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Interaction Between Ag^+, Nucleobases and Nucleotides in Solution

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The interaction between silver ion and adenine, guanine and guanosine monophosphate (GMP) has been followed by UV difference spectroscopy using the technique of continuous variation and high pressure liquid chromatography, combined with molecular modeling. Ag^+ is found to combine with adenine to form three adducts with binding ratios (Ag^+ : Adenine) of 2 : 1 and 1 : 1. It combines with guanine to form 1 : 1 adduct. It combines with GMP to form two adducts having the binding ratios (Ag^+ : GMP) of 2 : 1 and 1 : 1. The proposed structures of the complexes formed have been optimized based on molecular mechanics and semi-empirical calculations using HyperChem 7.

Key Words: Adenine, Guanine, Guanosine monophosphate, Silver ion, Interaction, Molecular modeling.

INTRODUCTION

Metal-nucleotide and metal-DNA interactions have been the subject of intensive study because metal ions such as Mg^{2+} act as cofactors of enzymes that utilize nucleotides as substrates and enzymes that synthesize or cleave DNA[1–3]. It has been proposed that divalent positive ions are required for the replication, transcription and translation of the genetic code[4]. Metal ions may interact with DNA non-covalently as counter ions to balance the negative charge of the phosphate backbone and/or covalently at a number of different binding sites including the nitrogen centres in nucleobases, sugars and the phosphates. Based on DNA melt studies, Eichorn[4] proposed that Ag^+ ion binds more strongly with nucleobases than phosphates. Although the exact molecular mechanism of action of *cis*-platin is not known, it is believed that its anticancer activity is associated with its covalent interaction with N7 centres of guanine and adenine in DNA[5]. Recently, an *ab initio* study[6] has been carried out on metal complexes of non-complementary base pairs.

The aim of the present study was to quantify the nature of interaction between Ag^+ and nucleobases and nucleotides in solution in water. Specifically, in the present paper, the interaction between Ag^+ and nucleobases and nucleotides in solution by UV difference spectroscopy has been investigated. Although it is commonly assumed that metal ions react with nucleobases and nucleotides in solution to form mainly 1 : 1 adducts, other adducts can also be formed. It has recently been reported that in solution in water Cd^{2+} combines with adenine to form 1 : 1, 1 : 2 and 1 : 3 adducts. It has been found that when Cd^{2} interacts with adenine a permanent chemical change is introduced in the nucleobase[7]. The findings about the interaction of Ag^+ with pBR322 plasmid, salmon sperm and calf thymus DNAs have also been reported previously[8].

EXPERIMENTAL

Analytical grade silver nitrate, silver acetate were purchased from Ajax Chemicals, NSW, Australia. Adenine, guanine and guanosine monophosphate (GMP) were purchased from Sigma-Aldrich Pty Ltd. NSW, Australia.

UV-Visible difference spectral analysis

Silver nitrate solution (0–4 mL, 1.0 mM) and 1.0 mm NB (adenine, guanine, GMP or dGMP), both made 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 900 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 100 nm per min and 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 or nucleotide at the same concentration was used as the blank. The absorbance values (given in Table-1) were then plotted against the added concentrations of reactants to determine the stoichiometry of the adducts formed.

TABLE-1

INTERACTION BETWEEN 1.0 mmol L^{-1} Ag^+ AND 1.0 mmol L^{-1} NB (ADENINE, GUANINE, GMP)

Solution	A	B	C	D	E	F	G	H	I
mL Ag^+	4.000	3.750	3.500	3.250	3.000	2.750	2.500	2.250	2.000
mL NB	0.000	0.250	0.500	0.750	1.000	1.250	1.500	1.750	2.000
[Ag^+] mM	1.000	0.940	0.880	0.810	0.750	0.690	0.630	0.560	0.500
[NB] mM	0.000	0.060	0.130	0.190	0.250	0.310	0.370	0.440	0500
Abs at 244 nm[1]	0.026	–0.189	–0.377	–0.977	–1.349	–1.676	–1.880	–1.925	–1.617
Abs at 304 nm[2]	–0.008	0.0672	0.089	0.154	0.167	0.154	0.153	0.205	0.251
Abs at 276 nm[3]	0.003	–0.161	–0.376	–0.588	–0.785	–0.925	–0.946	–0.795	–0.579
Abs at 299 nm[3]	0.011	0.129	0.264	0.428	0.559	0.683	0.708	0.647	0.574

