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Purine Nucleotide–Copper(II) Binary and Ternary Complexes. Synthesis, Electron Paramagnetic Resonance and Infrared Investigation

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Abstract

An X-band EPR and IR investigation was carried out on Cu(II) binary and ternary complexes involving ATP (adenosine 5′-triphosphate) or IMP (inosine 5′-monophosphate) and an aromatic base such as 2,2′-dipyridylamine (DPA), 2,2′-bipyridyl (BIPY) or 1,10-phenanthroline (PHEN) in the microcrystalline state or in aqueous solution. The measurements were performed at room temperature and at 100 K.

A new ternary complex containing Cu(II), IMP and DPA in a 1:1:1 molar ratio was prepared and characterized. The compound crystallizes as thin green plates in the monoclinic $P2_1$ space group with $a = 7.828(2)$, $b = 18.552(3)$, $c = 17.378(3)$ Å and $\beta = 91.16(2)°$. The Cu(II) ion interacts with phosphate oxygen atoms from IMP and with nitrogen atoms from DPA.

The interpretation of our data on the basis of the coordination sphere geometries suggests that EPR is a useful tool in investigating the structure and the nature of the close-lying donors of the Cu(II) ions. The EPR and IR data show that in the ternary complexes the aromatic bases BIPY, PHEN and DPA are bound to the Cu(II) centre, probably acting as bidentate ligands both in the solid state and in aqueous solution at pH = 7.

Introduction

Cu(II) ions proved to be very effective in promoting the phosphoryl transfer reaction on nucleoside triphosphates. Although the very important enzyme-catalyzed hydrolysis of ATP to adenosine 5′-diphosphate (ADP) and inorganic phosphate usually involves Mg(II) ions, the nature of the Cu(II)–nucleotide interaction is interesting owing to the Cu(II)-promoted large rate enhancement in the non-enzymatic ATP hydrolysis [1]. Zn(II) cations are less effective in catalyzing the process, while other cations such as Ni(II) and Mn(II) do not significantly accelerate the hydrolysis reaction. Binding of metal ions to the purine N(7) nitrogen atom is assumed to give an α,β-chelate, leaving the γ-phosphate free to undergo nucleophilic attack by water.

Addition of an aromatic base like BIPY able to form ternary complexes inhibits hydrolysis. This is probably due to the bond formation between the BIPY nitrogen atoms and the metal centre [2]. As a consequence, the N(7) purine nitrogen atom does not link to the Cu(II) cation and a β,γ or an α,β,γ-coordination preserves the terminal phosphate from hydrolysis.

In order to understand the nature of the Cu(II)–nucleotide interaction and the role of the aromatic base, the investigation of Cu(II)–nucleotide binary and ternary systems appears to be interesting both in the solution phase and in the solid state.

Recently we prepared a series of metal–ATP complexes cocrystallized with DPA [3]. This new class of compounds involves Mg(II), Ca(II), Mn(II), Co(II), Cu(II) and Zn(II) cations. The structures relevant to Mg(II), Ca(II), Mn(II) and Co(II) complexes [3–5] were characterized by a single-crystal X-ray diffraction technique. The aromatic base was found to be non-coordinated to M(II) for all the metal ions.

The compound containing the Cu(II) ion was found to have the same stoichiometry as the Mg(II)–ATP–DPA complex and an isomorphism could not be excluded in the solid state on the basis of X-ray powder measurements. Interestingly, the compounds containing Mg(II), Ca(II), Mn(II) and Co(II) showed the same α,β,γ-phosphate coordination type without any interaction between the purine moiety or DPA and the metal centre. Two ATP molecules are linked to a divalent cation forming an $M(HATP)_2^{4-}$ unit.

The EPR technique proved to be a valuable tool in revealing the coordination sphere geometry around the Cu(II) ion and the nature of the donor atoms.

