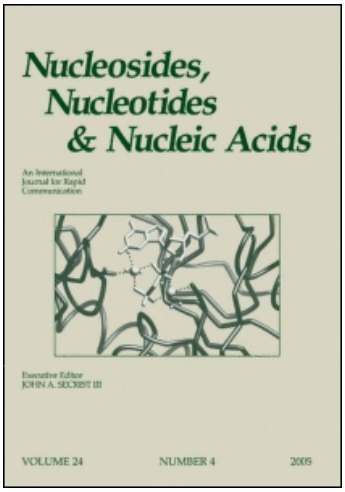

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Oligonucleotides Labeled by Fluorophores at the Sugar 2'-Positions for Fluorescence Energy Transfer Study of Nucleic Acid Structure

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OLIGONUCLEOTIDES LABELED BY FLUOROPHORES AT THE SUGAR 2'-POSITIONS FOR FLUORESCENCE ENERGY TRANSFER STUDY OF NUCLEIC ACID STRUCTURE

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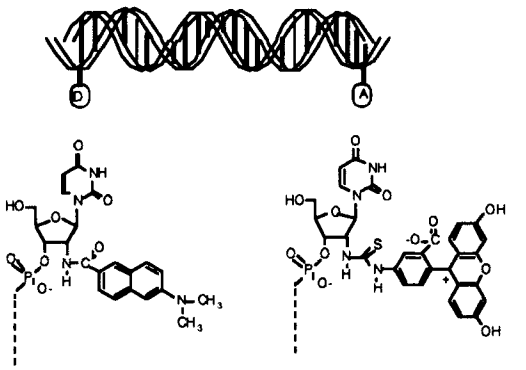
ABSTRACT : Oligonucleotides containing 2'-(6-dimethylamino-2-naphthamide)uridine have been shown to be useful as a donor fluorophore in FRET to oligonucleotides labeled with fluorescein at the 2'-position as an acceptor molecule.

INTRODUCTION

Several applications of fluorescence resonance energy transfer (FRET) have been reported to resolve the problems of helical four-way junctions in DNA topology,[1] geometry of bent DNA molecules,[2] and relative orientation of the helical segments in RNA hammerhead ribozyme.[3] In these studies, fluorophores have been introduced covalently via a tether into the 5'- or 3'-end or the phosphate backbone of oligonucleotides.



We have shown that the sugar 2'-position in oligonucleotides is a suitable site for covalent attachment of several fluorophores.[4] Very recently, the oligonucleotides

containing a new fluorescent nucleoside, 2'-(6-dimethylamino-2-naphthamide)uridine [U(DAN)], have been shown to exhibit an appropriate fluorescence that may be used as a donor fluorophore in FRET experiments to fluorescein as acceptor species.[5] The present report describes the FRET in DNA between U(DAN) and fluorescein labels at the 2'-positions.

RESULTS AND DISCUSSION

The synthesis of the U(DAN) containing oligonucleotides has been carried out on a fully automated DNA synthesis machine by using 5'-dimethoxytrityl U(DAN) 3'-phosphoramidite. The fluorescein labeling of oligonucleotides has been accomplished by the reaction of fluorescein isothiocyanate with oligonucleotides containing 2'-amino 2'-deoxyuridine.

The binding of U(DAN) containing oligonucleotides with complementary DNA segments was investigated with UV melting measurements. All the duplex exhibited typical sigmoidal curves in their melting profiles. Analysis of the tms obtained from the melting curves revealed that the oligonucleotide containing U(DAN) at the 5'-terminal end retains its normal affinity for DNA. The CD spectra showed that the 5'-end modified oligonucleotides exhibit the same profile as that for a B-form DNA double helix. Similar spectral analysis revealed that the fluorescein labeled oligonucleotides have similar characteristics.

The apparent fluorescence resonance energy transfer efficiencies (Eapp) depending on the oligonucleotide length were measured. Although a clear dependence of Eapp on the number of nucelotides in the DNA, the Eapp values decreased less rapidly with increase oligomer length than expected from the Forster expression. The results suggest that FRET is a useful qualitative indicator of distance in DNA molecules.

The analysis of structural features of DNA and RNA in solution by the present method is now under investigation.

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