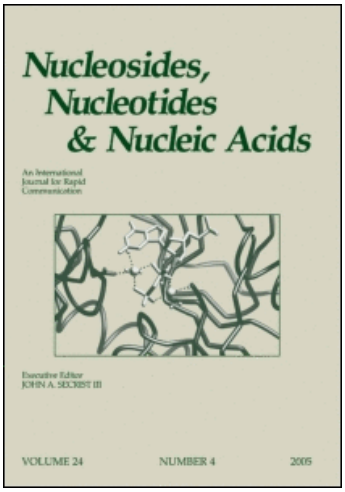

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SPECIFIC BINDING AND STABILIZATION OF DNA AND PHOSPHOROTHIOATE DNA BY AMPHIPHILIC α-HELICAL PEPTIDES

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ABSTRACT

Synthetic cationic peptides with amphiphilic α-helical structure were found to have DNA binding ability to stabilize double and triple stranded DNA. The stabilization effect was significant for cationic α-helical peptides indicating the importance of an electrostatic interaction of a positive charge of peptides and a negative charge of DNAs.

INTRODUCTION

Genetic medicines such as antisense or triple-helix forming oligonucleotides, ribozymes and decoy RNAs are attracting special interest from a medicinal and a biological aspects (1). Conjugation of oligonucleotides with functional peptides as well as chemical modifications on phosphodiester backbone, nucleobases, and/or sugar moiety is a fascinating way to add properties to antisense or triple-helix forming oligonucleotides such as enhanced membrane permeability, improved stability against cellular nucleases and increased affinity and specificity (2).

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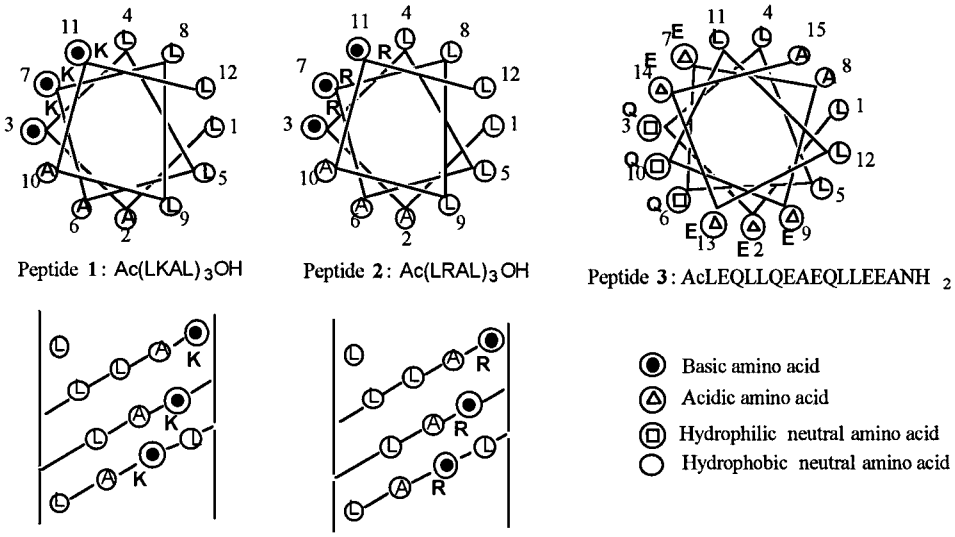
The present study describes the effect of hybridization of cationic amphiphilic α-helical peptides on the thermal stability of the hybrids of double or triple helical DNA containing phosphorothioate oligonucleotide.

RESULTS AND DISCUSSIONS

Two cationic peptides **1** and **2** (3) and an anionic peptide **3** which were designed to form amphiphilic α-helical structure were prepared on solid phase using standard Fmoc chemistry and characterized by ESIMS.

peptide **1**: $Ac(LKAL)_3OH$ (ESIMS m/z = 1338.0 (M+H))
peptide **2**: $Ac(LRAL)_3OH$ (ESIMS m/z = 1421.1(M+H))
peptide **3**: $AcLEQLLQEAEQLLEEANH_2$ (ESIMS m/z = 1798.0 (M+H))



Interaction of the peptides with dsDNA hybrids were characterized by CD spectra and UV melting study. UV melting analysis was performed at pH 7.0 in the presence of 100 mM NaCl. The results are shown in Table 1.