Here we report the results of X-band EPR and IR measurements carried out on some Cu(II)–purine nucleoside mono- and tri-phosphate systems in

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aqueous solution at room temperature or 100 K and in the microcrystalline state.

A new Cu(II)–IMP–DPA compound was isolated in the solid state.

Experimental

Synthesis of the Complexes

The disodium salt of adenosine 5′-triphosphoric acid (Na_2H_2ATP) and of inosine 5′-monophosphoric acid (Na_2IMP) were purchased from Sigma and used without any further purification. BIPY, DPA and PHEN were purchased from Fluka and recrystallized from ethanol. Cu(II) and Zn(II) sulphates were obtained from Carlo Erba and purified by crystallization from aqueous solutions. All the other materials were Reagent grade purchased from Merck.

The crystalline Cu(II)–ATP–PHEN, Cu(II)–ATP–BIPY and Cu(II)–ATP–DPA complexes were prepared as reported in ref. 6, 7 and 3, respectively. Cu(II)–IMP and Cu(II)–IMP–BIPY were prepared according to the procedure reported in ref. 8. Cu(II)–IMP–DPA was prepared by the following procedure: DPA (2×10^{-3} mol) was dissolved in ethanol (2 ml). The solution was added to an aqueous solution (5 ml) of Na_2IMP (2×10^{-3} mol). The resulting mixture was added to a solution (5 ml) of $CuSO_4 \cdot 5H_2O$ (2×10^{-3} mol). A green crystalline powder precipitated. The suspension was heated to 80 °C for 10 min, then it was cooled to room temperature. The solid was collected, then washed with water and ethanol. It was stored on silica gel.

Single crystals as tufts of very thin green plates were obtained by slow cooling of hot aqueous solutions of the complex.

Analytical data for all the complexes are reported in Table I.

EPR and IR Measurements

The X-band EPR spectra were recorded with an ER 200-SRCB Bruker spectrometer operating at $\omega_0 = 9.78$ GHz. The external magnetic field was calibrated with a microwave bridge ER041 MR Bruker wavemeter and the temperature was controlled with a ER 4111VT Bruker device (the accuracy was ±1 K). In order to estimate accurate g values at both room temperature and 100 K, the diphenylpicrylhydrazyl (DPPH) free radical was used as field marker (g_{iso} (DPPH) = 2.0036, $\omega_0 = 9.43$ GHz). To avoid Cu(II) dipole–dipole interactions in the solid state, the Cu(II) compounds were doped in Zn(II) at room temperature; spin–spin interactions in solution were ruled out by using Cu(II) concentrations of less than 5×10^{-3} mol dm^{-3}. The measurements were carried out in the solid state for all the complexes and in aqueous solution for the ATP species only as the IMP binary and ternary compounds are slightly soluble at neutral pH.

The simulations of the X-band spectra were carried out with the SIM14A program [9] implemented on a VAX/750 computer.

Infrared spectra, as Nujol mulls between CsI plates or as KBr pellets, were measured on a Perkin-Elmer Model 597 spectrometer.

Single crystal cell parameters were measured with a Philips PW1100 automatic diffractometer by the least-squares method applied to the setting angles of 25 reflections. A crystal measuring $0.15 \times 0.15 \times 0.02$ mm was used.

Results and Discussion

Table II reports the X-band EPR parameters for a number of Cu(II) binary and ternary complexes both in the solid state (microcrystalline, room temperature) and in frozen solution (100 K). The spectra of the crystalline powder at room temperature are practically superimposable with those recorded at 100 K. Selected EPR spectra are reported in Fig. 1 and Fig. 2.

