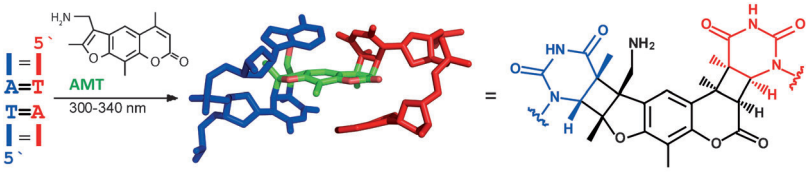
DNA Repair

DOI: 10.1002/anie.201103611

Photolyase-like Repair of Psoralen-Crosslinked Nucleic Acids**

Thorsten Stafforst and Donald Hilvert**

Psoralens are plant natural products that damage DNA and RNA in a light-dependent manner.[1] After intercalating into double-helical regions of nucleic acid, they react photochemically with thymidine or uridine residues to form cyclobutane adducts via [2+2] cycloaddition reactions. At 5′-d(TA) motifs, covalent interstrand crosslinks can result (Scheme 1). Linking the two strands in this way prevents unpairing and thus strongly impairs fundamental biological processes such as replication[2] and transcription.[3] These properties have made psoralens useful as probes of nucleic acid structure and function and also as agents for the treatment of psoriasis and other skin conditions.[1,4]



Scheme 1. Aminomethyltrisoralen (AMT) forms interstrand crosslinks with the 5′-TA motif in duplex DNA through two consecutive [2+2] photocyclizations. The configuration of the resulting crosslink is shown.[1b]

Repairing highly mutagenic psoralen crosslinks inside the cell is a difficult and complex task that involves several repair pathways.[5] In contrast, intrastrand cyclobutane pyrimidine dimer (CPD) lesions that arise when DNA is exposed to UV radiation are easily repaired either by nucleotide excision or by a light-dependent, flavoenzyme-catalyzed process.[6] In the latter case, photoinduced electron transfer (PET) from a reduced and deprotonated flavin cofactor in the DNA photolyase initiates [2+2] cycloreversion of the CPD, with subsequent back electron transfer to the cofactor to regenerate the pyrimidine monomers. Photolyase action has been successfully mimicked by simple model systems that position a flavin chromophore proximal to a CPD or related DNA lesion.[7] Although the cyclobutane rings formed between thymidine and the furan and pyrone psoralen moieties (Scheme 1) resemble CPDs, their repair by a photolyase-like mechanism has never been reported, nor has a psoralen-specific photolyase been identified. Since light-triggered release of psoralen crosslinks in DNA would be useful for a variety of photocaging applications,[8] we have investigated the feasibility of extending the PET strategy to the site-selective repair of such adducts.

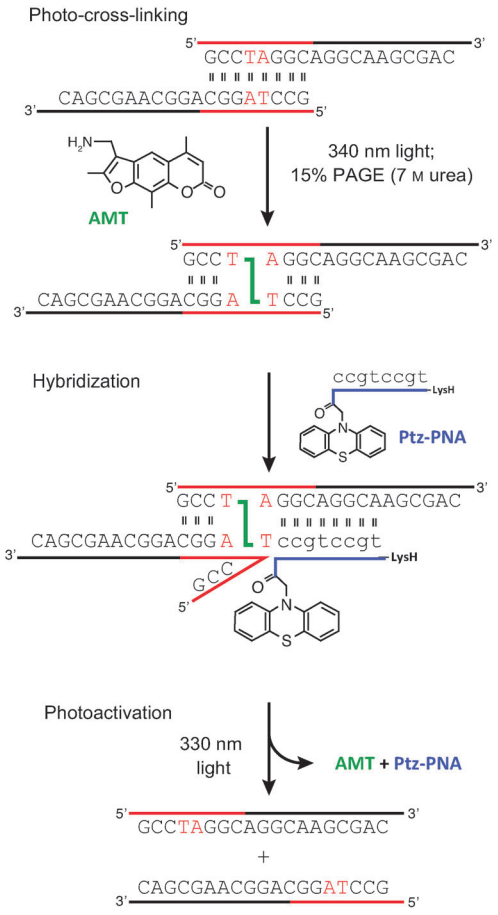
Electron-transfer reactions are strongly distance-dependent, so the design of a successful photolyase mimic requires a means of binding an electron donor near the psoralen crosslink.[9] For this purpose, peptide nucleic acids (PNAs) are an attractive option. PNAs are DNA mimics with a pseudopeptide backbone composed of neutral, achiral *N*-(2-aminoethyl)glycine units.[10] They hybridize with high affinity and selectivity to complementary sequences in single-stranded DNA and RNA and can even invade double-stranded secondary structures.[11] We therefore anticipated that a short PNA bearing a PET chromophore would allow site-selective delivery of the probe to a specific crosslink, thereby facilitating subsequent photorepair (Scheme 2). As electron donor we chose a phenothiazine (Ptz) derivative rather than the reduced flavin used by natural photolyases because it is more efficiently excited and does not require in situ reduction.[12] This chromophore was attached to an Fmoc-protected *N*-(2-aminoethyl)glycine building block that is easily incorporated into PNA oligomers by standard solid-phase peptide synthesis (for details of the synthesis, see the Supporting Information).[13]

