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DNA Strand Scission by a Cu(I)·Adenylated Polymeric Template: Preliminary Mechanistic and Recycling Studies

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Abstract—Cleavage of a model phosphate ester and supercoiled plasmid DNA by a Cu(I)·adenylated polymer template and preliminary mechanistic investigations are reported. A novel paradigm for the design of recyclable nucleolytic reagents allowing multiple use of this catalyst is also demonstrated

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The functional mimicry of biocatalytic pathways using artificial chemical systems has aided greatly our understanding of enzyme reaction mechanisms and assisted in the development of selective chemical catalysts. As an important theme in these endeavors, natural and synthetic transition metal complexes that emulate nuclease activity have been reported.[1] Some such systems generate reactive species resulting in oxidative nucleic acid strand scission, while others lead to hydrolytic cleavage of the phosphodiester backbone. Consequently, many complexes of transition metal ions and lanthanides have been investigated for the catalysis of phosphate ester cleavage and nucleic acid strand scission.[2–4] Of these, copper-based artificial nucleases have received considerable attention owing to their dual mode of action: they can induce oxidative strand scission[3] and also cleave DNA via hydrolytic pathways.[4]

We have reported previously the catalytic cleavage of model phosphate ester substrates using novel, copper-containing adenylated polymeric templates (Fig. 1).[5] Favorable kinetic profiles and recycling properties thus have confirmed the potential of our Cu·adenylated system in bioorganic investigations involving phosphate ester modification. Presently, given their documented activity, we have further evaluated one such polymeric matrix for its ability to effect supercoiled plasmid DNA cleavage.

Herein, we present the first evidence of a highly stable monovalent copper-impregnated adenylated polymeric template that promotes facile cleavage of supercoiled plasmid DNA in the absence of an exogenously added activating agent. Further, mechanistic investigations have revealed the apparent activation of molecular oxygen by these copper-metalated templates resulting in the formation of copper-oxo reactive species culminating in DNA strand scission. As an interesting addition, we have also exploited the heterogeneous nature of our polymeric catalyst in facile recycling for multiple cleavage cycles.

Synthesis of the Cu(I)·adenylated polymer template used in this study involved AIBN-initiated co-polymerization of 9-allyladenine with a 1,4-divinylbenzene (DVB) cross-linker in the presence of copper chloride dihydrate.[6] This in situ polymerization-metalation protocol is in contrast to other metal-containing adenylated constructs where polymer synthesis is followed by metalation by copper or uranyl salts in a separate reaction step.[7] Both of these methods yield satisfactory incorporation of metal ions, which have been characterized by atomic absorption spectroscopy or inductively coupled plasma analysis. Interestingly, metal loading was found to be higher for the in situ protocol compared to the post-synthetic metalation.

The cross-linked nature of the polymer prevents molecular weight estimation. However, the amount of copper incorporated in the polymeric matrix was determined by atomic absorption spectroscopy (63.6

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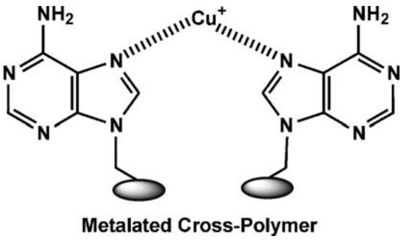

Figure 1. Representation of the copper(I)·adenylated polymeric matrix.

mg/g of polymer) and, interestingly, the metalated polymer was found to be EPR silent at both room and liquid nitrogen temperatures. This observation prompted us to further analyze the oxidation-state of coordinated copper and subsequent magnetic moment measurements revealed a negative gram susceptibility. Collectively, these observations indicate the presence of a diamagnetic Cu(I) species within the adenylated polymeric matrix. The metalated template was found to be insoluble in all solvents and displayed high stability in the aqueous buffers employed for nucleic acid and model substrate modification reactions. Moreover, there was no observable change in the copper content or EPR profile of the metalated template before and after cleavage reactions (supporting information) indicating that copper is not leached into the buffer during a reaction cycle. Although free Cu(I) species are usually unstable in water, a few coordinated systems display enhanced aqueous stability;[8] the compatibility and stability of monovalent copper in our adenylated polymer matrix is most likely due to its cross-linked and highly hydrophobic character.

