EPR and Electronic Absorption Studies of Interaction of Cu(II)–Glycylglycine Complexes with Nucleosides

S. V. DESHPANDE*, R. K. SHARMA** and T. S. SRIVASTAVA*

Department of Chemistry and R.S.I.C.**, Indian Institute of Technology, Powai, Bombay-400 076, India*

Received June 26, 1982

The interaction of Cu(II)GlyGly with four nucleosides has been studied by difference electronic absorption spectroscopy. The relative stability of binding of nucleosides to Cu(II)GlyGly is in the order: cytidine > adenosine ≅ guanosine > uridine. The EPR spectra of these copper complexes have also been recorded at room temperature and at liquid nitrogen temperature. Bonding parameters characterising the covalency from the EPR data have also been calculated and analysed. It is concluded that the decrease in $A_{\parallel}$ of copper complexes with increasing covalency in the coordination plane is due to a binding through nitrogen of nucleoside in the equatorial plane of Cu(II)GlyGly. The decrease in α^2 with small increase in β_1^2 supports the existence of competitive mechanisms of the in-plane σ- and π-bondings.

Introduction

The role of metal ions in the biological functions of nucleic acids in replication, transcription and translation has long been recognised [1]. The metal ions have also been implicated in mutagenesis [2] and carcinogenesis [3]. The unique properties of metal ions to probe into the functions of nucleic acids and nucleic acid constituents have been employed [4–7]. The recent use of *cis*-dichlorodiamminoplatinum(II) in treating human cancers [8] and the binding of this drug to DNA [9] have led to considerable recent interest in understanding of structural nature of interaction of metal complexes with nucleic acid constituents and nucleic acids [10, 11]. Recently, the results of X-ray diffraction analysis of the aquodiethylenetriaminecopper(II) complex of 5′-inosine-monophosphate in the ratio of 1:2 have suggested that the *cis*-$Pt(NH_3)_2Cl_2$ may interact with DNA by the intrastrand crosslinking of guanosines [9, 12]. In view of the importance of interaction of metal complexes with nucleic acids and nucleic acid constituents, we report here the results of interaction of Cu(II)–glycylglycine with nucleosides using electronic absorption and EPR spectroscopy.

Experimental

Materials

Copper(II)–glycylglycine·$3H_2O$ (Cu(II)GlyGly) was prepared by the method of Martell and coworkers [13]. Cu(II)GlyGly·(cytidine) was prepared by the method described earlier [14]. Other reagent grade chemicals were used.

Physical Measurements

Electronic absorption spectra of the complexes were measured using the Varian SuperScan-3 Spectrophotometer. The equilibrium studies of the complexes between Cu(II)GlyGly and nucleosides were obtained by measuring the difference spectra of mixed solutions of Cu(II)GlyGly (10^{-2} *M*) and an appropriate nucleoside in varying molar ratios of 0.5 to 2 against the same concentration of Cu(II)GlyGly at pH 7.5 in presence of 0.1 *M* KNO_3. These solutions were incubated at 30 °C for two hours before the difference spectra were recorded. The difference in the absorbance measured from the difference spectra of the above solutions at three wavelengths *i.e.*, 560 (cytidine), 580 (adenosine), 690 nm (uridine) were analysed to get equilibrium constant, K_{eq}, following the method of Drago and coworkers [15]. The final analysis of the data were carried out by the least squares method using the computer program LSMB, written by one of the authors (SVD).

The electron paramagnetic resonance (EPR) spectra of copper complexes were recorded using Varian E-112 EPR Spectrometer (X-band) and TCNE (g = 2.00277) was used as g marker. The Varian aqueous solution cell (E-248) was used to record the EPR spectra of solutions at room temperature. The EPR spectra at liquid nitrogen temperature (77 K) were measured as rigid glasses. The above solu-

0020-1693/83/0000-0000/$03.00

tions were prepared in a mixture of water and ethylene glycol in the ratio of 1:1 (v/v) and they were incubated at 34 °C for two hours before the EPR spectra were recorded.

