J|A|C|S
COMMUNICATIONS

Published on Web 01/17/2006

PNA-Based Reagents for the Direct and Site-Specific Synthesis of Thymine Dimer Lesions in Genomic DNA

J. Carsten Pieck, David Kuch, Friederike Grolle, Uwe Linne, Clemens Haas, and Thomas Carell*

Department of Chemistry and Biochemistry, Ludwig-Maximilians University, Butenandtstr. 5-13, 83177 Munich, Germany

Received September 15, 2005; E-mail: thomas.carell@cup.uni-muenchen.de

UV irradiation of cells furnishes large numbers of mutagenic cyclobutane pyrimidine dimer lesions (CPD, dT=dT) as a consequence of [2π + 2π] cycloaddition reactions between adjacent thymidines.[1−4] In recent years, the need for DNA containing such lesions at defined sites has steadily grown[5] in order to allow biochemical,[6] crystallographic,[7] and cellular studies.[8] These are needed to learn how DNA lesions induce mutations and how cells remove lesions from the genome.[9] Particularly, DNA with dT=dT lesions is needed because they are repaired by various pathways, shifting them into the center of DNA repair.[10] The chemical synthesis of DNA containing dT=dT dimers is a time-consuming process. It requires synthesis of a dimer lesion phosphoramidite building block,[11] chemical synthesis of DNA using this building block, purification of the prepared DNA, and, if required, ligation of the DNA into a larger DNA fragment. Alternatively, small DNA fragments with one dTpdT dinucleotide site can be irradiated in the presence of acetophenone to give dT=dT containing DNA and with side products that need to be separated by HPLC.[12,13] Both methods are labor intensive, and it is difficult to create DNA strands with more than one thymine dimer.[14]

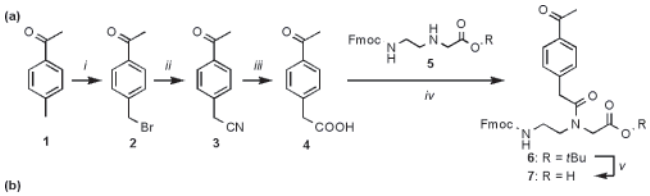
We thought that it would be a tremendous advantage if reagents could be created for the synthesis of a defined lesion in a defined position directly in large DNA strands or even in genomic DNA. For dT=dT, the incorporation of an acetophenone into peptide nucleic acids (PNA), which are able to bind tightly and sequence-specifically to complementary DNA sequences, might allow us to achieve this goal.

Two potential problems had to be solved. First, energy transfer from the light-excited acetophenone in the triplet state to the dTpdT dinucleotide must be faster than H-atom abstraction from DNA, which would give reactive DNA radicals.[15] Second, energy transfer is a rather long-range process, raising the question of how specific such reagents could potentially act.[16]

Syntheses of the acetophenone PNA building block and of the PNA strands needed for this study are depicted in Scheme 1. First, 4-methylacetophenone (**1**) was transformed via Wohl−Ziegler bromination (**2**), bromine to cyanide exchange (**3**), and hydrolysis of the cyanide group into compound **4**. Reaction of **4** with the Fmoc- and *t*-butyl-protected PNA backbone molecule **5** furnished, after acidic cleavage of the *t*-butylester, the acetophenone PNA monomer (**7**), denoted as ***A*** in Scheme 1. Synthesis of the peptide nucleic acid strands **PNA8**−**12** needed for this study was performed using a reported Fmoc synthesis protocol for peptide nucleic acids.[17,18] Two lysine residues at the N- and C-termini ensured excellent solubility (K).

We first analyzed the best position of the acetophenone relative to the dTpdT site to optimize the energy transfer step. To this end, the four PNA strands **8**−**11** were hybridized to the corresponding DNA strands (**DNA1** for **PNA8**, **-10**, **-11** and **DNA2** for **PNA9**) to give double strands, in which the ***A*** building block is (i) positioned opposite the 3′dT of the dTpdT dinucleotide (**PNA9:DNA2**); (ii) positioned opposite the 5′dT (**PNA8:DNA1**); (iii) positioned so that two ***A*** units face the dTpdT dinucleotide (**PNA10:DNA1**); and (iv) positioned so the ***A*** unit is shifted one nucleobase toward the 5′-end in order to allow the dTpdT to form two intact base pairs (**PNA11:DNA1**). Irradiation of these four PNA:DNA double strands (20 μM PNA:DNA duplex, 10 mM NaCl, 10 mM, phosphate buffer pH = 7.4) with λ = 340 nm was performed to induce dimer formation. The reaction mixture was analyzed before and after 3 h of irradiation by reversed-phase HPLC. Whereas irradiation of DNA with just acetophenone gave rise to a variety of products, irradiation of all PNA:DNA duplexes gave a clean conversion of the DNA strands to new DNA products of unchanged molecular weight as determined by MALDI-TOF. Addition of DNA photolyase enzyme, which specifically cleaves the naturally occurring *cis*−*syn* dT=dT dimers back into the dTpdT dinucleotides, converted the product strands back into **DNA1** and **DNA2**, respectively, proving that only *cis*−*syn* dT=dT dimers and no other products are formed during the experiment. Most interesting, however, is the chemical yield of the reaction. The best results were obtained with the duplex **PNA8:DNA1**, in which the ***A*** building block faces the 5′dT of the dTpdT site. Irradiation for only 3 h converted **DNA1** into the corresponding dT=dT dimer containing DNA strand in yields between 60 and 80%.

