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Thiazole Orange–Peptide Conjugates: Sensitivity of DNA Binding to Chemical Structure

Jay R. Carreon,[†] Kerry P. Mahon, Jr.,[†] and Shana O. Kelley*

Boston College, Eugene F. Merkert Chemistry Center, Chestnut Hill, Massachusetts 02467

shana.kelley@bc.edu

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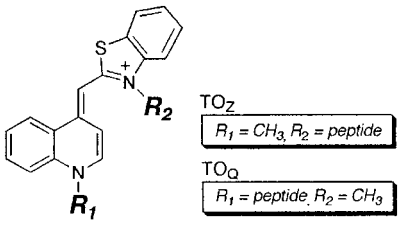
ABSTRACT



Derivatives of the highly fluorescent and DNA-binding dye thiazole orange (TO) are described that feature appended peptides. Functionalization of TO can be achieved at either of the endocyclic nitrogens, and the photophysical properties and DNA-binding modes are sensitive to the position of the tethered peptide. A series of TO–peptide conjugates are described, demonstrating the utility of a solid-phase synthesis approach to their preparation and illustrating how the photophysical and DNA-binding properties of the compounds are influenced by chemical structure.

The development of biomimetic molecules has provided useful probes of the structure and function of nucleic acids.[1] Peptide−intercalator conjugates, with an intercalating moiety serving as an anchor for nucleic-acid binding and an array of functional groups displayed on amino acids imparting increased sequence specificity or chemical reactivity, are a promising but relatively unexplored class of nucleic acid probes.[2,3] Only a few examples of peptidointercalator conjugates have been reported, but the successful isolation of compounds exhibiting RNA cleavage,[3a] DNA hydrolysis activity,[3b,c] or sequence specificity[2a,c−e] highlights the potential of this type of architecture.

Our efforts toward developing peptidointercalator conjugates focus on the use of thiazole orange (TO) as an intercalating scaffold that will deliver an appended peptide to the DNA helix. TO is an ideal photophysical probe for DNA binding, as it displays a high quantum yield when DNA is bound and is essentially nonfluorescent when uncomplexed in aqueous solution.[4,5] Derivatives of TO appended to peptide-nucleic acids have been prepared that provide fluorescence-based detection of triple helix formation,[6] but peptide appendages have not been explored systematically. Our initial studies of these compounds revealed that TO−peptide conjugates containing reactive aromatic residues exhibit photonuclease activity and provide model systems for amino acid promoted DNA damage.[7] To explore the DNA-binding properties of TO−peptide conjugates system-

[†] These authors contributed equally to this work.

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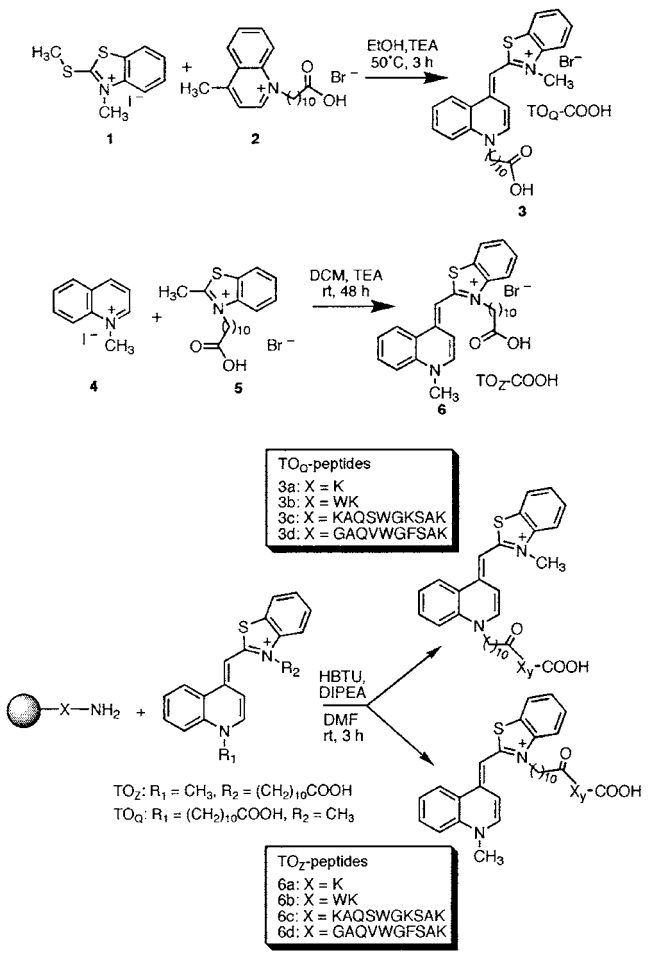
atically, we generated a series of compounds that feature peptides of different lengths and compositions, and we monitored the effect of appending peptides to the two endocyclic nitrogens within the heterocyclic TO structure. The photophysical properties of the DNA-bound conjugates reveal that the binding modes are altered by subtle structural changes.

