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Synthesis and Properties of Peptide Nucleic Acids Containing a Psoralen Unit

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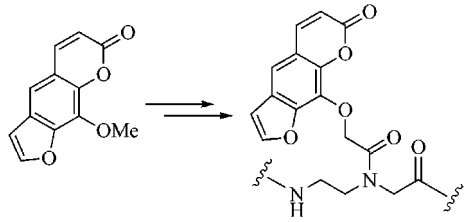
ABSTRACT



We prepared the psoralen PNA unit from 8-methoxypsoralen and synthesized various PNAs containing psoralen by a typical ^{t}Boc method. PNAs containing psoralen (P-PNA) at strand end formed a stable duplex with complementary DNA. The hybridization of P-PNA with complementary DNA resulted in a considerable decrease of the psoralen fluorescence.

Studies on the molecular basis for photosensitization induced by psoralen derivatives have attracted considerable attention in recent years.[1] These include investigations of the luminescence properties of their excited states,[2] the formation of psoralen−pyrimidine base cross-link via [2 + 2]photocycloaddition,[3] cell killing,[4] and the cure of skin diseases such as psoriasis.[5] Psoralens exhibit strong fluorescence by UVA irradiation, and the labeling of DNA and RNA by psoralen is been a promising tool for monitoring interactions with other biomolecules.[6]

Peptide nucleic acid (PNA) is a completely artificial nucleic acid consisting of a peptide backbone. PNA can recognize a complementary DNA sequence in a highly sequence-specific manner.[7] The PNA−DNA hybrid is thermodynamically more stable than DNA duplex,[8] and it is fairly independent of ionic strength in solutions.[9] Moreover, the incorporation of modified nucleobases into PNA is relatively easy. Therefore, incorporation of a psoralen unit into PNA seems to be very attractive. The sequence-specific DNA recognition by psoralen-containing PNA would potentially be used for the readout of a DNA sequence by monitoring characteristic psoralen fluorescence.

Herein we report the synthesis and properties of PNAs containing psoralen (P-PNA). We synthesized the psoralen PNA unit and then incorporated it into PNA by the ^{t}BOC method. The fluorescence of P-PNA considerably decreased upon forming a PNA−DNA hybrid.

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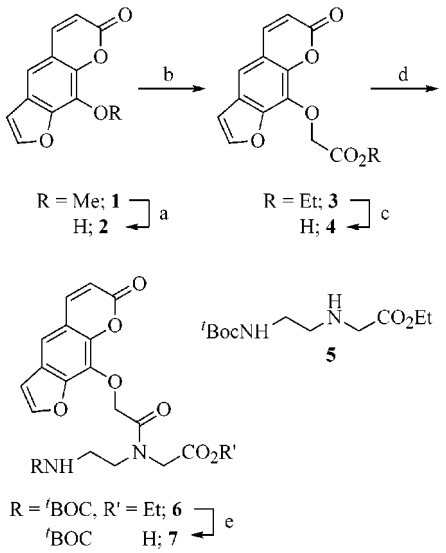
The synthetic route to the psoralen PNA unit is shown in Scheme 1. The methyl group of 8-methoxypsoralen (**1**) was

Scheme 1. Synthesis of Psoralen PNA Unit[a]



[a] Reagents and conditions: (a) boron tribromide, dichloromethane, 0 °C, 2 h, 71%; (b) ethyl bromoacetate, potassium carbonate, DMF, rt, 4 h, 81%; (c) lithium hydroxide, ethanol−water (2:1), 0 °C, 10 min, 99%; (d) EDCI, HOBt, DMF, rt, 1 h, and then **5**, rt, 4 h, 87%; (e) lithium hydroxide, ethanol−water (4:3), rt, 4 h, 86%.

removed (71%), and then the hydroxy group of **2** was coupled with ethyl bromoacetate to obtain **3** (81%). Ester **3** was hydrolyzed to **4** (99%), which was coupled with *N*-[2-(*tert*-butoxycarbonylamino)ethyl]glycine ethyl ester (**5**) (87%). Ester **6** was converted to free carboxylic acid to give the psoralen PNA unit **7** (86%). PNA oligomers were synthesized according to a typical ^{t}Boc solid-phase peptide synthesis using psoralen PNA unit **7**.[10] The crude P-PNA was purified by reversed phase HPLC. A typical example of the HPLC profile is shown in Figure 1. The composition of purified P-PNA oligomers was confirmed by MALDI-TOF mass spectrometry as shown in Table 1.

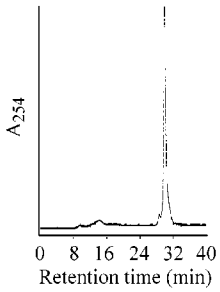


Figure 1. C_{18} reverse-phase HPLC profile of the crude reaction mixture of **P-PNA 5** obtained by solid-phase peptide synthesis (29.9 min; 0.05% TFA−water, 0−20% acetonitrile over 40 min).

Table 1. Mass Spectral Data of PNAs and T_m of Their Hybrids with Complementary DNA Strand

	PNA[a]	MALDI-TOF (M+H)$^+$	
	calcd	found	T_m (°C)[b]
PNA 1	H-GTTCCGC-NH_2		38.0
	1886.83	1886.18	
P-PNA 2	H-**P**GTTCCGC−NH_2		46.0
	2229.13	2229.62	
P-PNA 3	H-GTT**P**CGC-NH_2		*c*
	1977.89	1977.91	
P-PNA 4	H-GTTCC**P**C-NH_2		27.5
	1937.87	1938.69	
P-PNA 5	H-GTTCCGC**P**-NH_2		45.0
	2229.13	2229.61	(20.7)[d]

[a] "H" denotes a free amine end, "NH_2" denotes carboxamide end, and "P" denotes psoralin unit. [b] T_m value of hybrid with complementary DNA 5′-d(CGCGGAACC)-3′. [c] The sigmoidal melting curve was not observed. [d] T_m value of hybrid with 5′-d(CGC*C*GAACC)-3′ which forms a mismatched base pair.

