A Photochemical Approach to Highlight Backbone Effects in PNA

Pascale Clivio,*[,§] Dominique Guillaume,[‡]
Marie-Thérèse Adeline,[§] and Jean-Louis Fourrey*[,§]

Institut de Chimie des Substances Naturelles, CNRS
91198 Gif-sur-Yvette Cedex, France
Laboratoire de Chimie Thérapeutique (URA 1310)
Faculté de Pharmacie, Université René Descartes
4, Avenue de l'Observatoire, 75006 Paris, France

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In the past few years, artificial constructs have been designed to specifically recognize messenger RNA or double-stranded DNA (dsDNA) in view of modulating gene expression.[1] In particular, peptide nucleic acids (PNA) that are endowed of remarkable hybridization properties have attracted considerable attention.[2] PNAs are achiral and neutral DNA analogs in which the natural deoxyribose phosphate backbone has been replaced by a pseudopeptide chain. The latter is constituted of *N*-(2-aminoethyl)-*N*-(methylenecarbonyl)glycine units which provide the proper number of atoms between the nucleobases with respect to ribose (Figure 1). PNAs bind sequence-specifically to DNA and RNA with surprisingly high affinity.[2] Interestingly, these binding properties cannot be rationalized exclusively in terms of intrinsic base pair recognition since some artificial polymers of correct interbase distances are unable to hybridize with their complementary DNA.[3] Moreover, the lack of electrostatic repulsion with the phosphate backbone cannot account for their high affinity since some negatively charged synthetic polymers form even more stable duplexes with their complementary DNA than natural DNA.[4] This suggests that the backbone of PNAs plays a key role in their binding behavior.[5] A clear evidence for structuration of the PNA backbone is indeed provided by the formation of helical duplexes between either two complementary PNA strands or PNA/DNA and PNA/RNA hybrids.[6,7] Furthermore, binding orientation preferences of PNAs targeted to ssDNA, RNA, and PNA of mixed sequences and structural differences between duplexes of reverse orientation[6a,7] argue for the existence of a sense of the aminoethylglycine chain. However, conformation of the polyamide backbone has not yet been fully elucidated, and some contradicting results have appeared concerning the possibility of intramolecular hydrogen bonding.[8,5]

Some years ago, using simple dimers, we reported a sequence dependent effect on the photochemical behavior of single-stranded dinucleotides containing 4-mercaptopyrimidine bases which was ascribed to the conformation of the deoxyribose phosphate backbone.[9] Herein we proposed to take advantage of this photochemical approach to probe structural effects of the achiral backbone of PNAs. For this purpose, we have prepared single-stranded dimers **1** and **2**, which incorporate 4-thiothymine, either at their N- or C-terminal end[10] in view to compare their photochemical behavior in relation with that of the phosphate analogs. We have now demonstrated that the photochemistry of dimers **1** and **2** is modulated by the sense of the aminoethylglycine backbone and that none of them is a true mimic of the dinucleotide analogs.

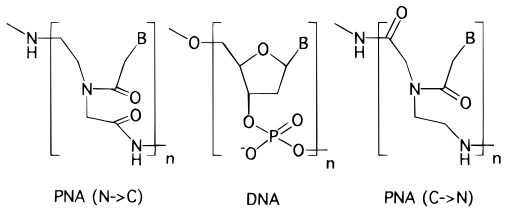

Figure 1.

Irradiation of **1** and **2** in aqueous solution at 366 nm led to their rapid disappearance with the concomitant formation of adducts **3** and **4**−**6**, respectively (Scheme 1).[13] Spectral data of these adducts are in full agreement with the proposed structures. The FAB mass spectra (positive mode) of **3** and **4** exhibited a peak at *m/z* 533 ($M + H^+$) in accordance with hydrogen sulfide elimination. Moreover the UV spectra of **3** and **4** (maxima at 268 and 317 nm) were superimposable with those of the corresponding 4-(α-thyminyl) adducts previously isolated in the phosphate series.[9] Comparison of the ^{1}H and ^{13}C NMR data of **3** and **4** with those of their respective starting materials revealed the absence of one methyl group signal and the presence of a new methylene system (δC in the 30−40 ppm region). In the case of **3**, the signal at 180 ppm (assigned from the NMR LR C−H correlation spectrum) could be attributed to C4 as expected for a 4-(α-thyminyl) adduct.[9] It is worth to notice, that for **3** and **4**, the pattern complexity of both their ^{1}H and ^{13}C NMR spectra indicated that in D_2O at 25 °C pH 7 they were present as 50/50 and 50/30/20 mixtures of conformers, respectively.[14]

