Dinuclear Zn^{2+} Complexes of Synthetic Heptapeptides as Artificial Nucleases

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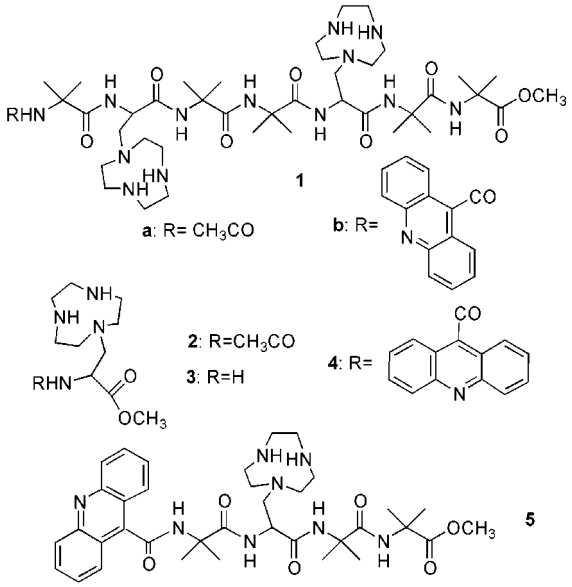
A substantial weight of experimental evidence indicates that many metallohydrolases contain and require a binuclear metal ion site for activity.[1] These include enzymes able to cleave RNA and DNA.[2] A number of research groups have reported suitably designed, simple dinuclear metal complexes quite effective in the cleavage of model substrate esters.[3] In some cases these dinuclear complexes proved effective in the cleavage of RNA[4] and, to a lesser extent, of DNA as well.[5] This latter biopolymer is a quite challenging target because of its very sluggish reactivity under physiological conditions.



We have recently reported[6] that the dinuclear Zn^{2+} complex of heptapeptide **1a** is active in catalyzing the intramolecular transphosphorylation of the RNA model substrate 2-hydroxypropyl-*p*-nitrophenyl phosphate. Analysis of the reaction mechanism indicated cooperativity between the two Zn^{2+} ions and a weak binding of the substrate to the catalyst. We speculated that such a weak binding could be the reason for our inability to evidentiate any significant rate acceleration in the cleavage of the DNA model substrate bis-*p*-nitrophenyl phosphate. However, we argued that polyanionic DNA, a substrate for which the interaction with the metal centers could be emphasized, would be an accessible hydrolytic target. We report here that dinuclear Zn^{2+} complexes of ligands **1** show, in fact, a remarkable activity in the hydrolytic cleavage of plasmid DNA with clear evidence of cooperativity between the two Zn^{2+} centers.

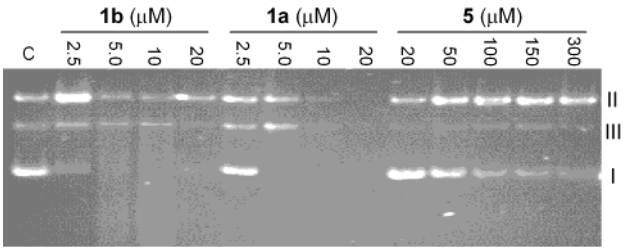


Figure 1. Cleavage of pBR 322 DNA (12 μM bp) in 20 mM HEPES, pH 7.0, 37 °C after 24 h incubation with the indicated concentration of Zn complexes. Lane C is DNA treated in the absence of ligands.

When plasmid DNA pBR322 (12 μM in base pairs) was incubated at 37 °C for 24 h in 20 mM HEPES at pH 7.0 in the presence of **1a**$-2Zn^{2+}$, it was converted from supercoiled (form **I**) to both nicked (form **II**) and linear (form **III**) (Figure 1) to an extent dependent on the concentration of the dinuclear complex. Activity was already observed with less than 2.5 μM **1a**$-2Zn^{2+}$. Introduction at the N-terminus of the heptapeptide of an acridine intercalating moiety[7] in place of the acetyl group (**1b**) did not result in major changes in the reactivity. However, **1b**$-2Zn^{2+}$ appears to be slightly more active than **1a** at very low concentration, while the opposite is true at the highest catalyst concentration explored. The higher activity at low catalyst concentrations is in line with a stronger binding than the one due just to phosphate coordination to the metal centers. This can be accounted for by an additional interaction of the acridine ring system with DNA. On the other hand, the lower activity at higher concentration could be reasonably related to the formation of dimers or larger aggregates of the peptide induced by the presence of the acridine moiety in association with the amphiphilic character of the helix. From Figure 1 it may also be appreciated that the relative amount of linear versus nicked plasmid increases upon increasing complex concentration. This suggest that DNA cleavage does not occur simultaneously on both strands. Hence, the linear form results from the statistical accumulation of hydrolytic events occurring on either strand of the double helix. Mononuclear complexes with ligands **2**−**5** are much less active (Figure 1 and Supporting Information) than the dinuclear ones. Their order of activity, **5**$-Zn^{2+} \approx$ **4**$-Zn^{2+} >$ **3**$-Zn^{2+} \gg$ **2**$-Zn^{2+}$, indicates that the strength of DNA binding is important. In fact, the two ligands incorporating an intercalating moiety are more active than ligand **3** devoid of the acridine group. In this latter the metal ion binding is reinforced by the electrostatic contribution of the free amino group of the amino acid (ammonium at the operational pH). Indeed, the activity of the complex of the acetylated ligand **2** is the lowest one.

