Inorg. Chem. **2009**, *48*, 2711−2713

Mononuclear Cu^{II}−Phenolate Bioinspired Complex is Catalytically Promiscuous: Phosphodiester and Peptide Amide Bond Cleavage

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Received November 18, 2008

In this work, the cleavage activity of the metal complex $[Cu(C_{21}H_{21}N_3O_2)(OH_2)_2]^{2+}$ is demonstrated to occur toward double-stranded DNA, in addition to its previously described amide bond cleavage activity, thus suggesting catalytic promiscuity for this complex.

Hydrolytic degradation of DNA by nuclease enzymes is an important biological reaction, where metal ions play a central role in mediating such cleavage pathways.[1] Nucleic acid cleavage in a nondegradative manner will permit the manipulation of gene expression and the development of gene therapies. Additionally, the biomimetic hydrolysis of nucleic acids is of increasing importance in biotechnology and medicine.[2]

Copper(II) complexes are known to be effective DNA interaction and cleavage agents and hydrolysis catalysts. These complexes are structurally well-defined and thus suitable for mechanistic studies. Recent examples include macrocycles, amino acid derivatives, and complex polysaccharides.[3] Metal coordination complexes have been described as good models of many natural enzymes that mediate this important cleavage reaction. Model complexes designed to perform either of these unique hydrolytic activities have already been described; however, very few examples of catalytic promiscuity in model systems are known to date.[4]

Catalytic promiscuity is the ability of an enzyme single active site to catalyze more than one chemical transformation. Hydrolysis of amide bonds, for example, is performed by aminopeptidase P; however, this enzyme also promotes phosphoester cleavage.[5] Metabolic and signaling biochemical pathways have numerous steps that involve the hydrolytic cleavage of peptide or phosphate ester bonds.[6] The growing interest in multitarget drugs to treat complex diseases and malignancies has motivated a reassessment of the therapeutic value of catalytic promiscuity,[7] since many successful drugs have been showed to be promiscuous.[8]

In this work, we demonstrate the catalytic promiscuity of a mononuclear Cu(II) complex which was initially synthesized as a structural and functional model for galactose oxidase.[9b] The cleavage activity of the metallic complex $[Cu(C_{21}H_{21}N_3O_2)(OH_2)_2]^{2+}$ (complex **1**) is demonstrated to occur toward double-stranded DNA, in addition to its previously described amide bond cleavage activity, with a preference for exposed loops in bovine serum albumin.[9a]

DNA cleavage is dependent on temperature, pH, ionic strength, and complex concentration. The reaction proceeds in an apparent hydrolytic way, and kinetic parameters for DNA degradation were also established. The complex given

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10.1021/ic802208v CCC: $40.75
Published on Web 03/02/2009

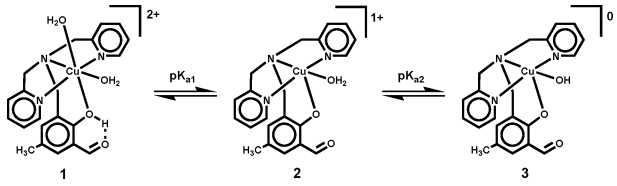

Figure 1. Proposed chemical equilibrium for the three species of complex **1** in solution (pK_{a1} 5.75 and pK_{a2} 9.23).

here is one of the few examples in the literature of a small molecule promoting different chemical reactions, with a half-life only a few orders of magnitude lower than enzyme-catalyzed reactions.

The mononuclear Cu(II) complex (**1**) described in this work (Figure 1) presents two cis-oriented labile sites suitable for coordinating the substrate, and at a pH near 9.0, a hydroxyl group is present, which may activate the substrate by nucleophilic attack. Thus, complex **1** presents the minimal requirements for phosphodiester cleavage.[9a,10]

Initial experiments demonstrated that ionic strength greatly influences the cleavage reaction (Figure S1, Supporting Information). Increasing the concentration of $LiClO_4$ in the reaction medium caused a marked decrease in the fraction of cleaved plasmid, suggesting that opposite charges are involved in the reaction mechanism.[11]

Plasmid DNA (pBSK II) was used to assay the cleavage activity of **1** at different pH's, as previously described.[12] Briefly, in a final volume of 20 μL, the following reagents were present: 30 μM DNA (bp) and different concentrations of **1** (10, 50, 100, 250 μM), in 100 mM Tris-HCl, at pH's of 7.0, 7.5, 8.0, and 9.0. Samples were incubated at 50 °C for 16 h. Agarose gel electrophoresis was used to determine the extension of DNA degradation.[12] Gel images were acquired using a photodocumentation system, and supercoiled DNA (FI) was quantified using LabWorks software (Figure 2).

