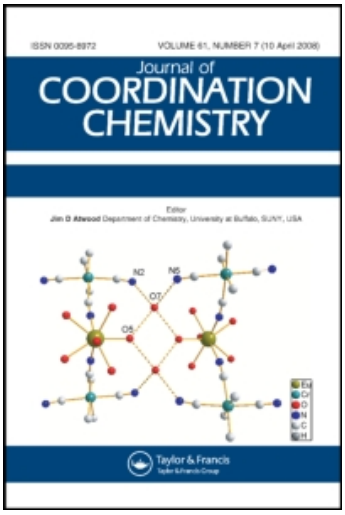

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Synthesis, structure, and DNA binding of three reduced amino-acid Schiff-base zinc(II), nickel(II), and cadmium(II) complexes

Chunyan Gao[a]; Xiaofang Ma[ab]; Jinlei Tian[a]; Dongdong Li[a]; Shiping Yan[a]
[a] Department of Chemistry, Nankai University, Tianjin 300071, China [b] Basic Medical College, Zhengzhou University, Zhengzhou 450001, China

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Synthesis, structure, and DNA binding of three reduced amino-acid Schiff-base zinc(II), nickel(II), and cadmium(II) complexes

CHUNYAN GAO†, XIAOFANG MA†‡, JINLEI TIAN†, DONGDONG LI† and SHIPING YAN*†

†Department of Chemistry, Nankai University, Tianjin 300071, China
‡Basic Medical College, Zhengzhou University, Zhengzhou 450001, China

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Three new reduced amino-acid Schiff-base complexes, $[Zn(HL)_2]\cdot H_2O$ (**1**), $[Ni(HL)_2]\cdot H_2O$ (**2**), and $[Cd(HL)_2]\cdot H_2O$ (**3**), where H_2L is a reduced Schiff base derived from condensation of *N*-(2-hydroxybenzaldehyde) and L-histidine, have been synthesized and characterized by elemental analysis, UV-Vis absorption spectra and single crystal X-ray diffraction. Complexes **1**–**3** are isostructural. All metal centers are six-coordinate with O_2N_4 donor sets in slightly distorted octahedra. Unlike its Schiff-base counterpart, the deprotonated monoanionic ligand HL^- has a more flexible backbone and two HL^- are tridentate to one metal. Moreover, the binding interactions of these complexes with calf thymus DNA (CT-DNA) have been investigated by UV-Vis spectra and fluorescence quenching, which show that the complexes bind in an intercalative mode.

Keywords: Transition metal complex; Crystal structure; Reduced Schiff base; DNA binding

1. Introduction

Binding of small molecules to DNA is very important in the development of DNA molecular probes and new therapeutic reagents. DNA-binding metal complexes have been extensively studied as DNA structural probes, DNA-dependent electron transfer probes, DNA footprinting and sequence-specific cleaving agents, and potential anticancer drugs [1].

Transition metal complexes containing Schiff-base ligands and their reduced products are often used as artificial chemical nucleases and some such complexes have proved to be efficient DNA cleavage reagents [2–6]. Most model studies of metal complexes of Schiff-base ligands containing salicylaldehyde and amino acids have focused upon the binding mode [7–14]. Structural studies on the metal complexes of reduced Schiff-base ligands, derived from various amino acids and salicylaldehyde,

*Corresponding author. Email: yansp@nankai.edu.cn

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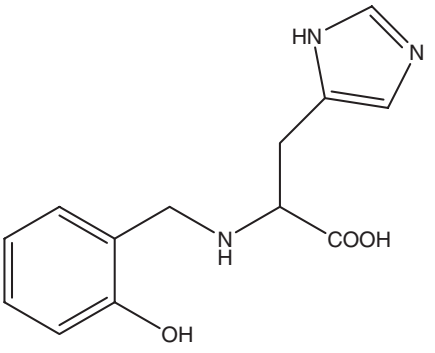

Scheme 1. Schematic structure of *N*-(2-hydroxybenzyl)-L-histidine (H_2**L**).

are well documented [15–25]. The Schiff-base ligand is tridentate, coordinating through the phenolato oxygen, imine nitrogen, and carboxylate oxygen, and is more flexible because of the reduction of the C=N bond of the Schiff base [26].

