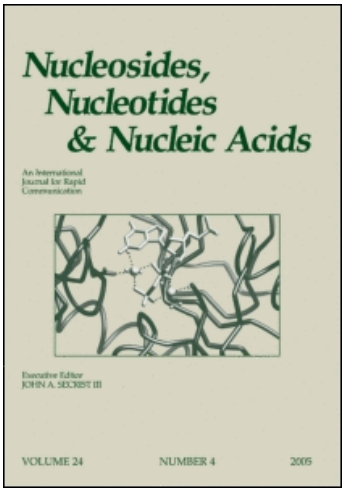

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Studies on the Interaction of Dna-Ligands with Modified Parallel-Stranded Duplex-Dna Oligomers

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STUDIES ON THE INTERACTION OF DNA-LIGANDS WITH MODIFIED PARALLEL-STRANDED DUPLEX-DNA OLIGOMERS

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ABSTRACT: We have investigated the minor groove binders netropsin (Nt) and related lexitropsins for possible interactions with parallel-stranded DNA (ps-DNA). The fluorescence emission spectra and their temperature dependence between 4°C and 30°C led to two conclusions: (i) The specific ligand Nt induces a conversion of the ps-DNA to an antiparallel-stranded DNA (aps-DNA) with mismatched base pairs, a reaction which is much less pronounced for the imidazole-containing analogs. (ii) The more weakly binding imidazole-bearing netropsin-analogs may bind to ps-DNA.

INTRODUCTION

The structure of parallel-stranded DNA (ps-DNA) has been demonstrated to exist as a linear duplex and in hairpin forms.[1-3] The secondary structure of ps-DNA, in which both strands have the same 5'-3' polarity, is maintained by reverse Watson-Crick base pairing.[1,2,4,5] This base pairing results in two equivalent major/minor grooves.[6,7] Conformations of this unusual DNA structure have been described for ps-DNA containing dA·dT, dG·dC, and dA·dU base pairs[8-10] as well as alternating d(G-A) with dG·dG and dA·dA base pairs.[7]

The interaction of ps-DNA with the minor groove binder netropsin (Nt) and distamycin (Dst) has been studied by infrared and fluorescence spectroscopy. A conformational change of the ps-DNA upon drug binding was suggested.[11] In this study we compared the binding ability to the parallel-stranded Duplex ps-D1D2 (FIG. 1) of two

lexitropsin drugs related to Nt (FIG. 1) by using fluorescence emission spectra and temperature-dependent measurements.

EXPERIMENTAL

Oligonucleotide syntheses were performed using Applied Biosystems Model 380B and 394 DNA synthesizers. Oligonucleotides for pyrene labeling reactions were synthesized with a primary amine residue linked to the 5'-end using the Aminolink-2™ reagent from Applied Biosystems as previously described.[10]

Fluorescence measurements were carried out on a SLM Ambiance 4800 spectrofluorimeter at 4°C if not mentioned in the text. For binding studies drugs were added in μL quantities with a microsyringe.

RESULTS AND DISCUSSIONS

The 5'-terminally pyrene labeled ps-duplex DNA shows an excimer fluorescence at 490 nm and the monomer-excimer fluorescence at 380 nm as it is shown in previous reports.[1,7] The former can be used to monitor any change in the strand orientation. As an example, the result for ps-D1D2 is presented in FIG. 2. Upon addition of Nt to the ps-form, the excimer band at 490 nm disappears after 24 h at a drug concentration of r'=0.2, indicating a change in the strand polarity. This can be interpreted in terms of a conversion of the ps-form into an aps-form with mismatched base pairs as previously proposed.[11] The ps- to aps-conversion is schematically shown in FIG. 3.

