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Interaction between Cu(II) and the Nucleobases in Solution

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The interaction between Cu^{2+} and nucleobases *viz.* adenine and guanine, has been followed by UV-difference spectrophotometry, high pressure liquid chromatography and molecular modeling. Cu^{2+} is found to form 1 : 1, 1 : 2 and 1 : 3 adducts with adenine and 2 : 1 and 1 : 1 adducts with guanine. In the adducts, the metal ion is believed in a distorted octahedral geometry being coordinated to adenine or guanine ligands and a number of water molecules. In all of the adducts except the 1 : 1 adduct between Cu^{2+} and guanine, there are one or more intramolecular H-bonds resulting into the formation of macrochelates.

Key Words: Adenine, Guanine, Copper(II) ion, UV difference spectra, Molecular modeling.

INTRODUCTION

Copper is a soft metal that can form coordinate covalent bonds with a number of donor centres[1] including O, N, S and Cl. Biomolecules including amino acids, proteins, nucleobases (NB), nucleotides, DNA and RNA provide excellent donor centres for Cu^{2+} and other metal ions. As a part of our project to investigate the interaction of metal ions with nucleobases, nucleotides and DNA, the interaction between copper(II) sulfate and adenine and guanine based on UV- difference spectroscopy using the technique of continuous variation combined with molecular modeling, were studied. Despite numerous studies on binding of divalent metal ions to nucleobases, nucleosides and nucleotides, in many cases there is controversy on the exact nature of the binding[2]. For example, it is often assumed that metal ions form 1 : 1 complexes with nucleobases in solution[3,4] although complexes with other binding ratios are quite likely[5]. The results of the present studies show that Cu^{2+} indeed binds with adenine and guanine in solution in 0.10 M $NaNO_3$ to form a number of adducts. Metal ions can also lead to base pairing. For example, Meggers *et al.*[6] showed that the addition of 1 equivalent of Cu^{2+} ions led to base pairing to the natural dA : dT base pair.

EXPERIMENTAL

UV difference spectral studies

0 to 4 mL of 1.0 mM solutions of $CuSO_4$ and 1.0 mM NB (adenine or guanine), both made in 0.10 M $NaNO_3$ dissolved in milli Q (mQ) water, were mixed in varying proportions and the total volume made up to 4 mL (Table-1). The solutions were placed in sealed plastic tubes and left at 37.0°C for 24 h. For each combination, the UV-visible spectrum from 190 to 400 nm of solution I (Table-1) was recorded using a Cary 1A UV-Visible spectrophotometer, to determine the wavelength (λ_{max}) at which the absorbance was a maximum. A scan rate of 200 nm per minute

TABLE-1
INTERACTION BETWEEN 1.0 mmol L^{-1} Cu^{2+} AND 1.0 mmol L^{-1} NUCLEOBASE (NB) (ADENINE OR GUANINE): ABSORBANCE VALUES AGAINST NB BLANKS

Solution	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
mL Cu^{2+}	4.00	3.75	3.50	3.25	3.00	2.75	2.50	2.25	2.00	1.75	1.50	1.25	1.125	1.00	0.875	0.75	0.50	0.25	0.00
mL NB	0.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	2.25	2.50	2.75	2.875	3.00	3.125	3.25	3.50	3.75	4.00
[Cu^{2+}] mM	1.00	0.94	0.88	0.81	0.75	0.69	0.63	0.56	0.50	0.44	0.38	0.31	0.28	0.25	0.22	0.19	0.13	0.06	0.00
[NB] mM	0.00	0.06	0.12	0.19	0.25	0.31	0.37	0.44	0.50	0.56	0.62	0.69	0.72	0.75	0.78	0.81	0.87	0.94	1.00
Abs at 278 nm[1]	–0.001	0.094	0.125	0.249	0.290	0.433	0.509	0.533	0.650	0.624	0.656	0.604	0.614	0.541	0.539	0.462	0.390	0.245	0.012
Abs at 289[2]	–0.003	0.063	0.378	0.594	0.706	0.802	0.804	0.566	0.599	0.55	0.506	0.375	0.313	0.292	0.285	0.258	0.129	0.069	0.00

[1] Cu^{2+} and adenine, [2] Cu^{2+} and guanine.

and a band width of 2 nm were used. The absorbance at λ_{max} was then measured for each solution A to S. The solution of the corresponding nucleobase at the same concentration was used as the blank. The absorbance values (Table-1) were then plotted against the added concentrations of the nucleobase and the metal ion to determine the stoichiometry of the adducts formed.

