Models for Enzyme–Copper–Nucleic Acid Interaction: Interaction of Some Copper Complexes Derived from Salicylaldehyde, Glycine and α-Alanine with Cytosine, Cytidine and Deoxycytidine

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The interaction of $[CuL(H_2O)]$ (H_2L = salicylideneglycine or salicylidene-α-alanine) with cytidine and cytosine has been investigated using UV–VIS, IR, EPR, ^1H NMR line-broadening and electrochemical techniques. Adducts of the type $[CuL(B)(H_2O)_n]$ (B = cytosine or cytidine, $n = 0$ or 1) have been isolated. Infrared spectra of these adducts and a ^1H NMR line-broadening study of the interaction of $[Cu(salgly)(H_2O)]$ with cytidine and deoxycytidine indicate the involvement of the N(3) and O(2) sites in co-ordination. EPR spectra and electrochemical studies show that the extra methyl group in the alanine complex leads to distortion of the co-ordination plane, thereby resulting in stronger interaction with bases. However, cytosine interacts with the glycine complex strongly as the planarity of the latter favours semi-chelation involving the O(2) site. The extent of interaction of the bases with the copper(II) complexes depends on the co-ordinative unsaturation and distortion from the co-ordination plane around Cu" as well as the basicity and *ortho* effect of the former. On the other hand, steric effects of the ligands are important in binding to Cu'.

The utilisation of genetic code for the eventual production of proteins specified by the code is controlled in some way by the presence of metal ions.[1] Metal ions promote the interaction of nucleic acids with amino acids in biological systems, leading to the formation of ternary complexes.[2,3] Bivalent metal ions are involved in nucleic acid–enzyme interaction during RNA synthesis and replication.[4,5] Frieden and Alles[6] observed that the oxidase activity of ascorbic acid oxidase was inhibited by deoxy- and ribo-nucleotides. They concluded that the latter form stable complexes with the enzyme. Metal ions are known to influence enzyme reactions that act on nucleic acids or their derivatives.[7] The enzymatic transfer of phosphoryl groups has been shown to proceed through the formation of enzyme–metal–ATP complexes.[8]

To elucidate the role of metal ions in such complex interactions, a systematic investigation has been undertaken in our laboratory; this would involve the isolation and study of models for enzyme–copper–nucleic acid interaction. In the present investigation aqua(*N*-salicylideneglycinato)- and aqua-(*N*-salicylidene-α-alaninato)-copper(II) were chosen as the starting models for copper–protein interaction. They contain biomimetic donors, *viz.* phenolic oxygen (as in tyrosinase[9]), imine nitrogen (similar to the imidazole nitrogen, a common biomimetic donor) and carboxylic acid oxygen. Polypeptides containing glutamic acid and tyrosine residues interact with polynucleotides[9] through copper(II) ion. Copper has been chosen because it not only destabilises the normal conformation of DNA by binding to bases and phosphate like other metal ions, but also differs in that it helps the regeneration of the double strand.[10] Since copper 'recognises' cytosine moieties[11] in its interaction with nucleic acids, the interaction of the above models with cytosine, cytidine[12] and deoxycytidine in aqueous solution was studied and the results are now reported.

Experimental

Reagents were used as received from BDH (India), Sisco, Aldrich (D_2O, deoxycytidine), Glaxo (glycine) and Merck (α-alanine). Deionised water was used for all experiments.

Synthesis of Copper(II) Complexes.—Aqua(*N*-salicylideneglycinato)copper(II) **1** and aqua(*N*-salicylidene-α-alaninato)-copper(II) **2** were obtained by the published procedure.[13]

Aqua(cytosine)(N-*salicylideneglycinato)copper*(II) **3.** Cytosine (0.11 g, 1 mmol dm^{-3}) dissolved in water (25 cm^3) was added to aqua(*N*-salicylideneglycinato)copper(II) (0.33 g, 1 mmol dm^{-3}) dissolved in water (50 cm^3) and stirred. The solution was heated on a water-bath. The pale green crystalline product obtained on cooling was collected by filtration and dried *in vacuo* over $CaCl_2$ (yield 0.45 g, 75%) {Found: C, 42.15; H, 4.25; Cu, 17.30. Calc. for $[Cu(salgly)(H_2O)(cyt)]$: C, 42.20; H, 4.25; Cu, 17.20%}.

(*Cytidine*)(N-*salicylideneglycinato)copper*(II) **4.** Cytidine (0.24 g, 1 mmol dm^{-3}) dissolved in water (2 cm^3) was added to aqua (*N*-salicylideneglycinato)copper(II) (0.33 g, 1 mmol dm^{-3}) dissolved in water (50 cm^3) and stirred. The solution was heated on a water-bath. The blue-green crystalline product obtained on cooling was collected by filtration and dried *in vacuo* over $CaCl_2$ (yield 0.52 g, 70%). {Found: C, 46.20; H, 4.20; Cu, 13.50. Calc. for $[Cu(salgly)(cyd)]$: C, 46.20; H, 4.25; Cu, 13.60%}.

Aqua(cytosine)(N-*salicylidene-α-alaninato)copper*(II) **5.** Cytosine (0.11 g, 1 mmol dm^{-3}) dissolved in water (20 cm^3) was added to aqua(*N*-salicylidene-α-alaninato)copper(II) (0.27 g, 1 mmol dm^{-3}) dissolved in water (30 cm^3) with constant stirring. The solution was heated on a water-bath for 15 min. The pale green crystalline product formed after cooling was collected by filtration, washed with water and dried *in vacuo* over $CaCl_2$ (yield 0.29 g, 75%) {Found: C, 43.70; H, 4.30; Cu, 16.45. Calc. for $[Cu(salala)(H_2O)(cyt)]$: C, 43.80; H, 4.15; Cu, 16.55%}.

