Hybridization properties of nucleic acid analogs containing β-aminoalanine modified with nucleobases

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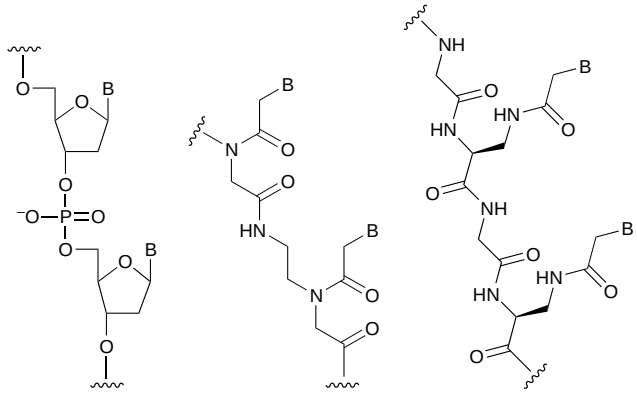
Oligopeptides containing *N*β-(thymin-1-ylacetyl)-β-aminoalanine and *N*β-(cytosin-1-ylacetyl)-β-aminoalanine moieties synthesized on a solid phase using standard Boc chemistry showed hybridization properties with single stranded DNA and RNA, and also with double stranded DNA, at pH 7.0.

The development of artificial regulatory molecules for specific gene expression is of special interest from the medicinal and biological points of view.[1] In particular, nucleic acids and their analogs, such as antisense or triple-helix forming oligonucleotides, ribozymes and decoy RNAs, are promising reagents as genetic medicines. In spite of intensive efforts to improve their chemical and biological properties, several problems still remain to be solved, which include degradability by cellular nucleases, impermeability through cell membranes and low hybridization affinity caused by electrostatic repulsion between phosphate backbones.[2]

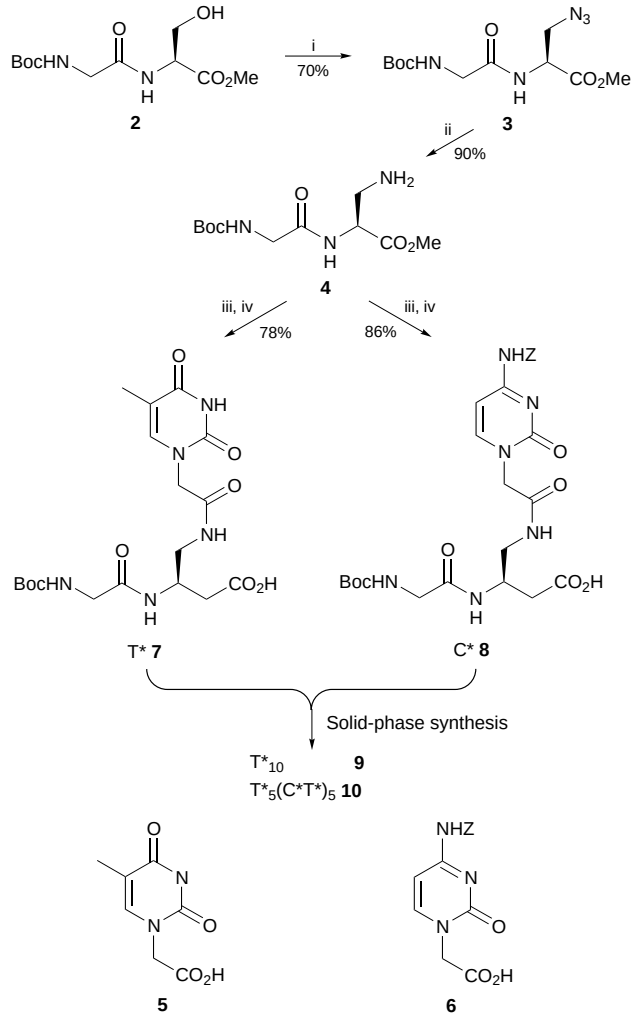
Among various chemical modifications that could be performed on such synthetic DNAs and RNAs, introduction of a peptide backbone into such molecules seems to be attractive because peptide compounds can be expected to have such preferable properties as nuclease resistance, membrane permeability and a good affinity and specificity to nucleic acids, as can be seen in a number of DNA binding proteins.