The interaction of supercoiled plasmid DNA (pBR322) and the Cu(I)·adenylated template was studied in sodium cacodylate buffer (10 mM, pH 7.5, 25 °C).[9] A time-dependent cleavage of the DNA substrate was evident and complete conversion of supercoiled DNA (Form **I**) to nicked DNA (Form **II**) was observed in 4.5 h (Fig. 2, Lanes 4–8) without added activating reagent. Interestingly, copper salt and unmetalated polymer alone lacked any effect on plasmid modification (Fig. 2, Lanes 2–3).

In addition to the above, the time-course gel indicates the formation of nicked DNA that occurs in a fashion that is highly dependent on the concentration (w/v) of the polymeric catalyst used and the incubation period. Longer reaction times (> 60 min), using 30 μg of the catalyst, produced a continuous smear on agarose gels rather than the appearance of discrete bands (data not shown). This clearly indicates that these catalysts have the potential to induce double strand DNA cleavage through an accumulation of single-strand cleavage acts, leading to an excessive degradation of DNA substrate.

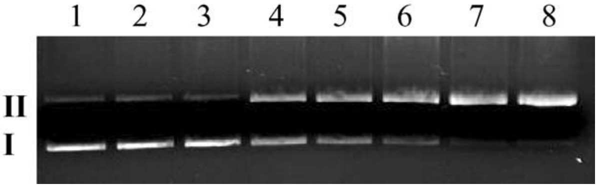

Figure 2. Cleavage of supercoiled plasmid DNA pBR322 by the Cu(I)·adenylated template. Each reaction contained 2 μg of polymer. Reaction conditions (ref 9). Lanes 1–3: pBR322; pBR322 with 100 μM Cu^{2+}; pBR322 with unmetalated polymer after 6 h incubation, respectively. Lanes 4–8: pBR322 with Cu(I)·adenylated polymer at 30, 90, 180, 270, 360 min, respectively.

It is important to note that the Cu(I)-metalated nucleobase polymer did ***not*** require an added co-oxidant and plasmid modification was catalyzed apparently through a non-diffusible free radical intermediate, as probed using a variety of radical scavengers.[10] As shown in Figure 3, DNA cleavage occurred unimpeded in the presence of radical quenchers such as ethanol, DMSO and D-mannitol (Lanes 6–8), which argues for the lack of involvement of a diffusible intermediate radical species. A singlet-oxygen scavenger (sodium azide)[11] and a hydrogen peroxide scavenger (catalase) partially inhibited the cleavage reaction, while a superoxide radical anion scavenger (superoxide dismutase, SOD) did not show any detectable inhibitory effect (Fig. 4). These observations indicated the likely activation of molecular oxygen by the Cu(I)·adenylated polymer, leading to in situ generation of hydrogen peroxide, subsequently resulting in the generation of reactive copper-oxo species leading to DNA strand scission.[12–14] It is pertinent to mention that activation of molecular oxygen by this metalated polymeric species was also implicated for the oxidation of various phenols to corresponding *o*-diquinone products.[6] Further, a completely uninhibited cleavage reaction in the presence of SOD rules out any role for superoxide radical; a similar cleavage mechanism has been invoked for DNA modification mediated by

