Interaction of Ni(II)-ethylenediamine/histamine with histidylglycine and investigation of their DNA cleavage abilities

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The interaction of Ni(II)-ethylenediamine (en)/histamine(hist) with histidylglycine(his-gly) and their DNA cleavage abilities have been investigated. The stability constants for the Ni(II)-en-his-gly (1) and Ni(II)-hist-his-gly (2) complexes have been determined at 298.0 K and 0.10 M (KNO₃) ionic strength. Both the 1:1:1 ternary complexes have been corroborated by species distribution curves and ESI-MS analysis. The complex geometries have been analyzed by UV-vis experiments and molecular mechanics simulations. The DNA binding abilities of these complexes have been established by UV-vis-absorption, thermal-denaturation and fluorescence spectroscopy. The intrinsic binding constants for the bound complexes, Ni(II)-en-his-gly:DNA and Ni(II)-hist-his-gly:DNA, have been determined to be 280 and 420 M⁻¹ respectively. Gel-electrophoresis experiments reveal that 1 and 2 cleave supercoiled DNA (type-I) to the nicked-circular (type-II) form hydrolytically at physiological pH. A tentative mechanism is proposed for the cleavage.

Keywords: Bioinorganic chemistry, Solution chemistry, Stability constants, DNA binding, DNA cleavage, Nickel (II)

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The selective modification of DNA and RNA by transition metal complexes has grown into a rich area of research¹. A number of approaches are now available towards oxidation or hydrolysis of sugar-phosphate backbone of nucleic acids using transition metal complexes². However, complexes that are capable of cleaving DNA hydrolytically (and selectively) would be highly desirable as they do not require any external agents. Further, the sequence specific nature of the DNA cuttings earlier provided strong evidence that a binding event is central to the scission mechanism³. This has resulted in the development of several artificial metallonucleases that bind to DNA for the cleavage of DNA. These (artificial metallonucleases) have potential applications as therapeutic agents, and as a versatile replacements for nucleases as laboratory tools⁴-⁷. In nature, many metalloenzymes that catalyze phosphate ester cleavage use amino acid side chains to enhance activity compared to the metal ion itself. Several metal ions have been used for the hydrolytic cleavage of DNA⁸-¹⁶, ⁴. However, there are only a few reports on the utilization of nickel complexes for this purpose¹⁷,¹⁸. Herein, we report the DNA binding and cleavage abilities of [Ni(II)(en)(his-gly)] (1) and [Ni(II)(hist)(his-gly)] (2) complexes at physiological conditions.

Materials and Methods

Ethylenediamine (en), histamine (hist), histidylglycine (his-gly) and ethidium bromide (EB) were obtained from Sigma (99.99% purity, Germany). Nickel nitrate was obtained from Merck (Germany) and was of analytical grade. CT-DNA (calf thymus) was obtained from Fluka (Switzerland) and pUC19 DNA, agarose and Tris-HCl were obtained from Bangalore Genei (India). All chemicals were used as supplied.

Concentrated CT-DNA stock solutions were prepared in buffer (5 mM Tris-HCl/50 mM aq. NaCl) and the concentration of CT-DNA solution was determined by UV absorbance at 260 nm by using ε = 6600 M⁻¹cm⁻¹. Solutions of CT-DNA in buffer (5 mM Tris-HCl/50 mM aq.NaCl) gave a ratio at 260 nm and 280 nm (A₂₆₀/A₂₈₀) of ca. 1.8-1.9, indicating that the DNA was sufficiently free of protein¹⁹. All stock solutions were stored at 4°C and used within four days. The concentration of ethidium bromide was determined spectrophotometrically²⁰.
using an extinction coefficient of 5680 M$^{-1}$ cm$^{-1}$ at 480 nm. Ni(NO$_3$)$_2$ stock solutions were prepared and standardized volumetrically by titration with the disodium salt of EDTA in the presence of a suitable indicator, as outlined by Schwarzenbach$^{21}$.