Complex **1** cleavage activity is highly dependent on the pH, and higher activity was observed at pH 8.0 (Figure 2). The crystal structures of the chloride derivatives of **1** and **2** and their corresponding species in solution at pH 6 and 8 have already been described in detail.[9a] At pH = 8.0, **2** (Figure 1) is the prominent species, in which the diester phosphate of DNA can easily substitute the equatorial Cu^{II}-bound H_2O molecule. Further activation of the substrate through hydrogen bonding with the free available carbonyl group in the ligand and concomitant nucleophilic attack of the adjacent Cu^{II}-bound phenolate oxygen is proposed as being the rate-determining step of DNA hydrolysis. This hypothesis is supported by the X-ray structure of the chloride derivative of **2**, in which the chloride atom is coordinated in the cis position relative to the phenolate (O_{ph}−Cu−Cl ≅ 96°), and by the fact that **2** is the thermodynamically most stable species in solution at the optimum pH of catalysis, which after the ligation of DNA (Michaelis complex) generates an intermediate in which the Cu phenolate is the available nucleophile for intramolecular hydrolysis. A mechanism in which a bound Cu alxoxide is the nucleophile in the transesterification of the model substrate 2,4-dinitrophenyl phosphate has already been reported.[9a,b]

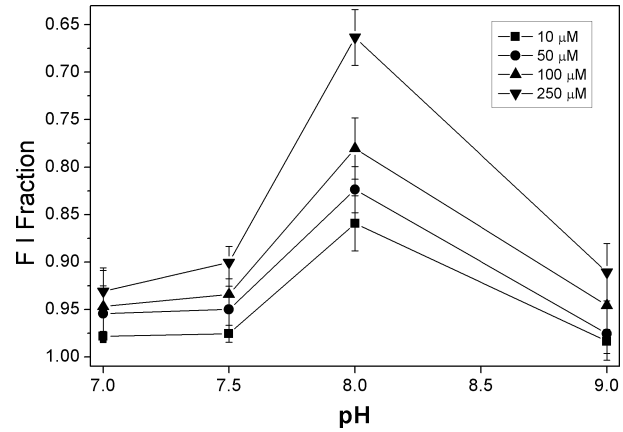

Figure 2. Fraction of supercoiled DNA (FI) as a function of pH. DNA quantification after treatment with complex **1** at 50 °C for 16 h in 100 mM Tris buffer varying pH as indicated.

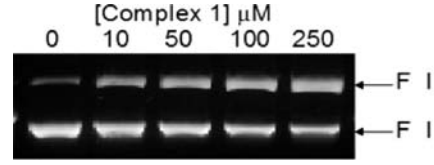

Figure 3. Agarose gel of plasmid DNA cleavage by **1** at different concentrations at optimum pH (100 mM Tris pH 8.0). Final concentrations of **1** are indicated in the figure.

Table 1. Estimated k_{obs} for Plasmid DNA Cleavage by Complex **1**

[complex **1**]	k_{obs} ($\times 10^{-3}$ min^{-1})[a]
1.0 mM	0.84
1.5 mM	1.33
2.5 mM	5.53
3.0 mM	13.0

[a] Pseudo-first-order reactions were used to estimate k_{obs}, on the basis of kinetic plots for plasmid FI fractions at different concentrations of complex **1**.

Our cleavage activity assays at the optimum pH 8.0 clearly indicated that the reaction is also dependent on complex concentration (Figure 3).

Mechanistic and kinetic parameters for the DNA cleavage reaction were also analyzed. Kinetic analysis of DNA cleavage was performed in 100 mM Tris (pH 8) in a range of 1−3 mM complex **1** and 30 μM DNA phosphodiester bonds in a final volume of 500 μL. Samples (15 μL) were collected in duplicate at different time intervals of incubation at 50 °C. The rate enhancement over that of the DNA uncatalyzed reaction was calculated (Table 1). We estimated a rate enhancement of 10^7 and a half-life of 50 min for the reaction with 1.5 mM complex **1**. These results are in agreement with other metal complexes previously described.[2,13]

The involvement of free radicals in DNA cleavage with copper complexes is not unusual, in particular, that of HO•.