Solution	J	K	L	M	N	O	P	Q	R	S
mL Ag$^+$	1.75	1.500	1.250	1.125	1.000	0.875	0.750	0.500	0.250	0.000
mL NB	2.25	2.500	2.750	2.875	3.000	3.125	3.250	3.500	3.750	4.000
[Ag$^+$] mM	0.44	0.380	0.310	0.280	0.250	0.220	0.190	0.120	0.060	0.000
[NB] mM	0.56	0.620	0.690	0.720	0.750	0.780	0.810	0.880	0.940	1.000
Abs at 244 nm[1]	–0.917	–0.660	–0.269	–0.134	0.026	0.144	0.059	–0.952	–0.013	0.022
Abs at 304 nm[2]	0.171	0.179	0.178	0.116	0.119	0.110	0.105	0.100	0.014	0.047
Abs at 276 nm[3]	0.063	0.035	0.113	0.087	0.159	0.147	0.091	0.037	0.085	–0.036
Abs at 299 nm[3]	0.535	0.419	0.341	0.309	0.274	0.232	0.193	0.132	0.076	–0.002

[1] Adenine; [2] Guanine; [3] GMP

HyperChem Calculations

The proposed structures of the adducts formed were optimized based on molecular mechanics and semi-empirical calculations using HyperChem 7 Molecular Visualization and Simulation program[9]. Geometry optimizations based on molecular mechanics (using MM$^+$ force field) and semi-empirical calculations (using ZINDO/1) were used to find the coordinates of molecular structures that represent a potential energy minimum. For geometry optimization using both molecular mechanics and semi-empirical calculations, Polak-Ribiere routine with a RMS gradient of 0.02 as the termination condition was used. For the structures optimized based on semi-empirical calculations, single point calculations were carried out to determine the total energies and heats of formation. The number of occupied and unoccupied orbitals in the single point CI calculations were both set equal to ten.

RESULTS AND DISCUSSION

Complexes between Ag$^+$ and adenine

The plot of absorbance *vs.* concentration at 244 nm (Fig. 1) applying to the continuously varying mixtures of $AgNO_3$ and adenine (Table-1) showed a broad complex minimum corresponding to the binding ratio (Ag$^+$: adenine) of 5 : 3. The minimum is believed to be produced from the partial overlap of two minima corresponding to the formation of 2 : 1 and 1 : 1 adducts between Ag$^+$ and adenine.

In the 1 : 1 adduct, it is believed that Ag$^+$ is bonded to N7 position of adenine and to a water molecule (Fig. 2), the coordination geometry around Ag$^+$ being linear.

In the 2 : 1 adduct, it is believed that adenine acts as a bidentate ligand being

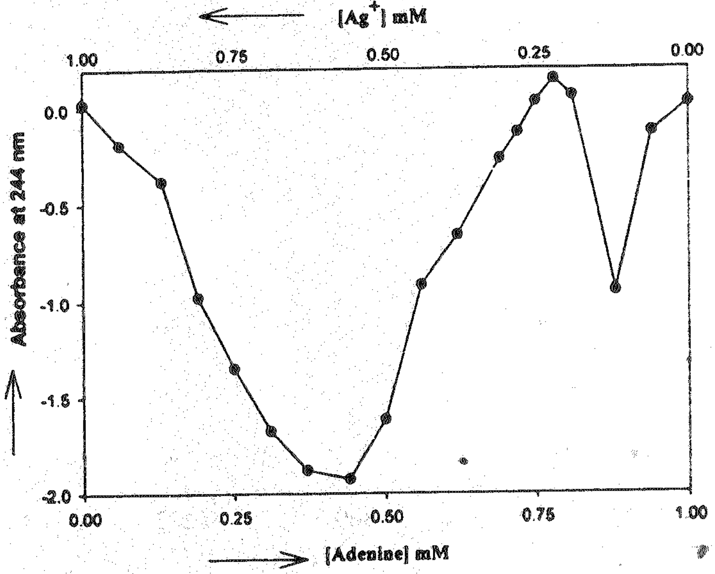

Fig. 1. Interacton between silver nitrate and adenine: absorbance *vs.* concentration plot at 244 nm

