COMMUNICATION

ChemComm
www.rsc.org/chemcomm

Reusable photonucleases: plasmid scission by a uranyl ion impregnated adenine homopolymer in the presence of visible light and sunlight

C. Madhavaiah and Sandeep Verma*

Department of Chemistry, Indian Institute of Technology-Kanpur, Kanpur 208016 (UP), India.
E-mail: sverma@iitk.ac.in; Fax: +91-512-259-7436

Received (in Cambridge, UK) 7th January 2003, Accepted 11th February 2003
First published as an Advance Article on the web 25th February 2003

Relaxation of supercoiled plasmid by uranyl ions impregnated on a adenylated polymeric support has been observed in the presence of visible light and sunlight. This insoluble polymer support permits facile reusability of the catalytic system and it failed to cleave lysozyme under the conditions employed for plasmid modification.

Photoinduced nucleic acid cleavage could be viewed as a powerful complementary approach to the more widely used hydrolytic or oxidative cleavage pathways.[1] The latter methodologies usually require involvement of a metal ion for nucleophile (water) activation or for the generation of reactive species, leading to nucleic acid modification.[2] Similarly, photochemical cleavage relies on the photophysical characteristics of ligands and their metal complexes and several examples of photonucleases are described in the literature.[3] Of these, uranyl-ion based photocleavage reagents have received considerable attention for biochemical applications,[4] due to the favorable photophysical properties of the uranyl cation.[5] We have been involved in developing novel catalytic reagents based on a nucleobase polymeric framework. Recently, we have reported copper, uranium and ruthenium containing adenylated polymers as synthetic dephosphorylation reagents using model phosphate ester substrates.[6] Our preliminary investigations with a uranylated polymer prompted us to evaluate the ability of uranyl ions contained within an adenylated homopolymer for light-induced plasmid modification.

AIBN initiated polymerization of 9-(4-vinylbenzyl)adenine to afford the homopolymer and subsequent metalation with uranyl acetate monohydrate has been previously reported (Fig. 1).[6b] The amount of impregnated uranium was determined to be 172.5 mg uranium (g polymer)$^{-1}$ by atomic absorption spectroscopy. Photoinduced supercoiled plasmid cleavage experiments were performed either under tungsten lamp illumination or under sunlight, in the presence or absence of the uranylated homopolymer.[7] A time-course investigation of plasmid DNA relaxation induced by tungsten lamp irradiation revealed the conversion of the supercoiled form **I** to nicked form **II** in 7 h (lane 6, Fig. 2). However, plasmid modification did not occur in the absence of uranylated polymer under identical conditions (lane 2, Fig. 2). In contrast, exposure of the plasmid to sunlight afforded conversion of form **I** to form **II** in 45 min in the presence of the uranylated polymer (lane 2, see Fig. 4A below).

Next, the cleavage reactions were performed in the presence of radical scavengers such as *tert*-butanol, D-mannitol and

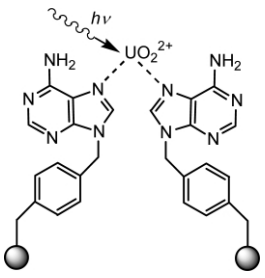

Fig. 1 Uranylated homopolymer for photoinduced DNA scission.

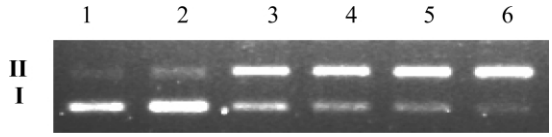

Fig. 2 Uranylated homopolymer mediated DNA cleavage experiment at different time intervals with tungsten lamp irradiation. Lane 1: DNA alone; lane 2: DNA under tungsten lamp (7 h); lanes 3–6: DNA + polymer (1 h, 3 h, 5 h, and 7 h respectively).

singlet oxygen quencher NaN_3, to ascertain the probable mechanism of DNA modification. The use of scavenger gel assay using *tert*-butanol and D-mannitol afforded similar results for both tungsten lamp and sunlight-induced plasmid cleavage (lanes 3, 4, Fig. 3; lanes 3–5, Fig. 4A). These results demonstrate that *diffusible* free radicals are probably not involved in the cleavage reaction. But the cleavage reaction was appreciably inhibited in the presence of singlet oxygen quencher NaN_3 (lane 5, Fig. 3; lane 6, Fig. 4A). This indicates a significant role of singlet oxygen species in plasmid relaxation for both illumination conditions, which was further confirmed by cleavage under anaerobic conditions.[8] Partial plasmid modification was observed under anaerobic conditions employing a tungsten lamp (lane 3; Fig. 4B). Taking these results together, it can be inferred that DNA cleavage occurred even in the absence of oxygen, which is in accordance with the observed effects of uranyl ions on DNA.

