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Ternary complexes of cobalt cysteinylglycine with histidylserine and histidylphenylalanine–stabilities and DNA cleavage properties

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Abstract. Interaction of cobalt cysteinylglycine with histidylserine and histidylphenylalanine was investigated in a 1 : 1 : 1 ratio at 35°C and 0·10 mol dm^{-3} ionic strength. Their stabilities and geometries were determined. Their DNA binding and cleavage properties were investigated. The intrinsic binding constants (K_b) for DNA bound **1** and **2** ($3{\cdot}03 \times 10^3$ M^{-1} for **1** and $3{\cdot}87 \times 10^3$ M^{-1} for **2**) were determined. Even though the negative charge on the complexes reduced their affinity for DNA, there was an enhancement of binding through specificity. The degradation of plasmid DNA was achieved by cobalt dipeptide complexes [Co^{II}(CysGly)(HisSer)] (**1**) and [Co^{II}(CysGly)(HisPhe)] (**2**). Cleavage experiments revealed that **1** and **2** cleave supercoiled DNA (form I) to nicked circular (form II) through hydrolytic pathway at physiological *p*H. The DNA hydrolytic cleavage rate constants for complexes **1** and **2** were determined to be 0·62 h^{-1}, for **1** and 0·38 h^{-1} for **2** respectively.

Keywords. Cobalt(II); dipeptides; ^1H and C^{13} NMR; DNA binding; DNA hydrolytic cleavage; kinetics.

1. Introduction

Nucleic acids are also chemically altered through strand scission of their polymeric backbones. This form of nucleic acid modification, which is fundamental to genetic engineering and biotechnology; can occur through several mechanisms including (1) oxidation of the ribose or deoxyribose sugar ring, (2) alkylation or oxidation of the aromatic nucleobase or (3) hydrolysis of the phosphodiester backbone. Unlike oxidative degradation, hydrolytic strand scission yields nucleic acid termini which are amenable to further enzymatic manipulations, allowing DNA to be specifically cut and re-ligated.

A significant effort in recent years has been directed towards finding suitable metalloreagents for hydrolytic cleavage of DNA as a replacement for conventional oxidative cleavage.[1–14] A survey of literature reveals that the most efficient cleavage agents involving hydrolytic mechanism happens to be the mononuclear complexes[15–22] as compared to the dinuclear,[23–27] trinuclear[28–31] and multinuclear complexes.[32,33] Enhancement of rate constants in the range of 0·09–0·25 h^{-1} for DNA hydrolysis by metal complexes are considered impressive[34,35] even though, they are still far from the rate enhancements produced by natural enzymes (between 40 and $1{\cdot}4 \times 10^4$ h^{-1}).[36,37] Recently, attention has been focused towards the development of cobalt complexes as DNA cleavage agents. However, most of the work on them was confined to oxidative and photocleavage.[38–44] To our knowledge, there is one report on the hydrolytic cleavage of DNA by cobalt complexes.[45] Therefore, it was thought important to develop cobalt complexes as hydrolase mimics for the degradation of DNA. We report, the interaction of cobalt with cysteinylglycine and histidylserine and/or histidylphenylalanine and their stabilities, DNA binding and cleavage properties.

2. Materials and methods

The ligands cysteinylglycine(CysGly), histidylserine (HisSer), histidylphenyl alanine(HisPhe), and ethidium bromide (EB) were obtained from Sigma (99·99% purity, USA). The calf thymus DNA (CT DNA) was obtained from Fluka (Switzerland). pUC 19 supercoiled plasmid DNA (CsCl purified), was obtained from Bangalore Genei (India) and cobalt nitrate was of Analar Grade (E-merck, Germany).

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2.1 *pH Titrations*

Potentiometric pH titrations of ligands in the absence and presence of Co^{II} were performed at 35°C. Fresh solid ligands were weighed out directly into the reaction cell. The stock solution of analytically pure cobalt nitrate was prepared and standardized volumetrically by titration with the disodium salt of EDTA in the presence of a suitable indicator.[46] Carbonate-free NaOH was prepared according to Schwarzenbach and Biederman,[47] and was standardized by titration with potassium hydrogen pthalate. The ionic strength was kept constant, using 0·1 M KNO_3 as supporting electrolyte, and with relatively low concentration of ligands and metal ion (10^{-3} M). During the course of titration, a stream of O_2 free N_2 was passed through the reaction cell to eliminate atmospheric CO_2. A *Digison DI- 707* digital pH meter fitted with a combined glass micro-electrode was used to determine hydrogen ion concentration. For the determination of pH values below 3·5 and above 10·5, the electrode system was calibrated with standard HCl and NaOH solutions respectively. Each experiment was repeated at least twice. Further details can be found elsewhere.[48]