Scheme 1[a]

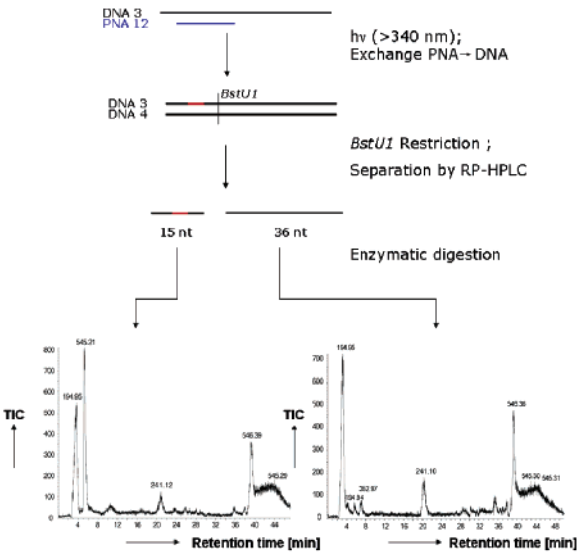


[a] Conditions: (a) *i* NBS, AIBN, MeCN, 1.5 h, 90 °C, 95%; *ii* NaCN, dioxane, H_2O, 2 h, reflux, 67%; *iii* H_2O, AcOH, H_2SO_4, 1.5 h, reflux; *iv* **5**, TBTU, HOBt, DIEA, DMF, 80 min, rt, 97%; *v* TFA/H_2O (95:1), 1 h, rt, 94%; (b) PNA (with ***A*** = acetophenone building block) and DNA (with ***TT*** = potential thymine dimers) strands.

To investigate the selectivity of the method, we prepared the PNA reagent **PNA12**, hybridized the reagent to a 51mer (**DNA3**) with two dTpdT sites (Scheme 2), irradiated the complex for 3 h, added the fully complementary DNA counter strand **DNA4** (5′-GCG-CAT-ACG-TGT-ATA-CTT-ACA-TGT-ATA-TAA-TAT-ACG-CGC-AAC-GTA-CTC-GCC-3′) to form the **DNA3:DNA4** duplex, and cut the duplex with the thermophilic restriction enzyme, *BstU1*, into a 15mer and a 36mer DNA strand. The 15mer and the 36mer were separated by rp-HPLC and analyzed by agarose gel

10.1021/ja056358i CCC: $33.50

Scheme 2[a]



[a] Procedure to create a CPD lesion (*red*) in a 51mer DNA. It is possible to generate the CPD lesion in only one of the two TT sites, which is analyzed by subsequent enzymatic digestion and HPLC−MS/MS.

electrophoresis. The strands were then fully digested with an enzyme mixture containing *nuclease P1*, *calf spleen phosphodiesterase II*, *alkaline phosphatase* (CIP), and *snake venom phosphodiesterase I*. The two digests were analyzed by HPLC−MS/MS (Scheme 2). The mass spectra show the peaks for the canonical nucleobases dA, dC, dG, and dT. Only for the small fragment was a large additional signal for a dT═dT dimer at $m/z = 545$ recorded. The fragmentation pattern of this signal was analyzed by HPLC−MS/MS and proven to be identical with reported data for a *cis*−*syn* dT═dT, proving the correct stereochemistry.[13] The results show that the PNA reagents bind specifically to the wanted sequence in the DNA strand in order to specifically direct the formation of *cis*−*syn* dT═dT. Formation of other lesions, such as (6−4) photoadducts, or *trans*−*syn* photo products can be ruled out based on the mass fragmentation data.

We finally investigated the ability to form site-specifically a dT═dT lesion directly in genomic DNA. This was tested with a M13 mp 18 phage genome (7249 nucleobases), which was prepared in the form of a cyclic single strand (Figure 1). The PNA reagent **PNA13** was prepared and hybridized to the genomic DNA, and the PNA:DNA ds was irradiated for 3 h. The PNA was then removed using reversed-phase HPLC at 55 °C. A biotin-labeled oligonucleotide (DNA^B, 5′-TCG-CCA-TTC-AGG-CTG-CGC-AAC-TGT-T^BGG-GAA-GGG-CGA-TCG-GT-3′ with T^B = biotin-labeled thymine) binding to the same sequence containing the potential thymine dimer was added and hybridized. The construct was bound to magnetic particles derivatized with streptavidin. Two restriction enzymes, *Bgl1* and *Pvu1*, were used to cut the immobilized construct into a small 28mer and a large 7221mer DNA piece. The small and large DNA fragments were spotted onto a positively charged nylon membrane and treated with a specific anti *cis*−*syn* dT═dT dimer antibody and a second antibody, HRP-labeled, to recognize the first one. As depicted in Figure 1, the chemiluminescence reaction is clearly limited to the spot containing the 28mer DNA, showing that the PNA reagent was able to recognize

