Modification of the Binding Affinity of Peptide Nucleic Acids (PNA). PNA with Extended Backbones consisting of 2-Aminoethyl-β-alanine or 3-Aminopropylglycine Units

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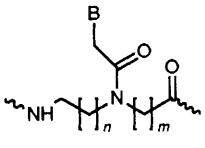
The binding affinity between peptide nucleic acids (PNA) and DNA is reduced by incorporation of PNA units with extended backbones.

We have recently reported the properties of peptide nucleic acids (PNA), oligonucleotide analogues in which the deoxyribosephosphate backbone is replaced by an achiral and neutral peptide backbone (**1**). These compounds were designed by means of computer modelling in order to obtain highly selective and efficient reagents for DNA and RNA recognition. Such reagents are of interest since they have the potential to serve as gene-targeted drugs, as tools in molecular biology, and in diagnostics.[1]

In our first approach towards a suitable PNA structure the backbone consisted of repeating (2-aminoethyl)glycine units, with thymine and cytosine attached through methylene carbonyl linkers.[2–4] Hybrids between such PNA oligomers and their complementary single stranded DNA oligomers with 2 PNA : 1 DNA stoichiometry have shown remarkably high stabilities (measured as melting temperatures).[3,4]

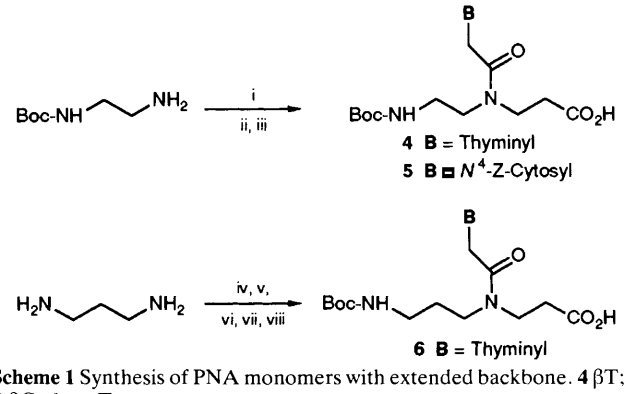
In order to investigate the variability of the PNA structure, we extended the backbone by one methylene group in a single unit in a PNA decamer (**2,3**). The extra methylene group was introduced by using thymine or cytosine monomers with either a (2-aminoethyl)-β-alanine or a (3-aminopropyl)glycine backbone rather than the original (2-aminoethyl)glycine, and we now report the synthesis and binding properties of these analogues. It is demonstrated that the binding affinity is lowered while the sequence specificity is preserved.

The syntheses of the thymine- or cytosine-containing monomers with extended backbones are outlined in Scheme 1. The PNA oligomers containing βT, βC and apgT were prepared by standard Merrifield solid phase peptide synthesis as previously described.[3,4] The oligomers were characterized by FAB mass spectrometry {H-T_4(βT)T_5-Lys-NH_2: *m/z* 2820.71 [M + 1] (calc. 2820.15); H-T_4(βC)T_5-Lys-NH_2: *m/z* 2806.11 [M + 1] (calc. 2806.16); H-T_4(apgT)T_5-Lys-NH_2: *m/z* 2820.92 [M + 1] (calc. 2820.15)}.



1 (*n* = 1, *m* = 1), the repeating unit in the initial PNA structure
2 (*n* = 1, *m* = 2), the unit with 2-aminoethyl-β-alanine backbone (βB)
3 (*n* = 2, *m* = 1), the unit with the 3-aminopropylglycine backbone (apgB)
B = thymin-1-yl or cytosyl.

The thermal stability of the hybrids between the resulting PNA oligomers H-T_4(X)T_5-Lys-NH_2 (X = βT, βC or apgT) and the complementary oligodeoxynucleotides or oligodeoxynucleotides with a single mismatch were determined by T_m measurements (Table 1). The unmodified PNA H-T_{10}-Lys-NH_2/$(dA)_{10}$ hybrid melts at 72 °C (Table 1, Row 1, X = T), whereas introduction of just a single monomer with extended backbone (Table 1, Row 1, X = βT or apgT) lowers T_m by 13 and 11 °C, respectively, indicating a less favourable geometry. The incorporation of a mismatch at position 5 (corresponding to the X site) in the oligodeoxynucleotide causes a further decrease in T_m of approximately 10 °C (Table 1, Rows 2–4). The same observation is made with cytosine attached to the (2-aminoethyl)-β-alanine backbone, thereby excluding the sequence specificity being due to any special structure of homothymine oligomers. The T_m for the H-T_4(βC)T_5-Lys-NH_2/$(dA)_4$(dG)$(dA)_5$-hybrid is 61 °C (Table 1, Row 4, X = βC) whereas any other base opposite to the βC unit resulted in significantly lower T_m values (Table 1, Rows 1–3, X = βC).