2.2 *Dissociation constants*

The dissociation constants of ligands viz. cysteinylglycine, histidylserine and histidylphenylalanine were determined by using the data from the experimental titration curves with the help of the computer programme PKAS.[49] The equilibria involved for the dissociation reactions are:

$$H_3L \overset{K_a}{\rightleftharpoons} H_2L + H^+ \text{ (L = CysGly)}$$

$$H_2L \overset{K_{2a}K_{3a}}{\rightleftharpoons} L + 2H^+$$

$$H_3A \overset{K_a}{\rightleftharpoons} H_2A + H^+$$

$$H_2A \overset{K_{2a}K_{3a}}{\rightleftharpoons} A + 2H^+ \text{ (A = HisSer/HisPhe).}$$

2.3 *Stability constants*

To determine the stability constants for the 1 : 1 binary [Co^{II}(HisSer)] and [Co^{II}(HisPhe)] complexes, the following equations were used in the buffer region between m = 1 and 3 (omitting charges):

$$M + A \rightleftharpoons MA$$

$$K_{MA}^{A} = \frac{[MA]}{[M]\ [A]} \quad (1)$$

Related equilibrium:

$$M + H_2A \rightleftharpoons MA + 2H^+$$

H_2A = HisSer/HisPhe respectively and M = Co^{II}.

The constant for [Co^{II}(CysGly)] complex could not be obtained due to the appearance of solid phase at an early stage of the reaction.

The stability constants for the ternary 1 : 1 : 1 Co^{II}-CysGly-HisSer (**1**) and Co^{II}-CysGly-HisPhe (**2**) were determined in the buffer interval m = 2-6 based on the following equation:

$$M + L + A \rightleftharpoons MLA$$

$$\beta_{MLA}^{M} = \frac{[MLA]}{[M][L][A]} \quad (2)$$

Related equilibria:

$$M + H_2L + H_2A \rightleftharpoons MLA + 4H^+.$$

The formation constants of binary and ternary complexes were determined with the help of computer programme BEST.[50] All the formation constants were subjected to computer refinement, considering all possible species present in the solution, i.e. H_2L^-, HL^{2-}, L^{3-}, HA, A^-, MA, MA_2, ML, ML_2 and MLA but excluding hydroxo and polynuclear species, as corroborated through concentration-dependent studies. The error limits in the constants were minimized (Sigma fit –0·01 to 0·001). The BEST program was also used to generate complete species-distribution curves as a function of pH.

2.4 *Spectral analysis*

ESI mass spectra were recorded on a *Micromass Quattro LC* triple -quadrupole mass spectrometer, using the MassLynx software. Samples were introduced into the source with *Harvard* infusion pump at a flow rate of 5 μL/min. The capillary and cone voltages were set to 3·5 kV and 10 V, respectively.

^{1}H NMR spectra of ligands in the absence and presence of varying concentrations of Co^{II} at pD 7·5 were recorded on a *Varian Gemini* FT–NMR spec-

trometer at 200 MHz in D_2O at 25°. The concentration of free ligands was set at 20 mM each, and Co^{II} was added such that the final concentration ratio for Co^{II}/CysGly/HisSer (or Co^{II}/CysGly/HisPhe) was 1 : 100 : 100, 1 : 10 : 10 and 1 : 1 : 1. The proton decoupled C^{13}-NMR spectra were recorded at room temperature (25°C) on a *Varian Gemini* – 200 MHz spectrometer operating in FT mode with 1,4-dioxane as an internal reference.

2.5 *Preparation of DNA solution*

Concentrated CT DNA solution was prepared in 5 mM Tris-HCl/50 mM NaCl in water (pH = 7·5). Its concentration was determined by UV absorption spectrum (molar absorption coefficient 6600 M^{-1} cm^{-1} as per.[51] The solutions were stored at 4°C and were used with in a week. Purity of DNA was checked by measuring the UV absorption ratio (A_{260}/A_{280} = 1·8–1·9), which is an indication for protein free DNA.[52] Concentration of ethidium bromide was determined spectrophotometrically[53] using the extinction coefficient of 5680 $M^{-1}cm^{-1}$ at 480 nm.

2.6 *DNA binding studies*

Absorption Spectra were recorded on *Jasco* V-530 spectrophotometer, using 1 cm quartz micro-cuvettes. The spectra of **1** and **2** were recorded at 25°in water (5 mM Tris-HCl/50 mM NaCl (pH = 7·5; λ_{max} 350 nm). Absorption titrations were performed at constant complex concentration (50 μM), and by varying [CT DNA] from 0–50 μM and the spectra were recorded after each addition of CT DNA. The binding constants (K_b) were determined according to the following equation 3:[54,55]

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f) \quad (3)$$

The 'apparent' extinction coefficient (ε_a) was obtained from A_{obsd}/[Co]. The terms ε_f and ε_b correspond to the extinction coefficients of free (unbound) and the fully bound complexes, respectively. From a plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs [DNA], the slope $(\varepsilon_b - \varepsilon_f)^{-1}$ with an intercept equal to $(K_b(\varepsilon_b - \varepsilon_f)^{-1})$ could be obtained and from their ratio K_b can be derived.