We used a partially self-complementary DNA oligonucleotide containing a single interstrand crosslink as the substrate for the repair experiments (Scheme 2). It was prepared by irradiating 5′-d(GCC**TA**GGCAGGCAAGCGAC) in the presence of AMT,[14] a commonly used psoralen derivative, in neutral Tris-HCl buffer (50 mM, 10 mM $MgCl_2$, 100 mM NaCl, pH 7.5) at (340±10) nm for 7 h at a constant temperature of 4 °C. The crosslinked duplex was purified by polyacrylamide gel electrophoresis (PAGE), and its identity was confirmed by mass spectrometry (Supporting Information, Figure S1). The sequence immediately downstream of the crosslink is complementary to the PNA derivative tgcctgcc-Ptz, so rapid hybridization ensues when the DNA substrate (20 ng μL^{-1} = 1.7 μM) is mixed with a 30% molar excess of the PNA per target site in phosphate buffer (10 mM, 100 mM NaCl, pH 7.0). Following initial binding to the exposed single-stranded nucleation site, strong PNA–DNA interactions allow the PNA probe to invade the adjacent duplex, even at physiological ionic strength, to place the phenothiazine chromophore near the lesion (Scheme 2).

[*] Dr. T. Stafforst, Prof. Dr. D. Hilvert
Laboratorium für Organische Chemie, ETH Zürich
Wolfgang-Pauli-Strasse 10, Zürich (Switzerland)
E-mail: thorsten.stafforst@uni-tuebingen.de
hilvert@org.chem.ethz.ch
Homepage: http://www.ifib.uni-tuebingen.de/forschung/stafforst.html
http://www.protein.ethz.ch

[**] This work was generously supported by the Deutsche Akademie der Naturforscher Leopoldina (Bundesministerium für Bildung und Forschung BMBF-LPD 9901/8-158) and the ETH Zürich.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201103611.



Scheme 2. Strategy for photoactivating psoralen-crosslinked DNA. The AMT crosslink (green) is introduced at a specific 5′-d(TA) motif in a partly self-pairing DNA oligomer (top). A complementary PNA molecule (blue) delivers an attached phenothiazine (Ptz) chromophore to the lesion site (middle). Irradiation of the resulting complex at 330 nm reverses the crosslink (bottom).

