)	13.20 (5)	3.54	Hg(HCTP)	15.17 (3)	3.02
	Hg(ATP)	9.66 (4)		Hg(CTP)	12.15 (3)	
	$Hg(ATP)_2$	12.42 (8)		$Hg(CTP)_3$	19.19 (4)	

All metals studied form ML type complexes with the nucleotides. The stability of the complexes decreases in the order of Hg(II) > Cu(II) > Cd(II) > Co(II) > Ni(II). Except Co(II) these ions form protonated complexes (Ni(II) only with CTP), Table 1. All the protonated species occur up to pH ~ 6 (Figs. 1a, b, c, d). Taking into regard the fact that two protons from the phosphate group dissociate off at pH < 2, and the proton from the endocyclic $–NH^+$ group at pH ~ 4.5 (Table 1) [28,29], the pH range of the protonated complexes occurrence indicates that the hydrogen cation remains at the phosphate group as it has been earlier observed [9].

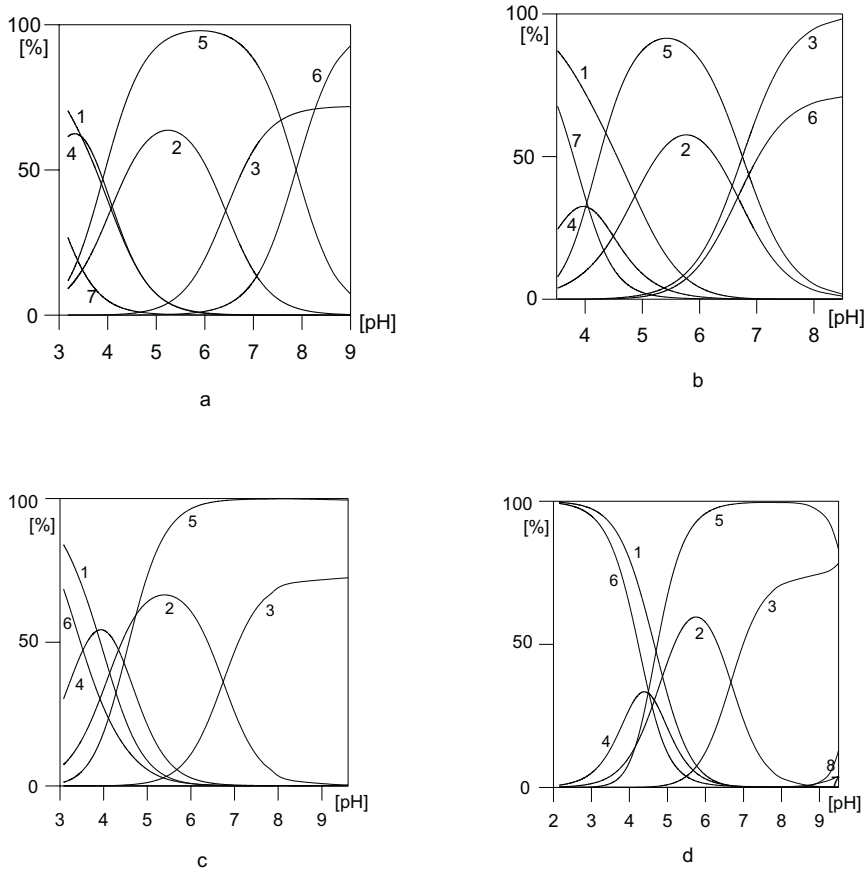


Figure 1. Distribution diagrams for the systems:
(a) Cu(II)/ATP: 1. H_2(ATP), 2. H(ATP), 3. ATP, 4. Cu(HATP), 5. CuATP, 6. Cu(ATP)(OH), 7. Cu^{2+}
$C_{ATP} = 1 \cdot 10^{-3}$ M, $C_{Cu^{2+}} = 4.2 \cdot 10^{-4}$ M;
(b) Cu(II)/CTP: 1. H_2(CTP), 2. H(CTP), 3. CTP, 4. Cu(HCTP), 5. CuCTP, 6. Cu(CTP)(OH), 7. Cu^{2+}
$C_{CTP} = 1 \cdot 10^{-3}$ M, $C_{Cu^{2+}} = 4.2 \cdot 10^{-4}$ M;
(c) Cd(II)/ATP: 1. H_2(ATP), 2. H(ATP), 3. ATP, 4. Cd(HATP), 5. CdATP, 6. Cd^{2+}
$C_{ATP} = 1.3 \cdot 10^{-3}$ M, $C_{Cu^{2+}} = 5.2 \cdot 10^{-4}$ M;
(d) Cd(II)/CTP: 1. H_2(CTP), 2. H(CTP), 3. CTP, 4. Cd(HCTP), 5. CdCTP, 6. Cd^{2+}, 7. $Cd(OH)^{+}$, 8. $Cd(OH)_2$
$C_{CTP} = 1.3 \cdot 10^{-3}$ M, $C_{Cu^{2+}} = 5.2 \cdot 10^{-4}$ M.