Absorption spectra were also recorded for thermal denaturation studies on Shimadzu 160A spectrophotometer. CT DNA (75 μM) was treated with **1** or **2** (75 μM each) in 5mM Tris-HCl/50 mM NaCl in water (λ_{max} 260 nm) pH = 7·5. The samples were continuously heated at a rate of 1°/min, and the change in absorption was monitored as a function of temperature. T_m values and the melting interval (ΔT) were determined according to the reported procedures.[56] Differential melting curves were obtained by numerical differentiation of experimental melting curves.

Fluorescence spectra were recorded with SPEX-Fluorolog 0·22 m fluorimeter equipped with a 450 W Xenon lamp. The slit widths were 2 × 2 × 2 × 2 and the emission spectral range was 560–700 nm. All fluorescence titrations were carried out in 5 mM Tris-HCl/50 mM NaCl (pH = 7·5, 25°C). Solutions containing DNA and ethidium bromide were titrated with varying concentrations of complexes **1** and **2**. The solutions were excited at 540 nm and fluorescence emission corresponding to 593 nm was recorded. The samples were shaken and kept for 2–3 min for equilibrium before recording the spectra. The DNA concentration was always 54 μM. The concentration of the complexes **1** and **2** was in the range of 0-77 µM and EB concentration was 43 μM.

Fluorescence spectra were used to obtain *Scatchard plots*. For this, titrations of DNA against EB in the absence and presence of cobalt complexes were performed. Initial concentration of DNA in 5 mM Tris-HCl/50 mM NaCl was 34 μM and EB was 70 μM. After each addition of EB to the solutions containing DNA and cobalt complexes, the emission spectra was recorded from 550 to 650 nm with 540 nm excitation at 25°C. The data was analysed by the method of *Lepec* and *Paoletti*[57] to obtain bound (c_b) and free (c_f) concentration of EB. *Scatchard plots* were obtained by plotting r/c_f vs r (where $r = c_b$/conc. of DNA).

2.7 *DNA cleavage*

The cleavage of pUC 19 DNA by complexes **1** and **2** was accomplished by mixing (in order) 16 μL of 5 mM Tris-HCl (pH 7·5) containing 5 mM NaCl buffer, varying concentrations of **1** and **2** and 2 μL of pUC19 (0·5 μg/μL, 10 mM Tris-HCl pH 8·0 and 1 mM EDTA). The concentration of **1** and **2** was varied from 125 to 250 μM. After mixing, the DNA solutions were incubated at 37°C for 4 h. The reactions were quenched by the addition of bromophenol blue and the mixtures were analysed by gel electrophoresis. The 1% agarose gels were run at 50 V for 4 h in TAE following electrophoresis. The gels were strained

with ETB solution at 0·5 μg/ml to observe the cleaved DNA products. The extent of DNA cleavage was determined by using the volume quantification method in UVI Doc Mw version 99.03. The relative amounts of the different forms of DNA were determined by dividing the fluorescence intensity for a particular band by the sum of the fluorescence intensities for each band in that lane. In order to examine if hydroxyl radicals were present, hydroxyl radical scavengers such as DMSO and glycerol were introduced. However, kinetic studies were performed at a fixed concentration of (312 μM) of **1** and **2** with different incubation time.

3. Results and discussion

Histidylserine and histidylphenylalanine contain two nitrogen atoms in the aromatic imidazole ring of the histidine moiety, one of which is protonated at the biologically relevant pH range of 5–7. This nitrogen atom can coordinate to metal ions, especially when histidine residue is a part of a protein chain. Cysteinylglycine by virtue of its thiol (SH) group, has a high affinity for both soft and borderline metal ions. HisSer and HisPhe may coordinate to metal ions through carboxylate, imidazole and amino groups, whereas cysteinylglycine through carboxylate, amino and thiol (SH) groups.

3.1 *Dissociation constants*

The free ligand titration curves of HisSer and CysGly (figure 1 a and b) and HisPhe showed an inflections at $a = 1$ and 3, where a is the mole of base added for mole of ligand, indicating step-wise and simultaneous dissociation of proton respectively. Corresponding dissociation constants were obtained with the help of computer programme pKAS and are presented in table 1.

