Synthesis and DNA Binding Property of a Novel Peptide Nucleic Acid that Contains *cis*-4-Adeninyl-L-prolinol Unit

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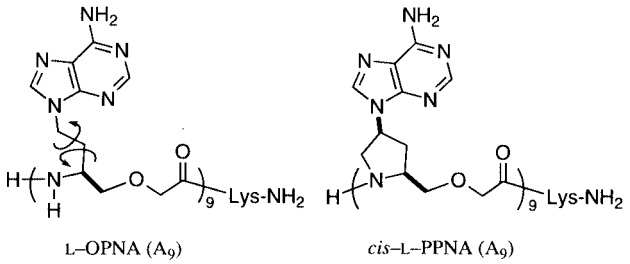
A novel oxy-peptide nucleic acid that contains a *cis*-4-adeninyl-L-prolinol unit in the main chain (PPNA) was synthesized. The peptide nucleic acid with nine adenine units [PPNA(A_9)] hybridized with the complementary DNA (T_9). The hybrid showed a very sharp melting curve with T_m = 34 °C.

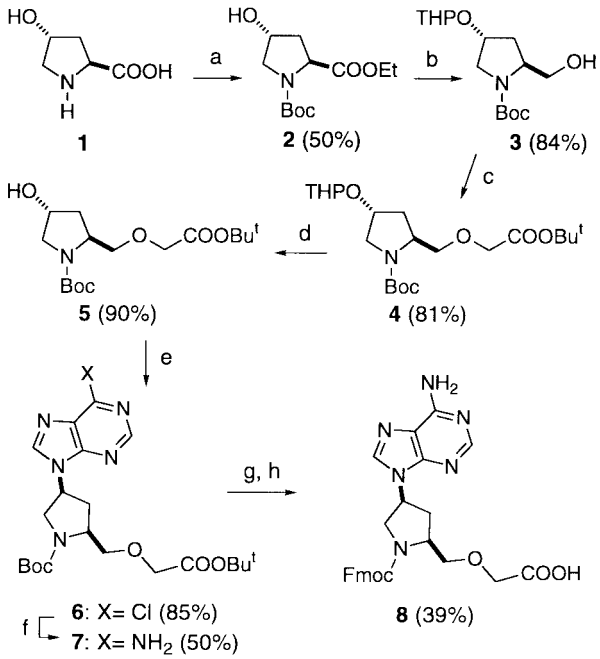
The completion of human genome analysis prompts chemists to develop substances that bind to DNAs of specific base sequences. Among a variety of attempts, peptide nucleic acids attract chemists' attention, because they are totally synthetic and cannot be decomposed by proteases or nucleases, but they bind to specific DNA sequences with higher affinity and specificity than the complementary DNA. A decade ago, Nielsen and coworkers[1] first demonstrated that the peptide nucleic acids of [–NH–CH_2–CH_2–N(CO–CH_2–Base)–CH_2–CO–]$_n$ main chain (hereafter called as PNAs) with various base sequences, hybridized with the complementary DNAs in vitro and in vivo. However, detailed study revealed that there remain some problems for their practical applications. First, the PNAs of purine-rich sequences show difficulty in its solubility and in their synthesis. Furthermore, in some cases the PNA favors triple helices rather than double helix.

To improve these drawbacks, several versions of peptide nucleic acids have been developed.[2] We have reported that a peptide of [–NH–CH(CH_2CH_2–Base)–CH_2–O–CH_2–CO–]$_n$ main chain (Oxy-PNA = OPNA) shows all-or-none-type hybridization with the complementary DNA.[3] The OPNA is superior to the PNA in its improved solubility and its ability to hybridize with pyrimidine-rich DNAs, but the pyrimidine-rich OPNAs cannot hybridize with the complementary DNAs. One of the possible reasons for the unsuccessful hybridization is inadequate side-chain orientation of the OPNA. In this letter, we report synthesis of a new version of oxy-peptide nucleic acid (*cis*-L-PPNA), in which the side-chain rotation is restricted by a pyrrolidine ring. By restricting the side chain rotations and partly the main-chain conformation, we may get information on the conformational requirements for the stable hybridization. Very recently, Altmann and coworkers also reported synthesis of PPNA that consists of *cis*-D-prolinol units with pyrimidine bases.[4]



