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Interaction of zinc and cobalt with dipeptides and their DNA binding studies

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Abstract. Interactions of zinc and cobalt with peptides cysteinylglycine and histidylglycine have been studied. The binding modes were identified and geometry assigned. Stabilities of these complexes and their ability to bind DNA have been investigated. It is demonstrated that only zinc complexes bind DNA as compared to cobalt complexes.

Keywords. Zinc; cobalt; cysteinylglycine; histidylglycine; DNA.

1. Introduction

Recent interest in zinc finger chemistry[1–13] has been oriented towards synthesis, characterization and solution studies of complexes mimicking the zinc core in zinc fingers and establishing the DNA binding.[14–18] As part of our efforts to create a simple small molecule model and its ability to recognize DNA, the interaction of metal ions with dipeptides was initiated.

Here the interactions of Zn(II) and Co(II) with cysteinylglycine and histidylglycine have been studied as models for the zinc core. Stabilities of the complexes, binding modes and their ability to bind DNA have been investigated.

2. Experimental

The ligands cysteinylglycine (CysGly) and histidylglycine (HisGly) were obtained from Sigma Chemical Company (USA). Zinc nitrate and cobalt nitrate were of analar grade from E Merck. Calf thymus DNA (CT DNA) was obtained from Fluka (Switzerland). They were used as supplied.

Concentrated CT DNA stock solutions were prepared in tris buffer (5 mM Tris-HCl/50 mM NaCl in water at pH = 7·5) and its concentration was determined by UV absorbance at 260 nm on a Shimadzu UV-160 A UV-Vis spectrophotometer using microcuvettes with a path length of 1 cm.

The molar absorption coefficient was taken as 6600 $M^{-1}\,cm^{-1}$.[19] Solutions of calf thymus DNA in 5 mM Tris-HCl/50 mM NaCl (pH = 7·5) gave a ratio of UV absorption at 260 and 280 nm A_{260}/A_{280} of ≈ 1·8–1·9, which indicates that the DNA was sufficiently free of protein.[20] Stock solutions were stored at 4°C and were used within a week. Double-distilled water was used to prepare all solutions.

2.1 *Potentiometric pH titrations*

The experimental method consisted of potentiometric titration of ligands at 35°C in the absence and presence of metal ions under the experimental conditions. For every titration fresh solid ligand was weighed out into the reaction cell to avoid possible hydrolysis. Stock solutions of analytically pure zinc nitrate and cobalt nitrate were prepared and standardized volumetrically by titrating against the disodium salt of EDTA in the presence of a suitable indicator as outlined by Schwarzenbach.[21] Carbonate-free sodium hydroxide was prepared by the method of Schwarzenbach and Biederman[22] and was standardized by titration against potassium hydrogen phthalate. The ionic strength was maintained constant using 0·1M KNO_3 as supporting electrolyte and relatively low concentration of ligand and metal ion (1×10^{-3}) were used. During the course of titration a steam of oxygen-free nitrogen was passed through the reaction cell to eliminate the adverse effect of atmospheric CO_2. A Digison model DI 707 digital pH meter fitted with combined micro glass electrode was used to

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determine hydrogen ion concentration. The pH regions below 3·5 and above 10·5 were calibrated by measurements in HCl and NaOH solutions respectively. Each experiment was repeated at least twice for accuracy. Further details can be found elsewhere.[23] The proton dissociation constants of ligands, CysGly and HisGly were determined from experimental data by the computer program PKAS[24] and the formation constants were determined by using computer program BEST.[25] The data are given in table 1.

2.2 *^{1}H-NMR spectra*

The ^{1}H NMR spectra of the ligands in the absence and presence of zinc ion were recorded at room temperature (25°C) on a Varian Gemini 200 MHz pulsed FTNMR spectrometer in D_2O. TMS was used as an internal standard. The values are given in table 2.

2.3 *^{13}C NMR spectra*

The ^{13}C NMR spectra were recorded in D_2O at room temperature (25°C) on a Varian 200 MHz spectrometer in FT mode with 1,4-dioxane as an internal standard with a chemical shift of d 66·5. The data are given in table 2.

