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DNA duplex as a scaffold for a ground state complex formation between a zinc cationic porphyrin and phenylethynylpyren-1-yl

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ABSTRACT

The attachment of one and two (*R*)-1-*O*-[4-1-(1-pyrenylethynyl)phenylmethyl]glycerol units (TINA, twisted intercalating nucleic acid) at the 5′-end of the 12-mer duplex led to decreased thermal stability at low (50 mM NaCl) salt concentration. This was circumvented by using buffers with high (1.0 M NaCl) salt concentrations, although the duplex with a three-unit 5′-tail (TINA-thymidine-TINA) was less stable than the unmodified DNA. UV–vis and fluorescence spectroscopy studies indicate that the cationic porphyrin ZnTMpyP4 has greater affinity to TINA-modified duplexes than to the unmodified 12-mer duplex at both low and high salt concentrations. An increase in duplex thermal stability (up to ΔT_m + 23 °C) was more pronounced upon addition of the porphyrin to duplexes having two TINA monomers at 5′-ends than for unmodified duplex and a duplex with single TINA monomer at each 5′-end. Complex formation resulted in a bathochromic shift observed in the UV–vis spectra for the porphyrin Soret and Q-bands (up to 8 nm) which was also accompanied by changes in their intensities. Energy transfer from TINA to porphyrin showed by fluorescence excitation spectra was accompanied by changes in TINA and porphyrin emission intensities. TINA fluorescence for both monomer and excited dimer was quenched when duplexes were further titrated with the porphyrin, which was attributed to the formation of the ground state complex (major component) and collisional quenching (minor component). Porphyrin fluorescence quenching by $K_4Fe(CN)_6$ showed that TINA-modified duplexes protect porphyrin from quenching slightly better than unmodified duplex but not to the extent of porphyrin protection shown by salmon testes DNA (stDNA).

1. Introduction

The supramolecular interactions of unmodified DNA with small molecules or ligands have been a subject of intense research for several decades [1–4]. This was mainly driven by the search for possible drugs interfering with biological processes on DNA [5]. However, interactions of ligands with modified DNAs bearing functional molecules have not been widely explored. This is despite the fact that recent advances in DNA chemistry allow us to introduce functional entities in any desired position along the DNA helix using a covalent anchoring strategy [6]. This forms a foundation for the creation of supramolecular helical nanoarrays based on modified DNA [7]. Combination of the supramolecular and covalent anchoring strategies has several advantages [8]. Firstly, interactions of ligands based on aromatic molecules with DNA possessing organic chromophores can lead to novel photoactive materials because properties of individual components may change after complex formation. Secondly, covalent attachment of certain compounds to DNA still remains challenging, requires significant efforts and is time-consuming. Moreover, creation of the tether in the desired molecule for the attachment to the DNA can, and often does, change spectroscopic properties of the molecule, which is usually detrimental for the array wanted. Thirdly, various fluorophores are either commercially available as DNA phosphoramidites or can be synthesised and attached to the DNA in a timely manner using available procedures. Fourthly, such systems can be quickly designed, self-assembled and evaluated spectrophotometrically using commercially available ligands and modified DNA. Finally, a prototype of photo-optical devices can be easily fabricated if needed.

The majority of the studies devoted to the interaction of ligands and DNA have been performed using cheap, commercially available DNAs of biological origin, such as salmon testes DNA (stDNA) and calf thymus DNA. These DNA duplexes are long (ca 2000 base-pairs) with more or less an even distribution of nucleotides, which

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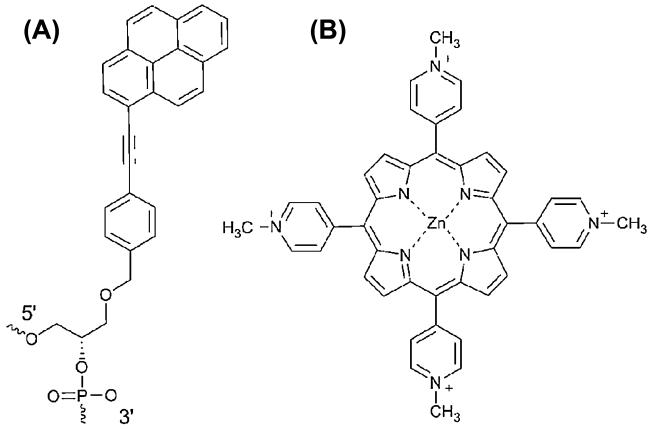

(C)

D1: 5'-CTCAAGCAAGCT
GAGTTCGTTCGA-5'

D2: 5'-**X**CTCAAGCAAGCT
GAGTTCGTTCGA**X**-5'

D3: 5'-**XX**CTCAAGCAAGCT
GAGTTCGTTCGA**XX**-5'

D4: 5'-**X**T**X**CTCAAGCAAGCT
GAGTTCGTTCGA**X**T**X**-5'

Scheme 1. Structures of twisted intercalating nucleic acid monomer (TINA, monomer **X**, A), a cationic porphyrin (B) and TINA-modified duplexes used in the present study (C).

preferentially adopt a B-type DNA helix. In contrast, DNA and RNA sequences with the length of 5–50 nucleotides can be designed to fold into topologies different from the classical A- and B-type DNA helices, which include DNA triplexes, G-quadruplexes, parallel duplexes, i-motifs and so on. It can be expected that interactions of ligands with such structures, especially if other organic chromophores are attached to DNA, are different from interactions with the long DNA duplex.

