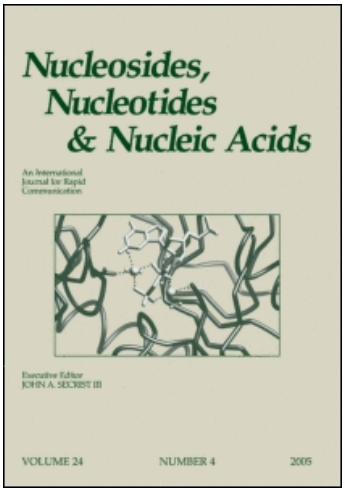

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AMPHIPHILIC β-SHEET PEPTIDES CAN BIND TO DOUBLE AND TRIPLE STRANDED DNA

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ABSTRACT

It was shown that synthetic peptides with amphiphilic β-sheet structure can bind to and stabilize double and triple stranded DNA. CD spectra indicated that β-sheet conformation of peptides were emphasized in the presence or absence of DNA and that no significant change of DNA conformation occurred. UV melting study at pH 7.0 revealed that interaction of peptides with DNA and its hybrids are sensitive and specific depending the host structure.

Conjugation of oligonucleotides with functional peptides is a fascinating way to add properties to antisense or antigene oligonucleotides such as enhanced cellular uptake, improved stability against cellular nucleases and increased affinity and specificity.

Gramicidin S (GS) (1, 2) is a cyclic decapeptide antibiotic which has intramolecular antiparallel β-sheet structure with four hydrogen bonds between L-Val and L-Leu residues and two type II′ β-turns around the D-Phe-L-Pro sequences (Fig.). GS shows an amphiphilic character because it has positively charged side chains of L-Orn oriented on one side of the β-sheet and hydrophobic ones of L-Val and L-Leu on the other side.

On the other hand, it has been shown that antiparallel β-sheet peptide can form right-handed double helices with helix parameters comparable to those of double

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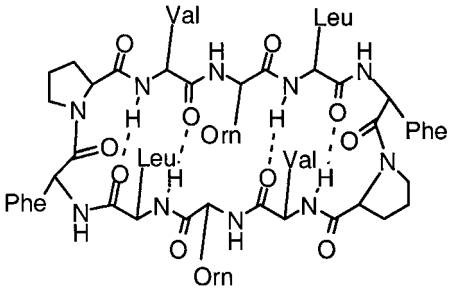

Figure. β-Sheet structure of Gramicidin S.

helical DNA, and the antiparallel β-sheet motif in a number of proteins recognizes the major groove of double-helical DNA (3).

The present study describes the effect of GS as an antiparallel β-sheet peptide on the thermal stability of double and triple stranded DNA.

CD spectra of mixed solution of GS and double stranded DNA (not shown) indicated that β-sheet conformation of GS was maintained in the presence or absence of dsDNA and that no significant conformational change of dsDNA occurred in the presence of GS.

As shown in Table 1, UV melting study at pH 7.0 revealed that interaction of GS with dsDNA and hybrid duplex are sensitive and specific depending on the structure of the target. Although GS stabilized dsDNA with mixed sequence evenin the presence of Mg^{2+} ($+6.0^{\circ}$C), it stabilized dsDNA with homosequence only in the absence of Mg^{2+} ($+6.0^{\circ}$C), i.e., no significant effect was observed in the presence of Mg^{2+}. These results were interpreted that the interaction of GS with dsDNA was mainly due to the electrostatic one between cationic side chains of GS and anionic phosphate backbone of DNA and that an antiparallel β-sheet structure of GS made the interaction very sensitive to the structure of minor or major groove of dsDNA. Competitive binding study of GS against distamycin A (Dis), which is known to bind to the minor groove of B-form dsDNA, indicated that prior addition of GS to the solution interfered with the binding of distamycin A to dsDNA. The unique feature of UV melting curves (not shown) suggested that distamycin displaced the prior added GS from the minor groove as temperature is increased. These results strongly suggest that GS interacted with dsDNA in the minor groove.