2.4 *DNA recognition measurements*

For DNA binding studies an increasing known amount of [Co(II)(CysGly)(HisGly)] and [Zn(II)(CysGly)(HisGly)] complexes were added to CT DNA until the ratio of these complexes with DNA reached ~1 : 1. The experiments were carried out at room temperature (25°C) and at pH 7·5 in 5 mM NaCl.50 mM Tris HCl. After each addition, the mixture was shaken and kept for ~ 5 min and then the absorbance was recorded.

2.5 *Electrospray ionization MS measurements*

Typically a 1·0 ml portion of [Zn(II)(CysGly)(HisGly)] solution, loaded into the injection valve of the Micromass Electrospray mass spectrometer QuattroLC unit, was injected into the mobile-phase solution (50% aqueous methanol) and then carried through the electrospray interface into the mass analyser at a rate of 60 ml min^{-1}. The potential employed at the electrospray was 4·0 kV, and the capilliary was heated to 100°C. The positive-ion mass spectrum was then obtained. Determination of the charge of the species was made by using the isotopic pattern.

Table 1. Stability constants* of [M(II)(CysGly)(HisGly)] systems at 35°C (m = 0·1 mol dm^{-3} KNO_3).

Complex	Composition of the complex		Log b^{M}_{MLA}
[Co(II)(CysGly)(HisGly)]	1 : 1 : 1 log b^{M}_{MLA}		15·23
[Zn(II)(CysGly)(HisGly)]	1 : 1 : 1 log b^{M}_{MLA}		17·02
pKa values at 35°C			
Ligand	COOH	SH	NH_3^+
CysGly	3·21	6·40	9·19
HisGly	2·35	5·38	7·18

*The values are accurate to ± 0·02 units

3. Results and discussion

Since the zinc core in zinc fingers is present in ternary mode, the interactions of metal ions in the presence of two dipeptides have been investigated. The proton dissociation constants of CysGly and

Table 2. ^{1}H and ^{13}C-NMR chemical shifts in [Zn(II)(CysGly)(HisGly)] system (at pH ~ 6).

System	CH(a)	CH$_2$(b)	CH(d)	CH(e)	CH$_2$(a')	CH(a_1)	CH$_2$(b_1)	CH$_2$(a'_1)
				^{1}H-NMR				
[(CysGly)(HisGly)]	3·65	2·90	6·90	7·65	3·70	3·32	2·70	3·75
[Zn(II)(CysGly)(HisGly)]	4·40	3·45	7·45	8·65	3·80	3·10	3·82	3·95

					^{13}C-NMR					
	COO^-	CO–NH	CH(a)	CH$_2$(b)	C(g)	CH(d)	CH(e)	CH(a_1)	CH$_2$(b_1)	CH$_2$(a'_1)
[(CysGly)(HisGly)]	178·5	172	54·38	26·99	132·2	120·01	137·49	54·81	29·85	40·24
[Zn(II)(CysGly)(HisGly)]	178·5	172	50·95	24·02	124·06	117·7	133·21	53·21	24·95	40·24

HisGly were determined which correspond to the dissociation of protons from COOH, SH, NH_3^+ and COOH, NH^+, NH_3^+ respectively.

The experimental titration curve of [M(II)(CysGly)(HisGly)] systems in a 1 : 1 : 1 ratio at 35°C (not shown) showed an inflection at $m = 2$ and $m = 6$, where m is the moles of base added per mole of metal ion. In the buffer region between $m = 0$ and $m = 2$ the titration curves of both the free ligands overlapped with that of mixed ligand complexes, indicating no interaction in this region. Since this region happens to be the region of carboxylate proton dissociation, it was assumed that the carboxylate groups are not involved in metal coordination. Coupled with this the trends of titration curves in the buffer region between $m = 2$ and $m = 6$ suggested a bidentate coordination of HisGly and CysGly with metal ions involving two protons from each of these ligands. Accordingly the thiosulphur, amino nitrogen, imidazole and amino nitrogen of CysGly and HisGly respectively, are proposed in metal coordination.