We have compared the effect of minor groove binding drugs on pyrene labeled aps-DNA. The results are given in FIG. 4. Interestingly, addition of Nt and Dst to aps-$^{5'Py}$D1$^{3'Py}$D3 (FIG. 1) significantly enhances the fluorescence band, which reaches a saturation level at r'=0.1 corresponding to one Nt per five base pairs (FIG. 4, inset). This agrees with the binding behavior of Nt to poly(dA)·poly(dT) derived from previous CD data and other findings.[12,14] Obviously, the end-labeled pyrenes are more separate from each other in the aps-DNA than in the fully drug-bound complex due to fraying ends of the drug-free AT-containing aps-duplex. This is not observed for the minor groove binder pentamidine, which has a lower AT base pair affinity than Nt and Dst (FIG. 4). The opposite drug effect on the pyrene labeled aps-DNA compared to the ps-$^{5'Py}$D1$^{5'Py}$D2

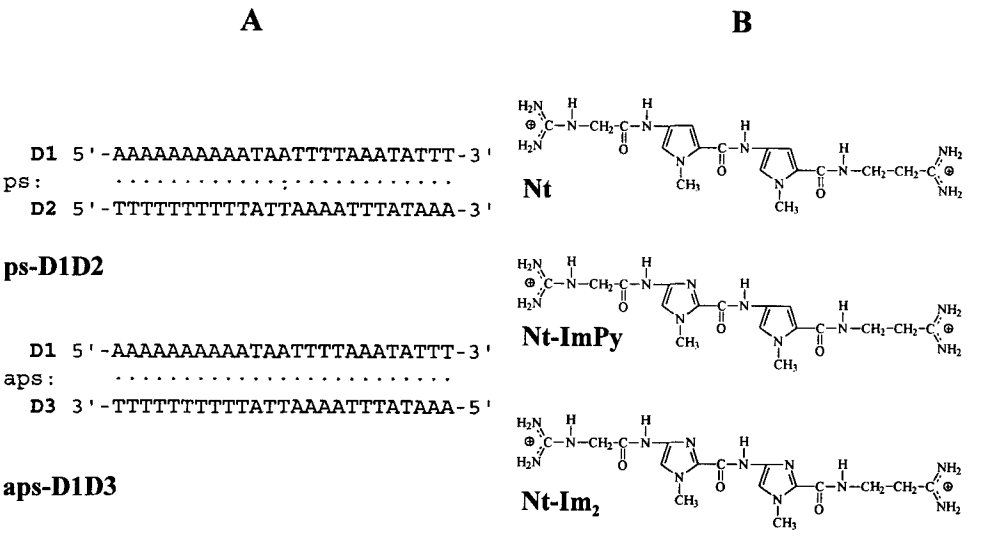

FIG. 1: A, sequences of DNA duplexes used in this study; B, chemical structure of netropsin (Nt) and related lexitropsins

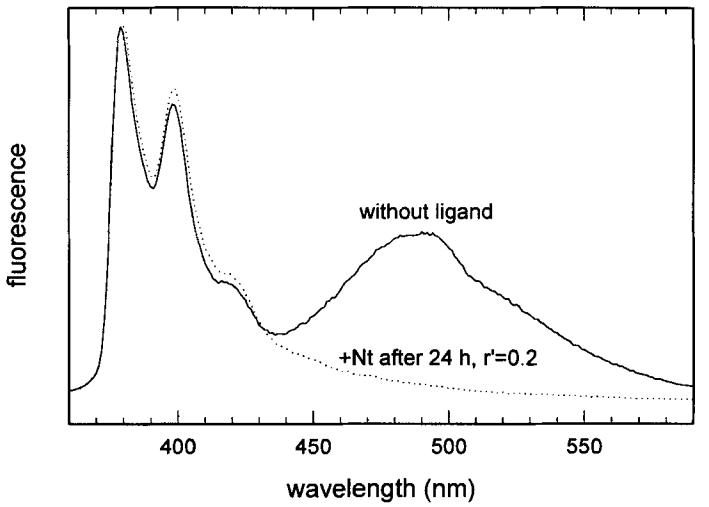

FIG. 2: Fluorescence spectra of the free duplex oligomer ps-$^{5'Py}$D1·$^{5'Py}$D2 and of the saturated complex with netropsin (Nt) measured after 24 h; λ_{exc}=340 nm; r', total ratio of ligand per nucleotide.

