Remarkable Stabilization of Duplex DNA Containing an Abasic Site by Non-Nucleosidic Phenanthroline and Pyrene Building Blocks

Simon M. Langenegger and Robert Häner*[a]

Abasic sites represent a common type of lesion in DNA. Loss of a base can take place as a spontaneous process,[1] it can happen as a result of base modification, or it can occur during an enzymatic repair process.[2,3] If not repaired, the resulting abasic site has a high potential for mutagenicity or might lead to cell death. Due to their biological importance, there is a strong interest in methods of recognizing abasic sites in DNA both for diagnostic and pharmaceutical use. Intercalating ligands[4–6] have been evaluated as inhibitors of enzymatic repair processes to increase the efficacy of cytotoxic agents.[7] Stabilization of abasic sites in duplex DNA has been achieved with complementary oligonucleotides carrying extended aromatic residues opposite to the abasic site. Thus, deoxyribofuranosides carrying pyrene and other polyaromatic hydrocarbons have been used as substitutes for missing nucleobases in order to maintain the aromatic stacking throughout the duplex.[8–10] We have recently reported that phenanthrene substituted with flexible aliphatic linkers can be used to stabilize abasic sites in a DNA duplex.[11] Such building blocks might present a considerable practical advantage over the synthetically more demanding sugar-derived analogues. From a structural point of view, an abasic site represents a discontinuity of

[a] *Dipl.-Chem. S. M. Langenegger, Prof. Dr. R. Häner*
Department of Chemistry and Biochemistry, University of Bern
Freiestraße 3, 3012 Bern (Switzerland)
Fax: (+41) 31-631-4382
E-mail: robert.haener@ioc.unibe.ch

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

  DOI: 10.1002/cbic.200400370

the DNA base stack and, thus, leads to a deviation from the regular DNA duplex structure.[7] Model considerations suggest that the aromatic building block replaces for the missing base by intercalation into the cavity resulting from loss of a nucleobase. Stacking interactions are influenced by factors such as the dipole moment and the surface of the aromatic compounds. Therefore, use of phenanthroline or pyrene instead of phenanthrene might have a further positive effect on the stability of abasic DNA. Here, we report on the effect of 2,9-disubstituted 1,10-phenanthrolines as well as 1,8-disubstituted pyrenes on the stability of double-stranded DNA containing an abasic site.

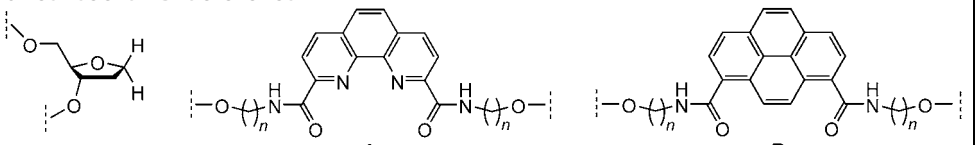
The synthesis of the phenanthroline- and pyrene-derived phosphoramidite building blocks containing linkers of different lengths is shown in Scheme 1. The preparation of the pyrene derivatives started from pyrene-1,8-dicarboxylic acid (**1**), which had been prepared according to a literature method.[12] Derivatization with the corresponding α,ω-aminoalcohols gave the amides **2a**–**d**. Subsequent phosphitylation provided the phosphoramidites **3a**–**d**. The synthesis of the phenanthroline building blocks followed the same synthetic scheme. Thus, the known 1,10-phenanthroline-2,9-dicarboxylic acid (**4**)[13] was transformed via the intermediates **5a**–**d** into the phosphoramidites **6a**–**d**. All compounds were characterized by conventional analytical methods (see Supporting Information).

The pyrene- and phenanthroline-derived phosphoramidite building blocks **3a**–**d** and **6a**–**d** were subsequently incorporated into oligonucleotides by standard oligonucleotide synthesis.[14,15] Coupling yields with the modified phosphoramidites were equal to the ones obtained with automated nucleoside building blocks. For synthesis of the abasic site-containing oligonucleotide (φ),[16] a commercially available, tetrahydrofuran-derived phosphoramidite was used. After deprotection (conc. ammonia, 55 °C), all oligonmers were purified by reversed-phase HPLC and characterized by high-resolution mass spectrometry (see Supporting Information).