Although GS showed no significant effect to stabilize the hybrid duplex of DNA and RNA in the presence of Mg^{2+}, it slightly stabilized the duplex of S-DNA and DNA (ΔTm $= +1.5^{\circ}$C) and more largely stabilized the duplex of S-DNA and RNA (ΔTm $= +6.0^{\circ}$C). In the absence of Mg^{2+}, the 15 mer phosphorothioate DNA containing homopyrimidine bases was not observed to form a stable duplex with the complementary homopurine phosphodiester DNA strand without GS. On the contrary, stable hybrid duplex formation between S-DNA and DNA was clearly observed in the presence of GS even in the absence of Mg^{2+}(Tm $= 23.0^{\circ}$C). These results imply that GS has greater affinity to phosphorothioate DNA compared with

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Table 1. Melting Temperature of Double Stranded DNA in the Presence of Gramicidin S

DNA	Ligands	Mg^{2+}	Tm (°C)	ΔTm (°C)
dsDNA	none	+	70.1	—
5′-GCTAGCTGAGCTGACAGTGCT-3′	GS (4 eq.)	+	74.3	+4.2
3′-CGATCGACTCGACTGTCACGA-5′	GS (16 eq.)	+	76.0	+6.0
	GS (21 eq.)	+	71.5	+1.0
dsDNA	none	+	64.0	—
5′-GCTAAAAAGAGAGAGAGATCG-3′	none	−	52.0	—
3′-CGATTTTTCTCTCTCTCTAGC-5′	GS(16 eq.)	+	64.0	+0
	GS (16 eq.)	−	58.0	+6.0
	GS (16 eq.) + Dis (16 eq.)[a]	+	64.0	+0
	GS (16 eq.) + Dis (16 eq.)[a]	−	52.5	+0.5
	Dis (16 eq.) + GS (16 eq.)[b]	+	69.0	+5.0
	Dis (16 eq.) + GS (16 eq.)[b]	−	67.0	+15.0
	Dis (16 eq.)	+	73.0	+9.0
	Dis (16 eq.)	−	68.5	+16.5
S-DNA/DNA	none	+	34.5	—
5′-S–(TTTTTCTCTCTCTCT)-3′	none	−	ND[c]	—
3′-AAAAAGAGAGAGAGA-5′	GS (16 eq.)	+	36.0	+1.5
	GS (16 eq.)	−	23.0	—
DNA/RNA	none	+	55.0	—
5′-TTTTTCTCTCTCTCT-3′	GS (16 eq.)	+	55.0	+0
3′-r(AAAAAGAGAGAGAGA)-5′				
S-DNA/RNA	none	+	39.0	—
5′-S-(TTTTTCTCTCTCTCT)-3′	GS (16 eq.)	+	45.0	+6.0
3′-r(AAAAAGAGAGAGAGA)-5′				

Conditions: 50 mM Tris buffer, pH 7.0, [Nacl] = 100 mM, [$Mgcl_2$] = 20 mM, [OligoDNA] = 3 μM.

a. GS was added first and Dis next.

b. Dis was added first and GS next.

c. Not determined.

normal phosphodiester DNA. This observation encourages the practical use of GS as a supplimental reagent for the application of phosphorothioate DNA to antisense therapy.

As shown in Table 2, UV melting study also revealed that GS could bind to and stabilized triple stranded DNA in the presence or absence of Mg^{2+}. It is quite interesting that GS showed a significant effect to stabilize the third strand (Hoogsteen base pair) but showed no or only slight effect to stabilize the double strand (Watson-Crick base pair) in the triple helix composed of three normal DNA strands as well as composed of the third strand of S-DNA and normal ds-DNA. In the interaction of GS and triple helical DNA, it also seems that GS has a

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Table 2. Melting Temperature of Triple Stranded DNA in the Presence of Gramicidin S

Target	Ligands	Mg^{2+}	Tm (°C)	ΔTm (°C)
tsDNA	none	+	39.0[b]/64.0[c]	—
3′-AAAAAGAGAGAGAGA-5′	none	−	35.0[b]/58.0[c]	—
5′-GCTAAAAAGAGAGAGAGATCG-3′	GS (16 eq.)	+	46.0[b]/65.0[c]	+7.0[b]/+ 1.0[c]
3′-CGATTTTTCTCTCTCTCTAGC-5′	GS (16 eq.)	−	41.0[b]/57.0[c]	+6.0[b]/− 1.0[c]
S-DNA/dsDNA	none	+	ND[a,b]/63.0[c]	—
5′-S-(TTTTTCTCTCTCTCT)-3′	GS (16 eq.)	+	34.0[b]/65.0[c]	—/+2.0[c]
5′-GCTAAAAAGAGAGAGAGATCG-3′				
3′-CGATTTTTCTCTCTCTCTAGC-5′				

Conditions: 50 mM Tris buffer, pH 7.0, [Nacl] = 100 mM, [$Mgcl_2$] = 20 mM, [OligoDNA] = 3 μM.
a. Not determined.
b. Melting temperature of Hoogsteen base pair.
c. Melting temperature of Watson-Crick base pair.

predominant affinity to phosphorothioate backbone compared with phosphodiester one.

The present study strongly suggests the potential of an antiparallel b-sheet peptide to function as a designed DNA/RNA binding motif and an intelligent accessory for antisense and antigene drugs.

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