Results and Discussion

Equilibrium Studies

The interaction of Cu(II)GlyGly with various nucleosides has been studied by electronic absorption spectroscopy. The stability constant, K_{eq} of 1:1 complexes was obtained using difference spectroscopy at pH 7.5. The K_{eq} values of the 1:1 complexes are 192 ± 22 (cytidine complex), 131 ± 7 (adenosine complex) and 34 ± 2 M^{-1} (uridine complex). The variations in K_{eq} values can be understood in terms of mode of binding of the nucleosides to Cu(II)-GlyGly which has a slightly distorted square based pyramidal geometry [16]. The four equatorial sites are occupied by a tridentate glycylglycine dianion and an oxygen of a water molecule, while the axial position is occupied by another water molecule. One or two water molecules in the Cu(II)GlyGly can be displaced by exocyclic or endocyclic donor atom or both. This can be used as a basis for explaining the variations in the stability constants. The unusually high stability constant found for binding of cytidine can be attributed to chelate formation by N-3 and O-2 of cytidine. The fairly stable complex formation between adenosine and Cu(II)GlyGly suggests that tridentate glycylglycine dianion and N-7 of the adenosine are occupying the four equatorial sites while the axial site is occupied by a water molecule. This axial coordinated water molecule forms an inter-ligand hydrogen bond with exocyclic amine on the adenosine. The binding of cytidine and adenosine are in accord with X-ray structural studies of these and related compounds [17]. The uridine binds relatively weakly to Cu(II)GlyGly possibly by O-4 of uridine by displacing one coordinated water molecule.

The stability constant of the guanosine complex of Cu(II)GlyGly cannot be determined by electronic absorption spectroscopy because of the low solubility of guanosine. The EPR spectra of 1:1 complexes of nucleosides to Cu(II)GlyGly were measured at liquid nitrogen temperature and the $g_{\parallel}$ values calculated from these data are given in Table I. The decrease of $g_{\parallel}$ values in the structurally related compounds can be related to the increase in covalency [18], and thus to the strength of binding of nucleosides to Cu(II)GlyGly. The $g_{\parallel}$ value of the guanosine complex is comparable to that of the adenosine complex, whereas the $g_{\parallel}$ value of the uridine complex is larger than that of the adenosine complex. The $g_{\parallel}$ value of the cytidine complex is smaller than those of the adenosine or guanosine complexes. The above variations in $g_{\parallel}$ values are in accord with the equilibrium studies. Therefore, the relative stability for binding of nucleosides to Cu(II)GlyGly can be written as cytidine > adenosine ≅ guanosine > uridine.

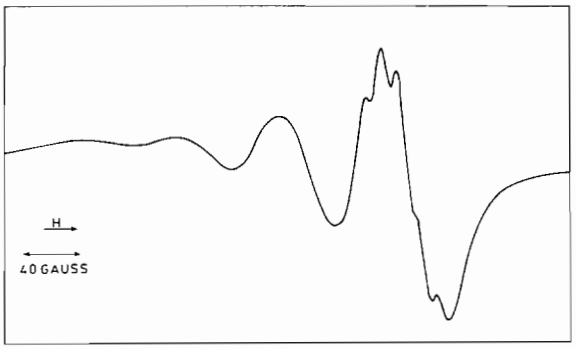

Fig. 1. Solution ESR spectrum of Cu(II)GlyGly (cytidine) complex at room temperature.

EPR Studies:

The EPR spectra of Cu(II)GlyGly and its nucleoside derivatives have been recorded in solutions at room temperature and as glassy solids at liquid nitrogen temperature. A random motion of the above molecules in solution at room temperature gives an isotropic spectrum from which g_o and A_o (nuclear hyperfine structure constant) can be measured. The former parameter is the g_o value at the centre of the four line pattern and A_o, expressed in cm^{-1}, is the spacing between these lines. A typical spectrum in solution is given in Fig. 1.

In the glassy state at liquid nitrogen temperature, all the orientations of the axis of molecules having axial symmetry are present but they are fixed. Therefore, an anisotropic spectrum is obtained, as shown in Fig. 2, from which the values of $g_{\parallel}$ and $A_{\parallel}$ can be accurately measured but the values of $g_{\perp}$ and $A_{\perp}$ are evaluated using the following relations [19]:

$$g_o = \frac{g_{\parallel} + 2g_{\perp}}{3} \text{ and } A_o = \frac{A_{\parallel} + 2A_{\perp}}{3}.$$

The EPR parameters thus obtained are given in Table I. The $g_{\parallel}$ and $A_{\parallel}$ values indicate the existence of an unpaired electron in either $d_{x^2-y^2}$ or d_{xy} ground state. The ground state in all square pyramidal complexes is assumed to be $d_{x^2-y^2}$ [20, 21]. Since the values of $g_{\parallel}$ indicate the covalent nature of the metal ligand bond, the bonding in these complexes can be explained in terms of molecular orbital theory with the unpaired electron in the $d_{x^2-y^2}$ molecular orbital in the ground state [21, 22].

