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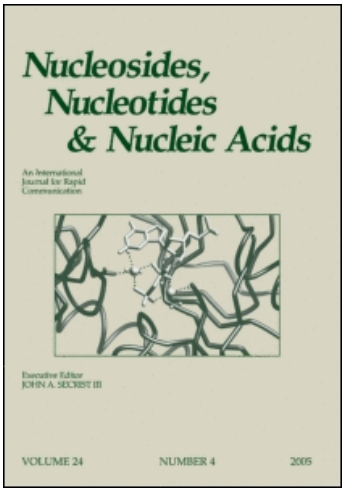

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DNA Labelling Topologies for Monitoring DNA-Protein Complex Formation by Fluorescence Anisotropy

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DNA LABELLING TOPOLOGIES FOR MONITORING DNA-PROTEIN COMPLEX FORMATION BY FLUORESCENCE ANISOTROPY

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□ *In this work, fluorescence anisotropy was used to study DNA binding of the DNA methyltransferase M.TaqI. For this purpose short DNA molecules labelled with three different fluorophores (Cy3, thiazole orange, and ethidium bromide) were prepared in various topologies and their suitability for detection of DNA-protein complex formation was investigated.*

Keywords DNA binding protein; fluorescence anisotropy; DNA methyltransferase; M.TaqI; directed intercalation; base surrogate; Cy3; thiazole orange; ethidium bromide

INTRODUCTION

Fluorescence anisotropy[1] is a powerful method to detect the association of biomolecules. In general, the smaller binding partner is fluorescently labelled to achieve an optimal signal change upon binding of the larger partner. For monitoring DNA-protein interactions short DNA oligomers are typically labeled with a suitable fluorophore. However, care must be taken that the fluorophore does not interfere with protein binding and that the fluorescence anisotropy change is large enough to monitor DNA-protein complex formation.

Surprisingly few systematic studies about general labelling strategies for short DNA have been reported.[2–6] Typically, 5′-end labelled DNA (Figure 1, top) is used for fluorescence anisotropy studies of DNA-protein interactions because the corresponding labelled oligodeoxyribonucleotides (ODN) easily can be obtained from a 5′-amino modified ODN. For

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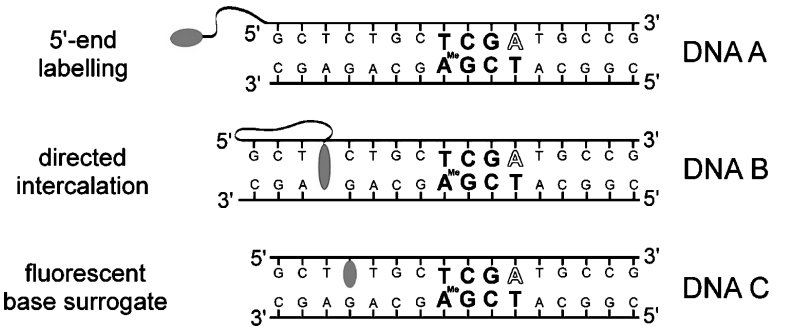

FIGURE 1 Three different topologies for the attachment of fluorophores to DNA. The 5′-TCGA-3′ recognition sequence of M.TaqI is highlighted and the target adenine in the nonlabeled ODN is methylated to create the natural hemimethylated substrate (A^{Me} = N6-methyl-2′-deoxyadenosine).

instance, this topology has been used for thermodynamic and kinetic analyses of DNA binding by DNA methyltransferases[7,8] and restriction endonucleases.[9] Other topologies for fluorescent dye-DNA conjugates like directed intercalation[10] and base surrogates[11] are known (Figure 1, center and bottom), but to the best of our knowledge they have not been utilized for the detection of DNA-protein complex formation by fluorescence anisotropy. Therefore, we decided to compare these three topologies using the adenine-specific DNA methyltransferase M.TaqI as a paradigm.[12]