In this article, we selected a biologically relevant reduced amino-acid Schiff base H_2**L** (H_2**L** = *N*-(2-hydroxybenzyl)-L-histidine) [27–30], scheme 1, and obtained three new transition metal (Zn, Ni, and Cd) complexes. H_2**L** is an unusual amino acid containing an imidazole and a phenolic group in addition to the imino and carboxylate, with a tendency to form an H-bonded network due to the presence of a complementary H-bond donor and acceptor groups within the same molecule. The interaction between calf thymus DNA (CT-DNA) and these complexes was investigated by UV absorption and fluorescence spectroscopy.

2. Experimental

2.1. *Materials and methods*

All reagents and chemicals were purchased from commercial sources and used as received. C, H, and N elemental analyses were obtained on a Perkin–Elmer analyzer model 240. Infrared spectra were recorded on KBr pellets using a Perkin–Elmer FT-IR spectrometer from 4000 to 400 cm^{-1}. Electronic spectra were measured on a JASCO V-570 spectrophotometer. Fluorescence spectral data were obtained on an MPF-4 fluorescence spectrophotometer at room temperature. CT-DNA and ethidium bromide (EB) were all purchased from the Sino-American Biotechnol Biotechnology Company. The tris-HCl buffer solution was prepared using deionized, sonicated, and triply-distilled water.

2.2. *Preparation of compounds*

2.2.1. Synthesis of the reduced Schiff base H_2L. H_2**L** was synthesized by reducing the corresponding Schiff base derived from the condensation reaction of L-histidine and 2-hydroxybenzaldehyde according to a literature method [17].

2.2.2. Synthesis of $[Zn(HL)_2]\cdot H_2O$ (1). A solution of H_2L (0.2 mmol) and 0.4 mmol LiOH in EtOH : H_2O (1 : 1 v/v, 10 mL) was added dropwise to a solution of 0.2 mmol $Zn(ClO_4)_2\cdot 6H_2O$ in EtOH : H_2O (1 : 1 v/v, 10 mL). After 24 h stirring, the solution was filtered and the filtrate left in air at room temperature. After 6 weeks, colorless needles suitable for X-ray diffraction were obtained. The crystals were collected by filtration, washed with Et_2O and dried over silica gel (yield 61%). Anal. Found (%): C, 51.78; H, 5.30; N, 13.77. Calcd (%) for $C_{26}H_{30}ZnN_6O_7$: C, 51.71; H, 5.01; N, 13.92. FT-IR bands (KBr phase): 1618 cm^{-1}, ν_{as}(COO); 1250 cm^{-1}, ν_s(COO); 3007 cm^{-1}, ν_s(N–H); 3417 cm^{-1}, ν_s(H_2O and/or OH).

2.2.3. Synthesis of $[Ni(HL)_2]\cdot H_2O$ (2) and $[Cd(HL)_2]\cdot H_2O$ (3). Complexes **2** and **3** were prepared by similar procedures to **1** using $NiCl_2\cdot 6H_2O$ or $CdCl_2\cdot H_2O$ instead of $Zn(ClO_4)\cdot 6H_2O$. Yield: 66% for **2**. Anal. Found (%): C, 52.31; H, 5.22; N, 13.89. Calcd (%) for $C_{26}H_{30}N_6NiO_7$: C, 52.29; H, 5.06; N, 14.07. Yield: 53% for **3**. Anal. Found (%): C, 48.20; H, 4.51; N, 12.79. Calcd (%) for $C_{26}H_{28}CdN_6O_7$: C, 48.12; H, 4.35; N, 12.95. FT-IR bands (KBr phase): for **2**, 1594 cm^{-1}, ν_{as}(COO); 1249 cm^{-1}, ν_s(COO); 3167 cm^{-1}, ν_s(N–H) and 3551 cm^{-1}, ν_s(H_2O and/or OH); for **3**: 1680 cm^{-1}, ν_{as}(COO); 1251 cm^{-1}, ν_s(COO); 3134 cm^{-1}, ν_s(N–H) and 3551 cm^{-1}, ν_s(H_2O and/or OH).