The bonding parameters, α^2, β_1^2 and β^2, which represent the in-plane σ-, in- and out-of-plane π-bond strengths for $3d^9$ configurations in approximate D_{4h}

TABLE I. Magnetic Parameters of Copper Complexes.

Compound	$g_\parallel$	$g_\perp$	g_o	$A_\parallel \times 10^4$ cm^{-1}	$A_\perp \times 10^4$ cm^{-1}	$A_o \times 10^4$ cm^{-1}
Cu(II)GlyGly	2.250	2.068	2.128	185	16	70
Cu(II)GlyGly·(cytidine)[a]	2.234	2.068	2.123	177	25	73
Cu(II)GlyGly + cytidine[b]	2.235	2.073	2.127	177	25	74
Cu(II)GlyGly + adenosine[c]	2.244	2.052	2.116	180	21	71
Cu(II)GlyGly + adenosine[d]	2.240	2.068	2.126	178	22	71
Cu(II)GlyGly + uridine[c]	2.252	2.049	2.117	184	20	71
Cu(II)GlyGly + uridine[d]	2.253	2.064	2.127	187	17	71
Cu(II)GlyGly + guanosine[c]	2.241	2.064	2.123	180	21	71
Cu(II)GlyGly + guanosine[e]	2.232	2.068	2.123	177	24	72

[a]Isolated complex. [b]Cu(II)GlyGly + nucleoside in molar ratio of 1:10. [c]Cu(II)GlyGly + nucleoside in molar ratio of 1:1. [d]Cu(II)GlyGly + nucleoside in molar ratio of 1:5. [e]Cu(II)GlyGly + nucleoside in molar ratio of 1:2.

TABLE II. Ligand Field Energies, Bonding Parameters and ϵ'^2 of Copper Complexes.

Compound	ΔE cm^{-1}	α^2	α'^2	β_1^2	β^2	ϵ'^2
Cu(II)GlyGly	15625	0.82	0.27	0.80	0.86	0.30
Cu(II)GlyGly·(cytidine)[a]	15780	0.75	0.35	0.83	0.93	0.19
Cu(II)GlyGly + cytidine[b]	15762	0.74	0.35	0.84	0.99	0.19
Cu(II)GlyGly + adenosine[c]	15718	0.79	0.30	0.81	0.77	0.23
Cu(II)GlyGly + adenosine[d]	15918	0.77	0.32	0.82	0.92	0.24
Cu(II)GlyGly + uridine[c]	15718	0.82	0.26	0.80	0.64	0.32
Cu(II)GlyGly + uridine[d]	15740	0.83	0.26	0.79	0.80	0.30
Cu(II)GlyGly + guanosine[c]	15792	0.78	0.31	0.82	0.85	0.25
Cu(II)GlyGly + guanosine[e]	15892	0.75	0.34	0.83	0.94	0.20

[a]Isolated complex. [b]Cu(II)GlyGly + nucleoside in molar ratio 1:10. [c]Cu(II)GlyGly + nucleoside in molar ratio 1:1. [d]Cu(II)GlyGly + nucleoside in molar ratio 1:5. [e]Cu(II)GlyGly + nucleoside in molar ratio 1:2.

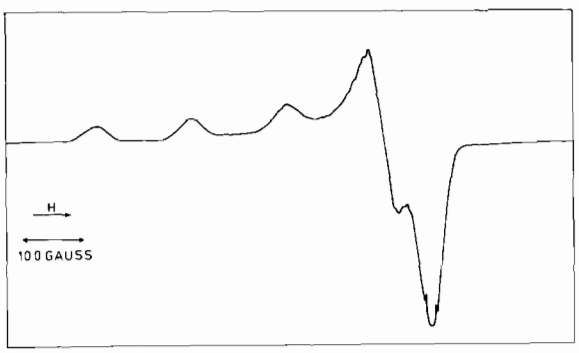

Fig. 2. Frozen solution ESR spectrum of Cu(II)GlyGly·(cytidine) complex at 77 K.

symmetry, can be calculated using known equations [22–24]. The Cu(II)GlyGly complexes and their nucleoside derivatives exhibit a broad band in the visible region between 15625 to 15918 cm^{-1} and these bands are used for the above calculations [22]. The α'^2, out-of-plane σ-bond strength, was calculated using the relationship $\alpha^2 + \alpha'^2 - 2\alpha\alpha' S = 1$ (where S is the overlap integral which is taken as 0.093 [22]). The Fermi contact term K is calculated using the relationship $K = A_o + P(g_o - g_e)$, where $g_e = 2.0023$ and $P = 0.036$ cm^{-1} [23]. The bonding parameters (α^2, α'^2, β_1^2, β^2) and ϵ'^2 [23] of the copper complexes are given in Table II.