Scheme 1 Synthesis of PNA monomers with extended backbone. **4** βT; **5** βC; **6** apgT.

Reagents and conditions: i, CH_2=$CHCO_2Me$ in MeCN, reflux, 20 h; ii, BCH_2CO_2Pfp,[3] Et_3N in DMF, 20 h; iii, aqueous NaOH, 10 min; iv, $ClCH_2CO_2H$; v, MeOH, HCl; vi, *p*-$NO_2C_6H_4OBoc$, H_2O–dioxane, pH 10; vii, BCH_2CO_2H, DhbtOH, DCC in DMF–CH_2Cl_2; viii, NaOH, MeOH, 1 h. Pfp = pentafluorophenyl; DMF = dimethylformamide; Boc = *tert*-butoxycarbonyl; Dhbt = dihydro-4-oxobenzotriazolyl; DCC = dicyclohexylcarbodiimide; Z = benzyloxycarbonyl.

Table 1 Melting temperatures (T_m) for the PNA–DNA hybrids

PNA	DNA	pH	T_m/°C[a] X = T	X = βT	X = βC	X = apgT
(1) H-T_4(X)T_5-Lys-NH_2	$(dA)_{10}$	7.0	72	59	45	61
(2) H-T_4(X)T_5-Lys-NH_2	$(dA)_4(dC)(dA)_5$	7.0	62	50	36	51
(3) H-T_4(X)T_5-Lys-NH_2	$(dA)_4(dT)(dA)_5$	7.0	62	50	34	50
(4) H-T_4(X)T_5-Lys-NH_2	$(dA)_4(dG)(dA)_5$	7.0	60	48	61	49
(5) H-T_4(X)T_5-Lys-NH_2	$(dA)_4(dG)(dA)_5$	5.0			67	
(6) H-T_4(X)T_5-Lys-NH_2	$(dA)_4(dG)(dA)_5$	9.0			58	

[a] The melting temperatures of the hybrids were determined as previously described.[3] The solutions were 10 mmol dm^{-3} in phosphate, 100 mmol dm^{-3} in NaCl and 0.1 mmol dm^{-3} in ethylenediaminetetraacetic acid.

This shows that the specificity of the PNA–DNA recognition is preserved even though the affinity is lowered.

The previously reported homopyrimidine PNA all showed a 2:1 stoichiometry (determined by UV titrations) upon binding to oligodeoxynucleotides,[3,4] and as expected the presently reported oligomers also bind in a 2:1 stoichiometry. We propose a triple helix structure for these complexes involving Watson–Crick and Hoogsteen base pairing. In accordance with this model PNA containing cytosine form more stable complexes with complementary oligonucleotides at acidic pH (Table 1, Rows 4–6, X = βC), indicating the importance of a protonated cytosine in the Hoogsteen strand for optimal binding. As expected, this effect is absent with adenine in the DNA strand opposite to βC in the PNA (data not shown).

As would be predicted by considering the dramatic lowering of T_m upon incorporation of a single unit of (βT), PNA H-$(\beta T)_{10}$-Lys-NH_2 showed no hypochromicity when mixed with its complementary DNA, indicating the absence of stable complexes.

To conclude, we have demonstrated that the stability of PNA/DNA complexes can be modulated by altering the PNA backbone. The stability was lowered significantly upon extension of just a single unit which shows the importance of having the correct interbase distance. The results are in agreement with the computer modelling predictions, and suggest that the binding affinity between PNA and DNA can be finely controlled.

This work was supported by the Danish Natural Science Research Council. Ms Vivi Hedeboe, Ms Annette W. Jørgensen and Mr Nikolaj Hansen are thanked for skilled technical assistance.

Received, 22nd December 1992; Com. 2/06792G

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