TABLE I. Analytical Data (%) for the Binary and Ternary Complexes[a]

Compound	C		H		N		Cu	
	Calculated	Found	Calculated	Found	Calculated	Found	Calculated	Found
Cu(II)–ATP–BIPY	31.56	31.73	3.44	3.41	12.88	12.64	8.35	8.04
Cu(II)–ATP–DPA	30.95	30.70	3.50	3.30	14.45	14.70	8.20	8.40
Cu(II)–ATP–PHEN	32.54	32.38	3.60	3.75	12.07	11.88		
Cu(II)–IMP	24.46	24.34	4.11	3.80	11.42	11.19		
Cu(II)–IMP–BIPY	36.18	35.62	3.49	3.75	14.77	14.32		
Cu(II)–IMP–DPA	37.83	38.20	4.13	4.15	15.44	15.50	10.01	9.95

[a] The calculated percentages were obtained on the basis of the following formulae: $Cu(H_2ATP)(BIPY) \cdot 2H_2O$, $C_{20}H_{26}N_7O_{15}P_3Cu$; $Cu(HATP)(HDPA) \cdot 2H_2O$, $C_{20}H_{27}N_8O_{15}P_3Cu$; $Cu(H_2ATP)(PHEN) \cdot 3.5H_2O$, $C_{22}H_{29}N_7O_{16.5}P_3Cu$; $Cu(IMP) \cdot 4.5H_2O$, $C_{10}H_{20}N_4O_{12.5}PCu$; $Cu(IMP)(BIPY)(H_2O) \cdot NO_3 \cdot H_2O$, $C_{20}H_{23}N_7O_{13}PCu$; $Cu(IMP)(DPA)(H_2O) \cdot 2H_2O$, $C_{20}H_{26}N_7O_{11}PCu$.

TABLE II. X-band EPR Parameters. Estimated Standard Deviations are: $g_\parallel$, 0.002; $A_\parallel$, 0.6[a]

Compound	Solid state[b]				Frozen solution[c]					
	$g_\parallel$	$A_\parallel$[d]	$g_\perp$	$A_\perp$[d]	$g_\parallel$	$A_\parallel$[d]	$g_\perp$	$A_\perp$[d]	g_0[e]	A_0[d, e]
Cu(II)–ATP–BIPY	2.316	144.0	2.074	12.8	2.287	165.5	2.069	12.4	2.142	63.4
Cu(II)–ATP–DPA	2.298	137.0	2.070	15.8	2.285	156.7	2.070	14.6	2.142	61.7
Cu(II)–ATP–PHEN	2.316	140.8	2.077	12.2	2.295	160.0	2.070	12.0	2.145	61.3
Cu(II)–IMP	2.343	138.0	2.078	12.4						
Cu(II)–IMP–BIPY	2.270	161.5	2.066	12.1						
Cu(II)–IMP–DPA	2.273	158.1	2.065	12.9						

[a] As none of the experimental spectra (Figs. 1 and 2) shows any hyperfine splitting of the $g_\perp$ bands, the $A_\perp$ values were obtained from a line-simulation procedure. However, we point out that accurate values for $A_\perp$ can be calculated only if hyperfine components are actually resolved and simulated. [b] Microcrystalline Cu(II) powders doped in Zn(II) species at room temperature. [c] The spectra were recorded at 100 K. The pH value at room temperature was 7.0. [d] The value of the A parameters are in gauss. [e] The isotropic g_0 and A_0 values were calculated on the basis of the formulae: $g_0 = 1/3(g_\parallel + 2g_\perp)$; $A_0 = 1/3(A_\parallel + 2A_\perp)$.

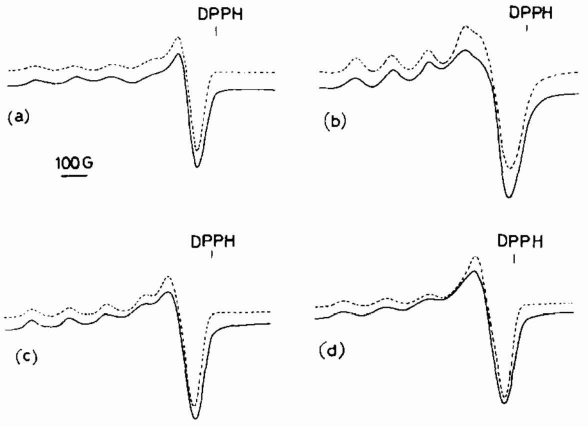

Fig. 1. X-band EPR spectra at 25 °C for selected microcrystalline ternary complexes: (a) Cu(II)–ATP–BIPY; (b) Cu(II)–ATP–DPA; (c) Cu(II)–ATP–PHEN; (d) Cu(II)–IMP–DPA. The Cu(II) species were doped into the Zn(II) compounds. Experimental: solid line; simulated: broken line.