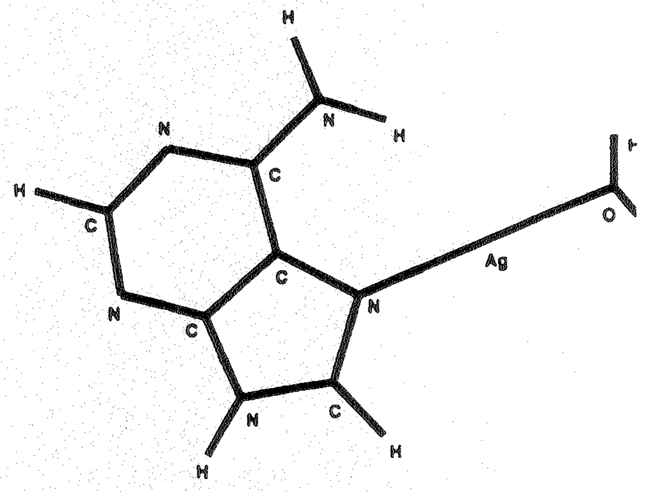

Fig. 2. Proposed structure of 1 : 1 adduct between Ag^+ and adenine in which the metal ion is bonded to N7 centre of adenine and to a water molecule so that the complex ion has the stoichiometry Ag (adenine) $(H_2O)^+$

coordinated to two Ag^+ ions, possibly at N1 and N7 positions. In addition to being bonded to the adenine ligand, each Ag^+ ion is believed to be bonded to a water molecule as well (Fig. 3) so that the coordination geometry around each Ag^+ is linear.

Complexes between Ag^+ and guanine

The plot of absorbance *vs.* concentration at 304 nm (Fig. 4) applying to the

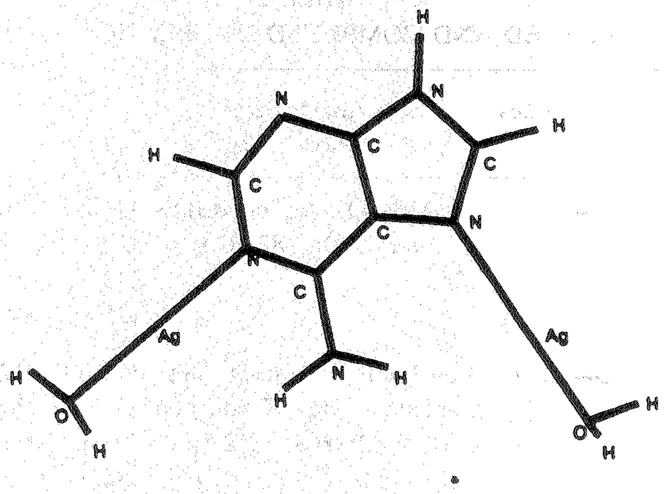

Fig. 3. Proposed structure of 2 : 1 adduct between Ag$^+$ and adenine in which adenine acts as a bidentate ligand being coordinated to two Ag$^+$ ions through the N1 and N7 position. In addition, each metal ion is believed to be bonded to a water molecule so that the complex ion has the stoichiometry $Ag_2(Adenine)(H_2O)^{2+}$

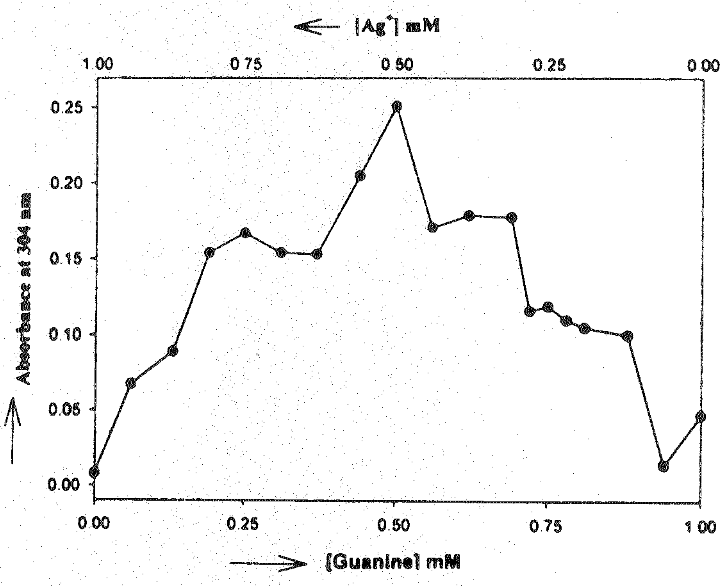

Fig. 4. Interaction between silver nitrate and guanine-absorbance *vs.* concentration plot at 304 nm

continuously varying mixtures of $AgNO_3$ and guanine (Table-1) showed a maximum corresponding to the binding ratio (Ag$^+$: guanine) of 1 : 1.