(*Cytidine*)(N-*salicylidene-α-alaninato)copper*(II) **6.** Cytidine (0.37 g, 1.5 mmol dm^{-3}) dissolved in water (3 cm^3) was added to aqua(*N*-salicylidene-α-alaninato)copper(II) (0.27 g, 1 mmol dm^{-3}) dissolved in water (50 cm^3) and stirred. The solution was heated on a water-bath. The blue-green crystalline product obtained on cooling was collected by filtration and dried *in vacuo* over $CaCl_2$ (yield 0.39 g, 78%). {Found: C, 45.80; H, 4.50; Cu, 12.65. Calc. for $[Cu(salala)(cyd)]$: C, 45.85; H, 4.40; Cu, 12.75%}.

Physical Measurements.—Elemental analyses were performed

Table 1 Infrared vibrational bands[a] (cm^{-1}) of the copper(II) complexes

Complex	ν(C=O) + δ(N–H)	$\nu_{asym}(CO_2)$	ν(N–H)	$\nu(NH_2)$	$\nu(H_2O)$[b]	ν_{ribose}(OH)
1 [Cu(salgly)(H_2O)]	—	1645s	—	—	3500s	—
2 [Cu(salala)(H_2O)]	—	1645s	—	—	3360s	—
3 [Cu(salgly)(H_2O)(cyt)]	1695s	1630s	3120w	3217ms	3400br	—
4 [Cu(salgly)(cyd)]	1707ms	1635vs	3320s	3340ms	—	3220br
5 [Cu(salala)(H_2O)(cyt)]	1685s	1640s	3100s	3225br	3450s	—
6 [Cu(salala)(cyd)]	1695s	1645s	3300s	3320ms	—	3250br

[a] As KBr discs. [b] As Nujol mull.

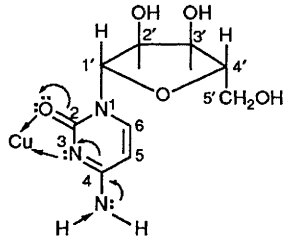


Table 2 Electronic spectral data (ν in cm^{-1}, ε in $dm^3\ mol^{-1}\ cm^{-1}$ within parantheses) for copper(II) complexes

Complex	Nujol	Methanol*
3	16 800	15 060(23)
	24 470	27 000(2 176)
4	16 500	15 150(141)
	26 500	27 250(3 813)
5	16 420	15 385(60)
	27 435	27 472(6 008)
6	16 366	15 270(120)
	26 450	27 140(11 436)

* Concentration range 5×10^{-3}–1.8×10^{-2} mol dm^{-3}.

by College Science and Analytical Instrumentation Centre, VHNSN College, Virudhunagar, India. The copper content of the complexes was estimated by ethylenediaminetetraacetate titration after decomposing the complexes with a mixture of concentrated HNO_3 and concentrated H_2SO_4 and extracting with concentrated HCl (2 cm^3).

Electronic spectral measurements were made on methanol solutions and Nujol mulls on filter paper with a Hitachi U-3400 double-beam UV/VIS/NIR spectrophotometer. IR spectra (400–4000 cm^{-1}) were obtained for KBr discs and Nujol mulls on a Shimadzu-435 spectrophotometer. EPR spectra were obtained on Varian E-12 and E-4 X-band spectrometers. The field was calibrated with diphenylpicrylhydrazyl. Frozen-solution spectra were obtained by preparing glasses of the complexes in methanol–acetone (1:4 v/v). The values of g_0 and A_0 were measured at ambient temperature and $g_{\|}$, $A_{\|}$ and $g_{\perp}$ at 77 K. Proton NMR spectra (90 MHz) were measured in D_2O at 28 °C on a Perkin-Elmer R 32 spectrometer. *tert*-Butyl alcohol (δ 1.17 with respect to $SiMe_4$) was used as internal reference and the chemical shifts are reported with reference to $SiMe_4$. Solutions (0.1 mol dm^{-3}) of cytidine and deoxycytidine were prepared in D_2O. The concentration of the copper complexes in D_2O was 0.006 mol dm^{-3}.

All voltammetric experiments were performed in a single-compartment cell with a three-electrode system on a EG & G PAR 273 potentiostat/galvanostat equipped with a IBM PS/2 computer and a HIPLOT DMP-40 series digital plotter. The working electrode was a glassy carbon disk and the reference electrode a saturated calomel electrode. A platinum plate was used as the counter electrode. The supporting electrolyte was 0.1 mol dm^{-3} $NaClO_4$. Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to measurements; during measurements, a stream of N_2 was passed over the solution. All experiments were carried out in aqueous solutions at 25 ± 0.2 °C maintained by a Haake D8-G circulating bath.