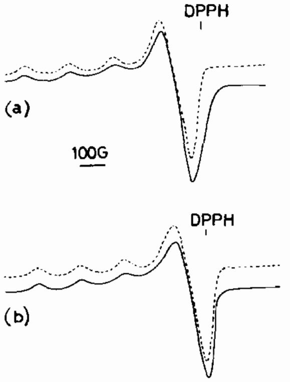

Fig. 2. X-band EPR spectra at 100 K of aqueous solutions (pH = 7 at 25 °C) of: (a) Cu(II)–ATP–BIPY and (b) Cu(II)–ATP–PHEN systems. The solutions were 1×10^{-3} mol dm^{-3} for all the components. Experimental: solid line; simulated: broken line.

Solid State EPR Spectra

The g as well as the A EPR parameters relevant to Cu(II)–ATP–BIPY and Cu(II)–ATP–PHEN ternary complexes are practically the same. In particular, the $g_\parallel$ values are equal to 2.316 for both compounds. The Cu(II)–ATP–BIPY complex is isomorphous with the corresponding Zn(II) species [10] and with the Cu(II)–ATP–PHEN compound [6].

It should be pointed out that these structures consist of dimeric molecules in which two metal atoms show an axially distorted octahedral coordination geometry formed by two oxygen atoms from two different γ-phosphate groups, one β-oxygen atom and two nitrogen atoms from the BIPY or PHEN systems. The sixth position of the coordination polyhedron is occupied by an α-phosphate oxygen atom at a larger distance.

The EPR parameters relevant to the Cu(II)–IMP complex show a higher $g_\parallel$ value (2.343) and a smaller $A_\parallel$ value (138.0 gauss) in comparison with the corresponding parameters for Cu(II)–ATP–BIPY and Cu(II)–ATP–PHEN. Cu(II)–IMP probably has a polymeric structure [8] and Cu(II) ions are coordinated to N(7) of the hypoxanthine base and to three phosphate oxygen atoms from three different IMP units.

Kivelson and Neiman [11] found that $g_\parallel$ and $A_\parallel$ values for Cu(II) complexes vary with the composition of the ligand atoms bound to the metal. Vänngård [12] suggested that an EPR technique might be useful to define the structure of the copper site in some copper proteins. Peisach and Blunberg [13] described how EPR spectra of copper proteins may be used to investigate the structure of the coordination polyhedron. In agreement with the literature, our data relevant to Cu(II)–ATP–BIPY, Cu(II)–ATP–PHEN and Cu(II)–IMP compounds show that the larger the number of nitrogen donors the smaller the $g_\parallel$ value and the larger the $A_\parallel$ value.

For the two ternary complexes the N/O ratio in the coordination sphere is 0.5, while in Cu(II)–IMP the N/O ratio is 0.33.

The Cu(II)–IMP–BIPY complex has $g_{\parallel}$ and $A_{\parallel}$ parameters equal to 2.270 and 161.5 (gauss) respectively. These dara are in agreement with the higher N/O ratio in comparison with the ternary and binary compounds reported above.

The crystal structure of Cu(II)–IMP–BIPY [8] contains the monomeric $Cu(II)(IMP)(BIPY)(H_2O)_2^+$ cation and the nitrate anion. The Cu(II) ion is in a square-pyramidal sphere with the N(7) of the purine system, the bidentate BIPY molecule and a water molecule in the equatorial position and a second water molecule in the axial position. The N/O ratio is 1.5 and this explains the small $g_{\parallel}$ and the high $A_{\parallel}$ values.