The following equations were used (omitting charges except for H^+) in the buffer region between $m = 2$ and 6.

$$M + H_2A + H_3L \rightleftharpoons MLA + 4H^+, \quad (1)$$

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

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

where M = Zn(II), and Co(II), and H_2L, and H_2A are CysGly and HisGly respectively.

The formation constants were subjected to refinement considering all possible species in solution, using computer program BEST. The error limits in these constants were minimized (sigma fit = 0·001 to 0·0001). BEST was also used to generate the complete species distribution curves as a function of pH. The data show the formation of mixed ligand complexes to the extent of 95% at biological pH, suggesting their exclusive formation. The constants are listed in table 1.

To further confirm these coordination modes the ^{1}H NMR and ^{13}C NMR spectra of free ligands and the ternary complex were recorded (figures 1 and 2). The data are compiled in table 2. The free ligand spectral values agree well with the literature.[26] Since the SH, NH and NH_2 hydrogen exchange with D_2O the changes in the chemical shifts were monitored for the neighbouring CH(a_1), CH(b_1) of the cysteine residue and CH(a) CH(e) and CH(d) of histidine residue and CH_2(a') of the glycine residue. In the ^{13}C NMR spectra of HisGly and CysGly the resonances due to C(a), C(b), C(g), C(d), C(e) of histidine residue, C(a') of glycine residue and C(a_1), C(b_1) of cysteine residue were monitored. The data confirm the non-involvement of carboxylate groups, and involvement of thiosulphur, amino nitrogen and imidazole nitrogen, amino nitrogen of CysGly and HisGly respectively in metal coordination. Thus a tetra-coordination around zinc was assumed (structure 1). Based on chemistry of the metal ions, a tetrahedral geometry was proposed.[27]

Since Zn(II) is a spectroscopically silent ion with electronic configuration of d^{10} Co(II) was used as a spectroscopic probe for Zn(II). The UV-Vis spectral data exhibited an intense absorption bands at 275 and 380 nm, which is indicative of the L → Co(II) LMCT bands (here L = S). The *d–d* transition bands observed at 620 and 580 nm is indicative of typical tetrahedral geometry.[28]

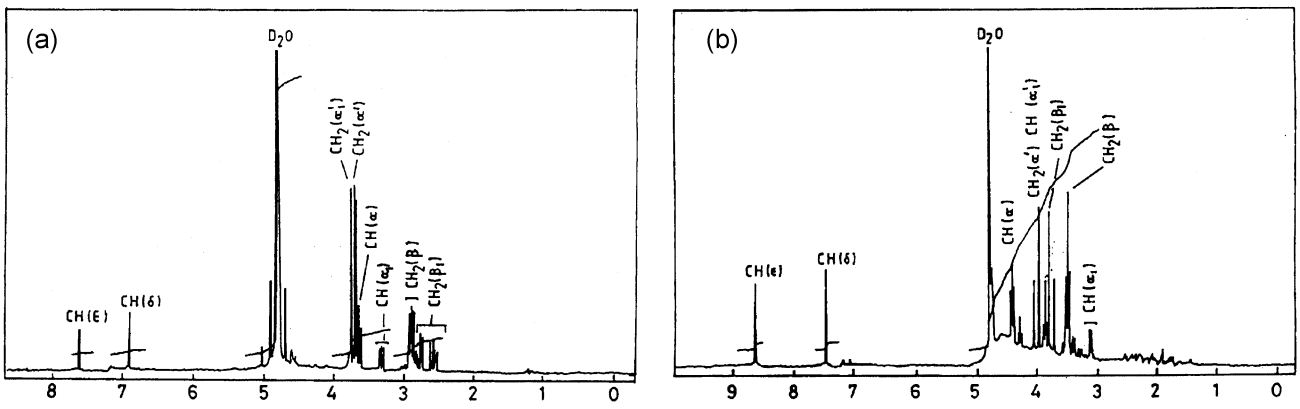


Figure 1. ^{1}H NMR spectra of [(CysGly)(HisGly)] (**a**) in the absence and (**b**) in the presence of zinc.