**pH Titrations**

Potentiometric pH titrations of ligands in the absence and presence of Ni(II) were performed at 298.0 K. For every titration, fresh solid ligand was weighed out directly into the reaction cell. Carbonate-free NaOH solution was prepared by the method of Schwarzenbach and Biederman$^{22}$ and standardized by titration with potassium hydrogen phthalate. The ionic strength was kept constant using 0.10 M KNO$_3$ as supporting electrolyte, and relatively low concentrations of ligands and metal ion (1×10$^{-3}$ M). During the course of titration, a stream 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 a combined micro glass electrode, was used to determine the hydrogen ion concentration. The pH regions below 3.5 and above 10.5 were calibrated by measurements in standardized HCl and NaOH solutions, respectively. Each experiment was repeated at least twice for accuracy. Further details can be found elsewhere$^{23}$.

**Stability constants of the complexes**

To determine the stability constants for the ternary, 1:1:1 [Ni(II)(en)(his-gly)], and [Ni(II)(hist)(his-gly)] systems in the buffer region between $m = 1$ and 5, the following equations were used; (omitting charges)

$$M + A + L \rightleftharpoons MAL$$

$$\beta_{MAL}^M = \frac{[MAL]}{[M][A][L]} \quad \ldots (1)$$

Related equilibria: $M + H_2A + H_2L \rightleftharpoons MAL + 4H^+$

The ternary stability constants were determined from experimental titration curves by BEST$^{24}$. The formation constants were subjected to computer refinement, considering all possible species present in the solution, i.e., $H_2L^+$, $HL^-$, $L^3^-$, HA, $A^-$, ML, $ML_2^-$, MA, $MA_2^-$, HA, MAL but excluding hydroxo and polynuclear species. 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.

**Spectral studies**

ESI mass spectra were recorded on a Micromass Quattro LC triple-quadrupole mass spectrometer, using the Mass Lynx software. Samples were introduced into the source with a Harvard infusion pump at a flow rate of 5 µL/min. The capillary 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 Ni(II), were recorded on a Varian Gemini FT-NMR spectrometer at 200 MHz in DMSO at 298.0 K. The concentrations of the free ligands was set to 10 mmol each, and Ni(II) was added such that the final concentration ratio for Ni/en/his-gly or Ni/hist/his-gly was 1:100:100, 1:10:10 and 1:1:1. TMS was used as an internal standard.

**DNA binding activity**

Absorption spectra were recorded on a Jasvo V-530 UV-visible spectrophotometer using 1 cm quartz microcuvettes at 298.0 K in Tris HCl buffer. Absorption titrations of 1 and 2 were performed by keeping the concentration of the complex constant (10 µM), and by varying the concentration of CT DNA from 0-10 µM. The binding constant ($K_b$), for the binding of the complex with DNA has been determined from the spectroscopic titration data using the following equation$^{25}$:

$$[\text{DNA}] / (\varepsilon_a - \varepsilon_i) = [\text{DNA}] / (\varepsilon_b - \varepsilon_i) + 1 / K_b (\varepsilon_b - \varepsilon_i) \quad \ldots (2)$$

The ‘apparent’ extinction coefficient ($\varepsilon_a$) was obtained by calculating $A_{obsd}$/[Ni]. The terms $\varepsilon_1$ and $\varepsilon_b$ correspond to the extinction coefficients of free (unbound) and of the fully bound form of the complex, respectively. From a plot of [DNA]/($\varepsilon_a - \varepsilon_i$) versus [DNA], $K_b$ is the ratio of the slope [1/(\varepsilon_b - \varepsilon_i)] to the intercept [1/$K_b (\varepsilon_b - \varepsilon_i)$].

The thermal denaturation studies were performed on a Shimadzu 160A spectrophotometer equipped with a thermostatic cell holder. DNA (30 µM) was treated with 1 and 2 (30 µM) in a 1:1 ratio in 5 mM Tris HCl/50 mM NaCl buffer. The samples were continuously heated with temperature increase of 1°C min$^{-1}$ while the absorption changes at 260 nm were monitored. Values for the melting temperature ($T_m$) and for the melting interval ($\Delta T_m$) were determined according to the reported procedures$^{26}$. Differential melting curves were obtained by numerical differentiation of experimental melting curves.
Fluorescence spectra were recorded with a SPEX-Fluorolog 0.22 m fluorimeter equipped with a 450W Xenon lamp. The slit widths were 2×2×2×2 and the emission spectral range was 550-700 nm. All fluorescence titrations were carried out in 5 mM Tris-HCl/50 mM NaCl at 290.0 K. The solution containing DNA and EB was titrated with varying concentrations of 1 or 2. The solutions were excited at 540 nm and fluorescence emissions, corresponding to 595 nm, were recorded. The samples were shaken and kept for 2-3 min to attain equilibrium, and then the spectra was recorded. The DNA concentration was always kept at 41 µM DNA-phosphate. The concentration of the complex was in the range of 0-196 µM and the EB concentration was 41 µM.

