Dye Binding

DOI: 10.1002/anie.200703251

Interaction of Malachite Green with Guanine-Rich Single-Stranded DNA: Preferential Binding to a G-Quadruplex**

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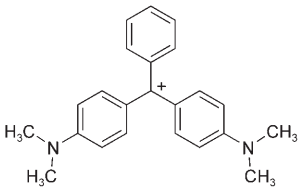
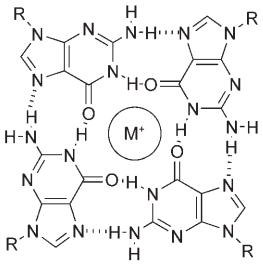
It is important to understand the molecular mechanisms by which chromophoric dye molecules interact with biological entities, such as proteins, DNA, and RNA, as these dyes have potential biological applications in photodynamic therapy,[1] drug delivery, catalysis,[2] and the recognition of specific sites and sequences of DNA and RNA.[3] The functional roles of biomolecules are often credited to their ability to adopt alternative structural forms to provide ligand-specific binding templates, which regulate the corresponding biochemical pathways.[4,5] These unique structures hold the key to the recognition of guest chromophores on the basis of size, structure, charge, and other properties. Oligonucleotide sequences that contain guanine-rich stretches can form mutually hydrogen bonded, internally folded, or interstrand G-quadruplexes,[6,7] which are active in chromosomal telomeres,[8] gene promoters,[9] and immunoglobulin switch regions.[6c] A considerable number of organic molecules have been evaluated for their ability to interact with and stabilize the G-quadruplex and/or influence the associated functions of the attached enzyme.[4,6,10,11] Joachimi et al. recently studied G-quadruplex-binding porphyrins and demonstrated that such interactions can be used to control the activity of functional aptamers that contain G-quadruplex elements.[12]

The application of triphenylmethane (TPM) dyes,[13] in particular malachite green (MG), in biological methodologies has been gaining momentum as a result of their aptness for photodynamic therapy,[14] the site-specific inactivation of RNA transcripts,[15,16] RNA-aptamer-based fluorescence sensors,[3,5] and the catalysis of chemical reactions.[2] We demonstrated recently the applicability of a supramolecular-enhancer strategy to improve the binding of the TPM dye Brilliant Green to proteins through cooperative binding.[17] Inspired by the enormous importance attributed to the structure and function of G-quadruplexes and the emerging biological applications of MG, we focused our attention on the interaction of MG with the guanine-rich single-strand oligomer $d(G_2T)_{13}G$. Herein, we demonstrate the effectiveness of the binding of malachite green to a G-quadruplex structure formed within the $d(G_2T)_{13}G$ sequence. This specific binding interaction leads to an approximately 100-fold enhancement in the radiative yield of the dye and thus forms the basis of a convenient method for the detection of the G-quadruplex. The enhanced excited-state activity of MG in the bound state and its modulation in the presence of a salt are directly relevant to the biological applications of MG. Furthermore, in the presence of the oligomer $d(G_2T)_{13}G$, a bathochromic shift of approximately 18 nm is observed for both the absorption and the fluorescence bands of MG (Figure 1).



malachite green



G-quadruplex

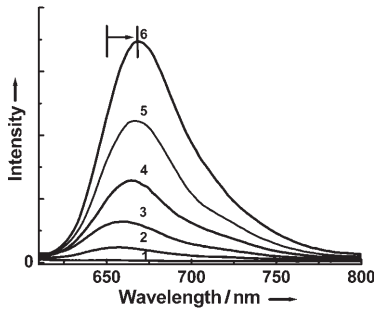


Figure 1. Enhancement of the fluorescence intensity of MG (1 μM) in an aqueous solution containing phosphate buffer (5 mM) at pH 7 with $d(G_2T)_{13}G$ at increasing concentrations: 1) 0, 2) 6, 3) 60, 4) 96, 5) 108, 6) 120 μM.

The large bathochromic shift and significant increase in the radiative yield indicate an increase in the rigidity of the dye structure upon binding to the oligonucleotide. A binding curve was constructed by monitoring the changes in the fluorescence intensity of MG at varying concentrations of $d(G_2T)_{13}G$ (Figure 2). The initial enhancement in the fluorescence intensity tends to a plateau at a $d(G_2T)_{13}G$ concentration of approximately 30 μM (region I). A further increase

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[**] Support and encouragement from the host institute is gratefully acknowledged.