L–OPNA (A_9) *cis*–L–PPNA (A_9)

The PPNA monomer was synthesized from *trans*-L-hydroxyproline in 10 steps (Scheme 1). The synthetic route is similar to that of the OPNA monomer,[5] except for steps from **3** to **4** and from **5** to **6**. In the former step, we used a phase-transfer condition for ether formation[6] and in the latter step 6-chloropurine was used instead of adenine base because of its high nucleophilicity.[7]



Scheme 1. Synthetic route of the PPNA monomer with an adenine base. (a) 1. $(Boc)_2O$, $NaHCO_3$/ dioxane–H_2O, rt,12 h, 2. EtBr/ DMF, rt, 50 h; (b) 1. DHP, PPTS/ CH_2Cl_2, rt,12 h, 2. $NaBH_4$/ EtOH, 0 °C, 16 h; (c) *tert*-Butyl bromoacetate, Bu_4NHSO_4 / benzene–50% aq-NaOH (3:1); (d) PPTS/ EtOH, rt, 17 h; (e) 6-chloropurine, DEAD, Ph_3P/ THF, rt; (f) NH_3/ EtOH, rt, 3 d; (g) HBr/AcOH; (h) Fmoc-OSu/ MeCN–H_2O.

The total yield of the Fmoc-δ-amino acid **8** was about 10% from *trans*-L-hydroxyproline. The δ-amino acid was identified by mass spectroscopy.[8] The PPNA with nine adenine bases [PPNA(A_9)=ppA_9] was prepared by solid-phase method as in the case of OPNA(A_9) (=oA_9).[3] A single L-lysine unit was attached at the C-terminal to increase the affinity to DNA. The ppA_9 was purified by preparative HPLC and identified by TOF-mass spectroscopy.[9]

CD spectra of the ppA_9-dT_9 mixtures of various molar ratios were measured. Spectra of mixtures with [ppA_9] > [dT_9] are shown in Figure 1 (left) and those with [ppA_9] < [dT_9] are in Figure 1 (right). In both sets of spectra, several isodichroic

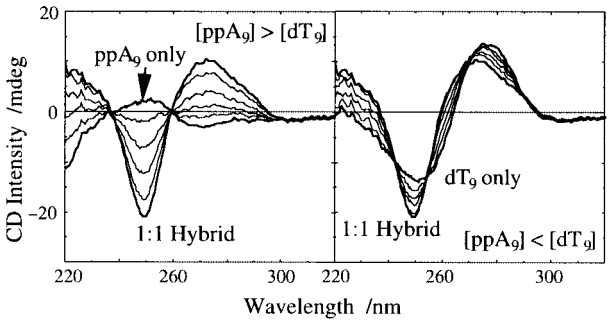


Figure 1. CD spectra of the ppA $_9$-dT$_9$ mixtures of different molar ratios: (left) [ppA$_9$]/[dT$_9$] = 10/0, 9/1, 8/2, 7/3, 6/4, and 5/5, (right) [ppA$_9$]/[dT$_9$] = 5/5, 4/6, 3/7, 2/8, 1/9, and 0/10.

points are observed, indicating that only the hybrid and free DNA are present under [ppA$_9$] < [dT$_9$] and only the hybrid and free PPNA under [ppA$_9$] > [dT$_9$]. The CD intensity at 249 nm showed a minimum when [ppA$_9$] = [dT$_9$]. These facts indicate that the ppA$_9$ hybridizes with dT$_9$ to form a 1:1 hybrid with very high affinity and no triplex is formed. Note that the CD spectrum of the 1:1 hybrid is very similar to that of the oA$_9$-dT$_9$ hybrid.[3]

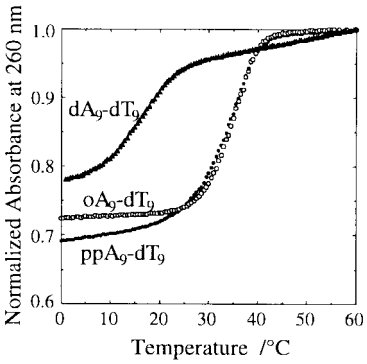


Figure 2. UV melting curves of the 1:1 mixtures of ppA$_9$-dT$_9$, oA$_9$-dT$_9$, and dA$_9$-dT$_9$ in 150 mM NaCl, 10 mM NaH_2PO_4, and 0.1 mM EDTA, pH 7.0. [ppA$_9$]=[dT $_9$]=[oA $_9$]=[dT $_9$]=5 μM. Temperature was raised at an interval of 0.5 °C/30 s.