The α^2 values quantitate the in-plane σ-bonding

within these complexes. The $\alpha^2 = 1$ represents totally ionic character and the $\alpha^2 = 0.5$ totally covalent character. The calculated α^2 values of copper complexes are in the range of 0.74 to 0.83. This is the range between the appreciable covalency to intermediate covalency [23]. The β_1^2 describes the in-plane π-bonding. The β^2 describes the out-of-plane π-bonding range of 0.79 to 0.84. This indicates appreciable covalent in-plane π-bonding within these complexes. The α^2 decreases with increase in β_1^2. This supports the competitive mechanism of in-plane σ- and π-bonding. The β^2 describes the out-of-plane k-bonding which varies in the range of 0.64 to 0.99. This indicates significant or little out-of-plane π-bonding.

The Cu(II)GlyGly has two ^{14}N nuclei bonded to Cu(II) and a pattern of 2 (2 X 1) + 1 = 5 superhyperfine lines is predicted. The room temperature spectrum of this compound shows the presence of five such ligand hyperfine structures superimposed on the high field copper hyperfine line. The room temperature spectra of Cu(II)GlyGly in presence of nucleosides show only five lines, even in presence of additional ^{14}N nucleus in the nucleosides. This can be explained in terms of rapidly exchanging ^{14}N nucleus of the nucleoside so that nuclear interaction does not occur [19].

As in-plane σ-bonding increases, there is a decrease in the α^2 value, as well as the $g_\parallel$ value [18, 24] on bonding of cytidine to Cu(II)GlyGly. Thus the N-3 of cytidine seems to bind at an equatorial site by displacing one H_2O molecule from equatorial plane, and thus the O-2 of the cytidine occupies the axial position by displacing the axial H_2O molecule. This chelate formation enhances the stability of binding of cytidine to Cu(II)GlyGly and thus the K_{eq} has highest value. The decrease of α^2 value on binding of adenosine to Cu(II)GlyGly is relatively less than cytidine. The N-7 of adenosine molecule binds the Cu(II)GlyGly at equatorial site by displacing the H_2O molecule. The axial water molecule of Cu(II)GlyGly can have interligand hydrogen bonding to exocyclic amine of adenosine. This bond is relatively weak and so the K_{eq} value of adenosine binding is smaller than that for cytidine. These modes of binding of nucleosides to Cu(II) are also in accord with the X-ray structural studies of these Cu(II)-GlyGly nucleoside complexes [17].

The α^2 values of 1:1 complexes of adenosine and guanosine are comparable. Therefore, the mode of binding of these nucleosides seems to be the same except O-6 in guanosine is involved in hydrogen bonding with axial water molecule of Cu(II)GlyGly rather than exocyclic amine of adenosine. There is virtually no change in α^2 value on binding of uridine to Cu(II)GlyGly. Thus, the uridine is weakly bonding possibly through O-4 to Cu(II)GlyGly. This weak bonding is also in accord with the lowest K_{eq} value of uridine binding to Cu(II)GlyGly.

An important feature of EPR parameters of the Cu(II) complexes is the decrease of $A_\parallel$ values with increase of covalency in equatorial plane whereas α^2 and $g_\parallel$ values reflect the expected trend [22]. The former trend can be explained if the mixing of 4s level is included in the ground state [23]. Various mechanisms for this mixing have been suggested but the spin polarisation model for mixing 4s level seems to be appropriate for these Cu(II) complexes. The support of this mechanism is obtained by a linear plot of α^2 against ϵ'^2 as shown in Fig. 3. The mixing of 4s with the ground state leads to either a static or dynamic distortion of D_{4h} symmetry. The static distortion seems to be operative over dynamic distortion. This is in accord with the EPR spectrum of the Cu(II) complexes showing rhombic distortion (see Fig. 2).

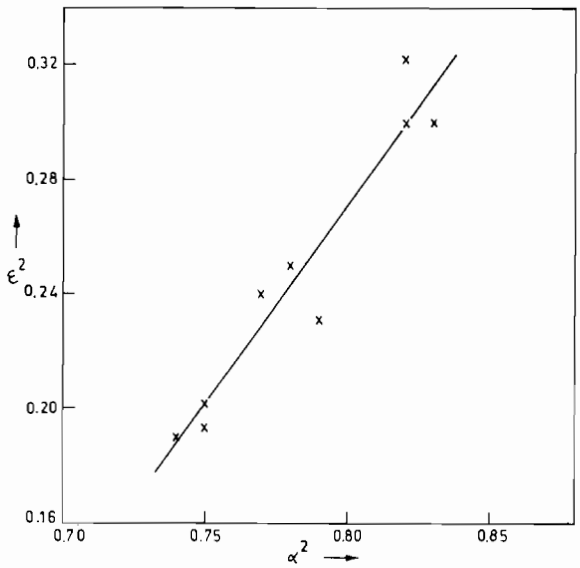

Fig. 3. Plot of α^2 against ϵ'^2 for the copper(II) complexes.