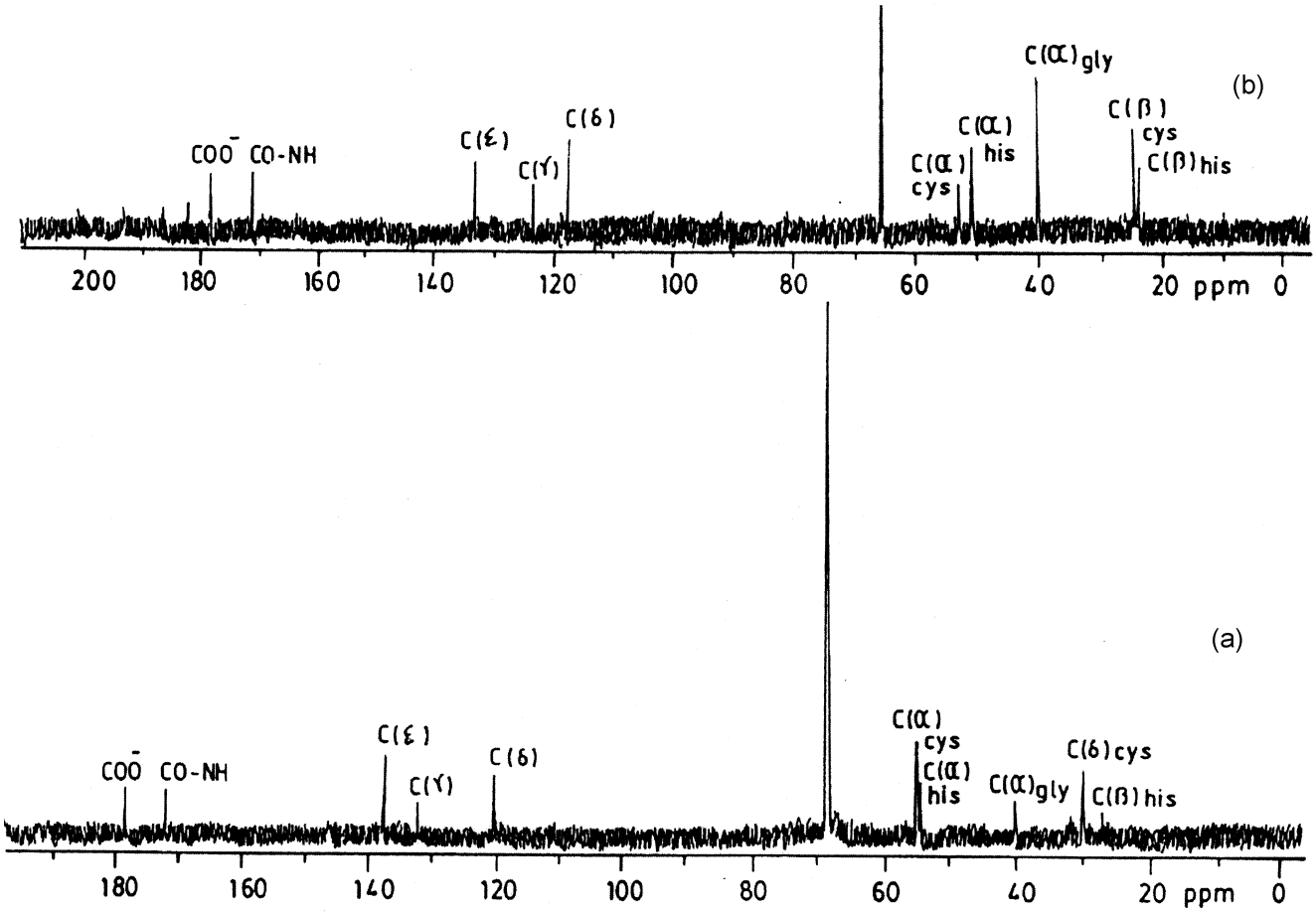


Figure 2. ^{13}C NMR spectra of [(CysGly)(HisGly)] (**a**) in the absence and (**b**) in the presence of zinc.