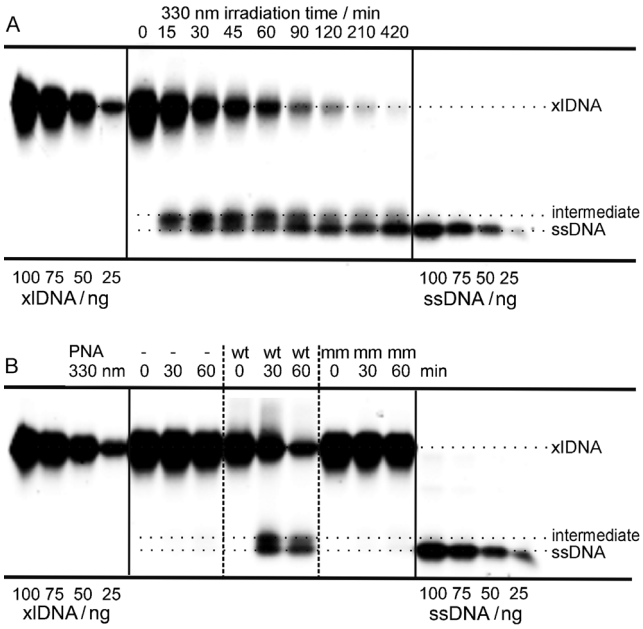
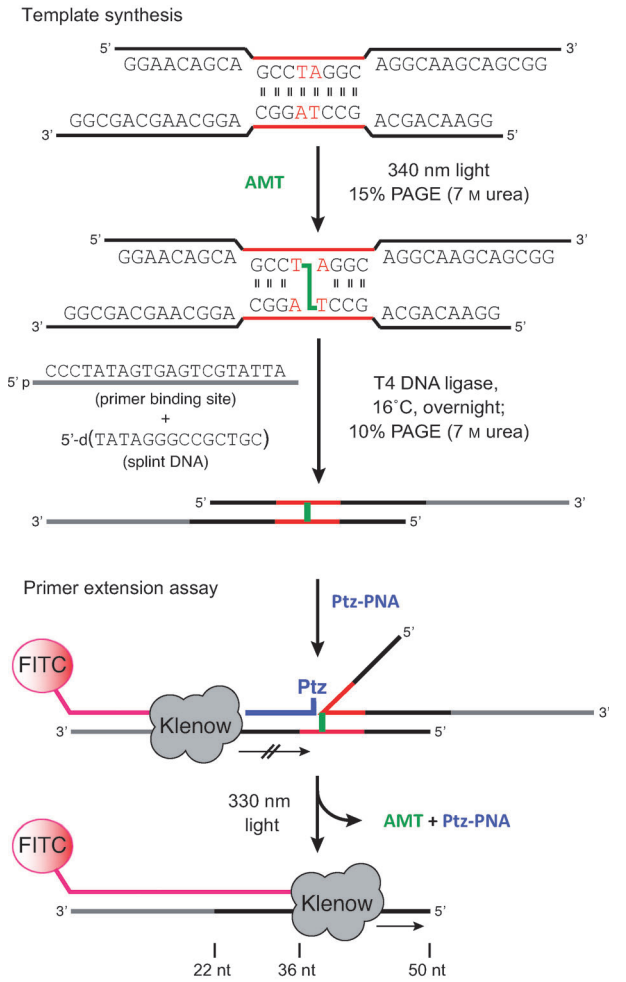


Figure 1. PET-induced DNA repair. A) After hybridizing crosslinked xlDNA (1.7 μM) with tgcctgcc-Ptz (4.5 μM) in 10 mM sodium phosphate, 100 mM NaCl, pH 7.0 buffer, samples were irradiated with monochromatic light ((330 ± 20) nm, 20 °C). Aliquots were removed periodically, analyzed by denaturing 15% PAGE in 7 M urea, and visualized with SYBR Gold stain. xlDNA is cleaved to give a fully uncaged oligonucleotide (ssDNA) and a psoralen monoadduct intermediate with a half-life of 26 min, as determined by densitometry (see Figure S3 in the Supporting Information). B) Whereas fully complementary wildtype (wt) tgcctgcc-Ptz repairs the crosslink, no detectable photoreversal was observed in control experiments lacking PNA (—) or with the mismatched sequence tgc***tc***gcc-Ptz (mm). For estimation of the repair yield, a dilution series (25–100 ng) of the starting material (xlDNA) and the final product (ssDNA) were loaded on each gel.

After incubation for 15 min, we initiated photoactivation by irradiating the sample at (330 ± 20) nm with a 75 W xenon arc lamp equipped with a 310 nm cut-off filter. At various time points, aliquots (5 μL = 100 ng) were removed from the mixture, and the progress of the reaction was monitored by PAGE (Figure 1 A). Densitometric analysis of the gels showed that cleavage of the crosslink follows first-order kinetics with a half-life of approximately 26 min (Figure S3 in the Supporting Information).