In the 1 : 1 adduct, it is believed that Ag$^+$ is bonded to N7 position of guanine and to a water molecule (Fig. 5).

Molecular modeling analysis showed that monodentate coordination of guanine to the metal ion through N7 position was more favourable energetically than its coordination through other positions such as N1, N3 or N9.

TABLE-2
OBSERVED AND COMPUTED UV SPECTRAL LINES

Complex	λ_{max} values (nm)	Structure/*Energy (kcal mol^{-1})/ΔH_f	Predicted spectral lines (nm)
$Ag(A)(H_2O)$	244, 279	$Ag(A)(H_2O)$: Ag^+ bonded to N7 and H_2O; –87881.9; –225.5	383.1 (0.0048); 342.6 (0.0045); 328.2 (0.0146); 324.5 (0.0091); 283.1 (0.0031); 269.1 (.0042); 235.8 (0.0052)
$Ag_2(A)(H_2O)_2$	244, 279	$Ag_2(A)(H_2O)_2$: each Ag^+ bonded to one N7 and one H_2O; –122606.8; –212.5	362.2 (0.0123); 346.1 (0.0320); 331.9 (0.0261); 313.5 (0.0045); 301.8 (0.0034); 291.2 (0.0125); 282.9 (0.0253); 280.2 (0.0299); 271.7 (0.0057); 268.9 (0.0125); 266.6 (0.0085); 264.1 (0.0092); 256.1 (0.0022); 253.1 (0.0129); 239.1 (0.0303)
$Ag(G)(H_2O)$	265, 304	$Ag(G)(H_2O)$: Ag^+ bonded to N7 and H_2O; –98329.3; –143.2	365.1 (0.0054); 353.2 (0.0056); 346.3 (0.0037); 335.7 (0.0039); 309.2 (0.0063); 270.1 (0.0026); 260.4 (0.0095); 242.0 (0.0052); 222.7 (0.0039)
$Ag(GMP)(H_2O)$	221, 276, 299	$Ag(GMP)(H_2O)$: Ag^+ bonded to N7 and H_2O; –197674.3; –536.9	329.3 (0.051); 322.4 (0.0047); 295.1 (0.0042); 289.8 (0.0055); 283.8 (0.0115); 277.9 (0.0076); 265.6 (0.0059); 247.5 (0.0068); 239.3 (0.0137); 223.4; 220.2; 219.0 (0.0188)
$Ag_2(GMP)(H_2O)_2$	221, 276, 299	$Ag_2(GMP)(H_2O)$: Ag^+ is bonded to N7's and H_2O's; –231881.3; –352.9	380.5 (0.0290); 338.1 (0.0084); 324.8 (0.0037); 312.4 (0.0023); 277.4 (0.0055); 247.6 (0.0027); 236.9 (0.0369); 233.8 (0.0043)

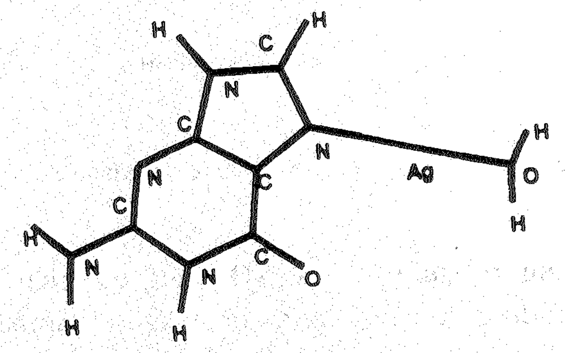

Fig. 5. Proposed structure of 1 : 1 adduct between Ag^+ and guanine in which the metal ion is bonded to N7 centre of guanine and to a water molecule so that the complex ion has the stoichiometry $Ag(guanine)(H_2O)^+$

Complexes between Ag$^+$ and GMP

The plot of absorbance *vs.* concentration at 299 nm applying to the continuously varying mixtures of $AgNO_3$ and GMP showed a maximum corresponding to the binding ratio (Ag$^+$: GMP) of 2 : 1 (Fig. 6).