Fluorescence spectra were also utilized to obtain Scatchard plots. For this, titrations of DNA against EB in the absence and presence of the nickel complex were performed. The initial concentration of DNA in 5 mM Tris-HCl/50 mM NaCl was 20 µM, the concentration of the complex was 50 µM. After each addition of EB to the solution containing DNA and the nickel complex, the emission spectra were recorded from 550 to 700 nm with 540 nm excitation at 298.0 K. Corrections were made to the data for the volume changes during the course of titrations. The data were analyzed by the method of Lepecq and Paoletti27 to obtain bound (c_b) and free (c_f) concentrations of EB. Scatchard plots were obtained by plotting r_{EB}/c_f Vs r_{EB} (where r = c_b / [DNA]).

DNA cleavage

The DNA cleavage experiments were conducted using supercoiled pUC19 DNA in Tris-HCl-buffered aqueous solution DNA cleavage was accomplished by adding varying concentrations of 1 or 2 (0-500 µM) to 2 µL of pUC19 DNA, while the total volume was increased to 16 µL by adding 5 mM Tris. HCl/5 mM NaCl buffer. After mixing, the DNA solutions were incubated at 37°C for 3 h and then the reaction was quenched by addition of loading buffer (0.25% bromophenol blue). The samples were then electrophoresed for 2 h at 50V on 1% agarose gel using Tris-acetic acid EDTA buffer (pH 8.0). The gel was stained using 1 µg/cm³ EB and photographed under UV light.

Results and Discussion

The metal complexes for our study were obtained by adding an appropriate amount of a solution of metal ion to solutions of the ligands. The molar ratio for the complexes MAL (1) and MBL (2) was 1:1:1 (A=en, B=hist and L=his-gly). The stability constants for the formation of [Ni(II)(en)(his-gly)]⁺ and [Ni(II)(hist)(his-gly)]⁺ were determined at 298.0 K and 0.10 M KNO₃ from experimental points (Fig. 1A) with the help of a computer program24, and are presented in Table 1. The pk values used in the calculation of stability constants were re-measured under our experimental conditions. These are in agreement with the literature values (Table 1)28. Some relevant distribution diagrams are shown in Fig. 1B.

All further studies were performed in the pH range 6.5-7.5.

The ESI-mass spectra of the complexes in the positive mode gave a single peak at m/z 332 for 1 and m/z 380 for 2 indicating that the complexes are in the form of mono positive species, i.e., [Ni(II)(en)(his-gly)]⁺ and [Ni(II)(hist)(his-gly)]⁺.
The 1H-NMR spectra of individual ligands (en, hist and his-gly) and of their mixtures both in the absence and presence of Ni(II), were recorded in DMSO (D6). The assignment of free ligand resonances was based on literature data and in combination with computer simulations (Chem3D Ultra 6.0). Both peak broadening and downfield shifts were observed for the imidazole H-atoms (Δ = ~0.86) of his-gly, the imidazole (Δ = ~0.82) and methylene H-atoms (Δ = ~0.20) adjacent to the amino group of hist, when the Ni(II)/hist/his-gly ratio was set to 1:100:1. The methylene H-atoms adjacent to the amino group of hist were obscured by DMSO (D6) peak. This indicates that the imidazole and amino N-atoms of both, his-gly and hist, are involved in metal coordination. The paramagnetic effect of the metal was clearly visible when the concentration of Ni(II) was increased (1:10:1); the signals disappeared fully when the reactants were in an equimolar ratio (1:1:1). Similar trends were observed with complex 1. Accordingly, the involvement of two N-atoms of his-gly and two amino N-atoms of en were considered in complex 1.