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(1) (a) Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435. (b) Sträter, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2024.
(2) Sam, M. D.; Perona, J. J. *Biochemistry* **1999**, *38*, 6576.
(3) Recent reviews: (a) Williams, N. H.; Takasaki, B.; Wall, M.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485. (b) Hegg, E. L.; Burstyn, J. N. *Coord. Chem. Rev.* **1998**, *173*, 133.
(4) (a) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Angew. Chem., Int. Ed.* **1999**, *38*, 3189. (b) Young, M. J.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 10577. (c) Chin, K. O. A.; Morrow, J. R. *Inorg. Chem.* **1994**, *33*, 5036. (d) Liu, S.; Luo, Z.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2678. (e) Irisawa, M.; Takeda, N.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1221. (f) Linkletter, B.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 472. (g) Chapman Jr., W. H.; Breslow, R. *J. Am. Chem. Soc.* **1995**, *117*, 5462.
(5) (a) Ragunathan, K. G.; Schneider, H.-J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1219. (b) Schneider, H.-J.; Hettich, R. *J. Am. Chem. Soc.* **1997**, *119*, 5638.
(6) Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **1999**, *121*, 6948.
(7) Armstrong, R. W.; Kurucsev, T.; Strauss, U. P. *J. Am. Chem. Soc.* **1970**, *92*, 3174.

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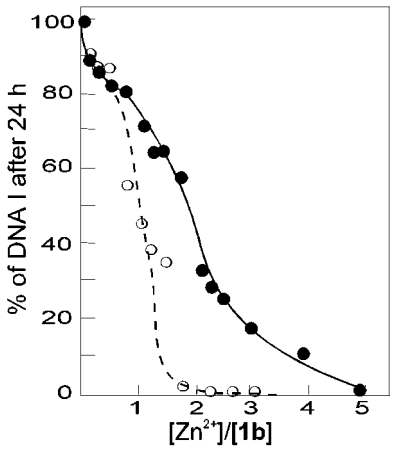

Figure 2. Percentage of supercoiled DNA (9.8 μM bp) after 24 h of incubation at 37 °C in a pH 7.0 solution containing 5.0 μM (○) and 2.5 μM (●) **1b** in the presence of increasing amounts of Zn^{2+}.[8]

A comparison between the activity of **5**−Zn^{2+} and **1b**−2Zn^{2+} provides clear evidence for the fundamental role of the two metal centers in triggering the efficiency of the peptide-based catalysts. While just 3.5 μM **1b**−2Zn^{2+} is required to cleave 50% of supercoiled DNA after 24 h at 37 °C, the concentration of **5**−Zn^{2+} necessary to provide the same effect is 80 μM. However, an even stronger support to a cleavage mechanism requiring both Zn^{2+} centers for enhanced efficiency is provided by the results illustrated in Figure 2. In these experiments pBR322 DNA (9.8 μM bp) was incubated with fixed amounts of **1b** (2.5 and 5.0 μM) in the presence of increasing aliquots of Zn^{2+}. The graph shows the amount of supercoiled (**I**) DNA remaining after 24 h at 37 °C as a function of the [Zn^{2+}]/[**1b**] ratio. The activity of the system does not depend on Zn^{2+} concentration in a simple way. A pseudoplateau is first reached, accounting for the modest activity present when only one of the macrocycles of the heptapeptide is complexed to Zn^{2+}. Then, when both Zn^{2+} binding sites are loaded with the metal ion, the catalyst displays its full activity.

Zn^{2+} appears to be one of the favorite metal centers present in phosphatases and nucleases.[9] Contrary to other metal ions used in many artificial systems (like Cu or Co) it does not exhibit a redox chemistry, and hence, it catalyzes only a hydrolytic cleavage process. Furthermore, the aquo ion is not hydrolytically active at pH values close to neutrality (data not shown), contrary to other high-valence metal ions (like Ce^{4+} and Zr^{4+}).[10] On this basis the activity of the dinuclear systems discussed here has to be related to: (i) a hydrolytic mechanism, (ii) the peculiar coordination of the triazacylononane moieties to Zn^{2+}, and (iii) the relative spatial position of the two ions. We have shown[6] that one water molecule bound to the Zn^{2+}-triazacyclononane complex has a pK_a of ∼7.7−7.9. This appears to be the case with the present system too as shown by the bell-shaped dependence of the DNA cleavage versus pH (Figure 3) which is consistent with a mechanism involving nucleophilic attack of a Zn^{2+}-coordinated OH^- to a facing metal-bound phosphate. At pH ∼7.8 (the maximum of the curve, close to the pK_a of Zn^{2+}-OH_2) half of the H_2O molecules bound to the metal ion are deprotonated and half are not. These may be easily displaced by a DNA phosphate as suggested with other dinuclear hydrolytic systems.[11] Since conformational studies on **1a**[6] (and analogous peptides)[12] have clearly demonstrated that the peptide