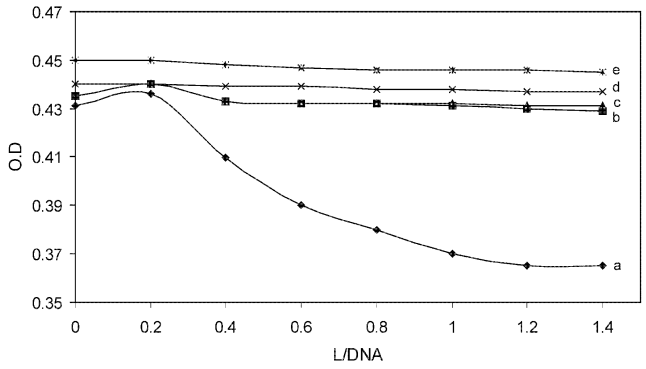
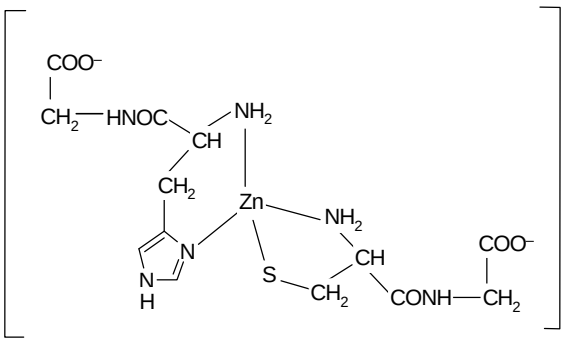


Figure 3. UV hypochromicity profile of interaction of DNA with (a) [Zn(II)(CysGly)(HisGly)], (b) [Co(II)(Cys-Gly)(HisGly)], (c) free [Co(II)], (d) [(CysGly)(HisGly)], (e) free [Zn(II)].



Structure 1. Geometry of the [Zn(II)(CysGly)(HisGly)] complex.

After establishing binding modes and geometry, which are known to play a critical role in DNA binding *in vivo*, DNA binding studies with these small molecule models have been investigated.

The changes in the hypochromicity profile are presented in figure 3. The initial increase in absorption is due to electrostatic interaction between DNA and metal complexes. The decrease in absorbance, which is an index of the interaction between the DNA and metal complex[29] was observed only in the