**Complex geometry**

The geometries of the Ni(II) complexes 1 and 2 were investigated by UV-vis spectroscopy. Two characteristic bands were observed at 14286 cm⁻¹ and 25381 cm⁻¹ indicating an octahedral geometry and, eventually D3h symmetry. The ground state of Ni(II) in an octahedral coordination is 3A2g. Thus, the above bands may be assigned to the spin-allowed transition (a) 3A2g (F) → 3T1g (F) ν1; 3A2g (F) → 3T1g (F) ν2; 3A2g (F) → 3T1g (F) ν3, respectively. The minimum energy conformers of 1 and 2 were calculated for the corresponding cis and trans isomers by means of the HYPER CHEM program package. The resulting optimized structures (including relevant bond lengths and relative energies) are shown in Fig. 2. As can be seen, the trans isomers of 1 and 2 are found to be more stable than cis isomers. The proposed structures for 1 and 2 resemble the X-ray structure of [Cu(his-gly)2(H2O)2]. 6H2O. The Cu(II) is in a tetragonally distorted octahedral coordination, where the basal plane is occupied by N-atoms belonging to the histidine portion. The apical positions are occupied by two molecules of water. The Ni-N distances are slightly shorter in 1 and 2 when compared to the Cu(II) complex. This may be due to the distortion in the latter.

**Table 1 – Dissociation and association constants of free ligands and metal complexes.**

<table>
<thead>
<tr>
<th>Ligands</th>
<th>COOH</th>
<th>ImH⁺</th>
<th>NH₃⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylenediamine</td>
<td>—</td>
<td>—</td>
<td>7.07; 9.15</td>
</tr>
<tr>
<td>Histamine</td>
<td>—</td>
<td>5.89 (6.01)</td>
<td>9.42 (9.57)</td>
</tr>
<tr>
<td>Histidyglycine</td>
<td>2.62 (2.32)</td>
<td>5.80 (5.39)</td>
<td>7.16 (7.62)</td>
</tr>
<tr>
<td>Metal complexes</td>
<td>Comp. and type of the complex</td>
<td>log k</td>
<td>Donor atoms involved in metal binding</td>
</tr>
<tr>
<td>[Ni²⁺(en)(his-gly)]</td>
<td>1:1:1</td>
<td>15.20</td>
<td>N,N,N</td>
</tr>
<tr>
<td>[Ni²⁺(hist)(his-gly)]</td>
<td>1:1:1</td>
<td>14.55</td>
<td>N,N,N</td>
</tr>
</tbody>
</table>

Error limits: ± 0.02; Lit. values (Ref. 28) in parentheses.

The UV-vis absorption spectra of 2 in the absence and presence of CT-DNA are illustrated in Fig. 3A. In the presence of DNA, a decrease of the peak intensities was observed for both 1 and 2, in combination with a small red shift. These data were thus, consistent with an intercalation of 1 and 2 into the DNA base stack. The intrinsic binding constants (Kb) are calculated as 280 M⁻¹ (1) and 420 M⁻¹ (2), respectively.

The thermal denaturation profiles of DNA in the absence and presence of 2 are shown in Fig. 3B. An increase of 3 to 4°C was observed in the Tm profile.
Fig. 2—The energy-minimised molecular structures, the relative energies and the calculated bond lengths of cis and trans-[Ni(II)(en)(his-gly)]$^{+}$ (1), cis and trans-[Ni(II)(hist)(hisgly)]$^{+}$ (2).

Fig. 3—(A) Absorption spectra of 2 in the absence (-----) and presence (---) of increasing amounts of DNA. [Cond.: [Ni] = 10 µM with increasing DNA concentration. Arrow (↓) shows the absorbance changes upon increasing DNA concentration. Inset: Linear plot for the calculation of the intrinsic DNA binding constant, $K_b$. (B) Thermal denaturation profiles of (a) free CT DNA, and, (b) after the addition of 2 (30µM). [The DNA concentration is fixed at 30µM].