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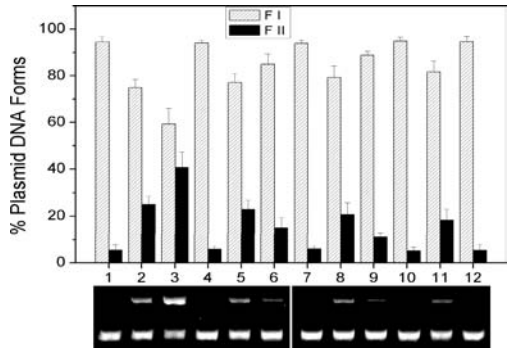

Figure 4. Plasmid DNA cleavage under aerobic (lanes 1−6) and anaerobic (lanes 7−12) conditions, in the absence (1−3 and 7−9) and in the presence (4−6 and 10−12) of DMSO [400 mM]. Lanes 1, 4, 7, and 10: control DNA (pBSK-II 24 μM pb). Lanes 2, 5, 8, and 11: DNA + complex **1** 500 μM. Lanes 3, 6, 9, and 12: DNA + $[Fe(EDTA)]^{2-}$ 25 μM + DTT 2.5 mM. Samples were incubated for 4 h at 50 °C, with 100 mM Tris-HCl, pH 8.0. Above the gel picture, the graph shows DNA quantification (forms I and II) for each sample.

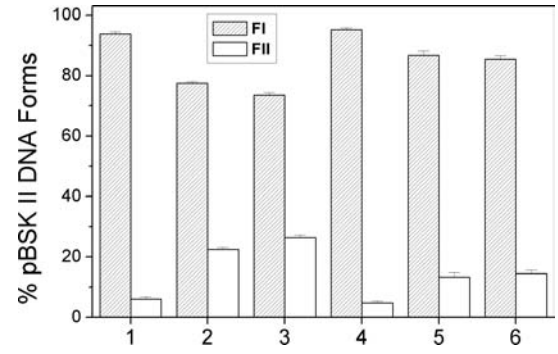

Figure 5. Plasmid DNA cleavage by complex **1** with distamycin. Samples were incubated for 2 h at 50 °C in a 100 mM Tris buffer, pH 8.0, without (lanes 1−3) and in the presence (lanes 4−6) of distamycin. Samples 1 and 4: pBSK II DNA control. Samples 2 and 5: DNA + 500 μM complex **1**. Samples 3 and 6: DNA + 1 mM complex **1**. Histogram shows the relative amounts of the two plasmid DNA forms.

Similarly, O_2 often has a key role in the reaction mechanism of these complexes, participating in redox processes. To understand the cleavage mechanism, the hydroxyl radical scavenger (DMSO) and anaerobic reaction conditions were tested in the cleavage reaction (Figure 4).[13e,14]

Neither DMSO nor an anaerobic atmosphere interferes with the cleavage reaction, thus suggesting a hydrolytic pathway to DNA cleavage promoted by **1**. Further assays carried out with glycerol and DMSO indicated that **1** hydrolytically cleaves peptide bonds, very efficiently (Figure S2, Supporting Information). Despite its catalytic promiscuity, **1** still acts as hydrolytic cleavage agent in both nucleic acids and proteins.

Absorption spectral titration following the changes in absorption of complex **1** was used to verify complex/DNA interaction (Figure S3, Supporting Information).[15] Maximum absorbance for the free complex was observed at 409 nm (complex **1**, [100 μM] 100 mM Tris, pH 8.0). The complex **1** absorbance spectrum was affected by the addition of increasing amounts of CT-DNA, with a decrease in molar absorptivity of **1** (hipochromism), resulting in an estimated intrinsic binding constant (K_b) of $(1.5 \pm 0.3) \times 10^4$ M^{-1}. As the interaction is not very strong and taking into account the previously mentioned negative salt effect, we can suggest that it occurs through electrostatic interactions. This interaction is not specific, as demonstrated in Figure 5, because the use of distamycin, a minor groove DNA binder, causes only a partial inhibition in DNA cleavage, indicating that **1** binds through the DNA major groove as well.[4]

In this work, complex **1** was shown to be able to hydrolyze DNA phosphodiester bonds in a dose-, temperature-, and pH-dependent manners. This complex interacts with DNA mostly by electrostatic interactions and may be considered a random cleavage agent hydrolyzing DNA. Interestingly, complex **1** is active in the hydrolysis of DNA and protein under similar experimental conditions (pH, complex concentration, and temperature), but the activity as a protease or nuclease can be switched by variation in the ionic strength of the medium.[9a] Thus, complex **1** is an interesting model for studying catalytic promiscuity.[16]

Acknowledgment. Financial support was received from CAPES, CNPq, MCT, FINEP, FAPESC. Patricia C. Severino is gratefull to PRODOC/CAPES for postdoctoral grant.

Supporting Information Available: Figures S1−S3, showing the effect of ionic strength, protein cleavage with radical scavengers, and spectral titration with CT-DNA, respectively (PDF). This material is available free off charge via the Internet at http://pubs.acs.org.

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