HPLC experiments

In this series of experiment, solutions of copper(II) sulfate and nucleobases (both 1.0 mM) were mixed at 1 : 1 molar ratio. The pH was adjusted to 7.0 in all cases and the mixtues were included for 24 h at 37°C. In the case of adenine, a number of experiments were done where the period of incubation was varied. At the end of the period, 50 μL aliquot was injected into a Waters HPLC (high performance liquid chromatography) system consisting of a Waters 600 pump and a Waters 600 controller, a Waters dual wavelength 2487 absorption detector set at 260 nm and a Waters 746 Data Module. Reverse phase method with a Waters Radial-Pak C_{18} column (8 × 100 nm, 10 μm particle size) was used. Ammonium acetate (0.1 M at pH 5.5) was used as eluent A whilst methanol (HPLC grade) was used as eluent B. The mobile phase consisted of 95% A and 5% B with a flow rate of 1.5 mL/min. The retention times for reaction mixtures and the components were noted. The Cu^{2+} contents (if any) of the eluted fractions were determined using a Varian Spectra A-20 plus Atomic Absorption Spectrophotometer (AAS) with a GTA-96 Graphite Furnace Tube Atomiser facility using the technique of standard addition. The nucleobase contents (if any) of the fractions were determined by UV-visible spectrophotometry. Based on the above results, the binding ratios of the adducts were calculated.

TABLE-2
OBSERVED AND COMPUTED UV-VISBLE SPECTRAL LINES

Complex	λ_{max} values (nm) observed in the difference spectra of continuously varying mixtures	Structure/*energy (kcal mol^{-1})/ΔH_f	Predicted spectral lines (nm)
$Cu(A)(H_2O)_5$	219, 278	$Cu(A)(H_2O)_5$—bonded to N7; −271186.5; −172582.5	232 (0.142); 240 (0.037); 261 (0.033); 277 (0.010); 287 (0.039); 293 (0.014); 335 (0.031)
$Cu(A)_2(H_2O)_4$	219, 278	$Cu(A)_2(H_2O)_5$—bonded to N7's; −370300.4; −238504.5	220 (0.042); 230 (0.011); 274 (0.012); 307 (0.016); 318 (0.008); 334 (0.008)
$Cu(A)_3(H_2O)_3$	219, 278	$Cu(A)_3(H_2O)_3$—bonded to N7's; −1022514.5; −857526.7	234 (0.011); 236 (0.008); 344 (0.011); 364 (0.012); 372 (0.022)
$Cu(G)(H_2O)_5$	224, 287	$Cu(G)(H_2O)_5$—bonded to N7; −314045.1; −208342.0	225 (0.157); 265 (0.0022)
$Cu(G)_2(H_2O)_4$	224, 287	$Cu(G)_2(H_2O)_4$—bonded to N7's; −1240773.7; −108697.5	223 (0.101); 271 (0.209); 284 (0.266); 323 (0.144)

HyperChem Calculations

The proposed structures of the complexes of Cu^{2+} with adenine and guanine were optimized and their electronic spectra generated based on molecular mechanics and semi-empirical calculations using HyperChem 5.[7] Geometry optimizations based on molecular mechanics (using MM^+ force field) and semi-empirical calculations (using ZINDO/1)[8, 9] were carried out. The electronic spectra were generated using the routine ZINDO/S.

RESULTS AND DISCUSSION

The plot of absorbance *vs.* concentration in the case of Cu^{2+} reacting with adenine (A), gave three maxima corresponding to the binding ratios (Cu^{2+} : A) of 1 : 1, 1 : 2 and 1 : 3 (Fig. 1). However, the only major peak found in the HPLC chromatogram did not have any copper although the retention time for the peak was

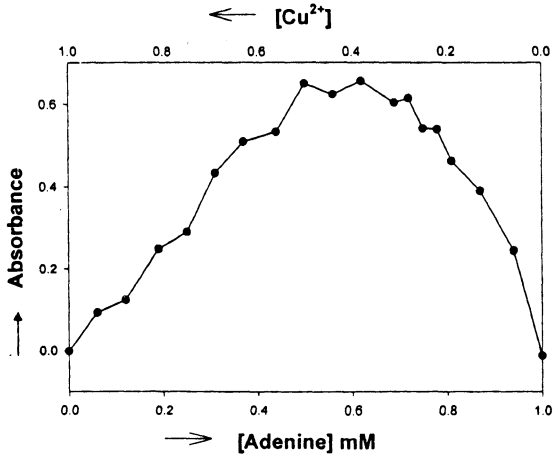

Fig. 1. Interaction between Cu^{2+} and adenine: Absorbance *vs.* concentration plot at 278 nm

different from that of adenine (6.31 min for adenine and 6.12 min for the major peak). Absence of copper in the peak fraction indicates the lability of copper-adenine adduct and change in retention time indicates a permanent change in adenine due to its binding with copper(II).