Temperature dependence of the absorption intensity at 260 nm of the ppA$_9$-dT$_9$ 1:1 mixture, together with those for the oA$_9$-dT$_9$ and dA$_9$-dT$_9$ mixtures are shown in Figure 2. The melting temperatures were 34 °C for the ppA$_9$-dT$_9$, 35 °C for the oA$_9$-dT$_9$, and about 15 °C for the dA$_9$-dT$_9$ hybrid, respectively. Similar to the oA$_9$-dT$_9$ case, the melting curve of the ppA$_9$-dT$_9$ hybrid showed a very sharp transition, suggesting that the ether linkage in the main chain is responsible for the sharp melting curve. Presumably, the flexible polyetheramide main chain causes a large entropy loss and a large enthalpy stabilization when it forms duplex with DNA, and results in the sharp melting curves. The sharp melting curve is very advantageous for the PPNA and OPNA when they were applied as antisense drugs.

Since the ppA$_9$-dT$_9$ and oA$_9$-dT$_9$ hybrids showed similar melting curves and CD spectra, the side-chain orientations of the two oxy-peptide nucleic acids in the hybridized state may be similar. The information will be helpful in designing conformationally matched oxy-peptide nucleic acids, especially for those with pyrimidine-rich sequences.

It is to be noted that the restriction of the side-chain orientation does not suppress the hybridization, if the orientation is appropriate. The flexible main chain and the restricted side chain of PPNA are common conformational characteristics to DNA and RNA.

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References and Notes

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6 Compound **3** (5.8 g, 16 mmol), *tert*-butyl bromoacetate (6.2 g, 32 mmol), tetrabutylammonium sulfate (1.4 g, 4 mmol) were dissolved into benzene/50% NaOH (3/1) mixture (56 mL). The mixture was stirred at 10 °C for 5 h. The organic layer was washed with 2M HCl, dried over anhydrous magnesium sulfate and evaporated. The crude product was purified by a silica gel column to give **4** (8.8 g, 81%).

7 Compound **5** (2.3 g, 6.94 mmol), 6-chloropurine (1.1 g, 6.94 mmol), and triphenyl phosphine (2.7 g, 10.4 mmol) were dissolved into anhydrous THF (20 mL) under argon atmosphere. The mixture was cooled to 0 °C and diethyl azodicarboxylate (1.6 mL, 10.4 mmol) was added dropwise. The reaction was continued overnight at room temperature. The product was purified by a silica gel column to give **6** (2.75 g, 85%). The latter was dissolved in ammonia-saturated ethanol and stored at room temperature for 3 days. The solvent was evaporated and the residue was purified by a silica gel column to give **7** (403 mg, 50%). ^{1}H NMR of **7** showed a single peak for each proton, indicating that the inversion at the 4th carbon was almost 100%; (300 MHz, $CDCl_3$,) δ = 1.52 (9H, s), 1.53 (9H, s), 2.76 (2H, m), 3.62 (1H, m), 3.80 (2H, m), 4.05 (2H, q), 4.21 (1H, br), 4.30 (1H, br), 5.19 (1H, m), 6.05 (2H, s), 8.22 (1H, s), 8.40 (1H, s).

8 TOF-mass data of **8**, found, *m/z* 514.8 $(M+H)^+$; calcd for $C_{27}H_{26}N_6O_5$, 515.2. ^{1}H NMR of **8** showed unresolved peaks that could not be assigned to individual protons.

9 TOF-mass data of ppA$_9$, found, *m/z* 2613.5 $(M+H)^+$; calcd for $C_{114}H_{142}N_{57}O_{19}$, 2613.2.