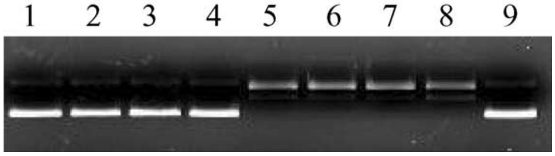

Figure 3. The effect of radical scavengers on the cleavage of pBR322 by the Cu(I)·adenylated template. Each reaction contained 2 μg of polymer. Reaction conditions (ref 9). Lanes 1–4: pBR322; pBR322 and 100 μM Cu^{2+}; pBR322 in presence of 100 μM Cu^{2+} and 1 mM EDTA; pBR322 and unmetalated polymer after 4 h incubation, respectively. Lane 5: pBR322 with metalated polymer after 4 h; Lanes 6–8: pBR322 in presence of 100 mM ethanol, dimethyl sulfoxide and D-mannitol after 4 h incubation, respectively. Lane 9: pBR322 in presence of metalated polymer and 1.0 mM EDTA after 4 h of incubation.

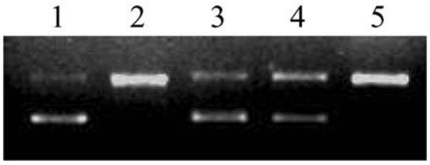

Figure 4. The effect of enzymatic oxygen scavengers on the cleavage of pBR322 by the Cu(I)·adenylated template. Cleavage reactions were performed in 20 μL of cacodylate buffer (10 mM, pH 7.5, 30 °C) containing pBR322 supercoiled plasmid DNA (9 ng/μL) and polymer weight was 30 μg. Lanes 1: pBR322 after 5 min; Lane 2: pBR322 with metalated polymer after 5 min; Lanes 3–5: pBR322 in presence of catalase (25 U/20 μL), sodium azide (100 mM) and SOD (25 U/20 μL) after 5 min incubation time, respectively.

copper(I)-phenanthroline,[12] copper-tambjamine[13] and copper-prodigiosin.[14]

The functional role of copper in the adenylated template was also investigated. It was found that plasmid cleavage was completely inhibited in the presence of EDTA (Fig. 3, Lane 9), thereby indicating a crucial role of coordinated copper ions. This observation was further tested through a reaction arrest experiment. In this assay, the metalated template was incubated with bNPP [bis(p-nitrophenyl phosphate)] and its cleavage was monitored as a function of release of the *p*-nitrophenolate anion for 10 h at 400 nm. The insoluble template was filtered after this time and the reaction was monitored for further 75 h (Fig. 5). A complete arrest of the reaction, as observed by the lack of change in the absorbance, reconfirmed the intricate association of cleavage activity with the metalated template and not by leached out metal ions.

The rarity of heterogeneous nucleolytic reagents and their possible application as recyclable systems prompted us to explore our Cu(I)·adenylated polymer in multiple cleavage reactions. Typically, reaction mixtures were centrifuged after completion of cleavage to leave catalysts in the form of a pellet. After the removal of supernatant, the polymer pellets were washed repeatedly with cacodylate buffer (4×100 μL) and then reused for subsequent plasmid modification, under similar conditions. To confirm the robust nature of our system, the Cu(I)·adenylated polymer was recycled for two consecutive reactions and each time a complete conversion of supercoiled form **I** to nicked form **II** was observed (Fig. 6). Though some phosphate ester hydrolyzing enzymes have been immobilized onto solid supports in order to achieve higher thermal stability,[15] our polymeric system presents a flexible paradigm towards reusable chemical nucleases.

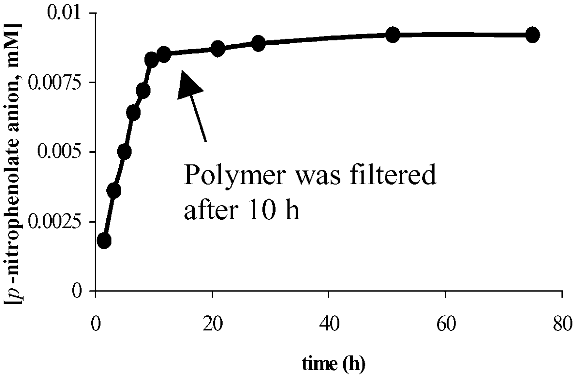

Figure 5. Reaction arrest experiment for bNPP cleavage.

