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Letter

Remarkable cooperative action of two zinc centers in the hydrolysis of plasmid DNA

F. Nihan Aka, Mahinur S. Akkaya[1], Engin U. Akkaya*

Department of Chemistry, Middle East Technical University, TR-06531 Ankara, Turkey

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Abstract

A novel binuclear zinc complex has been synthesized. The complex is highly efficient in the hydrolysis of plasmid DNA at pH 7.5. Furthermore, a comparison to a mononuclear complex reveals a high level of cooperativity between the two metal ion centers. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: DNA hydrolysis; Binuclear metal complexes; Artificial enzymes; Cooperativity

1. Introduction

Design and synthesis of artificial nucleases by employing metal ions in the form of various complexes is a vibrant area of research [1]. DNA/RNA hydrolysis can be artificially accelerated by either redox-active complexes [2–5] or by Lewis acid/metal bound-hydroxide (M^{n+}–OH) providing complexes [6–9]. In fact, in most cases, the overall process of hydrolysis involves contributions from both modes of action. The zinc ion or its complexes cannot participate in a redox process at or near physiological conditions, and yet it is the one metal ion most commonly employed in many hydrolytic metalloenzymes. It is also known that many metalloenzymes are in fact bi- and tri-nuclear [10–14]. The metal ions act in unison and cause rate acceleration as large as 10^{12}-fold [15]. This cooperative action of two or three metal centers has been mimicked in a number of model systems with varying degrees of success [16–21]. In our ongoing studies towards artificial nucleases, [22–24] we now report the synthesis and the hydrolytic activity of the zinc complex of the binucleating ligand **7**.

The exact coordination geometry and the nature of the donor atoms of the ligands may have profound effects on the hydrolytic activity of such metal complexes [25]. The Zn(II) complex of the ligand 1,5,9-triazacyclododecane was shown accelerate the hydrolysis reactions of a number of model substrates [26–31]. The pK_a of the water coordinated to zinc ion in this complex was determined to be 7.30 [32] which makes this complex a powerful hydrolytic agent supplying a metal-bound hydroxide at pH's near or above this pH.

2. Results and discussion

Considering the remarkable hydrolytic activity of the zinc complex of the parent ligand 1,5,9-triazacyclododecane, we targeted the binuclear

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* Corresponding author. Tel.: +90-312-210-5126; fax: +90-312-210-1280.

E-mail address: akkayaeu@metu.edu.tr (E.U. Akkaya).

[1] Co-corresponding author.

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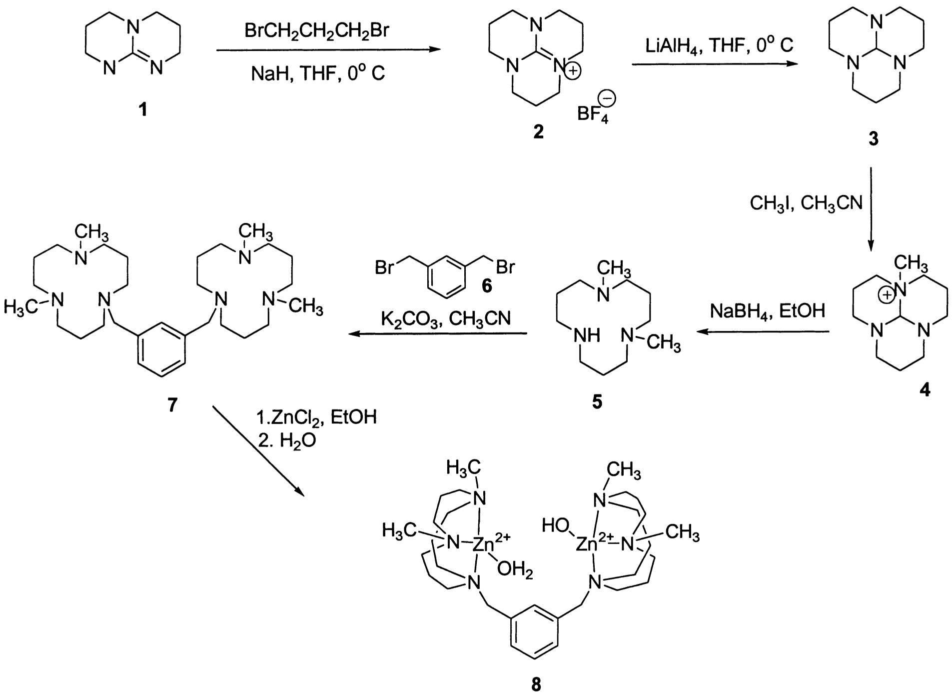

Scheme 1. Synthesis of the binuclear zinc complex **8**.