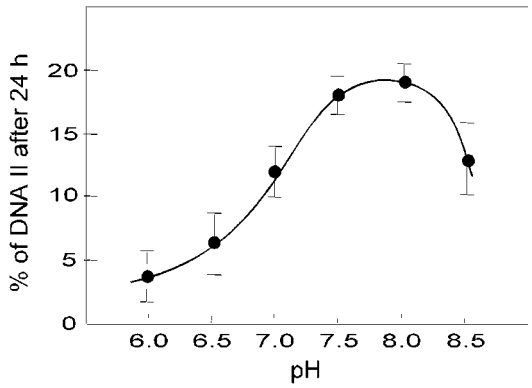

Figure 3. pH-dependence of the extent of nicked (II) DNA obtained by treatment of supercoiled pBR 322 (12 μM bp) with 2.0 μM **1b**−2Zn^{2+} in 20 mM buffer (MES or HEPES according to pH) at 37 °C after 24 h incubation. The figures are corrected for the hydrolytic effect occurring at acidic pH in the absence of metal complex.

is folded to a relevant extent in the 3_{10}-helical conformation under the experimental conditions used here, the two metal centers are facing each other after one complete turn of the ternary helix and are placed ∼6 Å apart (the pitch of the 3_{10}-helix) when the catalyst interacts with the phosphate. Since the distance between two consecutive phosphate groups in a B-DNA strand is ∼7 Å,[13] we may conceive a likely mode of binding of **1**−2Zn^{2+} in which the two macrocycles are inserted within three adjacent phosphate groups.[14] In this disposition the central phosphate may interact with the two metals, taking full advantage of their complementary roles for the hydrolytic cleavage.

The dinuclear complex **1b**−2Zn^{2+} is more than 20 times more active than mononuclear **5**−Zn^{2+} which remains more than 1 order of magnitude if one adjusts the relative rate for the number of metal centers present in the peptide. The rate constant measured for 3.6 μM **1b**−2Zn^{2+} at 37 °C and pH 7.0 with 12 μM (bp) pBR322 DNA is 1×10^{-5} s^{-1}, which is comparable to that recently reported for a binuclear cobalt complex by Schneider and Hettich.[5b] This value amounts to a 10 million-fold rate increase over that for the uncatalyzed DNA hydrolysis. A similar rate acceleration was also observed by Barton and Fitzsimons[15] with a mononuclear Zn^{2+}-binding peptide tethered to a rhodium intercalator.[16]

In conclusion, we have shown how a dinuclear Zn^{2+} complex of a tailored 3_{10}- helical heptapeptide may act as a powerful catalyst for the hydrolytic cleavage of plasmid DNA. The precise distance between the two metal centers, as defined by the pitch of the helix, which matches that between two adjacent DNA phosphate groups, allows one to take advantage of the cooperation between the two Zn^{2+} ions in binding and performing the hydrolytic process.

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Supporting Information Available: Synthesis and DNA cleavage experiments by Zn^{2+} complexes of ligands **2**, **3**, and **4** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The curve at lower peptide concentration indicates that the strength of Zn^{2+} binding is not such to allow the complete formation of the dinuclear complex when the stoichiometric amount of Zn^{2+} is added.

(9) Vallee, B. L.; Auld, D. S. *Acc. Chem. Res.* **1993**, *26*, 543.
(10) Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443.
(11) (a) Molenveld, P.; Kepsabelis, S.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1997**, *119*, 2948. (b) Chapman, W. H., Jr.; Breslow, R. *J. Am. Chem. Soc.* **1995**, *117*, 5462.
(12) Toniolo, C.; Crisma, M.; Formaggio, F.; Kaptein, B.; Broxterman, Q. B.; Rossi, P.; Scrimin, P. *Chem. Eur. J.* **2000**, *6*, 4498.
(13) Dickerson, R. E. *Methods Enzymol.* **1992**, *211*, 67.
(14) Our experiments do not indicate a specific base recognition as recently reported by Kimura: Kimura, E.; Kitamura, H.; Ohtani, K.; Koike, T. *J. Am. Chem. Soc.* **2000**, *122*, 4668.
(15) Fitzsimons, M. P.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 3379.
(16) Very effective DNA cleavage by a Cu^{2+}-aminoglycoside has been recently reported: Sreedhava, A.; Freed, J. D.; Cowan, J. A. *J. Am. Chem. Soc.* **2000**, *122*, 8814.