P-PNA was hybridized with the corresponding complementary DNA strand, and the stability of the P-PNA−DNA hybrid (2.5 μM duplex concentration) was examined by monitoring the melting temperature in 10 mM sodium cacodylate buffer (pH 7.0). The results are shown in Table 1. The P-PNA containing the psoralen unit at the strand end (**P-PNA 2** and **P-PNA 5**) showed 7−8 °C higher T_m values than that of the corresponding psoralen-free PNA oligomer (**PNA 1**). Stabilization of the duplex by psoralen at the strand end is probably due to the π-stacking of the hydrophobic aromatic ring of psoralen with the flanking base. However, the T_m values of the P-PNA−DNA hybrids containing the psoralen unit in the interior of the PNA strand (**P-PNA 3** and **P-PNA 4**) significantly decreased. It was known that a single mismatched base pair considerably destabilizes the PNA−DNA hybrid.[11] Actually, **P-PNA 5**-DNA duplex containing a single-mismatched base pair showed ca. a 24 °C T_m decrease. The reason for the destabilization of the duplexes of **P-PNA 3** and **P-PNA 4** is that the psoralen in the interior of the PNA strand cannot form base pairs.

The fluorescence spectra of P-PNAs were measured before and after addition of an equimolar amount of the complementary or mismatched DNA strand. The emission maximum of P-PNAs was 485 nm at 330 nm excitation. Single-stranded **P-PNA 4** and **P-PNA 5** exhibited strong fluorescence, but the fluorescence of **P-PNA 2** was very weak. The hybridization of **P-PNA 4** and **P-PNA 5** with a complementary DNA strand remarkably decreased their fluorescence intensities as compared with that of single-stranded P-PNA without a shift of the emission maximum. It is noteworthy that the fluorescence intensity of **P-PNA 5** decreased 43% by hybridization with complementary DNA (Figure 2). On the other hand,

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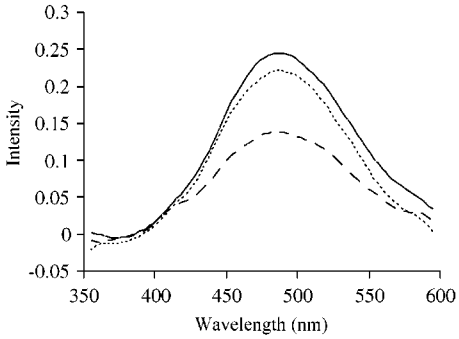

Figure 2. Fluorescence spectral changes caused by **P-PNA 5**−DNA hyblid formation. A solution of 20 μM **P-PNA 5** or **P-PNA 5**−DNA duplex in 10 mM sodium cacodylate (pH 7.0) was used. The fluorescence spectra were measured at 330 nm excitation at 21 °C. Solid line, single-stranded **P-PNA 5**; dashed line, **P-PNA 5**-5′-d(CGCGGAACC)-3′ (matched duplex); dotted line, **P-PNA 5**-5′-d(CGC*C*GAACC)-3′ (mismatched duplex).

the fluorescence intensity of the **P-PNA 5**−DNA hybrid containing a single-mismatched base pair decreased by only 7% as compared with that of single-stranded **P-PNA 5**. Thus, the full-matched P-PNA−DNA duplex is clearly distinguishable from the mismatched duplex or single-stranded P-PNA by monitoring their fluorescence intensities.

To understand the effect of the P-PNA sequence on the fluorescence intensity of P-PNA, we measured the fluorescence intensities of P-PNAs H-**P**T-NH_2, H-**P**C-NH_2, H-**P**A-NH_2, and H-**P**G-NH_2. The fluorescence intensities of H-**P**T-NH_2, H-**P**C-NH_2, and H-**P**A-NH_2 were close to that of H-**P**-NH_2, whereas almost no fluorescence of H-**P**G-NH_2 was observed (Figure 3). This result suggests that the low fluorescence intensity of **P-PNA 2** and the remarkable decrease of the fluorescence intensity by the hybridization of **P-PNA 5** and the complementary DNA

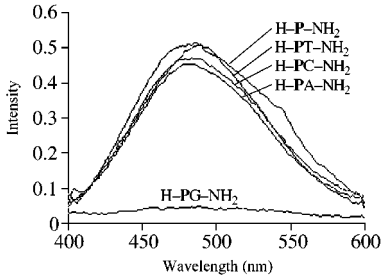

Figure 3. Fluorescence spectra of P-PNAs H-**P***N*-NH_2 (N = T, C, A or G) and H-**P**-NH_2. A solution of 20 μM P-PNA in 10 mM sodium cacodylate (pH 7.0) was used. The fluorescence spectra were measured at 330 nm excitation at 19 °C.

are due to the quenching of the fluorescence by the flanking G base.

In summary, we have disclosed a facile synthetic route to P-PNA from 8-methoxypsoralen. PNA containing a psoralen unit at strand end forms a stable duplex with complementary DNA. The fluorescence intensity of P-PNA considerably decreased by P-PNA−DNA hybrid formation because of the quenching of the fluorescence by a flanking G base. The fluorescence intensity change of P-PNA on hybridization makes it possible to use for monitoring PNA−DNA hybrid formation. P-PNA would also be useful as a tool for molecular biology and as a potent phototherapeutic drug for PUVA (psoralen plus UVA irradiation) therapy.[5b]

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

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