The results clearly showed that the cationic peptides **1** and **2** bound to dsDNA only in the absence of Mg^{2+} and stabilized the duplex by $+7.0^{\circ}C$ and $+9.5^{\circ}C$ in Tm, respectively, whereas the anionic peptide showed no effects. α-Helical structure of the peptides **1** and **2** were intensively observed in CD spectra (not shown). The extent of the stabilization by peptides **1** and **2** were larger than that by spermine, which suggests the importance not only of the electrostatic interaction between peptide side chains and DNA backbone but also of the interaction of hydrophobic face of α-helix of **1** and **2** with the major or minor groove of DNA. The difference in the effects of peptide **1** and **2** should be referred to the side chain strucutres and

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Table 1. Melting Temperature of Double and Triple Stranded DNA Containing S-DNA in the Presence of Amphiphilic α-Helical Peptides

DNA	Peptides	Mg^{2+}	Tm (°C)	ΔTm (°C)
dsDNA	none	+	64.5	—
5′-GCTAAAAAGAGAGAGAGATCG-3′		−	52.0	—
3′-CGATTTTTCTCTCTCTCTAGC-5′	peptide 1 (4 eq.)	+	63.5	−1.0
		−	53.0	+1.0
	peptide 2 (12 eq.)	+	66.0	+1.5
		−	59.0	+7.0
	peptide 2 (4 eq.)	+	65.0	+0.5
		−	53.0	+1.0
	peptide 2 (12 eq.)	+	66.0	+1.5
		−	61.5	+9.5
	peptide 3 (12 eq.)	+	64.0	−0.5
		−	52.5	+0.5
	spermine (12 eq.)	+	64.0	−0.5
		−	56.5	+4.5
S-DNA/DNA	none	+	34.5	—
5′-S-(TTTTTCTCTCTCTCT)-3′ 3′-AAAAAGAGAGAGAGA-5′	peptide 2 (12 eq.)	+	39.0	+4.5
DNA/RNA	none	+	57.5	—
5′-TTTTTCTCTCTCTCT-3′ 3′-r(AAAAAGAGAGAGAGA)-5′	peptide 2 (12 eq.)	+	58.5	+1.0
S-DNA/RNA	none	+	39.0	—
5′-S-(TTTTTCTCTCTCTCT)-3′ 3′-r(AAAAAGAGAGAGAGA)-5′	peptide 2 (12 eq.)	+	43.0	+4.0

Conditions: 50 mM Tris buffer, pH 7.0, [NaCl] = 100 mM, [$MgCl_2$] = 20 mM, [OilgoDNA] = 3 μM.

Table 2. Melting Temperature of Triple Stranded DNA and S-DNA/dsDNA Hybrids in the Presence of Amphiphilic α-Helical Peptides

Target	Peptide	Mg^{2+}	Tm_1/ ΔTm_1[a] (°C)	Tm_2/ ΔTm_2[b] (°C)
Parallel tsDNA 5′-TTTTTCTCTCTCT-3′	none	+	15.0/−	63.0/−
5′-GCTAAAAAGAGAGAGAGATCG-3′ 3′-CGATTTTTCTCTCTCTCTAGC-5′	2 (12 eq.)	+	33.0/+18.0	67.0+3.0
Anti-parallel is DNA 3′-AAAAAGAGAGAGAGA-5′	none	+	42.0/−	66.0/−
5′-GCTAAAAAGAGAGAGAGATCG-3′ 3′-CGATTTTTCTCTCTCTCTAGC-5′	2 (12 eq.)	+	43.5/+1.5	67.0+1.0
Parallel S-DNA/dsDNA 5′-S-(TTTTTCTCTCTCT)-3′	none	+	ND[c]	63.0
5′-GCTAAAAAGAGAGAGAGATCG-3′ 3′-CGATTTTTCTCTCTCTCTAGC-5′	2 (12 eq.)	+	45.0	63.0/+0

Conditions: 50 mM Tris buffer, pH 7.0, [NaCl] = 100 mM, [$MgCl_2$] = 20 mM, [OilgoDNA] = 3 μM.
a. Melting temperature of Hoogsteen base pair.
b. Melting temperature of Watson-Crick base pair.
c. Not determined.

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emphasized the stronger interaction of the guanidinium moiety in **2** and the DNA phosphodiester backbone.

It should be pointed out that in the presence of Mg^{2+}, peptide **2** interacted predominantly with the hybrids containing phosphorothioate DNA. Peptide **2** stabilized S-DNA/DNA hybrid by $+4.5^{\circ}C$ whereas it stabilized dsDNA only by $+1.5^{\circ}C$. Similarly, increase of Tm of S-DNA/RNA-peptide **2** complex ($+4.0^{\circ}C$) was larger than that of DNA/RNA hybrid-peptide **2** complex ($+1.0^{\circ}C$).

The interaction of the peptides and dsDNA hybrids were also characterized by CD spectra and UV melting study and the results are shown in Table 2. It is of a special interest that peptide **2** bound to tsDNA and stabilized very specifically only the third strand of S-DNA (Hoogsteen base pair), while it did not stabilize either the third strand of natural phosphodiester DNA (Hoogsteen base pair), or the second strand of DNA (Watson-Crick base pair).

The present study encouragingly suggests that cationic amphiphilic α-helical peptide permits the use of a phosphorothioate oligonucleotide as an antisense and an antigene drugs.

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