3.2 *Stability constants*

The binary titration curve for the interaction of Co^{II} with HisSer (not shown) and HisPhe (figure 1c) showed an inflection at $m = 1$ and 3 where m is the moles of base added per mole of metal ion. The constant for [Co^{II}(CysGly)] system could not be obtained due to the appearance of a solid phase. However, the titration curves between $m = 0$ and 1 exactly coincided with those of free ligand curves, indicating no interaction in that region. Thus, the formation of only normal binary (MA) complex was considered between $m = 1$ and 3 by assuming simultaneous dissociation of two protons. The constant K^{M}_{MA} was determined using (1) and the values are presented in table 2. The ternary titration curves for [Co^{II}(CysGly)(HisSer)] (**1**) (not shown) and [Co^{II}(CysGly)(HisPhe)] (**2**) (figure 1e) systems in a 1 : 1 : 1 ratio showed an inflection at $m = 2$ and 6. The titration curves between $m = 0$ and 2 were exactly coincided with those of independent free ligands suggesting no ternary complex formation in that region. Therefore, the formation of ternary complex was considered in the buffer region between $m = 2$ and 6 by assuming simultaneous dissociation of four protons. The constants β^{M}_{MA} were determined using (2). The percent-

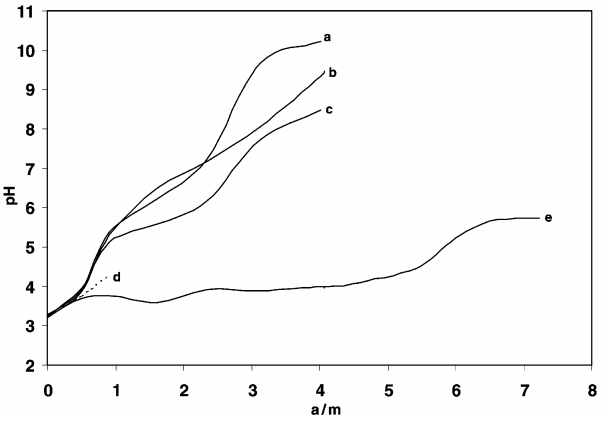

Figure 1. Potentiometric titration curves of free HisPhe (curve a), free CysGly (curve b), 1 : 1 [Co^{II}(HisPhe)] complex (curve c), 1 : 1 [Co^{II}CysGly] complex (curve d) and 1 : 1 : 1 [Co^{II}(CysGly)(HisPhe)] complex (curve e). Conditions: aqueous 0·10 mol dm^{-3} KNO_3 solution, at 35°C. The horizontal axis (m) states the number of (molar) equivalents of base added relative to Co^{II}. For abbreviations, see text.

Table 1. pK_a values of different amino acids and dipeptides [temp. = 35°C; μ = 0·10 mol dm^{-3} (KNO_3)].

Ligand	pK_a COOH	pK_{2a} ImH^+	pK_{2a} SH	pK_{3a} NH_3^+
Cys	2·31	–	8·07	9·94
Gly	2·48	–	–	9·53
His	2·22	6·04	–	9·03
Ser	2·18	–	–	8·84
Phe	2·31	–	–	9·02
CysGly	3·21	–	6·40	9·19
HisSer	3·22	6·53	–	8·32
HisPhe	3·64	6·28	–	7·95

The constants are accurate to ± 0·02 units

Table 2. Formation constants of binary and ternary complexes [temp. = 35°C; μ = 0·10 mol dm^{-3} (KNO_3)].

Complex	Composition and type of the complex	log *k*	Donor atoms involved in metal binding
Co(II)–HisSer	1 : 1 K^{M}_{MA}	6·20	N, N
Co(II)–CysGly	1 : 1 K^{M}_{ML}	ppt	N, S
Co(II)–CysGly–HisSer	1 : 1 : 1 β^{M}_{MLA}	15·55	N, N, N, S
Co(II)–HisPhe	1 : 1 K^{M}_{MA}	5·30	N, N
Co(II)–CysGly	1 : 1 K^{M}_{ML}	ppt	N, S
Co(II)–CysGly–HisPhe	1 : 1 : 1 β^{M}_{MLA}	15·00	N, N, N, S

The constants are accurate to ± 0·02 units

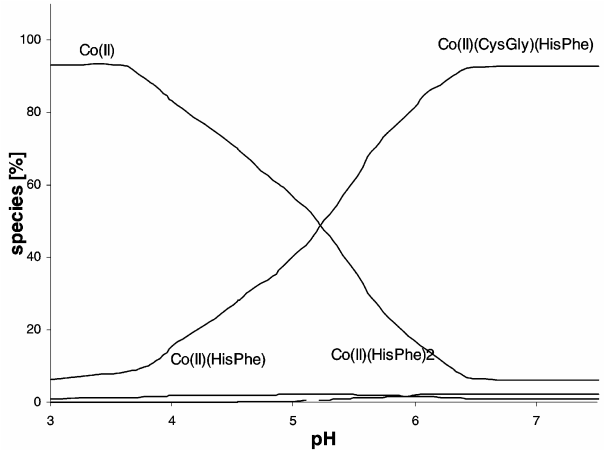

Figure 2. Species distribution curves for [Co^{II}(CysGly)(HisPhe)] in a 1 : 1 : 1 ratio.

age of various species present in solution was computed as a function of pH to identify the stable species at biological pH. The 1 : 1 : 1 ternary complex was the only major species present at the biologically relevant pH range of 6–7 (figure 2).