In this study, oligopeptides **1** containing β-aminoalanine bearing a nucleobase were synthesized and their hybridization properties with ssDNA, ssRNA and dsDNA were examined *via* T_m measurement.



Syntheses of *N*-*tert*-butoxycarbonylglycyl-*N*β-(thymin-1-ylacetyl)-L-β-aminoalanine **7** (T*) and *N*-*tert*-butoxycarbonylglycyl-*N*β-(cytosin-1-ylacetyl)-L-β-aminoalanine **8** (^{Z}C*) were achieved as shown in Scheme 1.[3] These protected amino acids **7** and **8** were readily applicable to solid-phase peptide synthesis using standard Boc chemistry on methylbenzhydrylamine (MBHA) resin.[4] The obtained 20-mer peptide T^*_{10} (**P**) and 30-mer peptide $T^*_5(C^*T^*)_5$ (**Q**) were purified by RP HPLC and confirmed by FAB mass spectrometry {**P**: $C_{122}H_{154}N_{52}O_{51}$ calc. 3164.9, found 3165.9 $[(M + H)^+]$; **Q**: $C_{177}H_{224}N_{82}O_{71}$ calc. 4636.2, found 4637.2 $[(M + H)^+]$}.

As shown in Table 1, formation of a hybrid double strand by **P** and dA_{10} was confirmed by observing a hypochromic effect at pH 7.0. It should be noted that the melting temperature (T_m) was higher than that for the natural DNA double strand by 13.5 °C (1.35 °C base^{-1}).[5] The stability of the hybrid was not affected by salt concentration. The peptide **P** also formed a double strand with rA_{10}, and the T_m was 21 °C. Moreover, it was shown that **P** could bind to double stranded DNA by triple helix



Scheme 1 *Reagents and conditions*; i, Ph_3P, NaN_3, CBr_4, DMF, room temp., 24 h; ii, Pd/C, H_2, MeOH, room temp., 20 h; iii, **5** or **6**, HOBt, DCC, CH_2Cl_2, 0 °C to room temp., 5 h; iv, 1 M NaOH, room temp., 12 h

Table 1 Double and triple helix formation by peptide DNA analogs **P** and **Q**

Strand	Target	T_m[a]/°C	Δ T_m/°C
dT_{10}	dA_{10}	23	—
PNA	dA_{10}	73[b]	+50
P	dA_{10}	36.5	+13.5
P	dA_{10}	36.2[c]	+13.2
P	dA_{10}	36.4[d]	+13.4
P	rA_{10}	21.4	—
dT_{10}	5′-GCTA_{10}TCG-3′/3′-CGAT_{10}AGC-5′	20.9	—
P	5′-GCTA_{10}TCG-3′/3′-GCAT_{10}AGC-5′	19.3	−0.4
5′-T_5-$(CT)_5$-3′	5′-GCT$A_5$$(GA)_5$TCG-3′/3′-CGA$T_5$ $(CT)_5$AGC-5′	21.3	
Q	5′-GCT$A_5$$(GA)_5$TCG-3′/3′-CGA$T_5$ $(CT)_5$AGC-5′	22.8	+1.5

[a] Measured in a buffer containing 50 mM Tris, pH 7.0, 20 mM $MgCl_2$, 100 mM NaCl, [Strand] = [Target] = 0.5 mM. [b] 140 mM NaCl, 10 mM sodium phosphate, pH 7.2. [c] 20 mM $MgCl_2$, 1.0 M NaCl, pH 7.0. [d] 0 M $MgCl_2$, 0 M NaCl, pH 7.0.

formation with comparable affinity (T_m = 19.3 °C, ΔT_m = −0.7 °C).