In the 1 : 1 adduct, Cu^{2+} is believed to be bonded to the N7 atom of adenine and five water molecules so that it has the stoichiometry $Cu(A)(H_2O)_5^{2+}$ (Fig. 2). In support of the idea that Cu^{2+} prefers to bind to nucleobases in DNA, it may be mentioned that based on the long wavelength shift and the change in the absorption band of UV-difference spectra, Bregadze[10] concluded that in solution in water Cu^{2+} interacts directly with nucleobases in DNA. And more to the point, current literature[4] suggests that Cu^{2+} ions prefer to bind to N7 position in purines as compared to the other positions such as N1 or N3.

In this adduct, there are two intramolecular H bonds involving the amino group of adenine and coordinated water molecules resulting into the formation of two macrochelates.

In the 1 : 2 complex between Cu^{2+} and adenine, Cu^{2+} is believed to be coordinated to two adenine ligands through N7 positions and four water molecules, so the complex has the formula $Cu(A)_2(H_2O)_4^{2+}$ (Fig. 3).

In this adduct, there is an intramolecular H bond involving N7 atom of adenine ligand and the amino group of the other adenine ligand resulting into the formation of a macrochelate.

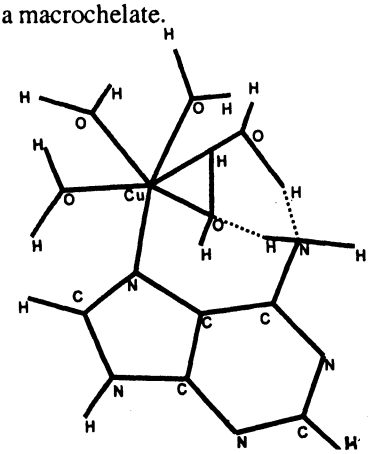

Fig. 2. The proposed structure of Cu(A)-$(H_2O)_5$ in which Cu^{2+} is believed to be bonded N7 centre of adenine and five water molecules in which there are two intramolecular h bonds

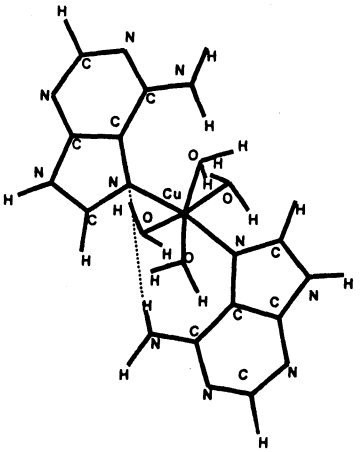

Fig. 3. The proposed structure of $Cu(A)_2$-$(H_2O)_4$ in which Cu^{2+} is believed to be bonded N7 centres of two adenine ligands and four water molecules in which there is an intramolecular H-bond between two adenine ligands

In the 1 : 3 complex between Cu^{2+} and adenine, Cu^{2+} is believed to be bonded to three N7 atoms and three water molecules so that it has the formula $Cu(A)_3(H_2O)_3^{2+}$ (Fig. 4). In this adduct, there is an intramolecular hydrogen bond resulting into the formation of a macrochelate. Alternatively, the observed 1 : 3 adduct between Cu^{2+} and adenine can be the result of stacking interaction between coordinated and uncoordinated adenines rather than actual coordination of all of the three adenine ligands with the metal ion. It has been suggested that head-to-tail stacking with five-membered and six-membered rings alternaing in the stack can occur in purines, especially adenine derivatives[11]. According to Mizutani *et al.*[12], the purine ring with a fused ring structure affords p-p stacking interaction in solution as well as in the solid state.

The plot of absorbance *vs.* concentration in the case of Cu^{2+} reacting with guanine (G) gave two maxima (Fig. 5) corresponding to the binding ratios of 2 : 1 and 1 : 1. However, there was only one major peak in the HPLC chromatogram corresponding to the formation of 1 : 1 adduct between Cu^{2+} and guanine. The observed retention time for guanine was 6.17 min and that for the major peak in the 1 : 1 incubated mixture of copper(II) sulfate and guanine was 5.48 min. Again absence of copper in the peak fraction indicates lability of copper-guanine adduct and change in retention time indicates a permanent change induced in guanine due to its interaction with copper(II).

In the 1 : 1 adduct, Cu^{2+} is believed to be the N7 atom of the guanine ligand and five water molecules so that it has the stoichiometry $Cu(G)(H_2O)_5^{2+}$ (Fig. 6). In this adduct, there is an intramolecular H-bond involving N7 atom of one adenine ligand and the amino group of a second adenine ligand.