Cu(II)–IMP–DPA has $g_{\parallel}$ and $A_{\parallel}$ values very close to those determined for the Cu(II)–IMP–BIPY complex. This fact suggests the two compounds could have similar structures. The X-ray diffraction studies on the green plates of Cu(II)–IMP–DPA show the complex crystallizes in the $P2_1$ monoclinic space group with $a = 7.828(2)$, $b = 18.552(3)$, $c = 17.378(3)$ Å and $\beta = 91.16(2)^\circ$. These cell parameters are very similar to those reported for Cu(II)–UMP–DPA (UMP = uridine 5′-monophosphate) [14] which crystallizes in the monoclinic $P2_1$ space group with $a = 7.739(3)$, $b = 18.248(6)$, $c = 17.473(7)$ Å and $\beta = 90.04(2)^\circ$. In addition, preliminary analysis of X-ray data indicates the two structures are similar. Thus we suggest the Cu(II)–IMP–DPA complex contains dimeric molecules where the two Cu(II) ions have almost identical square-pyramidal coordination spheres. A water molecule is further away from the metal ion than the two nitrogen atoms from DPA and the two phosphate oxygen atoms which constitute the equatorial ligands. The N/O ratio (0.67) is smaller than the corresponding value for Cu(II)–IMP–BIPY. The values of the $g_{\parallel}$ and $A_{\parallel}$ parameters for Cu(II)–IMP–DPA can be explained taking in account the total charge of the donor sets. In Cu(II)–IMP–DPA, the N_2O_3 donor group brings a −2 total charge owing to the presence of two phosphate oxygen atoms. In Cu(II)–IMP–BIPY, the N_3O_2 donor set has a formal charge equal to 0. It was shown that the higher the negative charge of the coordination sphere the smaller the $g_{\parallel}$ value and the higher the $A_{\parallel}$ value [13].

The $g_{\parallel}$ value (2.298) for the Cu(II)–ATP–DPA complex suggests the coordination sphere is similar to that found for the Cu(II)–ATP–BIPY and Cu(II)–ATP–PHEN species. Therefore we believe that Cu(II)–ATP–DPA is not isomorphous with the Mg(II)–, Ca(II)–, Mn(II)– and Co(II)–ATP–DPA species [3]. The DPA molecules are probably coordinated to the Cu(II) ions through the ring nitrogen atoms.

Infrared Spectra

Infrared absorption maxima are reported in Table III. Strong absorption at about 1600 and 1560 cm^{-1} have been found in most of the M(II)–ATP–DPA compounds. These absorptions can be assigned to C=C and C=N ring-stretching vibrations in DPA. An intense absorption at 1600 cm^{-1} was found in the spectrum of pure DPA but only a very weak band was revealed for Na_2H_2ATP. A medium absorption was also found at 1500 cm^{-1} for pure DPA. These data are in agreement with the presence of cocrystallized but noncoordinated DPA molecules in all the M(II)–ATP–DPA complexes studied by the single crystal X-ray diffraction technique [3].

The IR spectrum of the Cu(II)–ATP–DPA compound does not contain the absorptions at 1600 and 1560 cm^{-1}, but it shows a band at 1585 cm^{-1}. These data indicate that DPA interacts with the Cu(II) ion, probably acting as a bidentate ligand through the ring nitrogen atoms. The IR spectrum of Cu(II)–ATP–BIPY does not show the strong absorptions at 1580 and 1550 cm^{-1} present in the spectrum of pure BIPY. This is due to the bonding interaction between BIPY and the Cu(II) ion.

The intense bands at 1590 and 1540 cm^{-1} in the spectrum of Cu(II)–IMP–DPA are similar to the bands at 1590 and 1545 cm^{-1} found in the spectrum of free Na_2IMP. Therefore no useful information can be obtained about the absorptions relevant to the DPA molecule in this region.