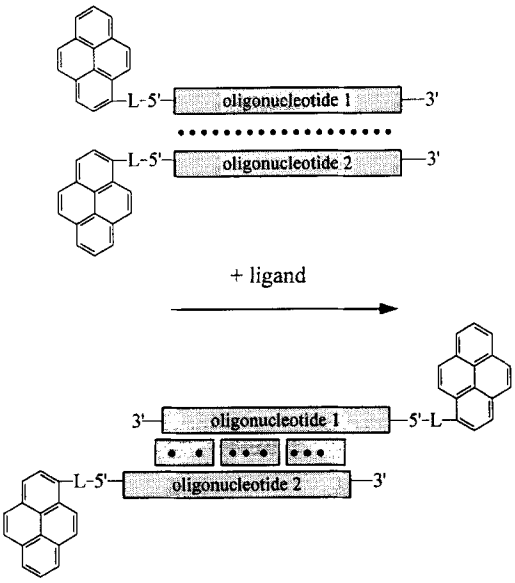

FIG. 3: Scheme of the conversion of the pyrene-labeled parallel-stranded (ps) duplex into the imperfectly matched antiparallel-stranded (aps) duplex. The aps-duplex with mismatched base pairs represents the more favorable structure for drug binding in the B-DNA minor groove.

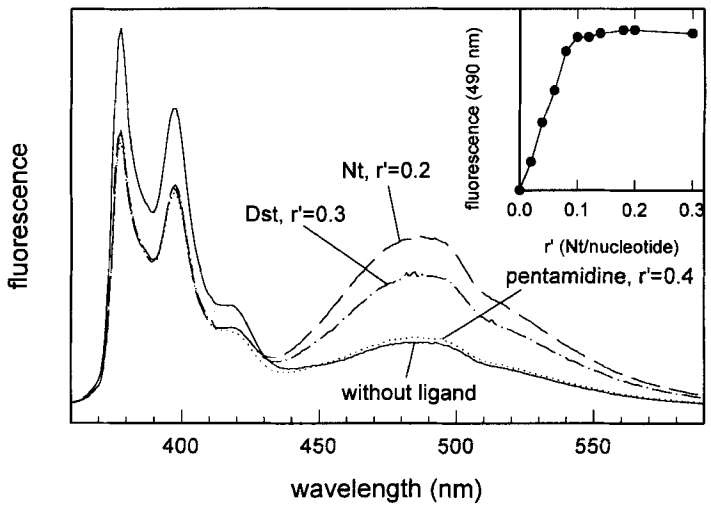

FIG. 4: Fluorescence spectra of the free duplex oligomer aps-$^{5'Py}$D1·$^{3'Py}$D3 and of the saturated complexes with Nt, Dst and pentamidine measured after 24 h. Inset: Titration curve for aps-$^{5'Py}$D1·$^{3'Py}$D3 with Nt, monitored at 490 nm; λ_{exc}=340 nm; r', total ratio of ligand per nucleotide.

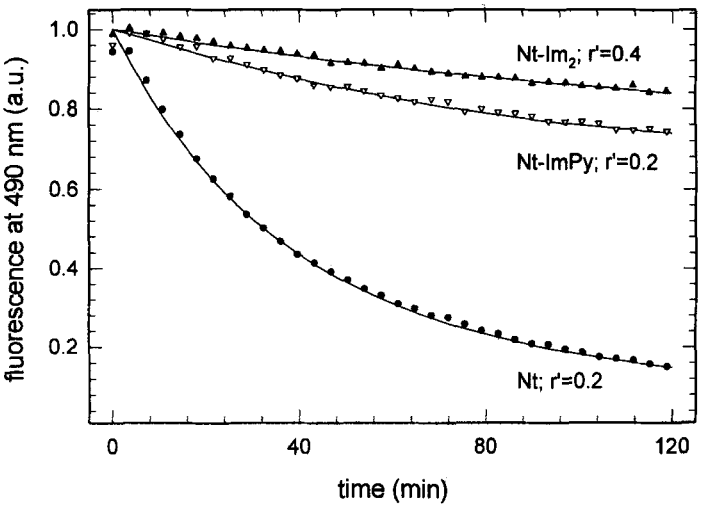

FIG. 5: Time course of the fluorescence emission of the pyrene-excimer at 490 nm on interaction of ps-$^{5'Py}$D1·$^{5'Py}$D2 with Nt, Nt-ImPy and Nt-Im_2 at 4°C; λ_{exc}=340 nm, r', total ratio of ligand per nucleotide.