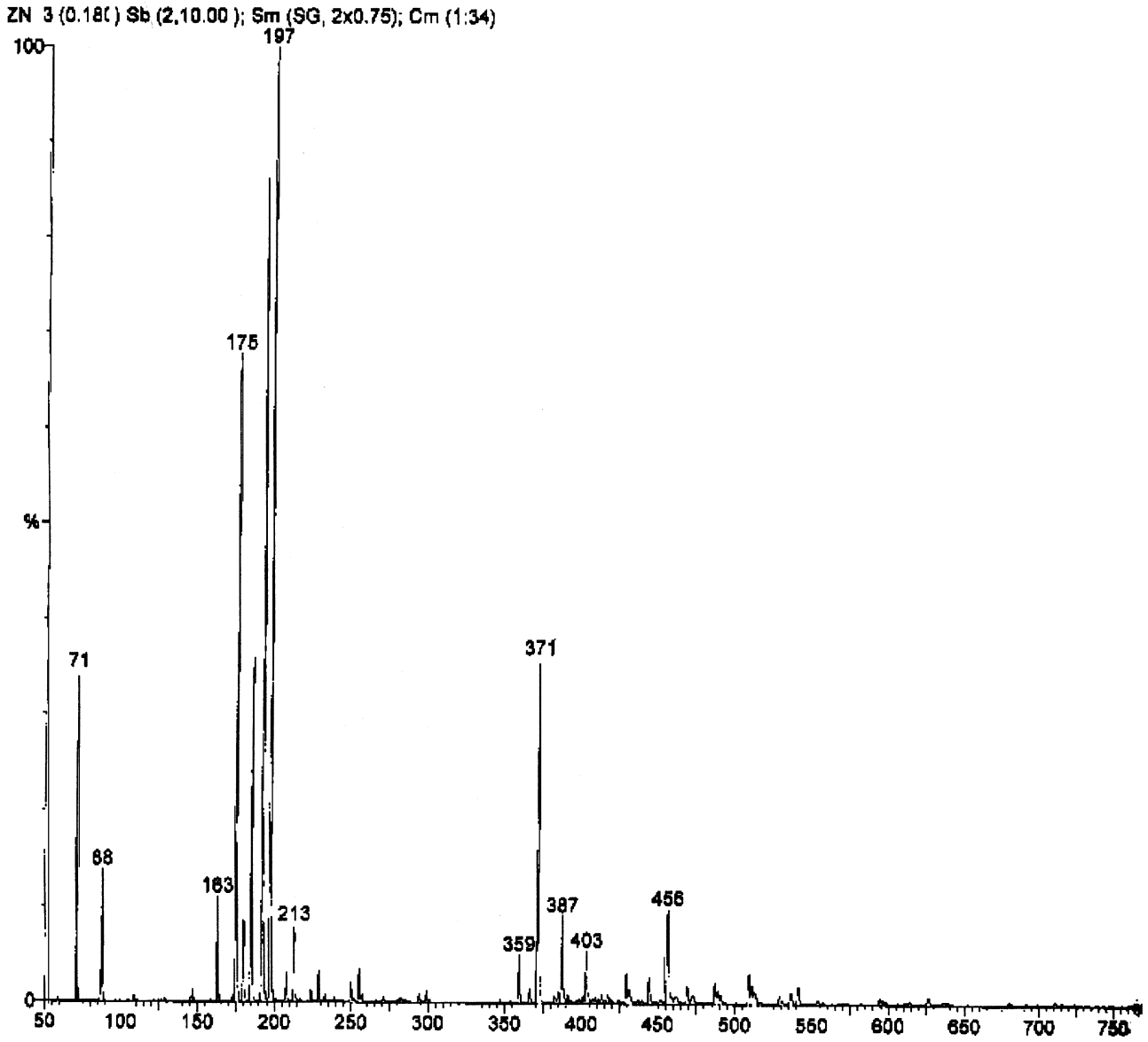


Figure 4. ESI mass spectrum of [Zn(II)(CysGly)(HisGly)] in aqueous solution.

case of zinc complex with the saturation point at 0·363. This was reached when the ligand concentration matched with that of DNA that is, at a ligand/DNA–phosphate ratio of 1 : 1. Virtually no effect was observed on the addition of free ligands and free zinc ion to DNA solution. Since the extent of hypochromicity[30–34] is a fairly accurate reflection of the extent of ordered secondary structure in DNA, the results obtained from the UV-Vis studies are significant and demonstrate that the zinc complexes bind to DNA leading to saturation of ~1 : 1 complexes through an interaction involving the amino group of zinc complex with the guanine base of DNA. It is presumed that the primary binding is between the phosphate oxygens and imdizole nitrogen through hydrogen bonding (N–H). The next step seems to involve the amine group of the zinc complex and the G (N–7) of the DNA. The assumption is based on X-ray crystallography data of zinc fingers.[1]

In the case of free cobalt ion there was slight decrease in the absorbance. This phenomenon, which was not observed in the case of free zinc ion, may be due to the preference of cobalt towards the phosphate backbone of DNA. However, in the case of cobalt complex, the extent of decrease was exactly similar to that observed in the case of free cobalt

ion. To make sure that the changes observed were not due to a competitive absorption by the complex, experiments were conducted by taking the same concentration of the complex in the reference cell. However, no appreciable changes were observed. This clearly indicates that there is no binding of cobalt complex with DNA.

Thus, it is interesting to note that the preference of zinc over other metals like cobalt seems to be governed by the basic principles involved in the selection of metal ions by nature.[35]

Since ESI-MS is a powerful new approach for analysing biomolecular complexes, the method was used for distinguishing among different types of chemical species that are present in solution that effect DNA binding. The spectrum of [Zn(II)(CysGly)(HisGly)] in aqueous solution is given in figure 4. The peak at *m*/*z* 456 is the molecular ion peak. The peak at *m*/*z* 213 is that of HisGly and the peak at *m*/*z* 197 is of CysGly with water molecule. Remaining peaks are impurities. These observations suggest that the main species that were present in aqueous solution were from the [Zn(II)(CysGly)(HisGly)] complex. Thus, it is clear from this investigation that the ternary zinc complex binds with DNA.

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