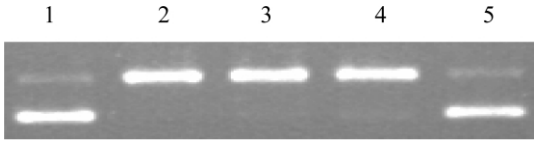

Fig. 3 pBR322 cleavage in the presence of free radical scavengers and singlet oxygen quencher mediated by uranylated homopolymer after irradiation for 7 h with a tungsten lamp. Lane 1: DNA alone; lane 2: DNA + polymer; lane 3: DNA + polymer + *tert*-butanol (100 mM); lane 4: DNA + polymer + D-mannitol (100 mM); lane 5: DNA + polymer + sodium azide (100 mM).

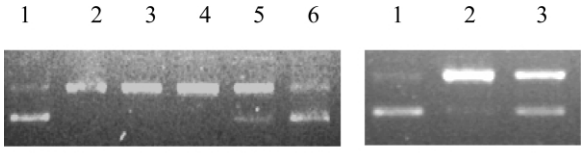

Fig. 4 pBR322 cleavage in the presence of free radical scavengers and singlet oxygen quencher, mediated by uranylated homopolymer in the presence of sunlight for 45 min (A) and cleavage in anaerobic conditions (B) with tungsten lamp irradiation for 7 h. (A): Lane 1: DNA alone; lane 2: DNA + polymer; lane 3: DNA + polymer + *tert*-butanol (100 mM); lane 4: DNA + polymer + D-mannitol (100 mM); lane 5: DNA + polymer + DMSO (100 mM); lane 6: DNA + polymer + sodium azide (100 mM). (B): Lane 1: DNA alone; lane 2: DNA + polymer; lane 3: DNA + polymer (anaerobic conditions).

DOI: 10.1039/b300134b

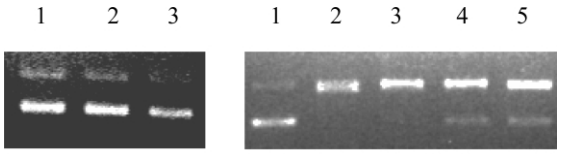

Fig. 5 pBR322 cleavage with unmetalated polymer (A) and reusability of metalated polymer with tungsten lamp irradiation for 7 h (B). (A): Lane 1: DNA alone; lanes 2, 3: DNA + unmetalated polymer with tungsten lamp and sunlight irradiation for 7 h and 45 min respectively. (B): Lane 1: DNA alone; lane 2: DNA + fresh polymer; lanes 3–5: first, second, third recycle experiments, respectively.

Unmetalated polymer failed to cleave DNA under both illumination conditions confirming the crucial role of uranyl ions for the photonucleolytic activity (lanes 2, 3, Fig. 5A).

A novel feature of our system is its facile reusability. Typically, the irradiated reaction mixture was centrifuged to leave uranylated polymer in the pellet form. After removal of the supernatant, the polymer was washed with buffer and reused for subsequent reactions. Gel electrophoresis tracking of wash fractions ensured complete removal of residual modified DNA. The uranylated homopolymer was then successfully reused for three consecutive cleavage reactions and each time satisfactory conversion of form **I** to form **II** was observed (Fig. 5B). These results are expected to provide a new paradigm for heterogeneously active, reusable photonucleases.

Curiously, this system proved to be ineffective towards protein modification, as probed by using lysozyme under tungsten lamp irradiation (Fig. 6).[9] No detecable cleavage of the protein was observed thus suggesting a greater degree of selectivity towards nucleic acid cleavage and even higher molecular weight cross-linked products were also not apparent from the gel analysis. Although the precise reasons are not clear at the present time, such discrimination should allow for specific degradation of nucleic acid contaminants.