In the 2 : 1 complex, it is believed that one Cu^{2+} is coordinated to the guanine

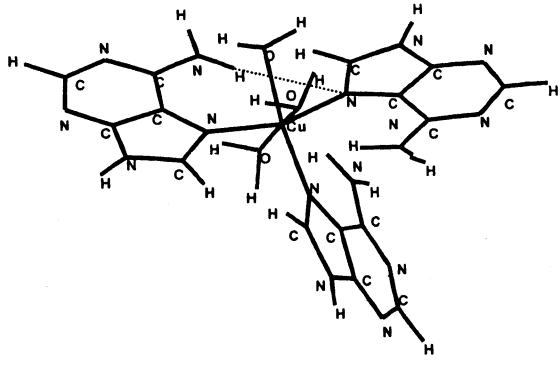

Fig. 4. The proposed structure of $Cu(A)_3(H_2O)_3$ in which Cu^{2+} is believed to be bonded N7 centres of three adenine ligands and three water molecules in which there is aa H-bond between two adenine ligands

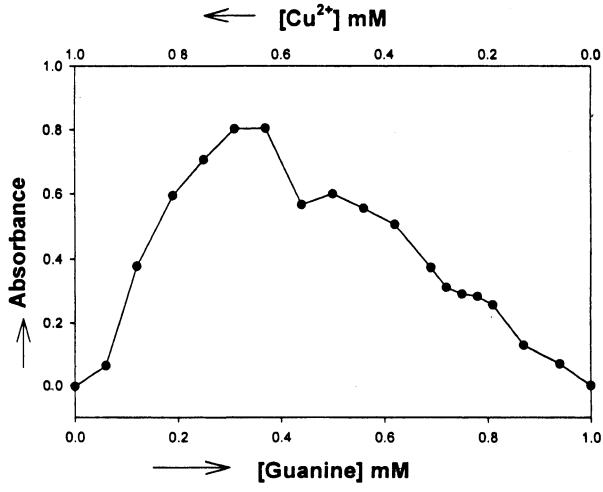

Fig. 5. Interaction between Cu^{2+} and guanine: Absorbance *vs.* concentration plot at 289 nm

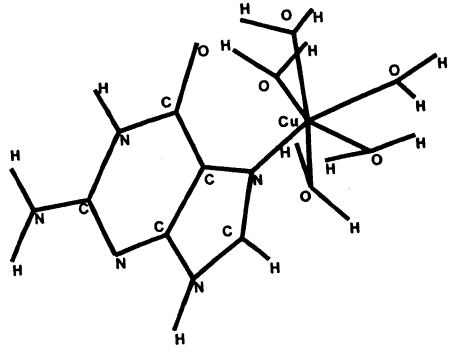

Fig. 6. The proposed structure of $Cu(G)(H_2O)_5$ in which Cu^{2+} is believed to be bonded N7 centre of guanine and five water molecules

ligand through the N7 atom and five water molecules and the other Cu^{2+} to the N3 atom of the guanine ligand and five water molecules so the complex has the formula $Cu_2(G)(H_2O)_{10}^{4+}$ (Fig. 7). In the structure, there are two intramolecular H-bonds resulting into the formation of two macrochelates.

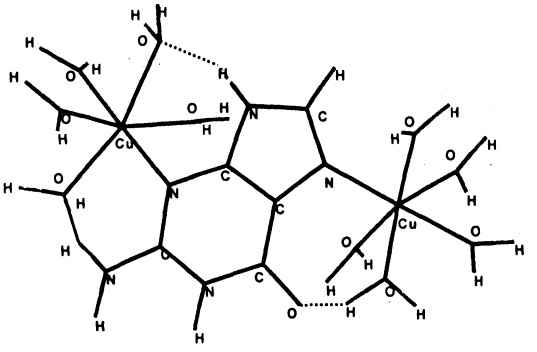

Fig. 7. The proposed structure of $Cu_2(G)(H_2O)_{10}$ in which one Cu^{2+} is believed to be bonded N7 centre of guanine and five molecules and the other Cu^{2+} is believed to be bonded N3 centre of guanine and five water molecules

Conclusion

In solution in water, copper(II) is found to form 1 : 1, 1 : 2 and 1 : 3 complexes with adenine, 1 : 1 and 2 : 1 complexes with guanine. The absence of copper in the HPLC peak fractions indicates lability of the adducts and changes in retention time indicate chemical changes introduced in the adenine and guanine due to binding with copper.

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