3.3 *Spectra analysis*

3.1a *^{1}H and ^{13}C spectra:* The ^{1}H NMR spectra of individual ligands CysGly, HisSer, HisPhe and ligands together (CysGly–HisSer and CysGly–HisPhe) in the absence and presence of Co^{II} were recorded. The assignments for the free ligand resonances were made based on the literature[58,59] and simulation (ChemDraw Ultra 7.0). The peak broadening and downfield shifts of CH(α_1), CH_2(β_1), CH(δ), CH(ε), CH(α), and CH_2(β) were observed when the concentration of Co^{II} was in the ratio of 1 : 100 : 100 with respect to CysGly and HisSer/HisPhe. This indicates that the thiol sulphur and amino nitrogen of CysGly imidazole nitrogen, amino nitrogen of HisSer and HisPhe respectively were involved in coordination with Co^{II}. The paramagnetic effect of the metal ion was clearly visible when its concentration was increased to tenfold excess (1 : 10 : 10) and total vanishing of signals, when the reactants were in an equimolar ratio (1 : 1 : 1). CH(α_1^1) in cysteinylglycine, CH ((α^1), CH_2(β^1) in histidylserine and histidylphenylalanine were not significantly changed presence of metal ion. This suggests the non-involvement of the serine part in histidylserine, phenylalanine part in histidylphenylalanine and glycine part in cysteinylglycine in binding with cobalt. In ^{13}C NMR spectra no change in chemical shifts of COO^{-} groups were observed in the presence of cobalt as compared to free ligand spectra (cf –179·2 ppm). This excludes the involvement of COO^{-} groups in metal coordination. Accordingly, a bidentate coordination of these ligands resulting in a tetra coordination around cobalt in both the systems were assumed.

To obtain information regarding the active chemical species that effect DNA damage, the existing multiple species in the systems were identified by means of electrospray ionization mass spectrometry (ESI–MS)(figure 3). The ES mass spectrum of complex **2** a molecular ion peak was observed at *m*/*z* 537 $(M + H)^{+}$, which was in agreement with the molecular weight of the proposed structure. In addition, the spectrum showed peaks due to [Co^{II}(CysGly)(HisPhe) + $Na]^{+}$ (*m*/*z* 559), and a few unidentified ions. The ES mass spectrum of complex **1** (not shown) showed a molecular ion peak at *m*/*z* 477$(M + H)^{+}$, which was in agreement with the molecular weight of the proposed structure. In addition, the spectrum shows ions due to [Co^{II}(CysGly)(HisSer) + $Na]^{+}$ (*m*/*z* 499), and a few unidentified ions.

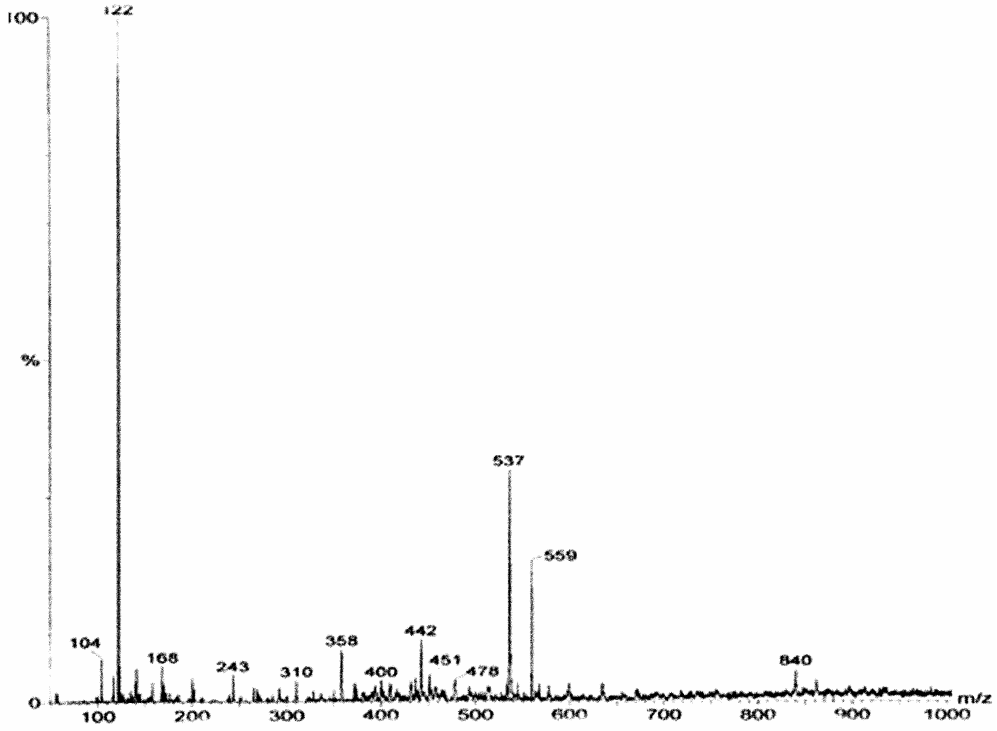

Figure 3. ESI mass spectrum of complex **2** in aqueous solution.