Results and Discussion

Infrared Spectra.—In Table 1 are collected the characteristic infrared bands most useful for establishing the co-ordination mode of the ligands. In all the cytosine and cytidine adducts the Amide I [coupled ν(C=O) and ν(N–H)] band occurs at higher frequencies, compared to cytosine and cytidine. This enhancement (20–47 cm^{-1}) suggests co-ordination of the carbonyl oxygen of the amide group and N(3) nitrogen to copper; this is explicable on the basis of the familiar amide resonance.[11] Such an enhancement has been noted for sodium (salicylideneglycylglycinato)cuprate(II) (1680 *vs.* 1655 cm^{-1} for free peptide)[13] and $[Cu^{II}(Hyt)_4Cl_2]Cl_4$ (10 cm^{-1} higher than for cytosine hydrochloride[14]). This mode of co-ordination is supported by the concomitant large decrease in ν_{asym}(N–H) for the cytosine[11] (−153, −145 cm^{-1}) and cytidine[11] adducts (−30, −50 cm^{-1}); $\nu(NH_2)$ is also affected because of this co-ordination.

Further, compared to [Cu(salgly)(H_2O)], $\nu_{asym}(CO_2)$ for the cytosine and cytidine adducts occurs at lower frequencies (−15 and −10 cm^{-1} respectively); this shows the weakening of copper–carboxylate bond, on adduct formation. For the adducts of Cu(salala), however, small or no lowering is observed.

A broad band around 3400 cm^{-1} for the cytosine adducts is characteristic of co-ordinated or most probably lattice water. On the other hand, for the cytidine adducts a very broad band occurs around 3200 cm^{-1}; this is ascribed to the differently hydrogen-bonded ribose hydroxyl groups.[11]

Ligand-field Spectra.—In Table 2 are presented the details of the electronic spectra of the solid complexes (Nujol) and their methanol solutions. All the compounds display a band in the 26 500–27 800 cm^{-1} region which may originate from a CO_2^- (π) ⟶ Cu^{II} charge-transfer transition.[15] The average energy of the ligand-field band for the solid adducts is 16 570 cm^{-1} which is typical of a tetragonal CuO_2N_2 chromophore;[16] this illustrates the co-ordination of the nucleobase, possibly through N(3).

The red shift (1400 cm^{-1}) of the ligand-field band observed on dissolution in methanol may be attributable to structural changes like axial solvent interaction. The higher molar absorption coefficient of the cytidine adducts compared to those of cytosine shows that the former have lower symmetry, probably distorted planar as a result of steric hindrance by the nucleoside.

Spectrophotometric titration of the aqua complexes **1** and **2** in aqueous methanol solution with *N*-methylimidazole (mim) showed well defined inflections in a plot of absorbance *vs.* mole ratio; this shows the formation of a 1:2 adduct with mim interacting both axially and equatorially. However, only poorly defined inflections corresponding to 1:1 adduct formation were observed in the case of cytosine and cytidine. The presence of O(2) and C(4) amino groups *ortho* to the N(3) site (*ortho* effect[17]) seems to hinder the formation of 1:2 adducts.

Table 3 Electron paramagnetic resonance spectral data for the complexes

Complex	Medium	g_0	A_0[a]	$g_\|$	$A_\|$[a]	$g_\perp$	G
1	Polycrystalline	2.179	30[b]	2.228	—	2.107	2.1
	CH_3OH–acetone[c]	2.129	73	2.259	188	2.063	4.1
2	Polycrystalline	2.125	48[b]	—	—	—	—
	CH_3OH–acetone[c]	2.131	72	2.270	189	2.065	4.2
3	Polycrystalline	2.116	83[b]	—	—	—	—
	CH_3OH–acetone[c]	2.122	79	2.252	184	2.057	4.5
4	Polycrystalline	2.122	63[b]	2.178	—	2.094	1.9
	CH_3OH–acetone[c]	2.126	74	2.238	180	2.057	4.2
5	Polycrystalline	2.064	22[b]	—	—	—	—
	CH_3OH–acetone[c]	2.112	73	2.241	183	2.048	5.0
6	Polycrystalline	2.096	60[b]	—	—	2.098	—
	CH_3OH–acetone[c]	2.124	74	2.254	183	2.064	4.0

[a] A values in 10^{-4} cm^{-1}. [b] One third of the width between the first-derivative spectrum's maximum and minimum. [c] 4:1 v/v.

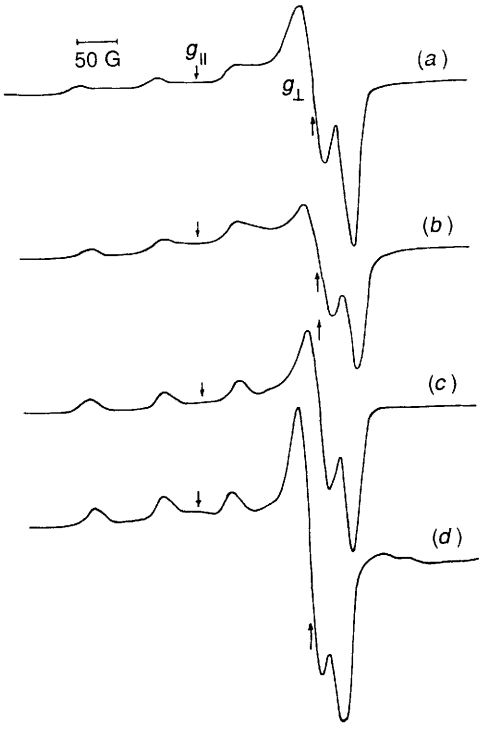


Fig. 1 EPR spectra of (*a*) [Cu(salgly)(cyd)], (*b*) [Cu(salgly)(H_2O)(cyt)], (*c*) [Cu(salala)(cyd)] and (*d*) [Cu(salala)(H_2O)(cyt)] at 77 K in methanol–acetone (4:1 v/v) glasses

