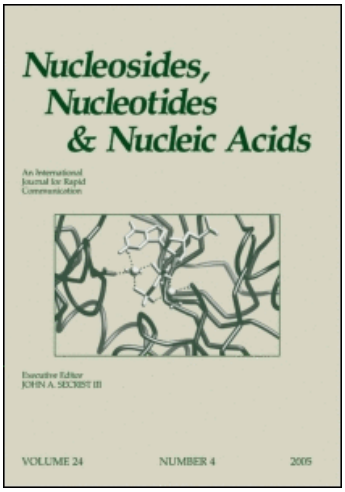


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# Suppression of Exonucleolytic Degradation of Double-Stranded DNA and Inhibition of Exonuclease III by PNA

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## ABSTRACT

Degradation of double-stranded DNA by Exonuclease III in the presence of complementary anti-parallel PNA was studied. It was found for the first time that the PNA suppresses the degradation of dsDNA in a sequence-specific manner as well as inhibits the activity of Exonuclease III in a non-specific way.

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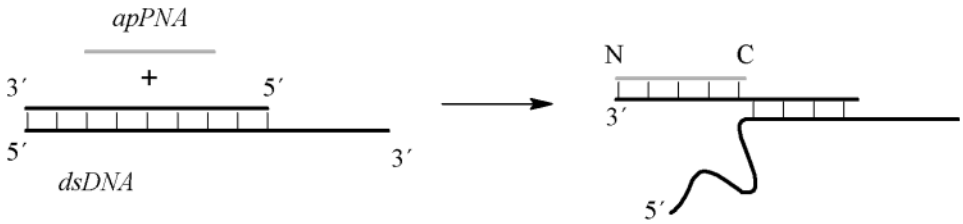


***Figure 1.*** System design. *dsDNA* consists of 30- and 45-mer[a] fragments (3′-overhang); *apPNA* is a 16-mer anti-parallel PNA.

As a part of a program directed towards the development of PNA-based antisense agents[1] and application of PNA in a novel sequencing approach,[2] we examined the ability of PNA to suppress degradation of dsDNA by $3' \rightarrow 5'$ Exonuclease III.

We reasoned that upon addition of PNA to dsDNA, a PNA-DNA duplex should be formed (Fig. 1), which would not be cleaved by Exonuclease III. To investigate this, two ssDNAs with different length i.e.30 and 45-mer were chosen. Their hybridization would create a 30 base-pair dsDNA and a 15-nucleobases containing single-stranded 3′-overhanging end. The 3′-overhanging end prevents the cleavage of the 45-mer by the enzyme that requires as the starting point a 3′-5′ duplex, hence, only the 30-mer would be digested by Exonuclease III (a 3′-overhanging end simplifies the experiment and the analysis of the results by HPLC). The ability of a 16-mer PNA fragment, complementary and anti-parallel to the 30-mer (i.e., the amino terminus of PNA is facing the 3′ of DNA), to hybridize to ssDNA and dsDNA was evaluated (Table 1). At first, a control experiment was performed, hence dsDNA (i.e., the 30-mer) was cleaved by Exonuclease III. The extent of this digestion by the enzyme was assigned as 100% degradation. The degradation was analyzed by HPLC and its extent determined by the amount of released dGMP.[b] The 45-mer, which remained intact, served as internal reference. Next, degradation of the dsDNA was studied at various concentrations of complementary (anti-parallel) PNA.[c]

We found that increasing the concentration of apPNA from 10 to 70 nM resulted in increased suppression of degradation (Fig. 2, a–c). Non-sequence-specific inhibition of the cleavage was observed when the dsDNA was degraded in presence of a 10-mer non-complementary PNA (70 nM; Fig. 2, d).

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[a]5′-TCT-TCA-CCT-CTC-TCT-CTT-TCT-GTC-TCT-CTC-TTT-CTT-TGT-CCT-CTT-3′.

[b]Buffer A - 0.1 M triethylammonium acetate (TEAA) in 1% aq. MeCN (pH 9.0), Buffer B - 0.1 M TEAA in 50% aq. MeCN (pH 9.0), gradient: isocratic A 10 min, 0-30% B in 45 min, 1 mL/min, 23°C, obs. 256 nm on ThermoHypersil HyPURITY® C18 ($150 \times 4.6$ mm).

[c]Cleavage experiments were performed in 66 mM TRIS + 6.6 mM Mg $Cl_2$ (pH 8.0) using 1 nmol dsDNA in 10 nM concentration. Exonuclease III (2 U) was added and samples incubated at 37°C for 60 min. Although these conditions may not be ideal for the hybridization of PNA to DNA or for strand invasion[3] they were chosen to be optimal for the enzyme activity (USB Corporation, Cleveland Ohio).





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***Table 1.*** UV thermal melting data.

| No. | Components[a] | $T_m$ (°C)[b] |
|---|---|---|
| 1. | DNA (45):DNA (30) | 57 |
| 2. | DNA (30):apPNA | 74 |
| 3. | dsDNA:apPNA | 37, 74 |

[a]0.3 μm in 66 mM TRIS + 6.6 mM $MgCl_2$ (pH 8.0).
[b]Heating/cooling rate 0.5°C/min, recorded at 260 nm.

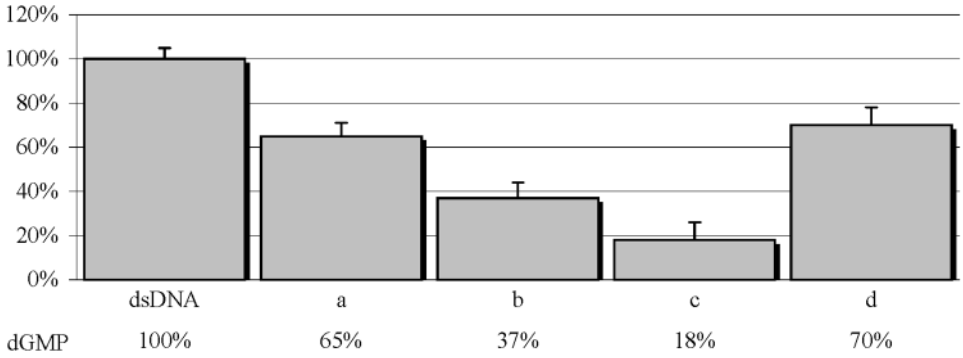


***Figure 2.*** Relative amount of degradation of dsDNA by Exonuclease III after 1 h (by the released amount of dGMP).

In conclusion, anti-parallel complementary PNA suppresses the degradation of dsDNA by Exonuclease III and the suppression is dependent on the amount and concentration of the PNA. The suppression occurs in a sequence specific fashion and is accompanied by a non-specific inhibition of Exonuclease III.[4] It was also found that the inhibition is less pronounced than the suppression.

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