complexes as compared to free DNA. It is well known$^{34}$ that the increase of $T_m$ indicates an intercalative and/or phosphate binding, whereby a decrease is an indicator of base binding. Thus, these results provide an evidence for intercalative and/or phosphate binding of the nickel complexes with DNA. It seems that the first interaction of DNA with complexes is due to an electrostatic interaction between the positively charged complexes and the negatively charged DNA-phosphate backbone, followed by an intercalative mode of binding. Similar trends were also obtained for 1.
Ethidium bromide (EB) strongly fluoresces in the presence of DNA due to complete intercalation, which results in electronic stabilization by π/π stacking and dipole/dipole interactions. Two mechanisms have been proposed in this context, the replacement of the fluorophore and/or electron transfer. To determine the binding strength of our complexes, we performed fluorescence-quenching experiments with EB-bound DNA. As can be seen from Fig. 4A, complex 2 gives rise to a typical quenching profile. Similar results are obtained for 1.

According to the Stern-Volmer equation,

\[ \frac{I_o}{I} = 1 + K_{sq} r \]  \hspace{1cm} \text{(3)}

where \( I_o \) and \( I \) are the fluorescence intensities in the absence and the presence of complexes, respectively, \( K_{sq} \) is linear Stern-Volmer quenching constant dependent on the ratio of bound concentration of EB to the concentration of DNA and ‘\( r \)’ is the concentration ratio of the complex to DNA. The quenching plots (Fig. 4A insets) illustrate that the quenching of EB bound to DNA by 1 and 2 are in good agreement with the linear Stern-Volmer equation, which indicates the DNA binding. In the plot of \( I_o/I \) versus \([\text{complex}]/[\text{DNA}]\), \( K_{sq} \) is given by the ratio of slope to intercept. The \( K_{sq} \) values for 1 and 2 are 0.15 and 0.19 respectively.

From the fluorescence-quenching data, Scatchard plots (Fig. 4B) were established as described previously, and \( r_{EB} \) values were determined. The term \( r_{EB} = [\text{EB}]_b/[\text{DNA}] \) is the concentration ratio of bound EB to total DNA and \([\text{EB}]_f\) is the concentration of free EB (i.e., \([\text{EB}] = [\text{EB}]_b + [\text{EB}]_f\)). As can be seen, the slope of the Scatchard plot is decreased with a slight reduction in the coordinate intercept upon addition of 2, (filled circles). These results suggest the following: (i) major interaction of the nickel complexes with DNA through intercalation, and, (ii) minor interaction with the bases (covalent binding), with the dissociation of water molecule. It seems that the first liaison between DNA and the complexes is due to an electrostatic interaction of the positively charged complexes with the negatively charged DNA-phosphate backbone, followed by an intercalative mode of coordination.

**DNA cleavage**

In agarose-gel-electrophoresis experiments, the conversion of supercoiled DNA (type-I) to a nicked-circular (type-II) form with increasing concentration of 1 and 2 was observed. When supercoiled pUC19 DNA was incubated at 37°C for 3 h in 5 mM Tris HCl-5 mM NaCl in the presence of 1 and 2, it was converted from a supercoiled (type-I) to a nicked (type-II) circular form (Fig. 5A). To ensure that the DNA cleavage is solely due to the Ni(II) complexes, the cleavage activities of free ligands and free Ni(II) were studied (Fig. 5B). It is interesting to note that they do not show any cleavage activity even at a concentration of 1.0 mM. As can be seen from Fig. 5A, the complex 2 cleaves DNA slightly more.
Conclusions
Interaction of Ni(II)-ethylenediamine/histidine with histidylglycine was investigated at 298.0 K and 0.10 molar ionic strength. The stability constants for the formation of Ni(II)-ethylenediamine-histidylglycine and Ni(II)-histidine-histidylglycine are 15.20 and 14.55 respectively. The geometry and binding sites in the complexes are established. The DNA binding abilities of the complexes were probed by UV-vis, thermal-denaturation and fluorescence spectroscopy. These complexes cleave supercoiled (SC) plasmid DNA to the nicked circular (NC) form hydrolytically at physiological pH.

Supplementary Data
ESI-MS spectra, UV-vis, $T_m$, fluorescence-quenching diagram and Scatchard plots for complex I may be obtained from the authors on request.

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References