The FAB mass spectra of adducts **5** and **6** displayed a peak at *m/z* 567 ($M + H^+$) as for dimer **2**. Inspection of their ^{1}H NMR spectra revealed the disappearance of a methyl group and of a H6 proton. In the case of **5** (80/20 isomer mixture in D_2O),[14] coupling of the remaining methyl signal [δH 1.42 ppm (J = 7.3 Hz) minor isomer; 1.37 ppm (J = 6.7 Hz) major isomer] indicated that it was linked to a C5 methine carbon. Saturation of the C5−C6 double bond of one of the two bases was confirmed by ^{13}C NMR data which revealed the presence

§ Institut de Chimie des Substances Naturelles.
‡ Université René Descartes.

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(10) Synthesis of **1** and **2** will be described elsewhere. Briefly, the methodology was adapted from Nielsen *et al.*[5,11] with slight modifications making use of 4-thiothymin-1yl acetic acid.[12]
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(13) Irradiation experiments were performed at pH 3.0−3.2 at a 0.26 mM concentration (100 mL) with an Original Hanau Quarzlampen Fluotest-Forte Ref. 5261, for 30 mn, under nitrogen bubbling. Progress of the reaction was monitored by UV and ^{1}H NMR spectroscopies. HPLC purification was performed on a Prep Nova-Pak HR C18 (6 μm, 60 Å) PrepPak cartridge (25 × 100 mm) using a gradient of acetonitrile in 0.05 M triethylammonium acetate (pH 4.7). Photoproducts **3**−**6** were isolated in 44, 18, 9, and 4% yield, respectively.
(14) Estimated from integration of the H6 protons.

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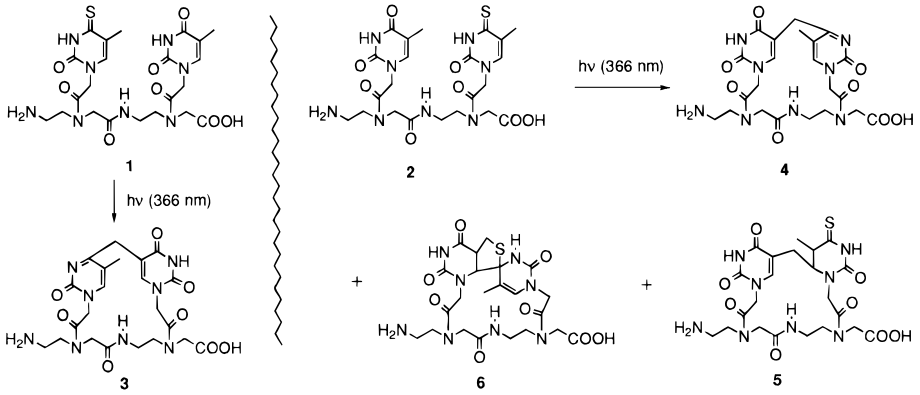
Scheme 1



of two sp^3 methine carbon signals (δC 63.5 and 47.8 ppm for **5** and δC 78.2 and 47.2 ppm for **6**) and that of a supplementary methylene (δC 27.4 ppm for **5** and 30.3 ppm for **6**). In the case of **5**, the presence of a characteristic quaternary carbon at δC 210 ppm and an UV absorption at 279 nm allowed attribution of a 5,6-dihydro-4-thiothymine structure substituted at C6 by a methylene.[9] In the case of **6**, a key sp^3 quaternary carbon (δC 83.0 ppm) assigned to C4 of the parent 4-thiothymine base and a shoulder at 255 nm on its UV spectrum were reminiscent of the spectral data recorded in the case of the tetrahydrothiophene-containing photoproducts which were isolated during photolysis of dinucleoside methylphosphonates containing N^3-methyl-4-thiothymine.[15]