A significant decrease of the protonation constant of the phosphate group in the complexes with Hg(II), *i.e*. Hg(HATP) and Hg(HCTP) relative the values for free ligands ($\log K^{H}_{Hg(HATP)} = 3.54$ and $\log K^{H}_{Hg(HCTP)} = 3.02$, while $\log K_{H(ATP)} = 6.50$ and $\log K_{H(CTP)} = 6.67$, Table 1), indicates that in these complexes an effective centre of metallation is the phosphate group, although some involvement of other groups in the metallation is also possible.

In the case of complexes with Ni(II), taking into regard the fact that the metallation of the ligand leads to an increased proton lability, relatively small decrease in the protonation constant of the phosphate group ($\log K^{H}_{Ni(HCTP)}$ = 5.07, $\log K_{HCTP}$ = 6.67, Table 1) suggests that the participation of this group in the coordination is much weaker. The analogous changes of the protonation constants of the phosphate group in the complexes with the other metals take intermediate values, Table 1.

In the ML complexes of Cu(II), Co(II), Ni(II), Cd(II) with ATP and CTP, the values of logβ are higher than for the ML complexes with AMP and CMP (Table 1) [14,15]. The greatest differences were noted for the species with Cu(II), $\log\beta_{CuAMP}$ = 3.02 and $\log\beta_{CuATP}$ = 6.63, while the smallest for the Ni(II) species, $\log\beta_{NiCMP}$ = 3.38, and $\log\beta_{NiCTP}$ = 4.35 (Table 1) [15]. An increase of $\log\beta_{ML}$ of the ATP and CTP complexes, relative to its values for monophosphate complexes, suggests a participation of a greater number of oxygen atoms from the triphosphate group in the interaction with the metal atom, which has been confirmed by the results of the spectral studies presented below. The stability constant of the complex Hg(ATP) is lower than that of Hg(AMP) ($\log\beta_{HgAMP}$ = 12.57, while $\log\beta_{HgATP}$ = 9.66) [14], which can be explained by a different character of Hg(II) ions and their tendency towards formation of linear complexes [14].

Spectral studies of Cu(II)/ATP, Cd(II)ATP and Co(II)/ATP systems: As already established in the AMP complexes with Cu(II), Co(II) and Cd(II), the oxygen atoms from the phosphate group and N(1) or N(7) are involved in the coordination [14,15]. The complexes occur as a mixture of isomers (coordination dychotomy) with the chromophores {N(1),O} and {N(7),O}. The results of the equilibrium study indicate a more effective participation of the oxygen atoms from the phosphate group of ATP complexes with Cu(II), Co(II) and Cd(II) than in the analogous complexes with AMP (Table 1) [14,15]. For all ATP complexes with Cu(II), occurring in the pH range from 4 to 9, λ_{max} is ~750 nm, (the maximum wavelengths are 743.5, 750.5, 745.5, 741.0 and 742.5 nm, for pH 4, 5, 6, 7, 9, respectively). This wavelength corresponds to the formation of the {N1,O1} chromophore [15,18,30,31]. The presence of {N1,O1} chromophore is confirmed by EPR study of Cu(II)/ATP system (*e.g.* at pH 5 and 6 $A_{||}$ equals 159 and 157, and $g_{||}$ 2.332 and 2.333, respectively). These parameters are in agreement with the results obtained for analogous coordination systems [32,33]. In the ^{13}C NMR spectra (up to pH 7) the signals assigned to the carbon atoms from the neighbourhood of the donor nitrogen atoms N(7), N(1) and the phosphate groups, (*e.g.* at pH 5 the signals of C(6), C(2), C(8), C(5), C(5′) are shifted by 0.073, 0.066, 0.955, 0.651 and 1.108 ppm, respectively). At pH above 7 only the signals assigned to the atoms from the neighbourhood of N(7) and the phosphate group are changed. For example, at pH 9, the shifts of the signals assigned to C(6), C(2), C(8), C(5), C(5′) are 0.018, 0.005, 0.966, 0.555 and 0.126 ppm. The participation of the phosphate groups in the coordination of Cu(II) ions in the whole pH range considered is confirmed by the shifts of the signals assigned to the phosphorus atoms in the spectrum of ^{31}P NMR (at pH 9 the signals of P_α, P_β and P_γ are shifted by 0.021, 0.267 and 0.602 ppm, respectively). Some literature data suggest that for the steric reasons the simultaneous inter-