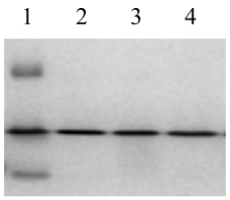

Fig. 6 Lysozyme cleavage experiment by using uranylated homopolymer under tungsten lamp (37 °C) and sunlight irradiation (30 °C). Lane 1: molecular weight markers (Da) (17000; 14200; 6500 from top to bottom); lane 2: lysozyme alone; lanes 3, 4: lysozyme + polymer under tungsten lamp (24 h) and under sunlight (8 h), respectively.

Though many heterogeneously active artificial nucleases and synthetic immobilized systems have been developed, directed attempts have not been made to study their reusability for DNA cleavage.[10] We have introduced a novel uranylated homopolymer as an artificial photonuclease for supercoiled plasmid DNA modification. This report details a singular example of a reusable photonuclease having the potential to be further developed for suitable investigations in chemical biology.

CM acknowledges the Council for Scientific and Industrial Research (India) for a Senior Research Fellowship. A grant from the Department of Science and Technology (India) to SV is acknowledged for the partial support of this work.

Notes and references

1 (*a*) T. Da Ros, G. Spalluto, A. S. Boutorine, R. V. Bensasson and M. Prato, *Curr. Pharm. Design.*, 2001, **7**, 1781; (*b*) P. M. Bradley, P. K. L. Fu and C. Turro, *Comments Inorg. Chem.*, 2001, **22**, 393; (*c*) M. P. Teulade-Fichou, D. Perrin, A. Boutorine, D. Polverari, J. P. Vigneron, J. M. Lehn, J. S. Sun, T. Garestier and C. Helene, *J. Am. Chem. Soc.*, 2001, **123**, 9283; (*d*))B. Armitage, *Chem. Rev.*, 1998, **98**, 1171.

2 (*a*) S. R. Korupoju, M. Nagarathinam, P. S. Zacharias, J. Mizuthani and H. Nishihara, *Inorg. Chem.*, 2002, **41**, 4099; (*b*) S. Franklin, *Curr. Opin. Chem. Biol.*, 2001, **5**, 201; (*c*) A. Patwardhan and J. A. Cowan, *Chem. Commn.*, 2001, 1490; (*d*) B. Chen, L. Milne, R. Landgraf, D. M. Perrin and D. S. Sigman, *ChemBioChem.*, 2001, **2**, 735; (*e*) R. Ren, P. Yang, W. Zheng and Z. Hua, *Inorg. Chem.*, 2000, **39**, 5454; (*f*) A. Sreedhara, J. D. Freed and J. A. Cowan, *J. Am. Chem. Soc.*, 2000, **122**, 8814; (*g*) B. C. Gilbert, S. Silvester, P. H. Walton and A. C. Whitwood, *J. Chem. Soc., Perkin Trans. 2*, 1999, 1891; (*h*) A. Roigk, R. Hettich and H.-J. Schneider, *Inorg. Chem.*, 1998, **37**, 751; (*i*) R. J. Hettich, A. Roigk and H.-J. Schneider, *Chem. Commn.*, 1996, 105; (*j*) M. Komiyama, *J. Biochem.*, 1995, **118**, 665.

3 (*a*) D. L. Mohler, E. K. Barnhardt and A. L. Hurley, *J. Org. Chem.*, 2002, **67**, 4982; (*b*) P. K. L. Fu, P. M. Bradley, D. Van Loyen and H. Durr, *Inorg. Chem.*, 2002, **41**, 3808; (*c*) S. Dong, P. P. Fu, R. N. Shirsat, H. M. Hwang, J. Leszczynski and H. Yu, *Chem. Res. Toxicol.*, 2002, **15**, 400; (*d*) L. Gude, M. J. Fernandez, K. B. Grant and A. Lorente, *Tet. Lett.*, 2002, **43**, 4723; (*e*) A. L. Hurley, M. P. Maddox, T. L. Scott, M. R. Flood and D. L. Mohler, *Org. Lett.*, 2001, **3**, 2761; (*f*) N. Yamakawa, Y. Ishikawa and T. Uno, *Chem. Pharm. Bull.*, 2001, **49**, 1531; (*g*) P. K. L. Fu, P. M. Bradley and C. Turro, *Inorg. Chem.*, 2001, **40**, 2476; (*h*) T. D. Maurer, B. J. Kraft, S. M. Lato, A. D. Ellington and J. M. Zaleski, *Chem. Commun.*, 2000, 69; (*i*) M. Fernandez-Saiz, P. T. Henderson, W. D. Wilson and G. B. Schuster, *Photochem. Photobiol.*, 1999, **70**, 847; (*j*) G. Mehta, S. Muthusamy, B. G. Maiya and S. J. Arounaguiri, *J. Chem. Soc. Perkin Trans. 1*, 1999, 2177.