3.4 *Absorption spectra*

The UV-vis spectra of [Co^{II}(CysGly)(HisSer)] and [Co^{II}(CysGly)(HisPhe)] complexes exhibited absorption maxima at 280 nm (ε = 2800 $M^{-1}cm^{-1}$) and 350 nm (ε = 2200 M^{-1} cm^{-1}), probably due to S → Co^{II} charge-transfer transitions.[60–64] Extinction coefficients for Co^{II} *d–d* transitions have been correlated previously with the co-ordination number such that a four co-ordinate ion has *d–d* bands with ε = 300 M^{-1} cm^{-1} or greater, a five coordinate ion has 50 M^{-1} cm^{-1} ε < 310 M^{-1} cm^{-1}, and a six coordiante Co^{II} exhibits peaks with ε < 50 $M^{-1}cm^{-1}$.[65] The weaker Co^{II} *d–d* transition bands at 625 nm (ε = 420 M^{-1} cm^{-1}) and 580 nm (ε = 310 M^{-1} cm^{-1}) clearly indicate a typical tetrahedral geometry.

Molecular mechanics, which is a tool of increasing importance for structural investigation of coordination and organometallic chemistry,[66–69] provides the minimized energy conformations. The minimum-energy conformers of complexes **1** and **2** were calculated by means of the MMFF force field, followed by PM3 optimization (Spartan 05 Programme package). The resulting optimized structures (including relevant bond lengths and relative energies) also supports the above conclusions (figure 4).

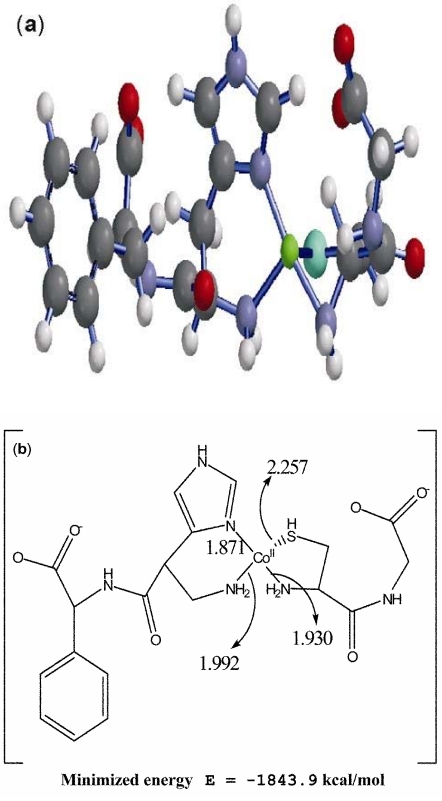

Figure 4. (**a**) The optimized structure of complex **2** (**b**) The structure with bond lengths and relative energy.