EPR Spectra.—The polycrystalline EPR spectrum of [Cu(salgly)(H_2O)] is less than axially symmetric[18] (Table 3) whereas that of [Cu(salala)(H_2O)] is isotropic. On dissolution in methanol, the $g_\|$ value of [Cu(salgly)(H_2O)] increases from 2.228 to 2.259, showing that structural changes like axial solvation have occurred. The higher $g_\|$ value for [Cu(salala)(H_2O)] than for [Cu(salgly)(H_2O)] shows[19] that the extra methyl group in [Cu(salala)(H_2O)] leads to some tetrahedral distortion[20] of the co-ordination plane. Their $g_\|$ and $A_\|$ values indicate that the chromophore is CuO_3N.[21]

For the axial spectra of copper(II) complexes ($g_\| > g_\perp >$ 2.03) the g values are related by the expression[22] $G = (g_\| - 2)/(g_\perp - 2) \approx 4.01$. While polycrystalline [Cu(salgly)(H_2O)] exhibits a very low G value (2.1) the cryogenic solution EPR spectra of [Cu(salgly)(H_2O)] and [Cu(salala)(H_2O)] exhibit a G value of ≈ 4. This indicates that in the solid state significant exchange coupling[23] and in solution appreciable misalignment of the local tetragonal axes are present. The quotient $g_\|/A_\|$ for the present complexes ranges from 120 to 124 cm showing that their geometry may be square planar.[18]

The EPR spectra of the polycrystalline adducts exhibit broad isotropic lines, indicating that the samples are not sufficiently dilute magnetically. The cryogenic spectra of all the complexes correspond to axial symmetry (Fig. 1) with $g_\| > g_\perp > 2.03$, indicating a $d_{x^2-y^2}$ ground state. Further, the G values for the cytidine adducts are ≈ 4, confirming the axial symmetry. The cytosine adducts, however, show higher G values (Table 3) indicating that the local tetragonal axes are aligned parallel or only slightly misaligned.[24]

The observed $g_\|$ and $A_\|$ values on the $g_\|$ *vs.* $A_\|$ map[25] lie in between the N_4 and O_4 delineators indicating that the donor atom set is O_2N_2. Again these values are well within the range (2.22–2.24) suggested[16,26] for a CuO_2N_2 chromophore. For the Cu(salgly) complexes the $g_\|$ values decrease in the order H_2O > cyt > cyd in accordance with the decreasing axial interaction[27] (solvent in the aqua complex). However, for the Cu(salala) complexes the order of $g_\|$ values is H_2O > cyt < cyd. The possible rationale for the higher value for [Cu(salala)(cyd)] may be that the interligand repulsion between the methyl group of α-alanine and the bulky cytidine effects a distortion from coplanarity[28] of the co-ordination bonds.

In line with the trend for the aqua complexes, $g_\|$ for [Cu(salala)(cyd)] is higher than for [Cu(salgly)(cyd)]. However, interestingly, unlike the aqua complexes and the cytidine adducts, [Cu(salgly)(cyt)] has a higher $g_\|$ value than for [Cu(salala)(cyt)]; this shows that, owing to the planarity of Cu(salgly), cytosine approaches Cu^{II} closely with the axial interaction of O(2) (semi-chelation) leading to a higher $g_\|$ value.[27] The possibilities of O(2) co-ordination by cytidine in glycine and α-alanine complexes as well as by cytosine in [Cu(salala)(cyt)] are decreased by both the bulkiness of cytidine and the distortion in the α-alanine complex. Thus, the small but significant variation in $g_\|$ values provides information about distortion in the geometry around copper(II). This is supported by the variation in the position of the ligand-field feature; the higher $g_\|$ the lower is $\bar{\nu}_{max}$. Similar observations made for copper(II) complexes[19,28] have been ascribed to increasing tetrahedral distortion.

However, for all the complexes no significant variation in $A_\|$ values is observed. This remarkable constancy of $A_\|$ is completely consistent with the ubiquity and constancy of the geometrical parameters for the Cu···O(2) intramolecular interaction in several cytosine or cytidine complexes of Cu^{II}. The calculated $A_\perp$ values are small and positive for all complexes. The failure to observe nitrogen superhyperfine structure cannot be ascribed to loss of tetragonality[27,29] but to the very weak coupling[18,30] of the aliphatic nitrogen donors.

NMR Spectra.—The line-broadening technique[31] provides the most direct evidence for the binding of copper to the nucleic acids and their components[32] and this has been employed in the present investigation. Since cytosine is not sufficiently soluble in solvents like D_2O, $(CD_3)_2SO$, *etc.*, it was not studied by this technique. In the line-broadening experiments the concentration of the copper(II) complex is much less than that of cytidine ($1:10^4$) and the rapid ligand exchange causes all molecules to be affected equally. The degree of broadening of a particular resonance peak in the ligand is related[33] to the distance of the bound paramagnetic metal ion from the group exhibiting that resonance.

In Fig. 2 is given the ^1H NMR spectrum of cytidine in D_2O. The lowest-field resonance at δ 7.84 [doublet, split by H(5)] is assigned to H(6) and the signals at δ 6.04 [doublet, split by H(6)] and 5.82 [doublet, split by H(2′)] are assigned to H(5) and H(1′) respectively. The broadening of the ^1H signals produced upon the addition of increased quantities of aqua(*N*-salicylideneglycinato)copper(II) in D_2O is also shown in Fig. 2. Complete broadening of the H(5) signal occurs upon the initial addition of the complex. The H(6) and H(1′) signals are broadened gradually.