action of metal ions (II) with the phosphate group oxygens and the nitrogen N(1) from ATP is impossible [7,10,34,35]. However, our NMR study of Cu(II)/ATP performed at pH below 7 revealed the shifts of the signals assigned to the carbon atoms C(6) and C(2) from the vicinity of N(1) (at pH 4, 5, 6 the signals were shifted by 0.057 and 0.088, 0.073 and 0.066, 0.083 and 0.097 ppm, respectively), which indicates that the nitrogen atom N(1) is also involved in the interactions of ATP with Cu(II) ions. On the other hand, the shift of the signal assigned to C(4) atom (not being in vicinity of any donor centres) is not observed (*e.g.* at pH 5 there is no change of the C(4) signal position, while at pH 9 it is changed by only 0.008 ppm). A similar model of the interactions with {N1, O1} chromophore has been proposed for the systems Co(II)/ATP and Cd(II)/ATP. For the complex Co(II)/ATP the wavelength λ_{max} at pH 8 and 10 is 518, 818 and 512, 827 nm, which testifies to the formation of {N1, O1} chromophore [15]. The NMR spectra of the complex Co(II)/ATP, apart from the shifts of the signals assigned to the atoms located close to N(7) and the phosphate group, reveal also the shifts of the signals from the vicinity of N(1), (*e.g.* at pH 8 C(6), C(2), C(8), C(5) and C(5′) the signals are shifted by 0.036, 0.031, 0.038, 0.030 and 0.027 ppm, respectively). A shift of the signal assigned to C(4) is only 0.004 ppm. For the complexes with diamagnetic Cd(II), the shifts of the signals in the ^{13}C NMR and ^{31}P NMR spectra also point to the formation of {N1,O1} chromophore including the nitrogen atom N(7) but also N(1), (*e.g.* at pH 6 at which the Cd(ATP) complex is dominant, signals for C(2), C(6), C(5), C(8) and C(5′) are shifted by 0.056, 0.405, 0.542, 0.712 i 0.065 ppm, Table 2).

Spectral studies on Ni(II)/ATP system: As has been established earlier, the donor nitrogen atoms N(1) and N(7) from the nucleotide are involved in coordination in Ni(AMP) complex, including (but only in the case of high pH of solution) oxygen atoms from the phosphate group [15]. In the case of the adenosine 5′-triphosphate system the positions of the d-d bands of Ni(II)/ATP at 386, 782 nm and 389, 788 nm for pH 8 and 10, respectively, suggest the involvement of the oxygen atoms from the phosphate group of the nucleotide in the coordination, which is confirmed by ^{13}C NMR and ^{31}P NMR studies (for instance the chemical shifts of C(6), C(2), C(4), C(8), C(5) and C(5′) at pH 7 are 0.027, 0.032, 0.009, 0.035, 0.031 and 0.018 ppm, respectively). The changes in the positions of the ^{31}P NMR signals (the shifts of P_α, P_β and P_γ at pH 7 are 0.003, 0.012 and 0.012 ppm, and at pH 9 they are 0.006, 0.018 and 0.016 ppm, respectively) indicate the participation of oxygen atoms mainly from the groups P_β and P_γ. Moreover, a small change in the signal positions, and a small increase of the Ni(ATP) stability constant relative to that of Ni(AMP) (Table 1) [15], suggest that the participation of the oxygen atoms from the phosphate group in the coordination with Ni(II) is of secondary importance.

Spectral study of the Hg(II)/ATP system: As follows from the results of NMR study, in the system Hg(II)/ATP (pH 2.5 and 5.0) the metallation involves the nitrogen atoms N(7) or N(1) of the ATP purine ring and the oxygen atoms from the phosphate group, Table 2. At pH 2.5 the signals assigned to C(2), C(6), C(5), C(8) and C(5′) are shifted by 0.304, 0.451, 0.140, 0.021 and 0.168 ppm, respectively. The changes in the

Table 2. ^{13}C NMR and ^{31}P NMR signal positions for the Cd(II) or Hg(II)/ATP or CTP systems and their changes in relation to free ligands* (ppm, in parentheses).

