Short communication

Synthesis, characterization and DNA binding studies of a zinc complex with 2,6-bis(benzimidazol-2-yl) pyridine

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Abstract

A zinc(II) complex having planar tridentate ligand, bzimpy, where bzimpy is 2,6-bis(benzimidazol-2-yl) pyridine was synthesized and characterized by UV, NMR, infrared spectroscopy and fluorescence spectra. The zinc complex acts as dibasic acids, in which N–H protons on benzimidazole moieties are responsible for a deprotonation site. Both the absorption spectra and reduction potentials are strongly dependent on the solution pH, which leads to the basis of a proton-induced molecular switch. The binding of this complex with calf thymus DNA has been investigated by absorption, luminescence titrations and viscosity measurements. The results suggest that the zinc(II) complex intercalates into DNA base pairs via the ligand bzimpy.

Keywords: 2,6-Bis(benzimidazol-2-yl) pyridine; Zinc complex; DNA binding

Benzimidazole derivatives and their metal complexes have been extensively investigated in recent years [1–3]. Studies on the interaction of transition metal complex with nucleic acid have gained prominence [4,5], because of their relevance in the development of new reagents for biotechnology and medicine. These studies are also important for the probes of nucleic acid structure and in determining the mechanism of metal ion toxicity [6]. There has been substantial interest in understanding the banding properties of metal complexes, particularly polypyridyl complexes of ruthenium, with biomolecules like DNA [7], but coordination compound of zinc was few used in metal-mediated DNA cleavage. Herein, we describe the DNA binding property and nuclease activity of zinc(II) complex of the planar tridentate ligand, 2,6-bis(benzimidazo-2-yl) pyridine(bzimpy). The results should be valuable for understanding the mode of the complex binding to DNA, as well as for the design of new probes of helical conformations and antitumor drugs.

Electronic absorption spectra and fluorescence spectra of the complex were determined at room temperature on Agilent-8453 spectrophotometer and RF-5301P spectrophotometer, respectively. Infrared spectra were obtained with a Nicolet WQF-200 spectrophotometer with KBr discs. Electrochemical experiment was performed on a CHI660A electrochemical system with a saturated calomel electrode (SCE) as reference, a platinum wire as counter electrode and a gasless carbon electrode as working electrode. Viscosity measurements were carried out using an Ubbelohde viscometer maintained at a constant temperature of 25 ± 1 °C in a big water bath. The DNA concentration per nucleotide and polynucleotide was determined by absorption
spectroscopy using the molar absorption coefficient (6600 M$^{-1}$ cm$^{-1}$) at 260 nm and (7400 M$^{-1}$ cm$^{-1}$) at 254 nm, respectively.

The ligand, bzimpy, was synthesized under microwave irradiation [8, 9]. The complex was prepared in high yield from a reaction of Zn(NO$_3$)$_2$·6H$_2$O (0.298 g, 1 mmol) in acetonitrile (10 ml) with bzimpy (0.311 g, 1 mmol) under stirring and refluxing for 1 h at 60 °C. The white precipitate obtained on cooling was filtered, washed with acetone and water, then dried in vacuo over CaCl$_2$. Yield: 0.0468 g (87%). IR (KBr disc): 3426, 3100, 1584 and 1610 cm$^{-1}$. Element analysis: Calc.: Zn, 13.0, C, 42.54, H, 3.17, N, 14.0. Found: Zn, 12.88, C, 42.75, H, 3.37, N, 14.35. That the conductance value is 129.5 Ω$^{-1}$ cm$^{-2}$ mol$^{-1}$ in DMF (N,N-dimethyl-formamide) implies that the complex is of 1:1 electrolyte type [10]. $^{1}$H NMR (400 MHz; solvent (CD$_3$)$_2$SO): δ 14.67 (s, N–H), 8.95 (sh, 2H), 8.77 (1H), 7.72 (3H), 7.57 (5H), 7.33 (4H), 7.12 (sh, 5H), 6.53 (6H). The complex is soluble in DMF and DMSO (dimethyl-sulthoxide), moderately soluble in ethanol and sparingly soluble in acetone and water (see Scheme 1).