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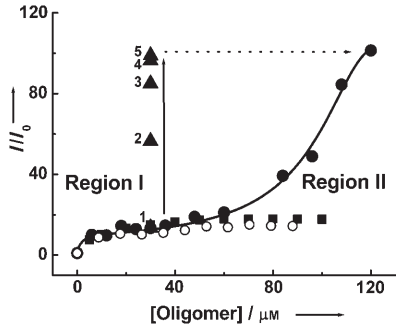

Figure 2. Binding curves for MG (1 μM) in the presence of oligomers at varying concentrations: ● $d(G_2T)_{13}G$, ■ $(dG)_{40}$, ○ ds-DNA_{40}. ▲ denotes the increase in emission intensity at $[d(G_2T)_{13}G] \approx 30$ μM upon the addition of NaCl: 1) 0, 2) 0.1, 3) 0.2, 4) 0.3, 5) 0.5 M.

in the oligomer concentration results in a remarkable second-stage enhancement of the MG fluorescence, which tends towards saturation at a concentration of approximately 120 μM in $d(G_2T)_{13}G$ (region II; see the Supporting Information). Analysis of the binding curve reveals that region I corresponds to typical 1:1 complexation, as confirmed by a Job plot. The binding constant was evaluated to be $3.1 \times 10^5\ M^{-1}$[18] by fitting the data up to a concentration of approximately 30 μM. Above this concentration, the curve deviates significantly from a typical binding curve expected for 1:2 complexation and suggests the formation of higher-order complexes. In separate experiments, we studied the interaction of MG with the single-strand homopolymers $(dG)_{40}$, $(dC)_{40}$, $(dA)_{40}$, and $(dT)_{40}$, and also with double-stranded DNA of a similar length ($(ds\text{-}DNA)_{40}$). Interestingly, in all cases a less than 14-fold enhancement in fluorescence was observed (see Figure 2 and Supporting Information). Thus, the significant enhancement observed is specific to the $d(G_2T)_{13}G$ strand.

The interaction of organic dyes with ds-DNAs has been well documented as intercalative, electrostatic, or groove binding.[19] For the single-stranded 40-mer used in this study, it is reasonable to rule out the possibility of intercalative or groove binding. Instead, an electrostatic or π-stacking interaction supported by the negatively charged phosphate backbone could be the preferred mode of binding.

In the case of an electrostatic interaction, an increase in the ionic strength of the medium would decrease the extent of dye binding and result in a reduction in the fluorescence yield. We investigated this effect at the two representative oligomer concentrations, which correspond to the two modes of binding observed (region I and region II in Figure 2). The addition of NaCl at concentrations of up to 1M to the solution in region II did not result in any significant change in the emission yield. However, in region I, a remarkable additional enhancement in the fluorescence yield (an increase from an initial 14-fold enhancement to an approximately 100-fold enhancement) was observed upon the addition of 0.5 M NaCl (Figure 2). This striking favorable modulation of the dye–oligomer interaction by the presence of a salt is fascinating, as a salt would usually be expected to have the opposite effect.

The radiative lifetimes of TPM dyes are very short (< 1 ps) in low-viscosity solvents,[20] but increase substantially upon binding to biomolecules, which therefore results in an enhancement in the fluorescence yield.[17] The fluorescence-decay traces for MG in the presence of $d(G_2T)_{13}G$ (Figure 3)

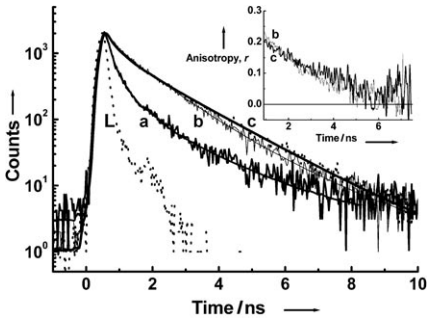

Figure 3. Fluorescence-decay traces ($\lambda_{ex} = 636$ nm, $\lambda_{mon} = 690$ nm) of a solution of MG (1 μM) containing phosphate buffer (5 mM) at pH 7 in the presence of $d(G_2T)_{13}G$: a) 30, b) 120 μM. Trace c represents the decay trace in the solution corresponding to trace a with NaCl (0.5 M) added. L represents the excitation pulse. Inset: Fluorescence-anisotropy traces under the conditions used to obtain traces b and c.