All the photoproducts isolated from the photolysis of **1** and **2**, namely **3**−**6**, derive from hydrogen abstraction of the methyl of thymine by the excited thiocarbonyl group to generate a biradical precursor. This biradical can undergo direct coupling or rearrangement prior coupling. The intermediates formed after coupling between the methylene and the C4 positions undergo H_2S elimination to give photoproducts **3** and **4** which are homologous, although of opposite orientation, with respect to the N- and C-ends. Rearrangement of the initially formed radical from the C4 to the C6 position and coupling with the methylene radical gave the 6-(α-thyminyl) adduct **5**. Similarly, photoproduct **6** must derive from radical allylic migration to position C6 leading to an exo methylene function onto which the thiol group adds *via* Michael addition after C6−C4 coupling.

Although **1** and **2** are both able to generate 4-(α-thyminyl) adducts (**3** and **4**), their photochemical behavior is not equivalent since the ratio of formation of **3** and **4** greatly differs (44 *versus* 18%). Indeed, the formation of the two other adducts **5** and **6** from **2** highlights a preferred conformation of the *N*-(2-aminoethyl)-*N*-(methylenecarbonyl)glycine backbone with respect to the N- and C-ends modulating the interactions between the two adjacent thymines.

With regard to the sequence dependence observed in the single-stranded dideoxynucleotide series, hydrogen abstraction is a feature of dinucleotides having a thiosubstituted nucleobase at the 5′-end (Figure 2).[9] In contrast, a major [2 + 2] photocycloaddition reaction leading to the formation of (6-4) adducts occurs when the 4-thiopyrimidine base is located at the 3′-end of the dimer (Figure 2).[9] Whereas, one would have expected (6-4) photoproduct formation, was the PNA backbone fully flexible, no such adducts have been isolated in the two PNAs series. This strongly suggests that the prerequisite conformation for the formation of (6-4) adducts, which play such an important role in DNA photodamage,[16] is not available in such PNAs and further supports the notion of constrained flexibility of the PNA backbone. Compared to that of the phosphate analogs, the photochemistry at the single strand level

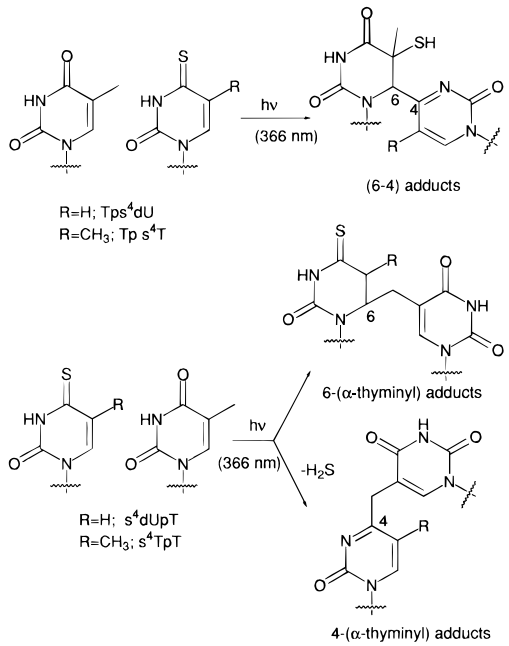


Figure 2. Photoproducts obtained in the dinucleoside phosphate series.[9]

of PNA **1** and **2** clearly revealed a different conformational behavior. Interestingly, competition between intramolecular [2 + 2] photocycloaddition and hydrogen abstraction reactions have been recently shown to depend of the conformations available to the excited molecules.[17] A very pertinent illustrating example is given by the photochemical behavior of the TpT motif. In cellular DNA, cyclobutane pyrimidine and (6-4) photoproducts are formed upon UV irradiation, whereas in bacterial spore DNA, 5-thyminyl-5,6-dihydrothymine derivative, resulting from hydrogen abstraction, is the major photoproduct to be observed.[18] This remarkable difference has been attributed to the conformation of DNA being B in cellular DNA and A in spore DNA.[19]

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Supporting Information Available: ^{1}H NMR spectra of **1**−**6** and the crude irradiation mixture of **1** and **2** and ^{13}C NMR and HRMS data of **1**−**6** (10 pages). See any current masthead page for ordering and Internet access instructions.

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