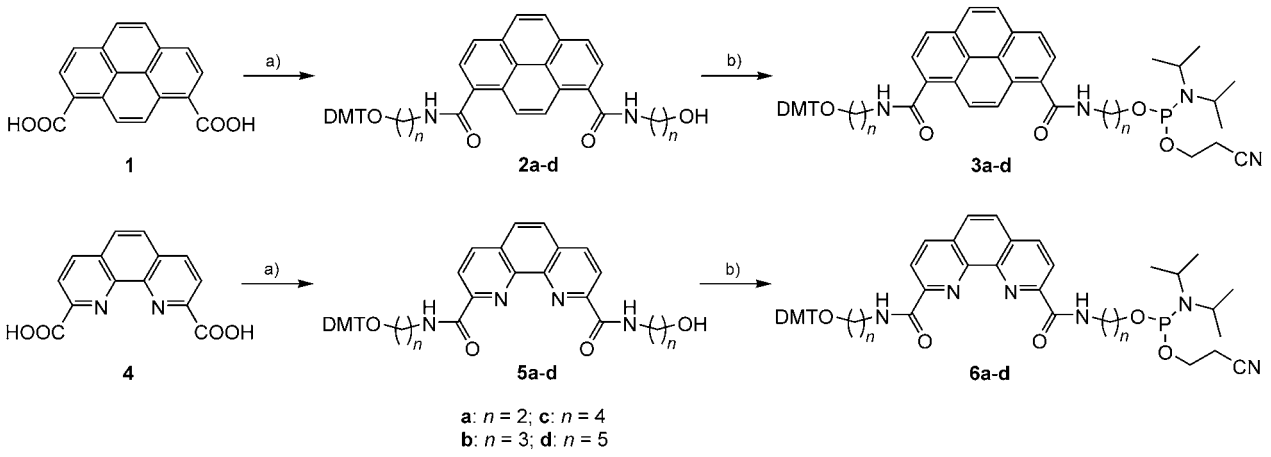
The effect of the different phenanthroline and pyrene building blocks on the stability of an abasic site-containing DNA duplex was analyzed by thermal-denaturation experiments. The modified building blocks were positioned opposite the abasic site. The melting temperature (T_m) data of the corresponding duplexes are summarized in Table 1. In comparison to a duplex containing a natural nucleotide (adenosine) oppo-

Table 1. *Influence of phenanthroline- and pyrene-derived, non-nucleosidic building blocks on duplex DNA containing an abasic site. Conditions: oligomer (1.0 μM), Tris-HCl (10 mM), NaCl (100 mM), pH 7.4. Experimental error: ±0.5 °C.*

Duplex	T_m [°C]	ΔT_m [°C][a]			
		$n=2$	$n=3$	$n=4$	$n=5$
5′ AGC TCG GTC A **T** C GAG AGT GCA 3′ TCG AGC CAG T **A** G CTC TCA CGT	67.7				
5′ AGC TCG GTC A φ C GAG AGT GCA 3′ TCG AGC CAG T **A** G CTC TCA CGT	56.3				
5′ AGC TCG GTC A φ C GAG AGT GCA 3′ TCG AGC CAG T **nAn** G CTC TCA CGT		4.2	6.2	5.9	8.4
5′ AGC TCG GTC A φ C GAG AGT GCA 3′ TCG AGC CAG T **nBn** G CTC TCA CGT		5.9	6.2	6.9	5.5



[a] Difference in T_m relative to the duplex containing an adenosine opposite to the abasic site (T_m = 56.3 °C).