follow triple-exponential decay kinetics with an average lifetime of 560 ps in region I and 1.1 ns in region II and clearly support the existence, depending on the composition of the solution, of two types of dye-binding templates. As expected, the decay trace observed in region I in the presence of 0.5 M NaCl clearly matches that observed in region II (Figure 3). These results suggest that in the presence of NaCl, the binding template in region I transforms into a template similar to that available in region II.

Guanine-rich sequences form G-quadruplexes in the presence of monovalent ions.[6] The proposed G-quadruplex model is based on Hoogsteen hydrogen bonding between four guanine residues in a square-planar array and is stabilized by cations, such as Na^+ or K^+.[6,7] Such tetrameric structures can be formed through either intrastrand or interstrand interactions.[6] In region II, that is, at higher oligomer concentrations, G-quadruplexes may be formed through an interstrand interaction.[6c] However, in region I, as a result of a lower oligomer concentration, the MG-binding template is mainly a single strand. In the presence of NaCl, a metal-ion-stabilized G-quadruplex structure is formed through an intrastrand or interstrand interaction. The electron-rich phenyl rings of MG can π stack effectively on the face of the G-quadruplex, whereby the planar structure of the dye becomes rigid, and the positive charge remains at the center of the quadruplex. The contention that NaCl modulates the structure of the binding template so that the binding mode that exists in region I in the absence of NaCl is transformed to resemble that of region II is supported further by fluorescence-anisotropy measurements. The anisotropy traces recorded for the solution in region I with 0.5 M NaCl and for the solution in region II without NaCl match well (inset of Figure 3) and thus confirm that the dye experiences a similar local environment in each case (see the Supporting Information).

To verify the existence of a G-quadruplex in $d(G_2T)_{13}G$, a melting curve was generated by monitoring MG fluorescence in a solution of $d(G_2T)_{13}G$ and MG upon heating. A sharp decrease in fluorescence intensity was observed at a T_m value of (21 ± 1) °C. This result is in good agreement with the T_m value reported for a similar G-tract strand.[21] Furthermore, the UV difference spectra of $d(G_2T)_{13}G$ in the presence of NaCl at 80 and 1 °C (see the Supporting Information) exhibit spectral features in agreement with those of a G-quadruplex.[21] Similar measurements with the known quadruplex-forming strand $(G_4T_4)_4$ (ca. 50 μM) showed an approximately 70-fold increase in fluorescence, which suggests that the observed enhancement may be more general with G-quadruplexes. Interestingly, the shift observed in the emission spectrum of $(G_4T_4)_4$ was approximately 8 nm larger than that observed for $d(G_2T)_{13}G$, and the emission band had a broader profile. As the folding topologies of these different G-tract sequences could be different,[7] the variations observed suggest a change in the orientation/rigidity of the dye on the quadruplex.

In summary, by using photochemical methods we have elucidated the binding interactions of the guanine-rich single-strand oligomer sequence $d(G_2T)_{13}G$ with the potentially biologically active chromophoric dye malachite green. Convincingly, MG forms a strong complex with the G-quadruplex, which results in an approximately 100-fold enhancement of its fluorescence yield. This specific interaction is not observed with other single/double-stranded DNA molecules and thus provides a convenient method for the detection of G-quadruplex formation. The cooperative effects of the binding of the dye to the G-quadruplex, the modulation of the binding by the addition of a salt, and the enhanced excited-state activity of the dye are relevant to the design of systems for controlled biomolecule binding, photodynamic therapy, drug delivery, and other phototherapeutic applications. We are currently using designed sequence-specific oligomers and structurally varied dyes to gain an understanding of the detailed mechanistic aspects of the binding interaction and explore its potential applications.

Received: July 20, 2007
Revised: September 3, 2007
Published online: October 30, 2007

Keywords: DNA · dyes/pigments · fluorescence · G-quadruplexes · oligonucleotides

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