2.3. *X-ray crystallography*

Diffraction measurements for **1**, **2**, and **3** were made on a Bruker Smart 1000 CCD area detector equipped with graphite-monochromated Mo-Kα radiation ($\lambda = 0.71070$ Å) using the ω-scan technique. Lorentz polarization and absorption correlations were applied using the multiscan program [31]. The structures were solved by direct methods and refined with full-matrix least-squares using the SHELXS-97 and SHELXL-97 programs [32]. Anisotropic thermal parameters were assigned to all nonhydrogen atoms. The hydrogen atoms attached to the organic groups were generated geometrically and allowed to ride along with the nonhydrogen atoms to which they were attached. Analytical expressions of neutral atom scattering factors were employed and anomalous dispersions were incorporated. A summary of the crystal data is given in table 1 and selected bond angles and distances are listed in table S1.

2.4. *DNA-binding experiments*

DNA-binding experiments were performed at room temperature in triply distilled water buffer containing 5 mM Tris-HCl/50 mM NaCl, adjusted to pH 7.2 with hydrochloric acid. Relative binding of the complexes to CT-DNA was studied by UV-Vis absorption and fluorescence spectroscopy. The solutions of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm, A_{260}/A_{280}, of 1.8–1.9, indicating that the DNA was sufficiently free of protein [33]. The stock solution of CT-DNA was prepared in Tris-HCL/NaCl buffer, pH = 7.2 (stored at 4°C and used in less than 4 days). The concentration of CT-DNA was determined by absorption spectroscopy using the known molar extinction coefficient value of 6600 $M^{-1}cm^{-1}$ at 260 nm [34]. UV absorption spectroscopy experiments were conducted by adding CT-DNA solution to

Table 1. Crystallographic data for **1**, **2**, and **3**.

Complex	**1**	**2**	**3**
Empirical formula	$C_{26}H_{30}ZnN_6O_7$	$C_{26}H_{30}NiN_6O_7$	$C_{26}H_{30}CdN_6O_7$
Formula weight	603.93	597.25	650.97
Temperature (K)	113(2)	113(2)	113(2)
Crystal system	Monoclinic	Monoclinic	Monoclinic
Space group	*P2(1)*	*P2(1)*	*P2(1)*
Unit cell dimension (Å, °)			
a	8.319(9)	8.3007(10)	8.3220(15)
b	11.425(12)	11.4658(14)	11.4425(19)
c	13.239(14)	13.197(18)	13.447(3)
β	95.302(14)	94.995(10)	94.638(9)
Volume (A^3), *Z*	1253(2), 2	1251.2(17), 2	1276.3(4), 2
Calculated density ($g\,cm^{-3}$)	1.601	1.585	1.689
F(000)	624	624	660
θ range for data collection (°)	2.36–25.01	1.55–27.84	1.52–25.02
Reflections collected	9322	15760	9676
Independent reflections	3958 [*R*(int) = 0.0491]	5814 [*R*(int) = 0.0504]	4395 [*R*(int) = 0.0446]
Goodness-of-fit on F^2	0.961	0.984	0.996
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0447$, $wR_2 = 0.0976$	$R_1 = 0.0432$, $wR_2 = 0.0858$	$R_1 = 0.0364$, $wR_2 = 0.0745$
R indices (all data)	$R_1 = 0.0480$, $wR_2 = 0.1000$	$R_1 = 0.0528$, $wR_2 = 0.0908$	$R_1 = 0.0391$, $wR_2 = 0.0761$

the complexes ($1.5 \times 10^{-4}\,mol\,L^{-1}$) at different concentrations. The binding constant, K_b, was determined using the following equation [35]:

$$[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_B - \varepsilon_F) \quad (1)$$

where ε_A, ε_F, and ε_B correspond to A_{obsd}/[complex], the extinction coefficient for the free complex, and the extinction coefficient for the complex in the fully bound form, respectively.