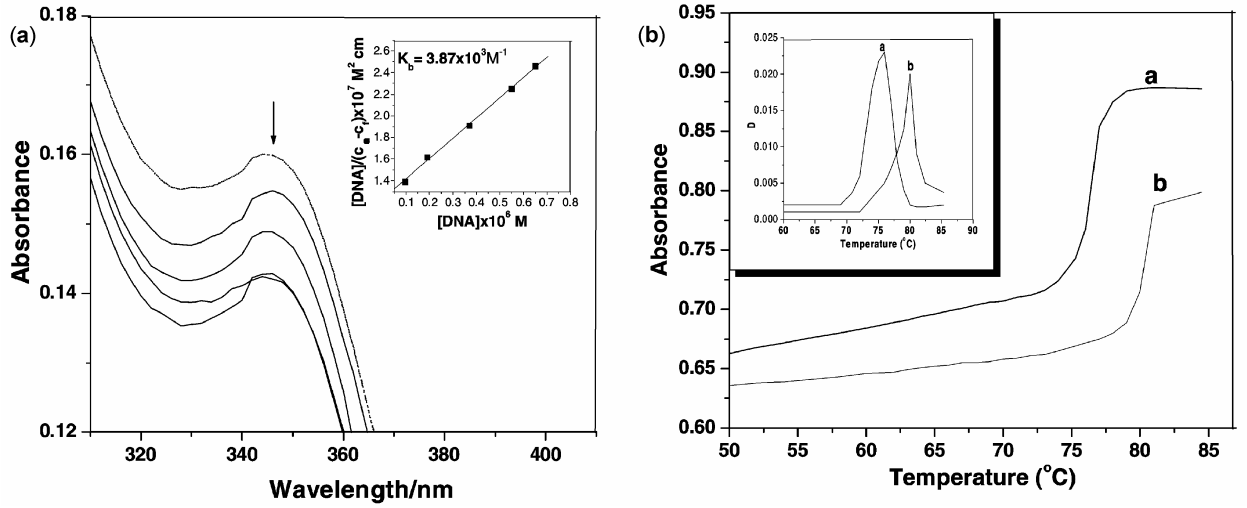

Figure 5. (**a**) Absorption spectra of complex **2** in the absence (----) and presence (—) of increasing amounts of DNA. [Co] = 50 μM. Arrow (↓) show the absorbance changes upon increasing DNA concentration. (**b**) Thermal denaturation profiles and differential melting curves (inset **a** and **b**) of calf thymus DNA before (**a**) and after (**b**) addition of complex **2** (75 μM) DNA concentration (75 μM).

3.5 *DNA binding studies*

The absorption spectra of complexes **1** and **2** in the absence and presence of calf thymus DNA are illustrated in figure 5a. In the presence of DNA, decrease of peak intensities (hypochromism) with slight increase of wavelength (Bathochromism) were observed for **1** and **2**. Hypochromism and Bathochromism were suggested to be due to the interaction between the electronic state of the intercalating chromophore and that of the DNA bases.[70–72] These spectral changes are consistent with the intercalation of complexes **1** and **2** into the DNA base stack. The plot of the absorption titration data according to (3) gave a linear plot and resulted in an intrinsic binding constant (K_b) of $3{\cdot}03 \times 10^3$ M^{-1} for complex **1** and $3{\cdot}87 \times 10^3$ M^{-1} for **2**. Even though the negative charge on the complexes reduced their affinity for DNA, there was enhancement of binding through specificity.

The thermal denaturation profiles of DNA in the absence and presence of complexes **1** and **2** are provided in figure 5b. An increase of 5 to 6°C was observed in the T_m profiles of complexes as compared to free DNA. It is well known that the increase of T_m is indicative of an intercalative and/or phosphate binding where as decrease is due to base binding. Since the complexes are negatively charged the results suggest that the mode of binding is through an intercalative process.

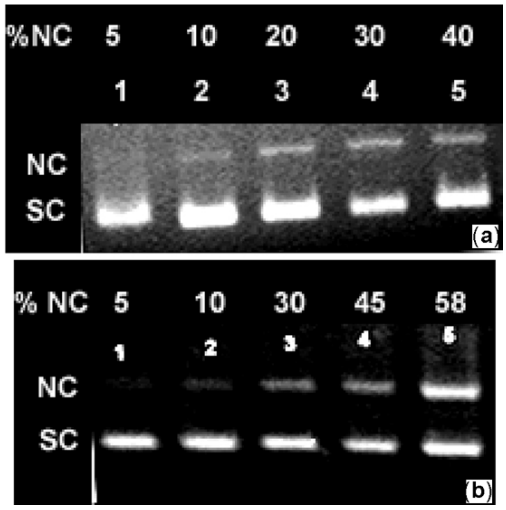

Figure 6. Agarose-gel-electrophoresis patterns for the cleavage of pUC19 DNA by (a) [Co^{II}(CysGly)(HisSer)] and (**b**) [Co^{II}(CysGly)(HisPhe)] respectively. (**a**) Lane 1, DNA control; Lane 2, (CysGly)(HisSer) (312 μM); Lane 3, **1** (125 μM); Lane 4, **1** (187 μM); Lane 5, **1** (250 μM). (**b**) Lane 1, DNA control; Lane 2, (CysGly)(HisPhe) (312 μM); Lane 3, **2** (125 μM); Lane 4, **2** (187 μM); Lane 5, **2** (250 μM).

Fluorescence quenching experiments were performed with ethidiumbromide (EB) bound DNA with increasing concentrations of complexes **1** and **2** to determine the extent of binding between the second molecule and DNA. Two mechanisms have been