The reaction initially afforded two products which were PAGE-purified and shown by mass spectrometry to be a psoralen monoadduct and the fully uncaged oligonucleotide (Supporting Information, Figure S4). Upon extended irradiation, the former is converted quantitatively to the latter, highlighting the excellent uncaging properties of this system. In control experiments, no repair was detected in the absence of the Ptz-PNA or with mismatched PNA sequences such as tgc***tc***gcc-Ptz (Figure 1 B). Efficient photoactivation evidently requires site-specific hybridization of the Ptz-PNA with the crosslinked DNA.[15]

A simple primer extension assay from a template containing a single psoralen crosslink illustrates the potential utility of psoralen caging/uncaging for controlling biochemical processes with light. As outlined in Scheme 3, we synthesized a 30 nucleotide (nt) long, partially self-complementary DNA oligonucleotide and crosslinked it with AMT as described above. The crosslinked species was purified by 15% PAGE (7 M urea) and then ligated at both 3′-ends to a 20 nt primer binding site using a splint ligation strategy (see the Supporting Information). The product—a symmetric 2 × 50 nt construct, specifically crosslinked 36 nt downstream of each 3′-terminus—was purified by 10% PAGE (7 M urea) and characterized by mass spectrometry (Supporting Information, Figure S2). It served as the template for extension of a 5′-FITC-labeled primer by the Klenow fragment (3′→5′ exo⁻, New England Biolabs). Since the polymerase cannot read through the crosslink, an approximately 36 nt truncated transcript is produced rather than the 50 nt run-off transcript obtained from its uncaged counterpart (Figure 2). When the crosslinked template was preincubated with the tgcctgcc-Ptz PNA probe and irradiated at 330 nm, however, primer extension yielded the full-length transcript as a result of PET-induced cleavage of the psoralen linkage. The relative amounts of truncated and full-length transcript that were obtained depend on the irradiation time and are consistent with the repair kinetics observed with the shorter template



Scheme 3. Psoralen photocaging/uncaging applied to a primer extension assay. Top: A 50 nt template, specifically crosslinked 36 nt downstream of the 3′ terminus, was generated with a splint ligation strategy. Bottom: After hybridization with a 5′-FITC-labeled primer (magenta, FITC = fluorescein isothiocyanate), transcription from the crosslinked template by the Klenow fragment results in premature transcription arrest after approximately 36 nt. In contrast, the full-length 50 nt run-off transcript is obtained after cleavage of the crosslink upon hybridization of the template with the Ptz-PNA (blue) and irradiation at 330 nm.

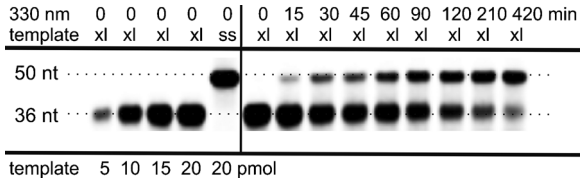


Figure 2. Primer extension assay. The crosslinked template was incubated with wt tgcctgcc-Ptz and irradiated with monochromatic light ((330 ± 20) nm, 20 °C) for 15 to 420 min prior to addition of the polymerase. As a control, primer extension was also carried out with crosslinked (xl) and free single-stranded (ss) templates in the absence of PNA and light. The resulting DNA products were separated by 15% PAGE (7 M urea). The amount of 50 nt long elongation product increased with increasing irradiation times, whereas the short product (≤ 36 nt) gradually disappeared, indicating conversion of the xl into the ss template. Densitometric analysis showed that the reaction obeys first-order kinetics with a half-life of approximately 85 min (Figure S5 in the Supporting Information). Conditions: see Reference [22].

(Figure 2 A). As expected, no detectable repair of the crosslinked template was observed with the mismatched tgc***tc***gcc-Ptz-PNA (Supporting Information, Figure S6). As a consequence, it should be possible to selectively repair lesions at complementary sites in mixtures containing multiple crosslinks.