Species	pH	C(2)	C(4)	C(5)	C(6)	C(8)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	P_α	P_β	P_γ
Cd(HATP)	4	150.989 (0.057)	149.339 (0.227)	118.667 (0.542)	153.950 (0.429)	142.310 (0.679)	88.358 (0.283)	71.137 (0.040)	75.691 (0.307)	84.896 84.783 (0.032) (0.041)	66.146 (0.112)	–10.962 –11.092 (0.145) (0.168)	–21.886 (0.848)	–9.397 –9.527 (1.115) (1.130)
Cd(ATP)	6	153.635 (0.056)	149.687 (0.259)	118.837 (0.542)	155.980 (0.405)	141.574 (0.712)	87.962 (0.283)	71.161 (0.081)	75.489 (0.348)	84.815 84.702 (0.007) (0.008)	66.025 (0.065)	–10.718 –10.871 (0.229) (0.236)	–20.604 –20.734 –20.871 (1.282) (1.297) (1.313)	–6.130 –6.267 (1.962) (1.978)
Cd(HCTP)	4	152.533 (0.521)	162.412 (0.400)	96.702 (0.112)	144.059 (0.204)	–	65.419 (0.112)	69.995 (0.009)	75.158 (0.041)	83.911 84.023 (0.093) (0.093)	90.209 (0.093)	–11.158 –11.317 (0.170) (0.182)	–22.108 –22.267 –22.413 (0.804) (0.804) (0.816)	–9.841 –9.987 (0.889) (0.902)
Cd(CTP)	6	158.430 (0.059)	166.395 (0.527)	97.464 (0.007)	142.275 (0.008)	–	65.577 (0.150)	70.060 (0.033)	75.037 (0.008)	83.632 83.511 (0.023) (0.144)	89.920 (0.005)	–11.560 –11.719 (0.426) (0.526)	–22.072 –22.230 –22.377 (0.142) (0.016) (0.163)	–8.561 –8.707 (0.252) (0.398)
Hg(HATP)	2.5	148.723 (0.304)	147.314 (0.818)	119.138 (0.140)	151.420 (0.451)	143.007 (0.021)	88.883 (0.275)	70.833 (0.134)	75.828 (0.074)	84.842 (0.151) (0.037)	65.847 (0.168)	–11.023 –11.145 (0.031) (0.091)	–22.451 (0.138)	–10.336 (0.275)
Hg(ATP)	5.0	153.099 (0.370)	149.755 (0.034)	119.384 (0.155)	155.922 (0.294)	141.072 (0.047)	87.808 (0.004)	71.121 (0.024)	75.274 (0.021)	84.841 84.725 (0.031) (0.053)	66.023 (0.083)	–11.000 –11.153 (0.031) (0.031)	–22.436 (0.198)	–10.000 (0.237)

Table 2 (continuation)

$Hg(ATP)_2$	7.5	153.754 (0.059)	149.895 (0.097)	119.388 (0.057)	156.451 (0.011)	140.877 (0.052)	87.694 (0.053)	71.004 (0.057)	75.132 (0.058)	84.788 84.681 (0.109) (0.105)	65.901 (0.045)	–10.794 –10.955 (0.061) (0.077)	–21.413 (0.412)	–6.359 –6.504 (0.664) (0.656)
Hg(HCTP)	2.5	151.037 (1.708)	161.363 (1.498)	96.778 (0.633)	144.050 (0.688)	–	65.260 (0.019)	69.842 (0.028)	75.186 (0.014)	83.992 (0.195) (0.078)	90.634 (0.410)	–11.223 –11.383 (0.060) (0.060)	–22.651 –22.811 –22.964 (0.137) (0.129) (0.136)	–10.529 –10.681 (0.167) (0.168)
Hg(CTP)	5.0	153.395 (3.952)	164.442 (1.612)	97.178 (0.152)	142.939 (0.288)	–	65.474 (0.080)	70.005 (0.089)	75.070 (0.001)	83.805 83.685 (0.066) (0.066)	90.080 (0.135)	–11.193 –11.487 (0.101) (0.193)	–22.690 –22.842 –23.010 (0.173) (0.021) (0.147)	–10.446 –10.598 (0.098) (0.250)

*[36–38].