By the fluorescence spectral method, the relative binding of the three complexes to CT-DNA was studied with an EB-bound CT-DNA solution in 5 mM Tris-HCl/NaCl buffer (pH = 7.2). Fluorescence intensities at 610 nm (510 nm excitation) were measured at different complex concentrations. The emission intensity showed a reduction upon addition of the complex.

3. Results and discussion

3.1. *Description of the crystal structures of 1, 2, and 3*

Complexes **1**–**3** have similar mononuclear structures, $[M(HL)_2]$ (M = Zn for **1**, Ni for **2**, and Cd for **3**); the scheme of the crystal structure is given in figure 1 and the drawings of the mononuclear unit of the three complexes are shown in figures S1–S3, respectively. All metal centers are six-coordinate with O_2N_4 donor sets in slightly distorted octahedra. The *cis* coordination angles vary from 73.41(17)° to 109.85(15)° for **1**, 78.39(10)° to 103.80(9)° for **2**, and 70.16(13)° to 118.48(12)° for **3**. Moreover, the M–N and M–O bond distances are within the range expected for such species (table S1).

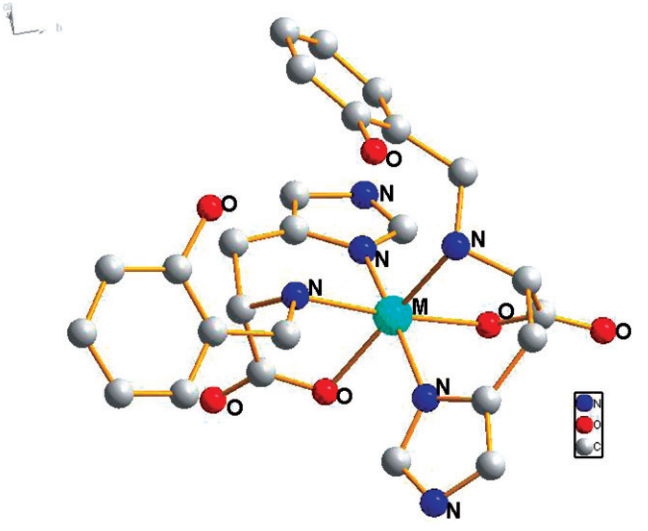

Figure 1. The labeled scheme of mononuclear unit of $[M(HL)_2] \cdot H_2O$ (M = Zn, Ni, Cd); hydrogens and H_2O are omitted for clarity.

Unlike its Schiff-base counterpart, the deprotonated dianionic HL^- is more flexible and two HL^- are tridentate ligands to one metal. In each HL^-, the chiral C-center has the absolute configuration S. After complexation with the metal, the nitrogen of HL^- becomes chiral. In all these complexes, the absolute configuration of nitrogen is R. Thus, the generation of the new chiral center is heterogeneous with respect to the original chiral C-center.

Hydrogen bond networks exist in crystals of the three complexes with complicated intramolecular and intermolecular hydrogen bonds among carboxyl oxygen, imidazole nitrogen, and phenolic oxygen. As an illustrative example the packing mode of **2** is given in figure 2 and some of the hydrogen bonding parameters are shown in table 2.

3.2. *DNA-binding studies*

DNA binding is the critical step for DNA cleavage in most cases. Therefore, the binding of **1**, **2**, and **3** to CT-DNA was studied by measuring their effects on UV and fluorescence spectra of DNA.

3.2.1. UV-Vis absorption spectroscopy. Electronic absorption spectroscopy is one of the most useful techniques for DNA-binding studies of metal complexes. As shown in figure 3, the potential CT-DNA binding ability of complexes was studied by following the intensity changes of the intraligand π–π^* transitions in the UV spectrum at 204–211 nm. Upon addition of increasing amount of CT-DNA (0–7.9×10^{-4} M) to the complexes (1.5×10^{-4} mol L^{-1}), 8.27–40.9% hypochromism and slight red shift (10–17 nm) were observed, indicating weak binding of the three complexes with DNA. The extent of hypochromism is consistent with the strength of intercalative interaction [36–38]. From the observed spectral changes, the values of the intrinsic binding