In conclusion, our results demonstrate the feasibility of cleaving interstrand psoralen crosslinks by photoinduced reductive electron transfer. This capability paves the way for selective photocaging applications that cannot be realized by currently available methods.[8] While mRNA, plasmids, and other nucleic acids can be caged by stochastic modification of nucleobases or backbone phosphates with multiple photolabile protecting groups,[16] unwanted residual activity and incomplete photoactivation limit the practicality of such approaches. Similarly, the ease with which intrastrand CPD-like lesions are repaired or bypassed[17] restricts their utility for photocaging purposes. In contrast, basic biochemical processes can be completely blocked by site-selective incorporation of a single psoralen lesion into plasmids[18] and chromosomes.[19] Employing an external PET chromophore for the repair of psoralen lesions provides a simple and selective means of restoring activity. Because PNA constructs have been designed for high-affinity recognition of a wide variety of single-stranded and double-stranded DNA and RNA structures,[10,11] it should be possible to create PNA PET probes that target virtually any psoralen lesion. Moreover, simple Watson–Crick pairing can be used to steer repair to specific lesions without affecting others, thus providing a level of control not possible with standard caging approaches. Extension of this strategy to other sequence-specific DNA binders, such as triplex-forming oligonucleotides[20] and polyamides,[21] and other chromophores, for example with more red-shifted absorbances or two-photon-absorbance capabilities, would further enhance its utility.

Received: May 26, 2011
Published online: August 29, 2011

Keywords: DNA repair · peptide nucleic acids · photoactivation · photolyases · psoralens

[1] a) G. D. Cimino, H. B. Gamper, S. T. Isaacs, J. E. Hearst, *Annu. Rev. Biochem.* **1985**, *54*, 1151; b) H. P. Spielmann, T. J. Dwyer, J. E. Hearst, D. E. Wemmer, *Biochemistry* **1995**, *34*, 12937.
[2] S. Diviacco, V. Rapozzi, L. Xodo, C. Hélène, F. Quadrifoglio, C. Giovannangeli, *FASEB J.* **2001**, *15*, 2660.
[3] a) Y.-H. Ping, T. M. Rana, *Biochemistry* **2005**, *44*, 2501; b) K.-H. Kim, X.-J. Fan, P. E. Nielsen, *Bioconjugate Chem.* **2007**, *18*, 567.
[4] J. F. Thompson, J. E. Hearst, *Cell* **1983**, *32*, 1355.
[5] P. A. Muniandy, J. Liu, A. Majumdar, S.-T. Liu, M. M. Seidman, *Crit. Rev. Biochem. Mol. Biol.* **2010**, *45*, 23.
[6] a) A. Sancar, *Chem. Rev.* **2003**, *103*, 2203; b) M. Müller, T. Carell, *Curr. Opin. Struct. Biol.* **2009**, *19*, 277.
[7] a) O. Wiest, C. B. Harrison, N. J. Saettel, R. Cibulka, M. Sax, B. König, *J. Org. Chem.* **2004**, *69*, 8183; b) T. Carell, J. Butenandt,

Angew. Chem. **1997**, *109*, 1590; c) R. Epple, E.-U. Wallenborn, T. Carell, *J. Am. Chem. Soc.* **1997**, *119*, 7440; d) M. K. Cichon, S. Arnold, T. Carell, *Angew. Chem.* **2002**, *114*, 793; *Angew. Chem. Int. Ed.* **2002**, *41*, 767; e) T. Stafforst, U. Diederichsen, *Angew. Chem.* **2006**, *118*, 5502; *Angew. Chem. Int. Ed.* **2006**, *45*, 5376; f) T. Ito, A. Kondo, S. Terada, S.-I. Nishimoto, *J. Am. Chem. Soc.* **2006**, *128*, 10934.

[8] a) G. Mayer, A. Heckel, *Angew. Chem.* **2006**, *118*, 5020; *Angew. Chem. Int. Ed.* **2006**, *45*, 4900; b) X. Tang, I. Dmochowski, *Mol. BioSyst.* **2007**, *3*, 100.

[9] R. A. Marcus, N. Sutin, *Biochim. Biophys. Acta Rev. Bioenerg.* **1985**, *811*, 265.