Oligopeptide **Q** containing mixed pyrimidine bases was also shown to form a triple helix with double stranded DNA (T_m = 22.8 °C, ΔT_m = +1.5 °C).[6]

Studies on the pH dependence of these hybridization properties revealed that the peptide **P**, which contains only thymine bases, binds to ssDNA and dsDNA with an affinity independent of pH, whereas the peptide **Q**, which contains thymine and cytosine bases, binds to dsDNA with less affinity as the pH value increased (Fig. 1). This pH dependency was interpreted to mean that triple helix formation by **Q** with dsDNA required protonation of the cytidine bases in **Q**. These results strongly suggested that **P** and **Q** are binding to dsDNA in the major groove by Hoogsteen hydrogen bonding.

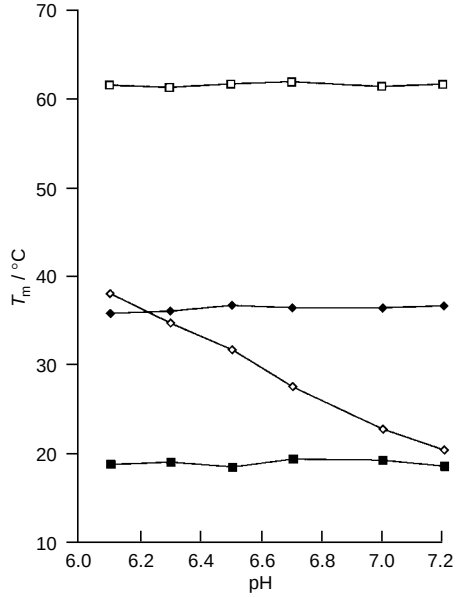


Fig. 1 pH dependence of hybridization: (□) dsDNA, (◆) **P**/ssDNA, (◇) **Q**/dsDNA and (■) **P**/dsDNA

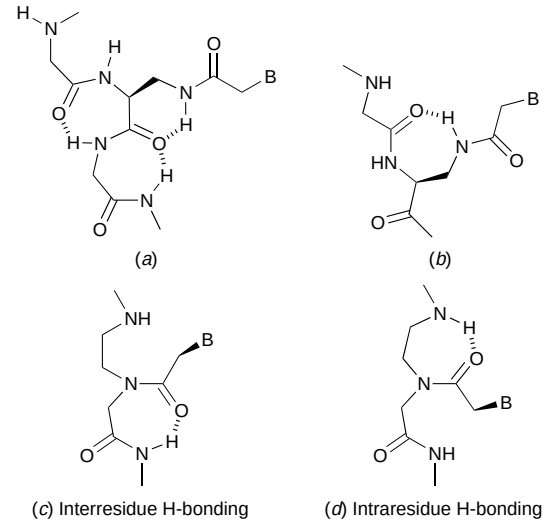


Fig. 2 Intramolecular hydrogen bonding in **1** [(*a*) and (*b*)] and PNA [(*c*) and (*d*)]

The oligopeptides **P** and **Q** were designed to have nucleobase moieties at an interval of six atoms on the backbone, which was previously demonstrated to be critical for hybrid formation with DNA or RNA by Nielsen.[7] The linkage between the nucleobase and the backbone in **P** and **Q** is longer than that in DNA or PNA by two atoms. It was also pointed out by Nielsen's group that the linkage is slightly flexible. It can be postulated that Watson–Crick base pairing in the duplex and Hoogsteen base pairing in triplex by **P** and **Q** is made possible because of the favorable orientation of the base moieties caused by intramolecular hydrogen bond, as shown in Fig. 2.[8]

The present study demonstrates that these novel peptide DNA analogs are promising candidates for antisense and triple helix forming molecules. Further studies to reveal the chemical and biological properties of the peptide DNA analogs are now in progress in our laboratory.

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Notes and References

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