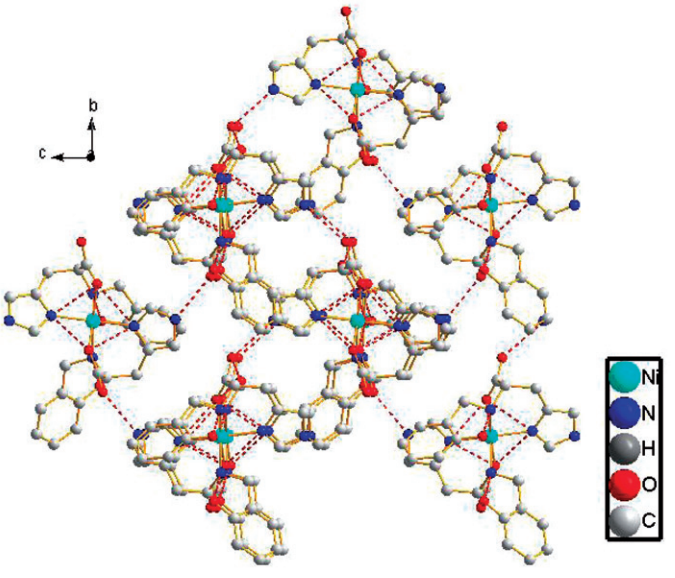

Figure 2. 3-D hydrogen bonding network for **2**.

Table 2. Hydrogen bond distances (Å) and angles (°) in **2**.

D–H···A	d(D–H)	d(H···A)	d(D···A)	∠(DHA)
N(2)–H(2A)···O(2)	0.78(3)	2.08(3)	2.791(5)	152(3)
N(3)–H(3)···O(3)	0.93	2.37	2.991(5)	124
O(5)–H(3A)···O(4)	0.84	2.00	2.838(5)	171
N(5)–H(5A)···O(5)	0.90(4)	1.98(4)	2.790(5)	150(3)
N(6)–H(6A)···O(6)	0.93(3)	2.13(3)	2.895(6)	139(3)
O(6)–H(6A)···O(5)	0.84	1.88	2.718(5)	172
O(7)–H(7D)···O(2)	0.85	2.13	2.975(5)	171

constants K_b ($3.08 \times 10^2\,M^{-1}$ for **1**, $4.68 \times 10^2\,M^{-1}$ for **2** and $4.56 \times 10^2\,M^{-1}$ for **3**, respectively) were determined by regression analysis using equation (1). These K_b values are comparable due to structural similarity and much smaller than those reported for classical intercalators (EB–DNA, $3.3 \times 10^5\,M^{-1}$ in 50 mM Tris-HCl/1.0 M NaCl buffer, pH 7.5) [39]; the results suggest that the interaction of three complexes with DNA is a weak intercalative mode.

3.2.2. Fluorescence spectroscopic studies. As a means for further clarifying the binding of these complexes, fluorescence spectral measurements were carried out on CT-DNA by varying the concentration of the complexes. The binding of the compounds to CT-DNA is evaluated by the fluorescence emission intensity of EB bound to DNA as a probe. EB shows reduced emission intensity in buffer because of quenching by solvent molecules and a significant enhancement of the intensity when bound to DNA. Binding of the complexes to DNA decreases the emission intensity and the extent of the