[10] a) M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Nordén, P. E. Nielsen, *Nature* **1993**, *365*, 566; b) P. Wittung, P. E. Nielsen, O. Burchardt, M. Egholm, B. Nordén, *Nature* **1994**, *368*, 561.

[11] For an overview see: *Pseudo-Peptides in Drug Discovery* (Ed.: P. E. Nielsen), Wiley-VCH, Weinheim, **2004**.

[12] C. Wagner, H.-A. Wagenknecht, *Chem. Eur. J.* **2005**, *11*, 1871.

[13] T. Stafforst, U. Diederichsen, *Eur. J. Org. Chem.* **2007**, 681.

[14] a) S. T. Isaacs, C.-K. J. Shen, J. E. Hearst, H. Rapoport, *Biochemistry* **1977**, *16*, 1058; b) H. Gamper, J. Piette, J. E. Hearst, *Photochem. Photobiol.* **1984**, *40*, 29.

[15] Additional mechanistic studies will be needed to determine whether the electron is transferred directly to the lesion or first injected into the DNA duplex: B. Giese, B. Carl, T. Carl, T. Carell, C. Behrens, U. Hennecke, O. Schiemann, E. Feresin, *Angew. Chem.* **2004**, *116*, 1884; *Angew. Chem. Int. Ed.* **2004**, *43*, 1848.

[16] a) W. T. Monroe, M. M. McQuain, M. S. Chang, J. S. Alexander, F. R. Haselton, *J. Biol. Chem.* **1999**, *274*, 20895; b) H. Ando, T. Furuta, R. Y. Tsien, H. Okamoto, *Nat. Genet.* **2001**, *28*, 317; c) S. Shah, S. Rangarajan, S. H. Friedman, *Angew. Chem.* **2005**, *117*, 1352; *Angew. Chem. Int. Ed.* **2005**, *44*, 1328.

[17] M. Ogino, D. Okamura, K. Fujimoto, *Sci. Technol. Adv. Mater.* **2007**, *8*, 318.

[18] a) W. A. Saffran, M. Goldenberg, C. R. Cantor, *Proc. Natl. Acad. Sci. USA* **1982**, *79*, 4594; b) M. Raha, G. Wang, M. M. Seidman, P. M. Glazer, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 2941; c) G. Wang, D. D. Levy, M. M. Seidman, P. M. Glazer, *Mol. Cell. Biol.* **1995**, *15*, 1759.

[19] a) A. Majumdar, A. Khorlin, N. Dyatkina, F.-L. M. Lin, J. Powell, J. Liu, Z. Fei, Y. Khripine, K. A. Watanabe, J. George, P. M. Glazer, M. M. Seidman, *Nat. Genet.* **1998**, *20*, 212; b) K.-H. Kim, P. E. Nielsen, P. M. Glazer, *Biochemistry* **2006**, *45*, 314; c) A. K. Thazhathveetil, S.-T. Liu, F. E. Indig, M. M. Seidman, *Bioconjugate Chem.* **2007**, *18*, 431; d) K.-H. Kim, P. E. Nielsen, P. M. Glazer, *Nucleic Acids Res.* **2007**, *35*, 7604.

[20] N. T. Thuong, C. Hélène, *Angew. Chem.* **1993**, *105*, 697; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 666.

[21] P. B. Dervan, B. S. Edelson, *Curr. Opin. Struct. Biol.* **2003**, *13*, 284.

[22] Experimental conditions for Figure 2: PET-induced repair: [xlDNA] = 1.7 μM, [PNA] = 4.3 μM in 10 mM Tris/HCl, 50 mM NaCl, pH 7.9. Transcription: 20 pmol 5′-FITC-labeled T7forw primer, 6 μL PET-repair mix, 250 μM of each dNTP, 1 mM DTT, 10 mM $MgCl_2$, 0.75 u Klenow fragment, all in 10 mM Tris/HCl, 50 mM NaCl, pH 7.9 in a total volume of 10 μL, 55 °C for 45 s, 42 °C for 1 h. PAGE: samples (10 μL) were mixed with 10 μL of 1 × TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA, 7 M urea, containing 3% formaldehyde) and heated to 90 °C (5 min) prior to gel analysis.

