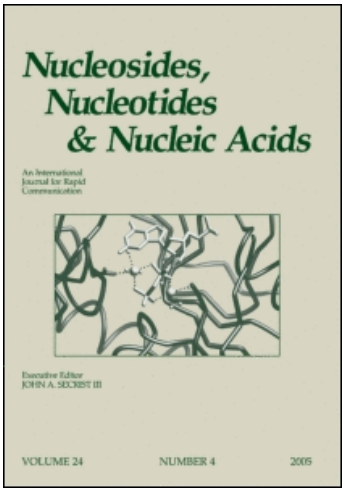

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Intercalating Insert into Internucleotide Linkages as Way for Stabilization and Detection of of Short DNA Duplexes

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INTERCALATING INSERT INTO INTERNUCLEOTIDE LINKAGES AS WAY FOR STABILIZATION AND DETECTION OF OF SHORT DNA DUPLEXES

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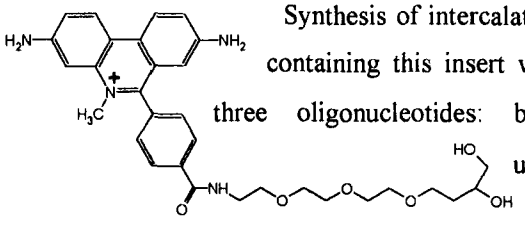
ABSTRACT: Simple and convenient method for stabilization and detection of duplexes of short oligonucleotides with DNA was developed. This method is based on use of oligonucleotides containing inercalating insert in internucleotide linkage. The linker is so long that dye can intercalate only into the same stacking contact. Additionally the method allows to introduce into oligonucleotide as one intercalator as well as several identical or different intercalating dye.



Synthesis of intercalating insert (see left) and oligonucleotides containing this insert was described early[1]. It was synthesized three oligonucleotides: basic strand (S_b) 5′ ATAGCCATGG, unmodified complementary strand (S_u) 5′ CCATGGCTAT, and modified complementary strand (S_m) 5′ CCATG(Met)GCTAT. Besides, amide of methidium-carboxylate (methidiumamide) was prepared.

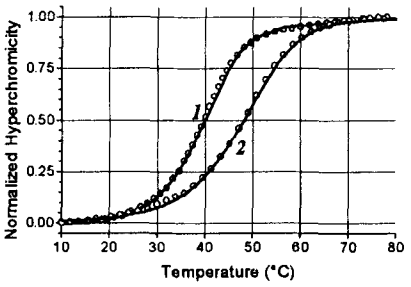


FIG. 1. Melting curves of duplexes D_u (*1*) and D_m (*2*). Conditions: 0.01 M phosphate buffer, pH 7 and 0.1 M NaCl. Fitting results are listed in **TABLE**.

Comparative study of binding of monomeric dyes (methidiumamide and ethidium) with DNA and unmodified duplex (D_u) formed by basic strand (S_b) and complementary unmodified strand (S_u) indicats that methidiumamide intercalates into two stranded DNA as well as into duplex D_u.

Properties of methidium in duplex D_m (S_b + S_m) is the same as properties of methidiumamid intercalated into D_u. This fact allows to conclude that attached methidium in D_m intercalates into

helix. Study of CD spectra shows that duplex $\mathbf{D_m}$ with intercalated dye has B-conformation.

Comparison of melting curves of duplexes $\mathbf{D_u}$ and $\mathbf{D_m}$ (**FIG. 1** and **TABLE**) demonstrates that intercalation of methidium stabilizes substantially two stranded structure.

TABLE. Thermodynamic parameters for duplexes formation calculated from melting curves (**FIG. 1**)

Duplex	Concentration (M)	ΔH (kcal/mole)	ΔS (cal·mole–1·K–1)	T_m^{exp} (°C)	T_m^{norm}* (°C)	ΔT_m^{norm} (°C)
$\mathbf{D_u}$	$2.4 \cdot 10^{-6}$	–59.3±2.4	–162.3±7.6	40.2±0.3	42.5±0.3	6.4
$\mathbf{D_m}$	$4.3 \cdot 10^{-6}$	–48.7±1.9	–125.8±5.8	48.3±0.3	48.9±0.3	

* Because melting temperature of short duplexes is depended on strand concentrations, the experimental T_m can not directly serve by extent of duplex stability. For correctness of comparison we use values of melting temperatures recalculated to equal concentration ($5.0 \cdot 10^{-6}$ M).

Finally, the most stable structure of $\mathbf{D_m}$ was computated by molecular mechanic (AMBER 4). Two results of conformation analysis are very important: (i) the methidium can intercalate only into the same stacking-contact in which methidium insert is incorporated and (ii) the most stable structure is corresponded intercalation on the side of major groove.

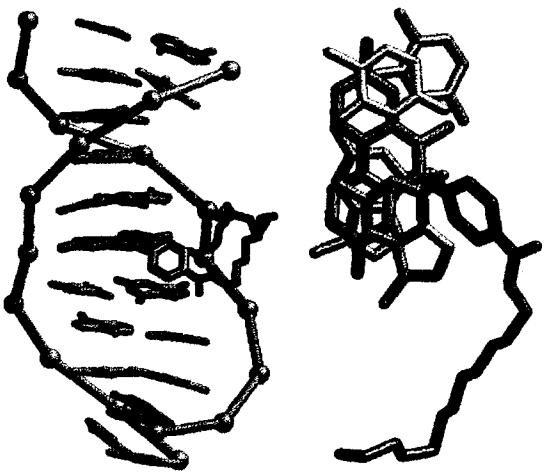


FIG. 2. Schematic representation of the most stable structure of modified duplex $\mathbf{D_m}$ (view perpendicular helix axes, left) and the stacking-contact in which methidium intercalated (view along helix axis, right). On the general view the bend of sugar-phosphate backbone in the point of attachment of methidium insert is clearly seen.

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1. Timofeev E. N., Smirnov I. P., Haff L. A., Tishchenko E. I., Mirzabekov A. D., and Florentiev V. L. *Tetrahedron Letters*, **1996**, *37*, 8467-8470.