Scheme 1. *Synthesis of the pyrene and phenanthroline phosphoramidites used in this study. Conditions: a) $HO(CH_2)_nNH_2$/DMTO$(CH_2)_nNH_2$ (DMT = 4,4′-dimethyltrityl; 1.0 equiv of each, in pyridine), (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium haxafluorophosphate (2.2 equiv), Hünig's base (5 equiv), DMF; b) 2-cyanoethyl diisopropylamidochloridophosphite (1.0 equiv), Hünig's base (3 equiv), CH_2Cl_2.*

site to φ, all building blocks led to a significant increase of the melting temperature. ΔT_m values between 4 °C and 8 °C were observed. A T_m increase of this extent is comparable to the contribution of 1–2 base pairs to the stability of an oligonucleotide of this length. In the pyrene series, the T_m is rather independent of the linker length. Thus, going from two to five methylene groups in both linker arms, the T_m's varied within a narrow range of approximately 1 °C. In the phenanthroline series, however, a stronger influence of the linker arms was observed. The T_m increased from 60.5 °C with two methylene groups to approximately 62.5 °C with three and four CH_2 groups and then to 64.7 °C with the pentamethylene derivative. The last T_m value corresponds to an increase of more than 8 °C, relative to the duplex with the adenosine opposite the abasic site. Compared to the unmodified duplex containing an AT base pair, this is a destabilization of 3.0 °C. All the different phenanthroline and pyrene building blocks cause a considerably larger stabilization than the corresponding reported phenanthrene analogues,[11] except for the case of the tetramethylene linker. In this case, the phenanthrene leads to a stabilization ($\Delta T_m = 5.7$ °C)[11] that is equal to the one effected by the phenanthroline with the same linker ($\Delta T_m = 5.9$ °C, Table 1, $n = 4$). All melting curves showed a sigmoidal shape with a single transition (see Supporting Information); this indicated a cooperative melting process. The hyperchromicities of the denaturation processes ranged from 15 to 20 %.

We have previously shown that non-nucleosidic phenanthrene building blocks are capable of stabilizing a DNA duplex containing an abasic site. The degree of stabilization was dependent on the length of the non-nucleosidic linker. A correlation between the linker and the structural stability of abasic site containing DNA was also found in the case of the present phenanthroline building blocks. The pyrene derivatives, on the other hand, are relatively insensitive to changes of the linker. With a given experimental error of ± 0.5 °C, no or only marginal differences are observed in the T_m values upon changing the number of methylene units in the linkers from two to five. NMR investigations of abasic DNA containing a *stacked-in* adenine[17,18] or thymine[19] revealed that the cavity arising from the missing nucleobase leads to a discontinuation of the stacking interactions within the helix. Figure 1 shows an Amber-minimized structure[20] of the abasic duplex containing the phenanthroline with the longest linkers. In this model, the phenanthroline is positioned between the base pairs adjacent to the abasic site. This arrangement of the polyaromatic residue allows for the continuation of stacking interactions in the absence of the nucleobase. The model agrees well with the NMR structure of a DNA duplex containing a sugar-derived pyrene opposite an abasic site.[9]

Conclusion

The influence of non-nucleosidic phenanthroline and pyrene dicarboxamide derivatives on the duplex stability of abasic DNA has been investigated. Different building blocks with flexible, aliphatic linkers have been incorporated opposite an abasic site. Thermal-denaturation experiments show that both types of polyaromatic hydrocarbons lead to a significant stabilization of the abasic DNA duplex. In comparison to the corresponding duplex with an adenosine opposite the abasic site, T_m's are increased by 4–8 °C. Thus, a considerable stabilization is observed even in the absence of a preorganized, continuous sugar phosphate backbone. The highest stabilization is effected by the 1,10-phenanthroline-2,9-dicarboxamide bearing two pentamethylene linkers. Model considerations suggest that the stabilizing effect arises from stacking interactions between the polyaromatic residues and the base pairs adjacent to the abasic site.