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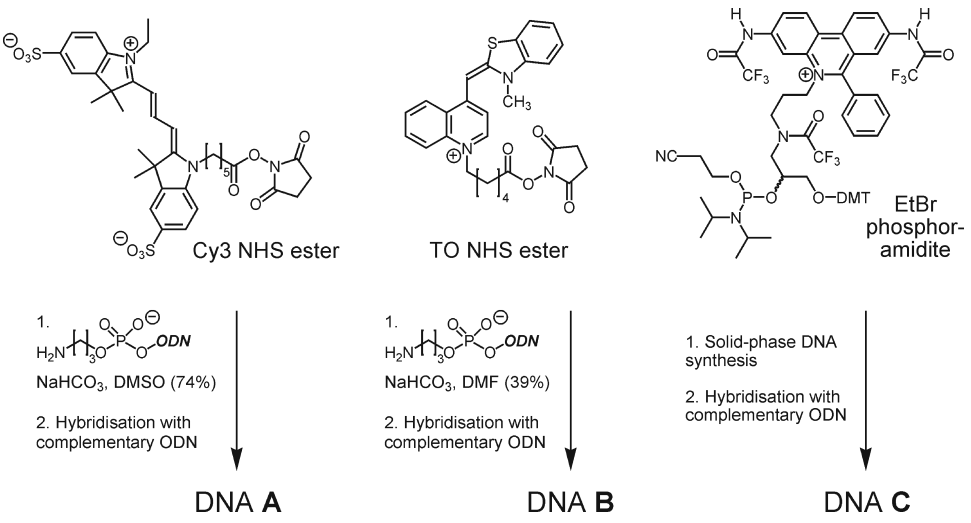
RESULTS AND DISCUSSION

Syntheses of fluorescent DNA molecules labelled with three different topologies (DNA **A**, **B**, and **C**) are shown in Scheme 1. 5′-End labeling with the non intercalating Cy3 fluorophore was achieved by reacting a 5′-amino modified ODN with Cy3 NHS ester followed by hybridization with a complementary ODN (DNA **A**). Similarly, directed intercalation was realised by covalent attachment of the intercalator thiazole orange (TO) to the 5′-amino modified ODN with TO NHS ester (DNA **B**).[10] Incorporation of ethidium bromide (EtBr) as fluorescent base surrogate (DNA **C**) was performed by solid-phase DNA synthesis as described before.[13]

Addition of M.TaqI to DNA **B** or **C** resulted in active site titration curves with respect to fluorescence anisotropy (Figure 2). Particularly the pronounced anisotropy change of DNA **C** enables convenient monitoring of M.TaqI-DNA complex formation. In addition, titration of DNA **C** showed an almost constant fluorescence intensity even after the addition of 5 equivalents of M.TaqI. This is in contrast to titrations of DNA **A** and **B** where a significant increase of the fluorescence intensity was observed after addition of more than 1 equivalent of M.TaqI. Such a behavior can be explained by nonspecific binding of a second M.TaqI molecule which could be assisted by an



SCHEME 1 Syntheses of fluorescently labelled DNA molecules with different topologies for the incorporated fluorophores.

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unwanted interaction with the fluorophore. However, this second binding apparently does not lead to a further increase of the fluorescence anisotropy of DNA **B**.

DNA **C** with a fluorescent base surrogate clearly gave the best results both in terms of absence of non-specific protein binding as well as fluorescence anisotropy increase. This can be readily explained by a good steric shielding of the fluorophore inside the DNA helix against interaction with the DNA binding protein. In addition, the fluorescent base surrogate is tightly incorporated in the DNA and faithfully reports the mobility of the whole DNA molecule and not a possible additional local motion of an attached fluorophore. Thus, we expect that DNA with fluorescent base

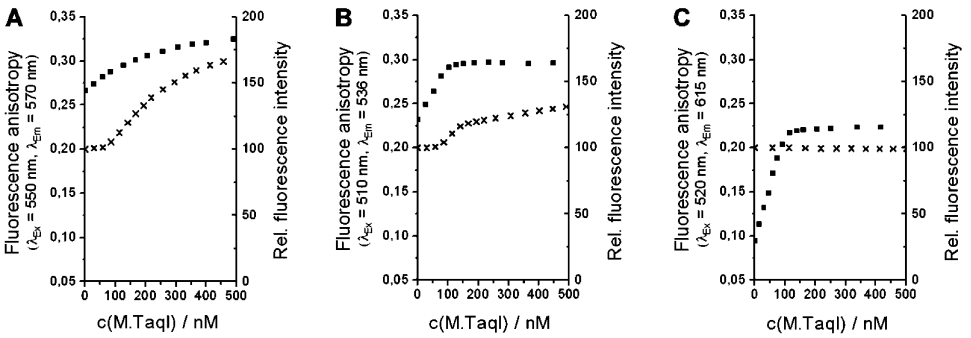

FIGURE 2 Titrations of DNA **A** (**A**), DNA **B** (**B**), and DNA **C** (**C**) at 100 nM concentration with M.TaqI. In each diagram the fluorescence anisotropy (■) and the relative fluorescence intensity (×) are given with respect to the M.TaqI concentration.

surrogates will find interesting applications in monitoring DNA-protein interactions by fluorescence anisotropy.

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