A plot of their half-linewidths (average of doublets) (Fig. 3) as

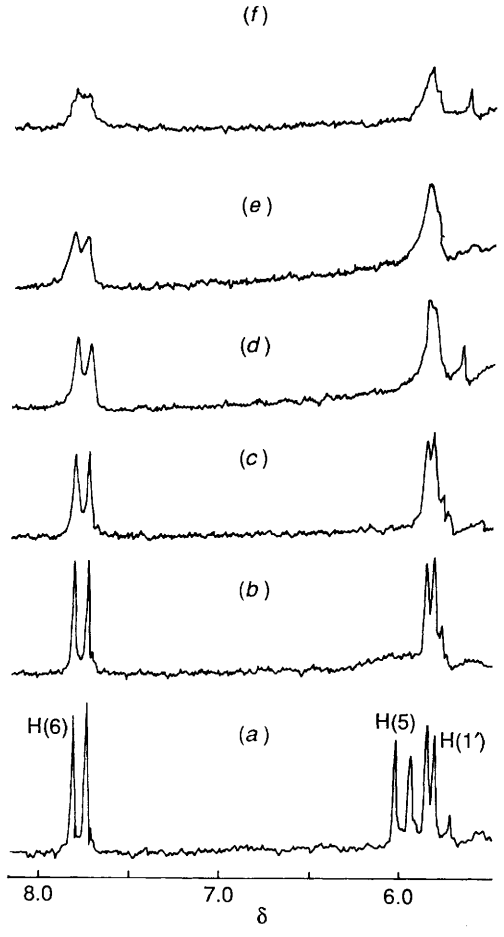


Fig. 2 Proton NMR spectra of cytidine (0.1 mol dm^{-3}, D_2O): (*a*) with no metal present, (*b*)–(*f*) with increasing amounts of [Cu(salgly)(H_2O)] present, 3.1 × 10^{-5}, 9.2 × 10^{-5}, 2.2 × 10^{-4}, 3.3 × 10^{-4} and 5.5 × 10^{-4} mol dm^{-3}

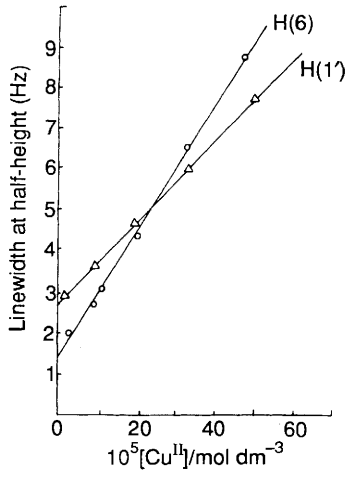


Fig. 3 Effect of [Cu(salgly)(H_2O)] on the ^1H NMR linewidth at half-height of 0.1 mol dm^{-3} cytidine in D_2O

a function of the copper concentration demonstrates elegantly that the order of broadening is H(6) > H(1′). The preferential broadening of the H(5) resonance demonstrates that copper(II) *in the* complex selectively binds to a site which is very near to H(5); this is N(3) of the base moiety. The broadening of the H(6) resonance indicates the binding of copper(II) to O(2) of cytidine. The binding of N(3) and O(2) is in agreement with the infrared spectral results. The hydrogens of the amino and ribose hydroxyl groups exchange with D_2O, making their detection impossible. However, one can rule out the possibility of N(4)

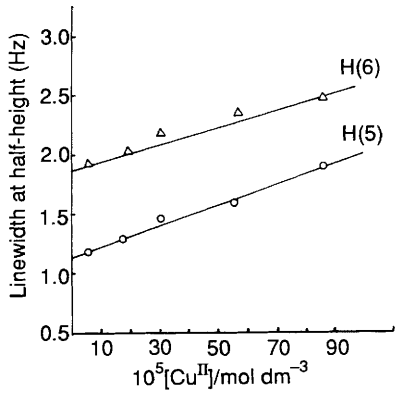


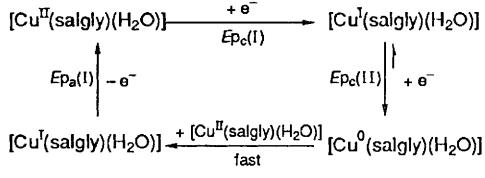
Fig. 4 Effect of [Cu(salgly)(H_2O)] on the ^1H NMR linewidth at half-height of 0.1 mol dm^{-3} deoxycytidine in D_2O

binding as it has little potential[34,35] to bind to copper. The 2′ and other ribose hydroxyls are poor monodentate or even bidentate ligands and hence are generally less involved in complex formation in neutral aqueous solution as well as in non-aqueous solvents.[36] The comparatively small broadening of the H(1′) signal shows that the possibility of binding of copper(II), at least at higher concentration, to the ribose moiety *via* the hydroxyl oxygen cannot be entirely ruled out.[37] A plot of the change in linewidth as a function of the concentration of the copper(II) complex is linear;[38] this shows that the line broadening is due to the paramagnetic copper(II) complex only.[39]

The interaction of cytidine with sodium (salicylideneglycylglycinato)cuprate(II) in D_2O has been also studied by the line-broadening technique.[40] It is found that the signals of H(5), H(6) and H(1′) are less affected by Na[Cu(salglygly)] than by [Cu(salgly)], indicating that the binding of the former is weaker compared to the latter. We suggest that this is because of the equatorial N(3) co-ordination in [Cu(salgly)], supported by weaker axial co-ordination of O(2); further, in this mode of co-ordination the C(4)(NH_2) group may be involved in hydrogen-bonding[41] with the co-ordinated carboxylate oxygen, encouraging the chelation. On the other hand, in Na[Cu(salglygly)] with a planar structure, only weak axial co-ordination through N(3) or O(2) is possible.