4 (*a*) N. E. Mollegaard and P. E. Nielsen, *Methods Enzymol.*, 2000, **318**, 43; (*b*) D. Wittberger, C. Berens, C. Hammann, E. Westhof and R. Schröder, *J. Mol. Biol.*, 2000, **300**, 339; (*c*) N. E. Mollegaard, A. I. Murchie, D. M. Lilley and P. E. Nielsen, *EMBO J.*, 1994, **13**, 1508; (*d*) P. E. Nielsen, C. Hiort, S. H. Sönnichsen, O. Buchardt, O. Dahl and B. Norden, *J. Am. Chem. Soc.*, 1992, **114**, 4967.

5 H. D. Burrows and M. D. Miguel, *Adv. Coll. Interface Sci.*, 2001, **89**, 485.

6 (*a*) S. G. Srivatsan and S. Verma, *Chem. Eur. J.*, 2002, **8**, 5184; (*b*) C. Madhavaiah, S. G. Srivatsan and S. Verma, *Catal. Commun.*, 2002, **3**, 299; (*c*) C. Madhavaiah, S. G. Srivatsan and S. Verma, *Catal. Commun.*, 2001, **2**, 95; (*d*) S. G. Srivatsan and S. Verma, *Chem. Eur. J.*, 2001, **7**, 828; (*e*) S. G. Srivatsan and S. Verma, *Chem. Commun.*, 2000, 515.

7 *Plasmid Photocleavage*: Reactions were performed in sodium cacodylate buffer (pH 7.5, 30 °C), irradiated with two tungsten lamps (200 W), and constant temperature (30 °C) was maintained with Julabo circulation bath (Julabo HC) or under sun light at 37 °C, in 1.5 mL ependorff tubes. The tungsten lamp source was kept 3 cm away from the sample container. Weight of the polymer was taken as 50 μg/20μL of cacodylate buffer and supercoiled plasmid DNA was 10 ng/ or 8 ng/μL. All cleavage reactions were quenched with 5 μL of loading buffer contains 100 mM EDTA, 50% glycerol in Tris-HCl, pH 8.0 and samples were loaded onto 0.7% agarose gel contains ethidium bromide (1 μg/ 1mL) and electrophoresed for 1h at constant current (80 mA) in 0.5X TBE buffer. Gels were imaged with a PC-interfaced Bio-Rad Gel Documentation System 2000.

8 *Plasmid cleavage under anaerobic conditions*: Oxygen-free nitrogen was bubbled through cacodylate buffer, which was then subjected to four freeze-thaw cycles. All reagents were transferred in argon filled glove bag and eppendorf tubes were tightly sealed with parafilm in the argon atmosphere, followed by tungsten lamp irradiation. Reactions were quenched with loading buffer and efforts were not made to ensure strict anaerobic conditions during irradiation and quenching.

9 *Lysozyme cleavage*: Same buffer and irradiation conditions were used as described in DNA cleavage experiments. Weight of the polymer was taken as 100 μg/20 μL of buffer contains 50 μM of lysozyme. All reactions were quenched with 20 μL of sample buffer containing 20 μg of bromophenol blue and 20% of glycerol in 0.01 M of Tris-HCl buffer. Samples were loaded onto polyacrylamide gel (concentrating gel 4.5% and separating gel 12.5%) and electrophoresed for 10 h at constant voltage (80 V) in Tris-glycine buffer contains 0.1% sodium dodecyl sulfate. Gel was stained with Coomassie blue and destained prior to imaging on Bio-Rad Gel Doc 2000 system.

10 (*a*) K. L. Smith, Z-F. Tao, S. Hashimoto, C. J. Leitheiser, X. Wu and S. M. Hecht, *Org. Lett.*, 2002, **4**, 1079; (*b*) C. S. Jeung, C. H. Kim, K. Min, S. W. Suh and J. Suh, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2401; (*c*) C. S. Jeung, J. B. Song, Y. H. Kim and J. Suh, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 3061.

Typeset and printed by Black Bear Press Limited, Cambridge, England