verifies the conversion of the latter to an aps-form. The ability of the lexitropsins (abbreviated by Nt-ImPy and Nt-Im_2) related to Nt to bind to the ps-form is demonstrated in FIGS. 5 and 6. Addition of Nt-ImPy and Nt-Im_2 to ps-DNA at low temperatures caused no comparable large decrease of the characteristic fluorescence at 490 nm (spectra not shown). As displayed in FIG. 5, the time course of the fluorescence change for ps-DNA exhibits a significant lowering of the curve for the Nt-ps-complex after 120 min, whereas both lexitropsins show only a slight decreasing fluorescence change. This is directly correlated to the number of imidazole residues of the drug. The influence of temperature on the fluorescence of pyrene labeled ps-DNA drug complexes further verifies the drug binding behavior.

From FIG. 6 it may be seen, that the excimer fluorescence at 490 nm of the drug-free ps-form decreases with increasing temperature up to 30°C. In presence of Nt-ImPy and Nt-Im_2, the temperature dependent curve is decreased in a similar manner but does not approach a complete loss of the fluorescence signal as in case of Nt (FIG. 6).

Thus, we are tempted to conclude that different from Nt, the two lexitropsins may bind to the ps-form of the AT-duplex at 4°C and at least partially at 20°C to 30°C. It seems, that Nt-ImPy and Nt-Im_2 have a lower tendency to convert the ps- to the aps-form

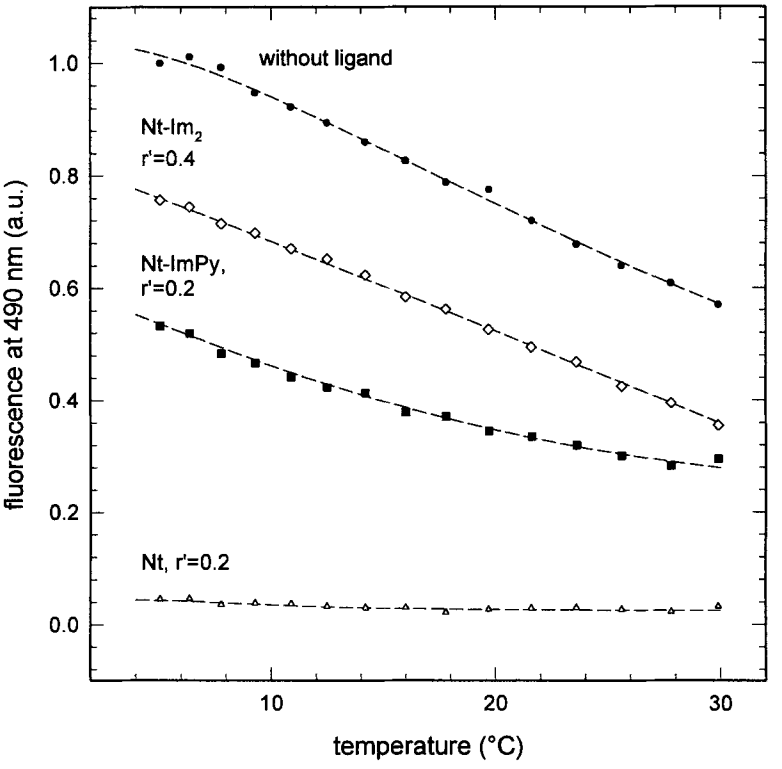

FIG. 6: Temperature dependence of the fluorescence emission (490 nm) of the pyrene-labeled duplex ps-$^{5'}$D1·$^{3'}$D2 in complex with Nt, Nt-ImPy and Nt-Im$_2$; λ_{exc}=340 nm; r', total ratio of ligand per nucleotide.

compared to Nt. This may be ascribed to the significantly reduced affinity to AT pairs in the minor groove due to the presence of imidazole residues.[15,16]

ACKNOWLEDGEMENTS

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