The interaction of deoxycytidine with [Cu(salgly)(H_2O)] in D_2O has been also studied by the line-broadening technique. The ^1H NMR spectrum of deoxycytidine shows H(6) and H(5) at δ 8.03 [doublet, split by H(5)] and 6.19 [doublet, split by H(6)] respectively.[34] The triplet signal of H(1′) at δ 6.10 partially overlaps with the doublet of H(5) and so its broadening was not investigated. A plot of linewidth at half-height as a function of the concentration of the copper complex (Fig. 4) is linear and the preferential broadening of the H(5) resonance as indicated by the greater dependence of its linewidth with metal-ion concentration shows that the copper(II) selectively binds to the N(3) site of the base moiety.[42]

Redox Properties.—Electrochemical behaviour of [Cu(salgly)-(H_2O)] *and* [Cu(salala)(H_2O)]. The cyclic voltammogram of [Cu(salgly)(H_2O)] (Fig. 5) obtained in aqueous solution at a glassy carbon electrode exhibits two reduction waves, corresponding to Cu^{II}–Cu^{I}, E_{pc}(I) and Cu^{I}–Cu^{0}, E_{pc}(II) reductions (Table 4). The former appears as a broad shoulder. The



Scheme 1

Table 4 Voltammetric behaviour of [Cu(salgly)(H_2O)] and [Cu(salala)(H_2O)] and their 1:1 adducts with cytosine, cytidine and *N*-methylimidazole

Complex	Solvent	$E_{p_c}(I)^b$/V	$E_{p_a}(I)$/V	$E_{p_c}(II)$/V	$E_{p_a}(II)$/V	$I_{p_c}(II)$/μA	$I_{p_a}(I)$/μA	$E_{\frac{1}{2}}^a$/V	$E_b^{o'} - E_f^{o'}$	K_{2+}/K_+
[Cu(salgly)(H_2O)]	Water	−0.34	0.09	−0.72	—	94.0	−140	−0.10	—	—
	Methanol	−0.52	0.02	−0.94	−0.40	56.1	−49	—	—	—
[Cu(salgly)(H_2O)] + mim (1:1)	Water	—	0.08	−0.67	—	69.1	−167	−0.03	0.07	0.06
[Cu(salgly)(H_2O)] + cyt (1:1)	Water	−0.16	0.27	−0.92	0.11	59.1	−72	−0.03	0.07	0.06
[Cu(salgly)(H_2O)] + cyd (1:1)	Water	−0.46	0.29	−0.72	—	46.8	−31	0.03	0.13	0.01
	Methanol	—	−0.04	−0.94	—	61.3	−62	—	—	—
[Cu(salala)(H_2O)]	Water	−0.30	0.102	−0.72	—	74.5	−148	−0.04	—	—
	Methanol	−0.53	−0.40	−0.88	—	—	—	—	—	—
[Cu(salala)(H_2O)] + mim (1:1)	Water	−0.12	—	−0.83	0.11	54.5	−80.0	−0.05	−0.01	1.75
[Cu(salala)(H_2O)] + cyt (1:1)	Water	−0.15	0.10	−0.69	—	48.2	−76	0.05	0.09	0.03
[Cu(salala)(H_2O)] + cyd (1:1)	Water	−0.12	0.20	−0.77	—	39.4	−33	0.04	0.08	0.04
	Methanol	−0.60	0.09	−0.88	—	31.4	−60	—	—	—

Supporting electrolyte 0.1 mol dm^{-3} $NaClO_4$. Sweet rate 20 mV s^{-1}, [complex] = 0.001, [cyd] = [mim] = 0.2, [cyt] = 0.04 mol dm^{-3}. [a] DPV measurements. [b] Appears as a shoulder.

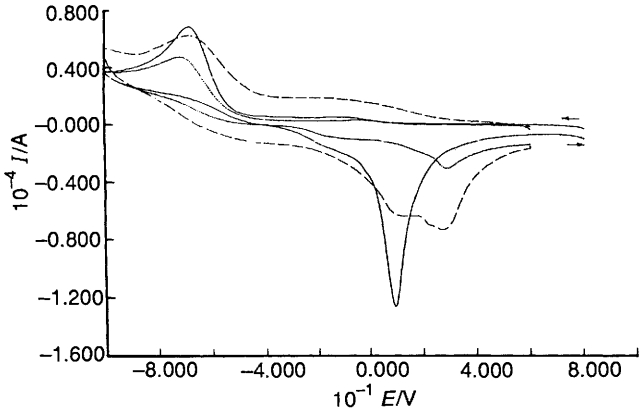


Fig. 5 Cyclic voltammograms of [Cu(salgly)(H_2O)] (——), [Cu(salgly)(H_2O)] + cytosine (R = 1, – – – –) and [Cu(salgly)(H_2O)] + cytidine (R = 1, · · · ·). Supporting electrolyte 0.1 mol dm^{-3} $NaClO_4$. Sweep rate 20 mV s^{-1}

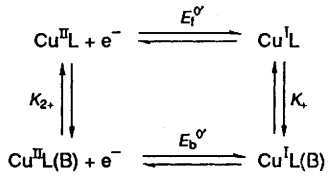
sharpness of the peak obtained during the reverse scan [Cu^I–Cu^{II}, $E_{p_a}(I)$] is indicative of adsorption[43] of Cu^I(salgly) on the electrode. On reversing the scan at −0.60 V the intensity of this wave is almost lost; this shows that most of the species adsorbed is produced at $E_{p_c}(II)$. This can be explained on the basis of Scheme 1.