complex **8**. The methyl groups on two amine nitrogens were placed to minimize multiple alkylations on the azacrown. The synthesis of this *N*,*N*′-dimethylated ligand has been described in the literature [33] but, we have slightly modified the published procedure to synthesize **5** (Scheme 1). The reaction of the macrocyclic triamine with the bis-electrophile 1,3-bis(bromomethyl)benzene was carried out in acetonitrile in the presence of anhydrous K_2CO_3. The zinc complex was obtained by mixing hot EtOH solutions of the ligand and the metal chloride. The complex starts to precipitate[2] on cooling to room temperature. DNA hydrolysis activity was studied as follows. Plasmid DNA (pKSHTat[3], 1 μg/ml) was treated with 5 mM complex in pH 7.5 buffer (0.1 M TRIS). Cooperative activity of the two metal centers was established by comparing the hydrolysis rate of the plasmid in the above reaction to the rate in the presence of 10 mM solution of the zinc complex of the ligand **5**. Aliquots were taken from both reaction mixtures and stored at −70°C. The DNA samples were applied to an agarose gel (0.7%) and the bands were separated electrophoretically. The isolated fragments were stained with Ethidium bromide. The photograph of the gel was scanned and the bands were analyzed digitally. The results show that the mononuclear complex of **5** at 10 mM concentration does not result in any detectable hydrolysis, but very efficient hydrolysis of the plasmid in the presence of 5 mM **8** is apparent. Scanned intensity values of the bands corresponding to the circular DNA were fitted by least squares analysis to a pseudo-first order kinetics to yield a rate constant of $2.3 \times 10^{-5}\,h^{-1}$. This rep-

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[2] Characterization data for 1,3-bis((5,9-dimethyl-1,5,9-triazacyclododecan-1-yl)methyl)benzene-2$ZnCl_2$: ^1H NMR (DMSO-d_6) δ 1.55–1.69 (m, 12H), 2.07–2.29 (m, 16H), 2.33–2.65 (m, 20H), 3.64 (br s, 4H), 7.21 (t 1H) 7.28 (s, 1H), 7.98 (d, 2H). ^{13}C NMR (DMSO-d_6) δ 29.3, 29.7, 39.8, 52.6, 55.5, 64.0, 128.8, 129.1, 139.2 MS (EI) 770 (M^+). Elemental analysis: Found: C, 46.55; H, 7.43; N, 10.81. $C_{30}H_{56}N_6{\cdot}2ZnCl_2$ requires C, 46.59; H, 7.30; N, 10.87.

[3] pKSHTat is Bluescript (KS^+) vector carrying the HIV-Tat gene.

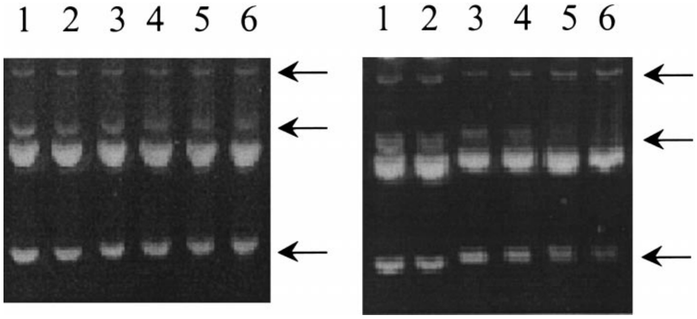

Fig. 1. Agarose gels showing the extent of hydrolysis with the mononuclear zinc complex of the ligand **5** (left) and the complex of **8** (right). The hydrolysis reactions were carried out in pH 7.5 TRIS (0.1 M), at 50°C. Plasmid concatamer in the form of a more intense band is apparent on both gels. Lane 1, plasmid DNA; lane 2, plasmid DNA kept at 50°C for 24 h; lane 3, reaction for 2 h; lane 4, reaction for 4 h; lane 5, reaction for 8 h; lane 6, reaction for 24 h. Bands marked with arrows are; linear, nicked and circular DNA (from top to bottom).

resents about 2.3×10^6-fold rate increase compared to the estimated [34,35] uncatalyzed hydrolysis rate of DNA. Both nicked and linear forms were apparent with the evidence of further degradation of the former as the reaction proceeds (Fig. 1). The pH optimum of 7.5 also suggests a special role for the complex with the protonation state indicated in the structure **8** (aqua-hydroxo complex, i.e. one metal carrying a metal-bound-hydroxide whereas the other having a labile water ligand).

3. Conclusion

Our results demonstrate that when cooperativity between two metal centers is achieved the rate accelerations to be obtained in the hydrolytic reactions will be much greater. This is in accordance with the earlier results obtained in the phosphodiester hydrolysis using Co(III) complexes [25]. Our work in the refinement of multinuclear functional metalloenzyme models is in progress.

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