The broad band at 1090 cm^{-1} in the spectrum of Na_2IMP is attributable to P–O stretching vibrations. In the spectrum of Cu(II)–IMP–DPA, two intense bands were found at 1150 and 1100 cm^{-1}. This difference between the two spectra is in agreement with the metal coordination to phosphate oxygen atoms in the ternary complex. The spectrum of Cu(II)–IMP–BIPY is similar to that of Na_2IMP in the P–O region, in agreement with the X-ray analysis which shows the phosphate oxygen atoms are not directly involved in the coordination to the metal.

The triphosphate region in the spectrum of Cu(II)–ATP–DPA does not show large differences in comparison with the spectra of the Mg(II)–, Ca(II)–, Mn(II)–, and Co(II)–ATP–DPA species. This could be explained with a α,β,γ-coordination also for the Cu(II)–ATP–DPA compound.

EPR Spectra in Solution

The EPR parameters relevant to the ternary species in frozen solution are again very similar for all the three ATP compounds. The pH was adjusted at 7 as the distribution diagrams for the ternary systems indicate that the $Cu(II)(ATP)(BASE)^{2-}$ species is the prevailing one (more than 95%) when Cu, ATP and the aromatic base (BASE) are in equimolar ratio [2, 15]. Therefore the EPR values we measured are probably relevant to the monomeric

TABLE III. Infrared Absorption Maxima (cm^{-1}) of Metal–Nucleotide Complexes[a]

Mg(II)–ATP–DPA	Cu(II)–ATP–DPA	Cu(II)–ATP–BIPY	Cu(II)–IMP–DPA	Cu(II)–IMP–BIPY	Cu(II)–IMP	Na_2H_2ATP	Na_2IMP	DPA	BIPY
1690(sh)	1690(s)	1705(s)	1680(s)	1680(s)	1690(s)	1705(s)	1680(s)		
1660(s)	1640(s)	1610(m)	1660(s)		1630(m)				
1600(s)	1585(m)	1600(m)	1590(m)	1600(m)	1600(m)		1590(m)	1600(s)	1580(s)
1560(s)			1540(m)	1550(m)	1580(m)		1545(m)	1560(m)	1550(s)
								1525(s)	
		1290(s)						1310(m)	
		1270(s)							
		1260(s)							
1245(s)	1230(s)	1230(s)	1240(w)			1255(s)			1250(s)
			1220(w)		1215(w)		1220(m)		
1120(s)	1120(s)	1110(m)	1150(s)	1120(s)	1120(s)	1110(s)		1145(m)	
1080(s)	1080(s)	1075(s)	1100(s)	1080(m)		1065(s)	1090(s)		1085(m)
1000(m)	1000(m)	1005(w)	1000(m)	980(m)	995(m)	980(s)	980(s)	985(w)	990(m)
		950(s)		950(m)		910(s)	940(m)		
		920(s)	900(w)						
895(m)	895(s)	900(m)							
820(w)	820(w)	815(m)	820(m)		830(w)	815(m)	800(m)		
770(m)	760(m)	775(m)	770(m)	780(m)				765(s)	755(m)

[a] Abbreviations: sh = shoulder; s = strong; m = medium; w = weak.

species in which the Cu(II) ion is coordinated to ATP and to the aromatic base molecule.

To summarize, this investigation confirms that in ATP ternary complexes, Cu(II) ions usually link to the aromatic bases both in the solid state and in aqueous solution. Thus the Cu(II)–ATP–DPA compound probably has a dimeric structure (in the solid state) in which the triphosphate chain and the aromatic base nitrogen atoms are linked to the metal.

In the IMP ternary compounds, the Cu(II) ions link to the aromatic base nitrogen atoms, but the existence of the bonding interactions with the phosphate moiety depends on the nature of the aromatic base itself.

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