positions of the signals in the ^{31}P NMR spectrum indicate that the main metallation centres of the phosphate group are the oxygen atoms at P_β and P_γ (the shifts of the signals corresponding to P_β and P_γ at pH 2.5 are 0.138 ppm and 0.275 ppm, respectively, whereas the shifts of the signals at P_α are significantly smaller: 0.031 and 0.091 ppm, Table 2). At higher pH values there are no position changes in the signals assigned to the carbon atoms located close to N(1) and N(7) of the nucleotide. However, significant shifts (at pH 7.5) of the signals assigned to P_β and P_γ (P_β 0.412 ppm, P_γ 0.664 and 0.656 ppm in the spectrum ^{31}P NMR) indicate that main centres of metallation in ATP complexes with Hg(II) are the oxygen atoms from the phosphate group. A decrease of the value of $\log\beta_{HgATP}$ relative to that of $\log\beta_{HgAMP}$ (Table 1, [14]) corresponds to a diminished contribution of the atoms N(1) and N(7) in coordination of Hg(II) with ATP. Such a model of the interactions is in agreement with our hitherto analysis of ternary systems including polyamines. In these systems the main centre of metallation is the phosphate group, whereas the donor nitrogen atoms from the purine and pyrimidine rings are involved in noncovalent interactions with other bioligands [16–21,39].

Spectral studies on Cu(II)/CTP, Co(II)/CTP, Ni(II)/CTP, Cd(II)/CTP and Hg(II)/CTP systems: According to some literature data, the main metallation centre of CTP are the oxygen atoms from the phosphate group, and less effective participation of the nucleotide nitrogen atom N(3) in the coordination is also possible [8,9,10,40]. However, our analysis of the spectral results suggests that the Cu(II), Hg(II), Cd(II) and Co(II) ions bind effectively both the oxygen atoms of the CTP phosphate group and as well the nitrogen atom N(3) from the nucleotide ring. For instance in the system Cu(II)/CTP in the whole pH range studied, that is from 3 to 10, the position of the d-d band indicates the formation of {N1,O1} chromophore (at pH 5, 6, 7 λ_{max} is 766, 744 and 774 nm, respectively). The ^{13}C NMR spectra show significant shifts of the signals assigned to the carbon atoms C(2) and C(4) in the neighbourhood of the nucleotide atom N(3) and the signal assigned to C(5′), being in the neighbourhood of the CTP phosphate group (*e.g.* at pH 5 the shifts of the signals assigned to the carbon atoms C(2), C(4) and C(5′) are 1.225, 0.584 and 0.127 ppm, while the shifts of the atoms C(5) and C(6), not in the vicinity of the donor centres are only 0.008 and 0.009 ppm). The participation of the phosphate group in the coordination has been supported by the analysis of ^{31}P NMR observation, (at pH 5, the signals at –11.294, –22.863 and –10.348 ppm assigned to P_α, P_β, P_γ of the free ligand, become one broad band with a maximum at –12.184 ppm as a result of metallation). IR studies exclude the coordination by oxygen atom from carbonyl group. The stretching vibrations bands both for the metal free nucleotide and complexed ligand occur at the same position (1457 cm^{-1}). The formation of {N1, O1} chromophore has been confirmed by EPR results, as at pH 8 the values $g_{||} = 2.3310$, and $A_{||} = 143$ (compare analogous values from [32,33]). For the systems of CTP with Co(II), Cd(II) and Hg(II), a similar character of interactions was obtained and the main metallation centres were concluded to be the oxygen atoms from the phosphate group and N(3) donor atom. For instance, in the spectrum of the system Co(II)/CTP in the whole pH range studied the

position of the d-d band with a maximum at about 820 nm indicates the formation of {N1, O1} chromophore [15]. An analogous coordination mode was found for the systems with diamagnetic Cd(II) and Hg(II) ions as it can be concluded from NMR results, Table 2. The positions of the d-d transition bands in the spectra of CTP with Ni(II) suggest a participation of the donor nitrogen N(3) and the oxygen atoms from the CTP phosphate group (λ_{max} at pH 7 is 385 and 699 nm) in the coordination. On the other hand, a small effect of Ni(II) ions on the lability of the proton from the phosphate group ($\log K^{H}_{Ni(HCTP)} = 5.07$, $\log K_{HCTP} = 6.67$) suggests that the participation of the oxygen atoms in the metallation is of minor importance, contrary to the interactions with the other investigated metals, and the main metallation centre remains the nitrogen atom N(3) from CTP, Table 1.

Considering biological systems including nucleotides and metal ions, the competitive effect of such bioligands as *e.g.* polyamines should be taken into regard. The changes in the character of the nucleotides donor centres as a result of the presence of polyamine have been shown earlier for the systems of metal (II) ions with adenosine 5′-monophosphate or cytidine 5′-monophosphate. The main and sometimes the only metallation centres in AMP and CMP are the oxygen atoms from the phosphate group of the nucleotide, while the donor nitrogen atoms from the purine and pyrimidine ring are more effective in the interligand non-covalent interactions [16–21,39]. As a continuation of this observation, a study of solving an analogous problem of the interactions in ternary systems with triphosphates, metal ions and polyamines is under way.

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