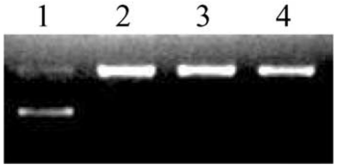

Figure 6. Recycle experiment for the cleavage of plasmid DNA by Cu(I)·adenylated template. All cleavage reactions were performed in 20 μL cacodylate buffer (10 mM, pH 7.5, 30 °C) for 5 min and polymer weight was 30 μg. Lane 1: pBR322; Lane 2: pBR322 with metalated polymer (fresh); Lane 3 and 4: recycle 1 and recycle 2, respectively.

The results presented above indicate that our water-stable Cu(I)-metalated adenylated template oxidatively cleaves supercoiled plasmid in a time-dependent fashion. Preliminary mechanistic investigations using radical scavengers support the involvement of non-diffusible radicals in plasmid modification. Their prolonged and sustained reactivity, as demonstrated by the recycle experiments and reaction arrest assay, indicate that they can retain metal ion-dependent catalytic properties for a long duration, without altered reaction rates. Further studies, which will employ these reagents for DNA and RNA structural probing, are being contemplated in order to broaden the scope of such insoluble templates for nucleic acid manipulation.

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References and Notes

1. Reviews: (a) Sreedhara, A.; Cowan, J. A. *J. Biol. Inorg. Chem.* **2001**, *6*, 337. (b) Chen, C. H. B.; Milne, L.; Landgraf, R.; Perrin, D. M.; Sigman, D. S. *Chembiochem* **2001**, *2*, 735. (c) Kimura, E. *Curr. Opin. Chem. Biol.* **2000**, *4*, 207. (d) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev.* **2000**, *29*, 75. (e) Komiyama, M.; Takeda, N.; Shigekawa, H. *Chem. Commun.* **1999**, 1443. (f) Krämer, R. *Coord. Chem. Rev.* **1999**, *182*, 243. (g) Long, E. C. *Acc. Chem. Res.* **1999**, *32*, 827. (h) Trawick, B. N.; Daniher, A. T.; Bashkin, J. K. *Chem. Rev.* **1998**, *98*, 939. (i) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089. (j) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109. (k) Hegg, E. L.; Burstyn, J. N. *Coord. Chem. Rev.* **1998**, *173*, 133. (l) Chin, J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 514.
2. (a) Branum, M. E.; Tipton, A. K.; Zhu, S.; Que, L., Jr. *J. Am. Chem. Soc.* **2001**, *123*, 1898. (b) Sissi, C.; Rossi, P.; Felluga, F.; Formaggio, F.; Palumbo, M.; Tecilla, P.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **2001**, *123*, 3169. (c) Chand, D. K.; Bharadwaj, P. K.; Schneider, H.-J. *Tetrahedron* **2001**, *57*, 6727. (d) Bodsgard, B. R.; Burstyn, J. N. *Chem. Commun.* **2001**, 647. (e) Abraham, A. T.; Zhou, X.; Hecht, S. M. *J. Am. Chem. Soc.* **1999**, *121*, 1982. (f) Wall, M.; Linkletter, B.; Williams, D.; Lebuis, A.-M.; Hynes, R. C.; Chin, J. *J. Am. Chem. Soc.* **1999**, *121*, 4710. (g) Liu, S.; Luo, Z.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2678.
3. (a) Patwardhan, A.; Cowan, J. A. *Chem. Commun.* **2001**, 1490. (b) Gilbert, B. C.; Silvester, S.; Walton, P. H.; Whitwood, A. C. *J. Chem. Soc., Perkin Trans. 2* **1999**, 1891. (c) Perrin, D. M.; Mazumder, A.; Sigman, D. S. *Prog. Nucl. Acid Res. Mol. Bio.* **1996**, *52*, 123.
4. (a) Ren, R.; Yang, P.; Zheng, W.; Hua, Z. *Inorg. Chem.* **2000**, *39*, 5454. (b) Rammo, J.; Hettich, R.; Roigk, A.; Schneider, H.-J. *Chem. Commun.* **1996**, 105.
5. (a) Srivatsan, S. G.; Verma, S. *Chem. Eur. J.* **2001**, *7*, 828. (b) Madhavaiah, C.; Srivatsan, S. G.; Verma, S. *Catal. Commun.* **2001**, *2*, 95. (c) Srivatsan, S. G.; Verma, S. *Chem. Commun.* **2000**, 515.