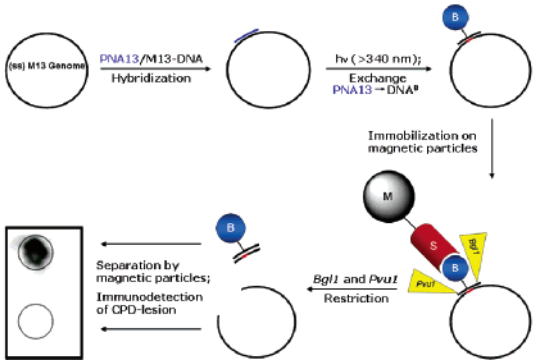


Figure 1. Schematic illustration of generating and analyzing a defined CPD lesion (*red stretch* in the plasmid strand) in genomic M13 mp 18 ss DNA.

specifically the targeted sequence in a large genomic DNA fragment in order to generate selectively upon irradiation the desired *cis*−*syn* dT═dT lesion. In summary, we have created novel reagents that allow synthesis of *cis*−*syn* dT═dT lesions directly in single-stranded DNA and even in large genomic DNA.

Acknowledgment. We thank the Deutsche Forschungsgemeinschaft (DFG), the Volkswagen Foundation, the EU Marie Curie Training and Mobility Program (CLUSTOXDNA), and the Fonds der Chemischen Industrie for generous financial support of this research. We thank D. Hammond for proofreading the manuscript.

Supporting Information Available: Experimental protocols and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Taylor, J. S. *Acc. Chem. Res.* **1994**, *27*, 76−82.
(2) Friedel, M. G.; Cichon, M. K.; Carell, T. In *CRC Handbook of Organic Photochemistry and Photobiology*, 2nd ed.; Horspool, W., Lenci, F., Eds.; CRC Press: Boca Raton, FL, 2004; pp 141/141−141/122.
(3) Begley, T. P. In *Comprehensive Natural Products Chemistry*; Poulter, C. D., Ed.; Elsevier Science B. V.: Amsterdam, The Netherlands, 1999; Vol. 5, pp 371−399.
(4) Taylor, J. S. *Pure Appl. Chem.* **1995**, *67*, 183−190.
(5) Schaerer, O. D. *Chem. Inf.* **2003**, 34.
(6) Greenberg, M. M.; Barvian, M. R.; Cook, G. P.; Goodman, B. K.; Matray, T. J.; Tronche, C.; Venkatesan, H. *J. Am. Chem. Soc.* **1997**, *119*, 1828−1839.
(7) Karplus, M.; Yang, W.; Banerjee, A.; Verdine, G. *Nature* **2005**, *31*, 612−618.
(8) de Padula, M.; Slezak, G.; Auffret van Der Kemp, P.; Boiteux, S. *Nucleic Acids Res.* **2004**, *32*, 5003−5010.
(9) Friedberg, E. C.; Walker, G. C.; Siede, W. *DNA Repair and Mutagenesis*; ASM Press: Washington, D.C., 1995; Vol. 1.
(10) Jans, J.; Schul, W.; Sert, Y.-G.; Rijksen, Y.; Rebel, H.; Eker, A. P. M.; Nakajima, S.; van Steeg, H.; de Gruijl, F.-R.; Yasui, A.; Hoeijmakers, J. H. J.; van der Horst, G. T. *J. Curr. Biol.* **2005**, *15*, 105−115.
(11) Taylor, J.-S.; Brockie, I. R.; O'Day, C. L. *J. Am. Chem. Soc.* **1987**, *109*, 6735−6742.
(12) Smith, C. A.; Taylor, J.-S. *J. Biol. Chem.* **1993**, *268*, 11143−11151.
(13) Douki, T.; Court, M.; Sauvaigo, S.; Odin, F.; Cadet, J. *J. Biol. Chem.* **2000**, *275*, 11678−11685.
(14) Carty, M. P.; Lawrence, C. W.; Dixon, K. *J. Biol. Chem.* **1996**, *271*, 9637−9647.
(15) Gut, I. G.; Wood, P. D.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 2366−2373.
(16) Förster, T. *Discuss. Faraday Soc.* **1959**, 7−17.
(17) Nielsen, P. E.; Egholm, M. *Peptide Nucleic Acids—Protocols and Applications*; Horizon Scientific Press: Wymondham, UK, 1999.
(18) Thomson, S. A.; Josey, J. A.; Cadilla, R.; Gaul, M. D.; Hassman, C. F.; Luzzio, M. J.; Pipe, A. J.; Reed, K. L.; Ricca, D. J.; Wiethe, R. W.; Noble, S. A. *Tetrahedron* **1995**, *51*, 6179−6194.

JA056358I