The species Cu^0(salgly) could not be detected by cyclic voltammetry (CV) as it reacts immediately with Cu^{II}(salgly) to yield Cu^I(salgly). At higher scan rates, however, an anodic peak corresponding to its oxidation develops. The value of $E_{p_c}(II)$ shifts cathodically with increase in scan rate, supporting an electrochemical–chemical (e.c.) mechanism;[44] $E_{p_c}(I)$ shifts anodically showing a chemical–electrochemical (c.e.) mechanism as illustrated in Scheme 1. A similar redox behaviour at a glassy carbon electrode (Scheme 1) is exhibited (Fig. 8) by [Cu(salala)(H_2O)] in aqueous solution. The $E_{\frac{1}{2}}$ values for the Cu^{II}–Cu^I couple (Table 4) were determined from the differential pulse voltammetry (DPV) peak potentials.[45] They show that Cu(salala) stabilises Cu^{II} less than does Cu(salgly) by +64 mV. The tetrahedral distortion[21] effected by the CH_3 group in the former raises the $E_{\frac{1}{2}}$ value; this corroborates the EPR results.

Effect of addition of bases. On the addition of cytidine or cytosine to Cu(salgly), $E_{p_c}(II)$ becomes more negative (Fig. 5, Table 4), showing that both cytidine and cytosine stabilise Cu^I more than Cu^0. The value of $E_{p_a}(I)$ is shifted anodically on adding cytidine, with loss in its sharpness, showing that the adsorption of Cu^IL(cyd) (L = salgly or salala) is prevented possibly by the bulkiness of cytidine. The case with cytosine is similar. On addition of mim to Cu(salgly), however, $E_{p_c}(II)$ becomes more positive (from −0.78 to −0.65 V) at R = 2 (R = ratio of the total concentration of cytidine or cytosine to that of Cu^{II}) and then shifts negatively to −0.67 V at R = 3.

The addition of each base shifts $E_{\frac{1}{2}}$ to more positive potentials, indicating stabilisation[46] of Cu^I more than of Cu^{II}. When $E_{\frac{1}{2}}$ is plotted as a function of R (Fig. 6) an inflection around R = 1 is noted in the case of cytidine and cytosine indicating the formation of 1:1 adduct. In contrast, a well defined inflection at R = 2 in the $E_{\frac{1}{2}}$ *vs.* R plot (Fig. 7) for mim indicates the formation of a 1:2 adduct, clearly confirming the spectrophotometric results. Plots of I_{p_c} and E_{p_c} *vs.* R also indicate the formation of a 1:1 adduct. Similar observations have been made for Cu(salala) (Table 4).

The net shift in $E_{\frac{1}{2}}$ can be used to estimate the ratio of the equilibrium constants for the binding of the 2+ and 1+ ions to bases. For a Nernstian electron transfer in a system in which both the oxidised and reduced forms associate with a third species (base) in solution, Scheme 2 analogous to the treatment of the association of small molecules with micelles[47] is applicable.



Scheme 2

Here Cu^{II}L and Cu^IL represent the oxidised and reduced forms of Cu(salgly) and Cu(salala) and $Cu^{I/II}$L(B) represents $Cu^{I/II}$L bound to a base B. The parameters E_f° and E_b° are the formal potentials of the +2/+1 couple, in the free and bound forms (for mim the potential corresponding to the formation of the 1:1 adduct is taken for comparison), respectively, and K_{2+} and K_+ are the corresponding binding constants for the +2 and +1 species to bases. For a one-electron redox process (assuming reversibility), $E_b^\circ - E_f^\circ = 0.059 \log(K_+/K_{2+})$.

The preference for Cu^{II} relative to Cu^I can be discussed on the basis of K_{2+}/K_+ values. These values for Cu(salala) are higher (except with cytosine) than for Cu(salgly); the distortion from planarity in the former allows the bases to approach Cu^{II} more closely along the equatorial direction and raise the K_{2+} values. The order of interaction of the bases with Cu(salala) varies as

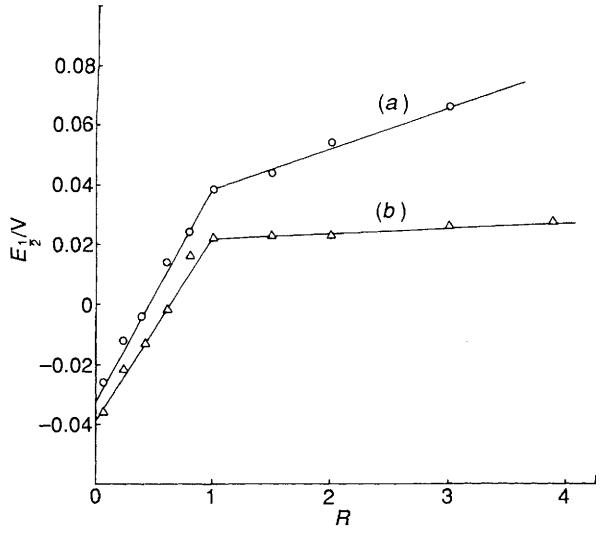


Fig. 6 Dependence of $E_{\frac{1}{2}}$ on the ratio of cytidine:copper complex, R, for (*a*) [Cu(salgly)(H_2O)] and (*b*) [Cu(salala)(H_2O)]