$^{1}$H NMR show that the deprotonation of the N–H proton in [Zn(bzimpy)NO$_3$]NO$_3$ induces an upfield shift. In particular, benzimidazol ring proton show a large upfield shift (−0.6 ppm), which indicates that the deprotonation leads to the accumulation of electron density on the carbon-5H and -4H. UV spectra (zinc(II) complex, 20 μM in DMSO-buffer (1:1 v/v)) are very sensitive to solution pH. From pH 2.94 to 9.76, the absorption maximum at 311 nm decreases and appears red shift, then the two split absorption bands collapse to a single absorption band at 321 nm. In addition, a new shoulder appears at 362 nm for the ligand π–π* transitions. From simulation of the titration curve for the plots of absorbance vs. pH, the pK$_a$ values are obtained as pK$_{a1}$ = 3.32 and pK$_{a2}$ = 9.12, because the free bzimpy ligand itself can act as a Lowry–Bronsted acid, which can be titrated, so the zinc complex also acts as dibasic acids, in which N–H protons on benzimidazole moieties are responsible for a deprotonation site [11]. The reduction potentials of the zinc(II) complex in DMSO-buffer (1:1 v/v) show strong pH dependence, too. $E_{pc}$ of ligand reduction is −0.94 V in pH = 2.1, −1.42 V in pH = 7.2, and −1.86 V in pH = 12 (scan rate, 100 mV/s), while $E_{pc}$ of Zn$^{2+}$ → Zn$^0$ at −0.56 V is little shift. These results imply the complex might have some potential applications as a proton-induced molecular switch.

The application of electronic absorption spectroscopy in DNA-binding studies is one of the useful techniques. The absorption spectra of this complex in the absence and presence of DNA (at a constant concentration of the complex) are given in Fig. 1. As the DNA concentration is increased, the π–π* transition bands of this complex at 311, 328 nm exhibit hypochromism of about 60.9%, and bathochromism of about 2 nm, respectively. A similar result was found for the complex of [Co(bzimpy)$_2$]$^{2+}$, which have the same ligand bzimpy [12]. These spectral characteristics may suggest a binding mode involving an intercalative interaction between the benzimidazoyl chromophore and the DNA base pairs. The binding constant was determined using [DNA]/(ε$_A$ – ε$_F$) = [DNA]/(ε$_A$ – ε$_F$) + 1/κ, where ε$_A$ and ε$_F$ correspond to A$_{obsd}$/[Zn], the extinction coefficient for the free zinc complex, and the extinction coefficient of the fully bond zinc complex, respectively. A plot of [DNA]/(ε$_A$ – ε$_F$) vs. [DNA], give κ as the ratio of the slope to the intercept. From the plot of [DNA]/(ε$_A$ – ε$_F$) vs. [DNA], the intrinsic binding constant of the complex with DNA was calculated to be (6.53 ± 0.02) × 10$^5$ M$^{-1}$, which is lower than that reported for classical intercalator, such as ethidium bromide and [Ru(phen)$_2$DPPZ]$^{2+}$ (phen = phenanthroline, DPPZ = dipyridophenazine), whose binding constants have been found to be in the order of 10$^6$–10$^7$ M$^{-1}$ [13].

When the complex [Zn(bzimpy)NO$_3$]$^+$ in DMSO-buffer is excited with 305 nm, there is an emission band at about 404 nm. Fig. 2 shows the result of [Zn(bzimpy)$^+$NO$_3$]$^+$ both in the absence and presence of DNA. The solution of this complex exhibits an obvious enhancement in emission intensity when DNA is added, the intensity being about 2.8 times of that of abserent one when [DNA]/[Zn] = 25. This may be due to that the bound [Zn(bzimpy)NO$_3$]$^+$ cations are protected from the anionic water-bound quencher by the array of negative charges along the DNA phosphate backbone.

![Scheme 1. Left: bzimpy; right: [Zn(bzimpy)NO$_3$]NO$_3$.](image)

![Fig. 1. Absorption spectra of [Zn(bzimpy)NO$_3$]$^+$ [0.2 mM in DMSO-buffer (1:1 v/v) pH = 7.2] in the absence and presence of increasing amounts of CT-DNA ([DNA] = 0–100 μM).](image)
So it could suggest that the complex intercalate into the DNA base pairs, which is consistent with the absorption spectral result. In contrast, the emission intensity of \([\text{Co(bzimpy)}_2]^2+\) decrease at 402 nm, which was considered as through surface binding (groove binding) with DNA [12].

Optical photophysical probes generally provide necessary, but not sufficient, clues to support an intercalative binding model. The viscosity measurement was introduced to furthermore support this interaction between the complex and DNA. A classical intercalation generally results in lengthening of the DNA helix as base pairs are separated to accommodate the binding ligand, then leading to an increase of DNA viscosity. At the other hand, a partial or non-classical intercalation of ligand may bend (or kink) the DNA helix, resulting in a decrease of its effective length and, concomitantly, its viscosity [14]. The effects of the zinc(II) complex on the viscosity of DNA are depicted in Fig. 3. The relative viscosity of DNA increases with increasing in the concentration of the zinc(II) complex, which is similar to that of the classical intercalators (i.e., ethidium bromide, [Ru(phen)_2DPPZ]^2+). In the case of Cobalt(III) complex having the same ligand, the viscosity of DNA was found to be decrease with the concentration of the complex [12]. This is not surprising, since the Zn(II) complex is square planar and planarity of the ligand is retained. So the viscosity results could also show that zinc(II) complex binds with DNA by intercalative mode.

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References