It should be emphasised that DNA as a scaffold allows controllable arrangement of chromophores, which leads to the appearance of remarkable spectroscopic and electronic effects ranging from formation of excimers, J- and H-aggregates to efficient energy transfers between several dyes [6–8]. Some photochromic systems can only be reliably obtained using DNA scaffolding [9]. We anticipate that future efforts will be devoted to study interactions between various organic chromophores tethered to DNAs with free ligands in solution which will provide even wider range of photochromic assemblies useful for material science applications.

It is a general assumption that ligands form a ground state complex with chromophores attached to the DNA which supposed to result in the effective communication between the ligand and the chromophore. However, lack of the systematic studies in the creation of such modular assemblies hinders the rational development of them. In the present article we evaluated the interactions of a cationic porphyrin ZnTMpyP4 (Scheme 1B), which is one of the most studied DNA ligands [2,10], with DNA duplexes bearing (*R*)-1-*O*-[4-1-(1-pyrenylethynyl)phenylmethyl]glycerol (TINA, twisted intercalating nucleic acid, Scheme 1A), which has been used recently in the structure of short DNA duplexes, DNA triplexes and G-quadruplexes [11]. ZnTMpyP4, DNA duplexes, and TINA monomer do not have significant overlaps in the UV–vis spectrum and hence each of them can be easily monitored in the mixture using a variety of spectrophotometric methods. In addition, ZnTMpyP4 and TINA are highly fluorescent, which makes it

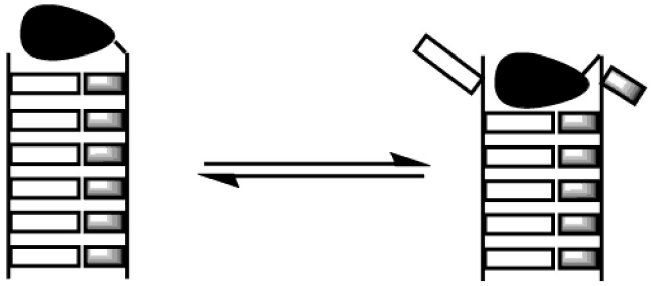

Fig. 1. Schematic representation of different positioning of an organic chromophore attached at the end of the duplex at low salt concentration.

easy to determine the change in their environment in the mixture by fluorescence spectroscopy. This study shows that the collisional interactions of a free porphyrin and a free TINA in solution are converted into the dominant ground state complex when TINA is attached at the terminal positions of the duplex. This is accompanied by significant changes in thermal stabilities of duplexes and in UV–vis and fluorescent characteristics of a porphyrin and TINA. Despite the fact that effective communication between organic chromophores was detected, the breathing of duplexes and insufficient engagement of the porphyrin with modified DNA indicate that assemblies based on other DNA architectures should be considered in future.

2. Results and discussion

2.1. Interactions of free TINA and ZnTMpyP4 in solution

Stern–Volmer analysis of solutions containing free TINA monomer and a porphyrin in DMSO/H_2O (9:1) revealed that fluorescence of these chromophores is quenched as a result of dynamic interactions with a Stern–Volmer dynamic quenching constant of 857.0 M^{-1} (see Supporting Information). These dynamic interactions did not result in any changes in the UV–vis absorption spectra for both TINA and a porphyrin (data not shown), which suggests that there is no ground state complex formation between ZnTMPyP4 and TINA monomer. These properties justify our choice of chromophores to be studied when TINA is attached to the DNA scaffold.

2.2. DNA duplex design

TINA-modified duplexes based on a dodecameric sequence **D1** (Scheme 1C) were synthesised for our study. It has been shown that incorporation of TINA as a bulge in the middle of the Watson–Crick-type duplex is detrimental for the duplex stability (ΔT_m = −8.0 to −15.5 °C). Instead, chromophore attachment to the 5′-terminal position on the DNA does not disrupt duplex formation and can potentially maximise the interaction between the phenylethynylpyrene moieties with the cationic porphyrin ZnTMpyP4. We hypothesised that by increasing the content of lipophilic pyrene moieties in the duplex the porphyrin–DNA interaction will become stronger and the cationic porphyrin will reside next to TINA. This will lead to the supramolecular complex with significantly increased duplex stability whereas TINA and a porphyrin will interact electronically giving a rise to new photonic systems. Single and double 5′-TINA modifications as well as double TINA modification with a thymidine sandwiched between two TINA monomers were employed (Scheme 1C). Several DNA systems based on multiple insertions of pyrenes at terminal positions have been designed in the past and used for detection of DNA and RNA sequences [12]. The appropriate duplexes (1.0 μM) were prepared in 10 mM sodium phosphate buffer (pH 7.0, 0.1 mM EDTA) in