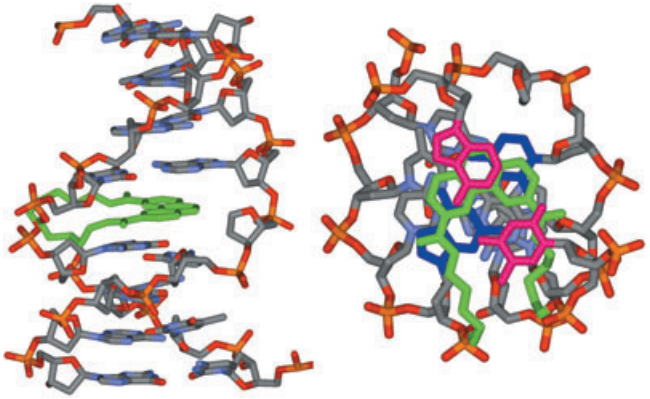


Figure 1. *Amber-minimized structure of the duplex containing a phenanthroline building block with pentamethylene linkers (green) opposite an abasic site. Left: view perpendicular to the helical axis. Right: view along the helical axis; the base pairs adjacent to the phenanthroline are shown in dark blue and pink.*

Acknowledgements

Financial support by the Swiss National Foundation (Grant 31-63380.00) is gratefully acknowledged.

Keywords: abasic sites · DNA · phenanthroline · pyrene · stacking interactions

[1] T. Lindahl, B. Nyberg, *Biochemistry* **1972**, *11*, 3610–3618.
[2] B. Demple, L. Harrison, *Annu. Rev. Biochem.* **1994**, *63*, 915–948.
[3] O. D. Scharer, *Angew. Chem.* **2003**, *115*, 3052–3082; *Angew. Chem. Int. Ed.* **2003**, *42*, 2946–2974.
[4] N. Berthet, J. F. Constant, M. Demeunynck, P. Michon, J. Lhomme, *J. Med. Chem.* **1997**, *40*, 3346–3352.
[5] N. Berthet, J. Michon, J. Lhomme, M. P. Teulade-Fichou, J. P. Vigneron, J. M. Lehn, *Chem. Eur. J.* **1999**, *5*, 3625–3630.
[6] K. Yoshimoto, S. Nishizawa, M. Minagawa, N. Teramae, *J. Am. Chem. Soc.* **2003**, *125*, 8982–8983.
[7] J. Lhomme, J. F. Constant, M. Demeunynck, *Biopolymers* **1999**, *52*, 65–83.
[8] T. J. Matray, E. T. Kool, *J. Am. Chem. Soc.* **1998**, *120*, 6191–6192.
[9] S. Smirnov, T. J. Matray, E. T. Kool, C. los Santos, *Nucleic Acids Res.* **2002**, *30*, 5561–5569.
[10] E. T. Kool, J. C. Morales, K. M. Guckian, *Angew. Chem.* **2000**, *112*, 1046–1068; *Angew. Chem. Int. Ed.* **2000**, *39*, 990–1009.
[11] S. M. Langenegger, R. Häner, *Chem. Biodiversity* **2004**, *1*, 259–264.
[12] H. Vollmann, H. Becker, M. Corell, H. Streeck, G. Langbein, *Justus Liebigs Ann. Chem.* **1937**, *531*, 1–159.

[13] C. J. Chandler, L. W. Deady, J. A. Reiss, *J. Heterocycl. Chem.* **1981**, *18*, 599 – 601.
[14] S. L. Beaucage, M. H. Caruthers, *Tetrahedron Lett.* **1981**, *22*, 1859 – 1862.
[15] N. D. Sinha, J. Biernat, J. McManus, H. Koster, *Nucleic Acids Res.* **1984**, *12*, 4539 – 4557.
[16] The building block φ is commonly used as a chemically stable analogue of an abasic site. We refer to this building block as an "abasic site" although it is not one in the strict sense.
[17] S. T. Hoehn, C. J. Turner, J. Stubbe, *Nucleic Acids Res.* **2001**, *29*, 3413 – 3423.
[18] R. D. Beger, P. H. Bolton, *J. Biol. Chem.* **1998**, *273*, 15 565 – 15 573.
[19] Y. Coppel, N. Berthet, C. Coulombeau, C. Coulombeau, J. Garcia, J. Lhomme, *Biochemistry* **1997**, *36*, 4817 – 4830.
[20] HyperChem, Hypercube, Inc., Gainesville, FA, USA.

Received: October 18, 2004
Published online on March 18, 2005