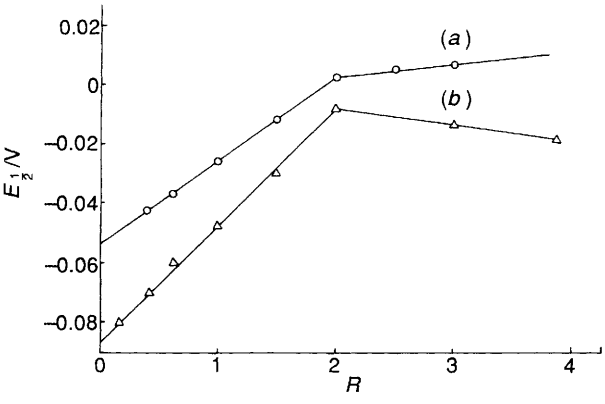


Fig. 7 Dependence of $E_{\frac{1}{2}}$ on the ratio of mim:copper complex, R, for (*a*) [Cu(salgly)(H_2O)] and (*b*) [Cu(salala)(H_2O)]

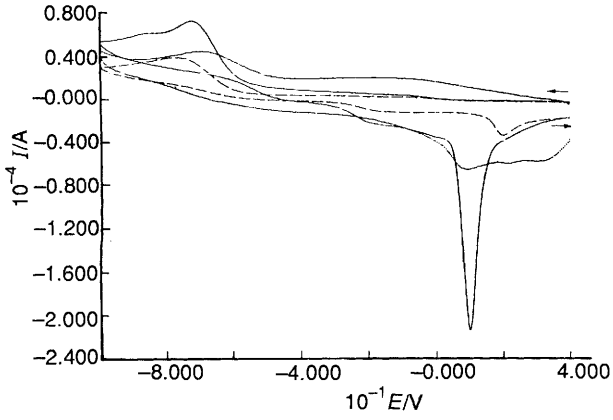


Fig. 8 Cyclic voltammograms of [Cu(salala)(H_2O)] (——), [Cu(salala)(H_2O)] + cytosine (R = 1, – – – –) and [Cu(salala)(H_2O)] + cytidine (R = 1, · · · ·). Supporting electrolyte 0.1 mol dm^{-3} $NaClO_4$. Sweep rate 20 mV s^{-1}

mim > cyt ≈ cyd and with Cu(salgly) as mim ≈ cyt > cyd. This reveals that the basicity and the *ortho* effect or the steric effect near the binding site rather than the bulky ribose is important in the interaction with Cu^{II}. With Cu(salgly), the interaction of cyt is as strong as that of mim; this is explicable on the basis of the additional interaction from O(2) (semi-chelation). This is not possible in Cu(salala) as the planarity required for the additional interaction is less.

Implications for the Binding of Enzymes with DNA.—The present EPR and electrochemical studies indicate that cytosine is bound strongly to copper and the possibility of co-ordination of O(2) is greater if Cu^{II} possesses square-planar geometry. However, when distortion from planarity occurs due to steric effects as in Cu(salala) the possibility of co-ordination of O(2) decreases. In the light of this observation, it may be suggested that if copper(II) in proteins or enzymes possesses square-planar geometry then the additional stability from O(2) co-ordination of the cytosine moiety in nucleic acids, though small, becomes important. Also the basicity and the *ortho* effect of the binding sites are important while the ribose moiety and thus the length of the DNA are not.

Further, our investigation reveals that copper, co-ordinated to tridentate salgly and salala moieties, can further be co-ordinated equatorially to only one cytosine/cytidine molecule because of the steric hindrance from the *ortho* oxo and amino groups and the binding may occur through N(3) and possibly O(2) as well. However, when binding to the simple Cu^{2+} ion, four cytosine molecules are co-ordinated as revealed in the crystal structure of $[Cu(cyt)_4][ClO_4]_2 \cdot 2H_2O$.[48] However, if Cu^{II} is co-ordinatively saturated as in $[Cu(salglygly)]^-$, only axial bonding of N(3) [or O(2)] is possible. If significant co-ordination affinity exists along the open axial positions the adducts may take up octahedral geometry, without any chelation to O(2). Thus it may be suggested that if copper becomes co-ordinatively saturated, as in some copper proteins or enzymes, its interaction with N(3) of the cytosine moiety in nucleic acids may not be strong and co-ordination to O(2) may not be possible; thus the effect of copper ion may be only a template one. However, hydrogen bonding[41] between the protein and nucleic acid moieties can facilitate the interaction.

When copper(II) interacts with DNA it binds to a guanosine–cytidine pair in preference to an adenosine–ribosylthymine pair and selectively ruptures it; this may be related to its binding to N(3) and in some way the C=O group of cytosine as suggested.[14,49] Several crystal-structure determinations[39,50] of copper(II) complexes of cytosine and cytidine show a normal Cu–N(3) bond length and a much weaker and longer apical chelation to O(2). According to Martin and Sook-Huikim,[17] however, since the O(2) atoms of the cytosine bases are bound at an average distance of 2.85 Å from the copper ion, they could not be thought of as forming a true bond. These workers found that though the *ortho* oxo and amino groups in cytosine and cytidine are not as hindering for binding of Cu^{2+} as are methyl and larger groups in other *o*-substituted pyridines, the *o*-oxo group in cytidine does not appear to strengthen binding of Cu^{2+} by chelation.

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