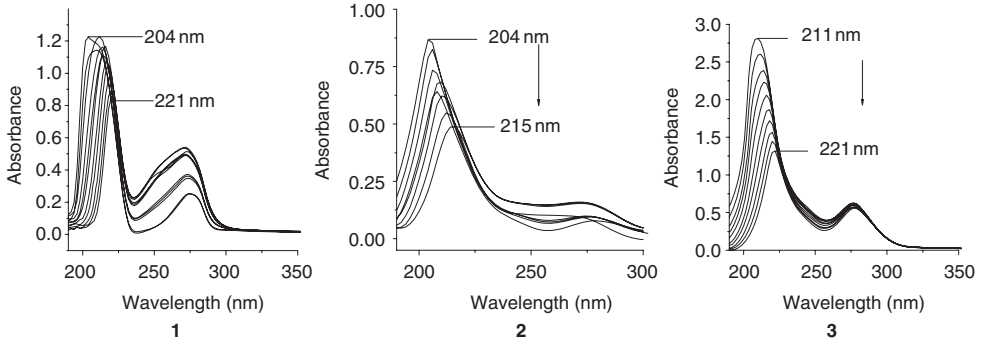

Figure 3. Electronic absorption spectra of the three complexes in the absence (dashed line) and presence (solid line) of increasing amounts of CT-DNA ($0–7.9 \times 10^{-4}$ mol L^{-1}) at room temperature in 5 mM Tris-HCl/NaCl buffer (pH 7.2). The dashed lines indicate the free complexes.

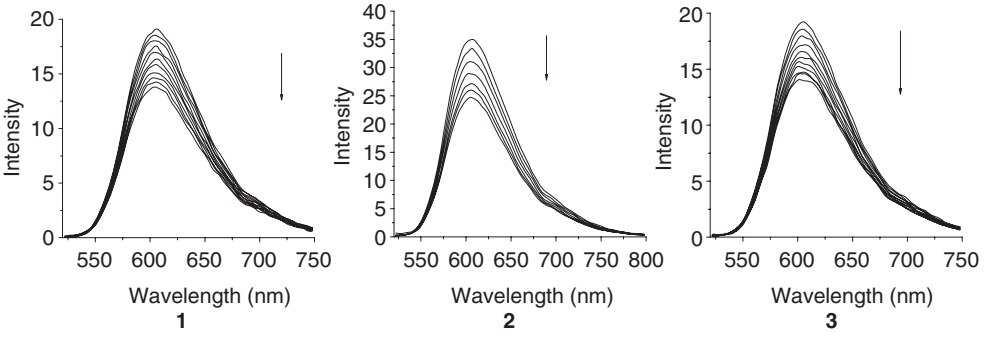

Figure 4. Fluorescence emission spectra for **1**, **2**, and **3** ($\lambda_{ex} = 510$ nm) of the EB–DNA system (2.4×10^{-6} mol L^{-1} EB, 4.0×10^{-3} mol L^{-1} CT-DNA) in the absence and presence of 1.5×10^{-4} mol L^{-1} complex (40 μL per scan) and plot of I_0/I *vs.* [complex], I_0 is the emission intensity of EB–DNA in the absence of complex, I is the emission intensity of EB–DNA in the presence of complexes.

reduction of the emission intensity gives a measure of the DNA binding propensity of the complexes and stacking interaction (intercalation) between the adjacent DNA base pairs [40]. The fluorescence quenching of EB bound to DNA by zinc(II), nickel(II), and cadmium(II) complexes is shown in figure 4; fluorescence intensities at 610 nm (510 nm excitation) were measured at different complex concentrations. The emission intensity showed a reduction upon addition of the complex.

4. Conclusions

New zinc(II), nickel(II), and cadmium(II) complexes with reduced amino-acid Schiff base have been synthesized and structurally characterized. Three complexes are isostructural and all metal centers are O_2N_4 slightly distorted octahedra. The binding with CT-DNA of the three complexes have been studied by UV-Vis spectra and

fluorescence quenching, suggesting that the complexes bind to CT-DNA with intercalative mode. The intrinsic binding constants K_b obtained from spectral titration are much smaller ($3.08 \times 10^2\,M^{-1}$ for **1**, $4.68 \times 10^2\,M^{-1}$ for **2** and $4.56 \times 10^2\,M^{-1}$ for **3**, respectively) than those reported for typical classical intercalators (EB–DNA, $3.3 \times 10^5\,M^{-1}$), indicating that the interaction of these complexes with DNA is a weak intercalative mode.

Supplementary materials

Crystallographic data of the three complexes (excluding structure factors) for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre with the CCDC No. 635060 (**1**), 672076 (**2**), 649468 (**3**). Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44 1223 336 033; Email: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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