Sometimes appreciable variations have been observed in the g factors and hyperfine parameters of Cu(II)GlyGly in presence of one mole or more than one mole of nucleoside. These variations are probably due to weak bonding interactions, such as hydrogen bonding between uncoordinated nucleoside and coordinated ligands. These interactions collectively may give rise to the above variations [25].

The relative stability of binding of nucleosides to Cu(II)GlyGly has been found to be cytidine > adenosine ≅ guanosine > uridine. This trend in the stability is governed by two factors, namely chelate formation and interligand hydrogen bonding. The modes of binding of nucleosides to Cu(II)GlyGly are in accord with the available X-ray structural studies of some nucleoside complexes of Cu(II)-GlyGly.

Acknowledgement

We thank Dr. T. K. Gundu Rao for recording the EPR spectra.

References

1 G. L. Eichhorn, in 'Inorganic Biochemistry', Vol. 2, G. L. Eichhorn, Ed., Elsevier, New York, 1973, p. 1191.
2 M. A. Sirover and L. A. Loeb, *Science, 194,* 1434 (1976).
3 G. N. Schrauzer, Ed., 'Inorganic and Nutritional Aspects of Cancer', Vol. 91 of Advances in Experimental Medicine and Biology', Plenum Press, New York, 1977.
4 M. L. De Pamphilis and W. W. Cleland, *Biochemistry, 12,* 3714 (1973).
5 R. M. K. Dale and D. C. Ward, *Biochemistry, 14,* 2458 (1973).
6 S. J. Lippard, *Acc. Chem. Res., 11,* 211 (1978).
7 C. Formoso, *Biochem. Biophys. Res. Commun., 53,* 1084 (1973).
8 B. Rosenberg, in 'Metal ions in Biological systems', Vol. 11, H. Sigel, Ed., Dekker, New York, 1980, pp. 127–196.
9 A. J. Thomson, *Platinum Met. Rev., 21,* 2 (1977).
10 L. G. Marzilli, *Prog. Inorg. Chem., 23,* 255 (1977).
11 D. J. Hodgson, *Prog. Inorg. Chem., 23,* 211 (1977).
12 C. C. Chiang, T. Sorrell, T. J. Kristenmacher and L. G. Marzilli, *J. Am. Chem. Soc., 100,* 5102 (1978).
13 R. Manyak, C. B. Murphy and A. E. Martell, *Arch. Biochem. Biophys., 59,* 273 (1955).
14 D. J. Szalda, L. G. Marzilli and T. J. Kristenmacher, *Biochem. Biophys. Res. Commun., 63,* 601 (1975).
15 R. S. Drago and R. M. Guidry, *J. Am. Chem. Soc., 95,* 6645 (1973).
16 B. Strandberg, I. Lindqvist and R. Rosenstein, *Z. Kristallogr., Kristallgeometrie, Kristallphys., Kristallchem., 116,* 266 (1961).
17 L. G. Marzilli and T. J. Kristenmacher, *Acc. Chem. Res., 10,* 146 (1977).
18 R. Barbucci and M. J. M. Campbell, *Inorg. Chim. Acta, 16,* 113 (1976).
19 G. F. Bryce, *J. Phys. Chem., 70,* 3549 (1966).
20 R. Barbucci, A. Mastroianni and M. J. M. Campbell, *Inorg. Chim. Acta, 27,* 109 (1978).
21 B. A. Sastry, S. Md. Asadullah, G. Ponticelli and M. Massacesi, *J. Chem. Phys., 70,* 2834 (1979).
22 D. Kivelson and R. Neiman, *J. Chem. Phys., 35,* 149 (1961).
23 A. Rockenbauer, *J. Mag. Res., 35,* 429 (1979).
24 L. Casella, M. Gullotti, A. Pasini and A. Rockenbauer, *Inorg. Chem., 18,* 2825 (1979).
25 K. E. Falk, E. Ivanova, B. Roos and T. Vanngard, *Inorg. Chem., 9,* 556 (1970).