6. Srivatsan, S. G.; Nigam, P.; Rao, M. S.; Verma, S. *Appl. Catal. A: General* **2001**, *209*, 327.

7. (a) Madhavaiah, C.; Verma, S. *Chem. Commun.* **2003**, 800. (b) Srivatsan, S. G.; Parvez, M.; Verma, S. *Chem. Eur. J.* **2002**, *8*, 5184.

8. (a) Zhang, J.; Xiong, R.-G.; Zuo, J.-L.; Che, C.-M.; You, X.-Z. *J. Chem. Soc., Dalton Trans.* **2000**, 2898. (b) Doyle, G.; Eriksen, K. A.; Van Engen, D. *Organometallics* **1985**, *4*, 830.

9. *Kinetics of DNA cleavage*: DNA cleavage was performed by suspending 2 μg of adenylated polymer in 20 μL of 10 mM cacodylate buffer (pH 7.5, 25 °C), containing 11 μM of pBR322 supercoiled plasmid DNA. The copper concentration impregnated in the polymer amounts to 100 μM, if the polymer (2 μg) were to be completely soluble in the buffer. Individual reactions were quenched by adding 6 μL of EDTA (120 mM) containing loading buffer at regular time intervals. Control reactions consisted of DNA alone, DNA with an equivalent concentration of Cu^{2+} and DNA with an equivalent mass of non-metalated polymer, all in cacodylate buffer.

10. (a) Ananias, D. C.; Long, E. C. *J. Am. Chem. Soc.* **2000**, *122*, 10460. (b) Liang, Q.; Ananias, D. C.; Long, E. C. *J. Am. Chem. Soc.* **1998**, *120*, 248. (c) Hettich, R.; Schneider, H.-J. *J. Am. Chem. Soc.* **1997**, *119*, 5638. (d) Hegg, E. L.; Burstyn, J. N. *Inorg. Chem.* **1996**, *35*, 7474. (e) Mack, D. P.; Dervan, P. B. *Biochemistry* **1992**, *31*, 9399.

11. (a) Li, Y.; Trush, M. A. *Carcinogenesis* **1993**, *14*, 1303. (b) Yamamoto, K.; Kawanishi, S. *J. Biol. Chem.* **1989**, *264*, 15435.

12. Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295.

13. Borah, S.; Melvin, M. S.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **1998**, *120*, 4557.

14. Melvin, M. S.; Tomlinson, J. T.; Saluta, G. R.; Kucera, G. L.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 6333.

15. (a) Gast, F. U.; Franke, I.; Meiss, G.; Pingoud, A. *J. Biotech.* **2001**, *87*, 131. (b) Sweeney, R. Y.; Kelemen, B. R.; Woycechowsky, K. J.; Raines, R. T. *Anal. Biochem.* **2000**, *286*, 312. (c) Pant, H. K.; Warman, P. R. *Biol. Fert. Soils* **2000**, *30*, 306. (d) Varshney, H.; Iqbal, J.; Saleemuddin, M. *Enzymes Microbial Technol* **1999**, *25*, 172. (e) Reddy, L. G.; Shankar, V. *Crit. Rev. Biotechnol.* **1993**, *13*, 255.