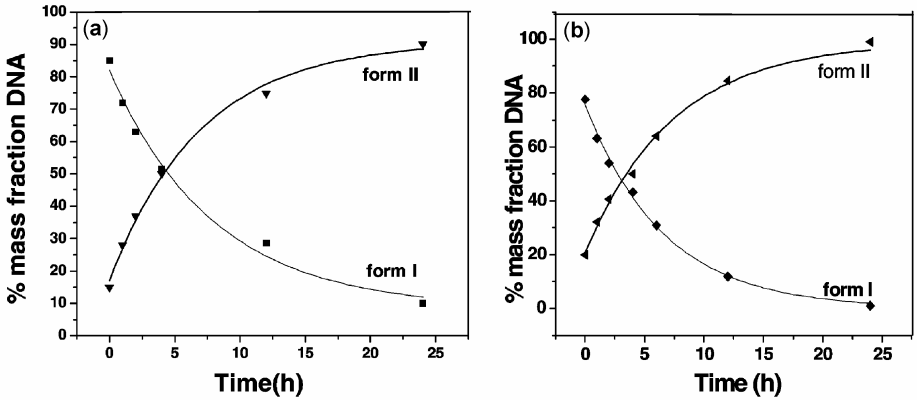

Figure 7. (**a**) Time course of DNA cleavage by complex **1** at 37°C. (♦) form I (supercoiled) (▲) form II (nicked circular). (**b**) Time course of DNA cleavage by complex **2** at 37°C. (♦) form I (supercoiled) (▲) form II (nicked circular).

proposed to account for the quenching the replacement of molecular fluorophores and/or electron transfer.[73,74] Accordingly, the fluorescence profile of DNA bound EB in 5 mM Tris-HCl/50 mM NaCl at pH 7·5 was monitored with increasing addition of complexes **1** and **2**. Quenching of fluorescence of EB bound DNA on addition of complexes **1** and **2** demonstrated the binding of Co^{II} dipeptide complexes with DNA.

The binding isotherms of EB and CT DNA in the absence and presence of **1** was determined experimentally and presented. The *Scatchard* plot in the presence of **1** resulted in the decrease of slope as compared to the complex free plot. Similar results were obtained with **2**. This confirms an intercalative binding of these complexes with CT DNA.[75,76]

3.6 *DNA cleavage*

After establishing the binding abilities of Co^{II} dipeptide complexes with DNA, cleavage experiments were performed with pUC 19 DNA. Figures 6a and b depict agarose gel electrophoresis patterns for the cleavage of pUC 19 DNA. The conversion of form I (supercoiled) to form II (nicked circular) was observed with increase in concentrations of complexes **1** and **2**. It was noted earlier that a single cut or nick on a strand of supercoiled DNA relaxes the supercoiling and leads to form II. Therefore, it is clear that **1** and **2** have cut the DNA to convert it from form I to form II. The extent of DNA cleavage was also quantified via fluro imaging.

Although, **1** and **2** did not require the addition of external agent, the possibility of DNA cleavage occurred via a hydroxyl radical-based depurination pathway was also considered. When pUC 19 DNA was incubated with **1** and **2** in the presence of DMSO and glycerol ($HO^{\cdot}$ radical scavenger), only slight inhibition of DNA cleavage was observed. This suggests that the **1** and **2** cleave the DNA in the absence of external agent and does not proceed via hydroxyl radical pathway. The possible explanation for the degradation of DNA is the formation of a three centered hydrogen bond involving the NH_2 group of guanine, the lone pair of electrons on pyrrole nitrogen of imidazole and the COO^- groups of HisSer/HisPhe and CysGly. Bruice *et al*[77] in their study of *bis*-(2-carboxy phenyl) phosphate, found that carboxyl groups could participate in phosphodiester hydrolysis. Recently Yang *et al*[78] in their study on copper-L-Histidine system have reported that the Cu^{II}-histamine and Cu^{II}-imidazole complexes do not produce measurable extent of DNA cleavage. Thus, they concluded that COO^- group in L-Histidine plays a key role in the cleavage of phosphodiester backbone of DNA. Our findings support this.

The cleavage of pUC 19 DNA by **1** and **2** has been kinetically characterized by quantification of supercoiled and nicked DNA. The observed distribution of supercoiled and nicked DNA in an agarose gel provides a measure of the extent of hydrolysis of phosphodiester bond in each plasmid DNA, and the data were used to perform simple kinetic analysis. Figures 7a and b are the time course plots for the decrease of form I and formation of form II during the reaction under mild conditions by **1** and **2**, respectively. The decrease of form I fits well to a single exponential decay curve and the increase of form

II also fits well to a single exponential curve. From these curve fits, the hydrolysis rate constants at 37°C and at a complex concentration of 312 μM were determined to be 0·62 h^{-1} (0·924) for **1** 0·38 h^{-1} (0·926) for **2**.

Enhancement of rate constants in the range of 0·09–0·25 h^{-1} for DNA hydrolysis by metal complexes are considered impressive[34,35] even though they are still far from the rate enhancements produced by natural enzymes. Thus the nuclease activity of **1** and **2**, which are $1{\cdot}72 \times 10^7$ and $1{\cdot}05 \times 10^7$ fold rate enhancement over unhydrolyzed double stranded DNA respectively, is impressive considering the nature of the ligands involved. The increased rate of hydrolysis by the complex **1** compared to **2** may be due to the presence of hydroxyl group in the former which is known for its contribution towards cleavage activity.

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