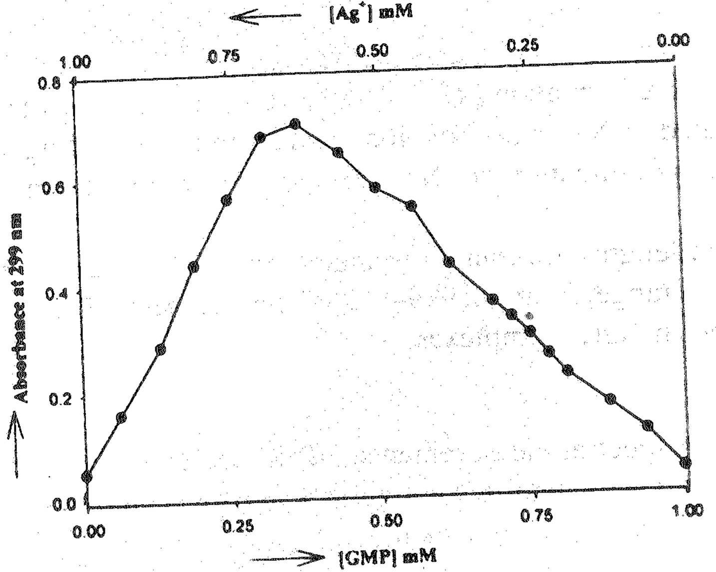

Fig. 6. Interaction between silver nitrate and GMP-absorbance *vs.* concentration plot at 299 nm

In the 2 : 1 adduct, it is believed that guanine moiety of GMP acts as a bidentate ligand being coordinated to two Ag$^+$ ions, possible at N1 and N7 positions. In addition to being bonded to the adenine ligand, each Ag$^+$ ion is believed to be bonded to one water molecule as well (Fig. 7).

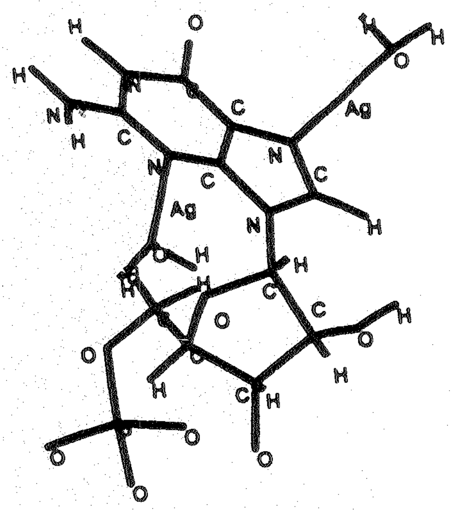

Fig. 7. Proposed structure of 2 : 1 complex between Ag$^+$ and GMP in which GMP acts as a bidentate ligand being coordinated to two Ag$^+$ ions through N1 and N7 positions. In addition, each metal ion is believed to be bonded to a water molecule so that the complex ion has the stoichiometry $Ag_2(GMP)(H_2O)_2^{2+}$

The plot at 276 nm gave a minimum also corresponding to the binding ratio (Ag$^+$: GMP) of 2 : 1 (plot not given).

The increased absorption around 300 nm observed in the incubated mixtures of Ag^+ and guanine, Ag^+ and GMP is believed to be associated with the formation of 1 : 1 adduct. Since in the 1 : 1 adduct it is believed that the metal ion is bonded to N7 position, it follows that binding of Ag^+ to N7 position results into an increase in absorption around 300 nm as compared to that in free ligand. It was also observed that there was a decrease in absorption around 240–280 nm. Since the minimum observed in the wavelength range 240–280 nm is considered to be associated with the formation of 2 : 1 (Ag^+ : Ligand) adduct with the metal ion being coordinated to N1 or N3 position in addition to its binding to N7 position, it follows that coordination to N1 or N3 position results in a decrease in absorption.

Ag—N bond lengths are found to range from 0.2007–0.2018 nm and Ag—O bond lengths to range from 0.1999–0.2007 nm, respectively, in line with the values observed in Ag(I) complexes.

Conclusion

UV-difference spectral measurements, HPLC and graphite furnace AAS have been carried out to show that in solution in water Ag^+ combines with adenine to form 2 : 1 and 1 : 1 adducts, with guanine to form 1 : 1 adduct, with GMP to form 2 : 1 adduct and with dGMP toform 2 : 1 and 1 : 1 adducts. Molecular modeling analyses using HyperChem 7.0 have been carried out to explore the possible structures of the adducts formed.

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