Two derivatives of TO suitable for peptide coupling were prepared (Scheme 1) using an adaptation of previously

Scheme 1. Synthesis of TO_Z and TO_Q Derivatives and TO−Peptide Conjugates[a]



[a] See the Supporting Information for synthetic protocols and compound characterization.

developed methods.[6,8] One derivative featured a carboxylate-terminated tether attached to the quinoline nitrogen (TO_Q, **3**), and the other featured the same linker attached to the benzothiazole nitrogen (TO_Z, **6**). Derivatives of quinoline and benzothiazole were modified with 11-bromoundecanoic acid (selected to minimize cyclization reactions that occurred with shorter linkers for TO_Z) and subsequently coupled with the appropriate heterocyclic quaternary salt to yield the cyanine dye containing a carboxylate functionality. The motivation for preparing these two isomers comes from the analysis of the NMR structure of a dimeric TO derivative, which shows the two nitrogens pointing toward different faces of the DNA helix.[9] The quinoline nitrogen points directly into the DNA minor groove, while the benzothiazole nitrogen is oriented toward the major groove. Therefore, the isolation of the TO_Q and TO_Z derivatives may permit the delivery of appended functional groups to different regions of DNA's structure.

To study the effects of peptide length, peptide sequence, and linkage position on the DNA-binding characteristics of the peptidointercalators, a series of compounds was synthesized (Scheme 1) using TO derivatives functionalized with a carboxylate-terminated tether and a solid-phase synthetic approach. Peptides were prepared on solid support functionalized with a Wang linker, capped with the TO derivative using standard Fmoc synthetic methods, and cleaved from the resin. The individual sequences selected for this study were designed to include a diverse array of functional groups.

The synthetic procedure produced highly pure materials, with purities of ~95% as judged by HPLC. The conjugates were then characterized using MALDI-TOF mass spectrometry, UV−vis spectroscopy, and fluorescence spectroscopy, and DNA binding affinities were measured by fluorescence. The characterization methods and spectral data are described in the Supporting Information.

All of the TO−peptide conjugates displayed the same DNA-dependent fluorescence as the parent compound (Table 1). Fluorescence quantum yields in the absence of DNA were very low (<0.0001), with 80- to 1300-fold increases in intensity observed upon the addition of calf thymus (CT) DNA (Table 1). TO is only emissive when the monomethine bridge connecting the two heterocyclic structures is rigidified through intercalation and exhibits a quantum yield of 0.11 when DNA bound.[5] Therefore, the observation of comparable quantum yields for most of the TO−peptide conjugates indicates that an intercalative binding mode is maintained. The conjugates with the lowest quantum yields are the decapeptide derivatives featuring the GAQVWGFSAK sequence. Compounds with this sequence appeared to display very weak DNA binding, as evidenced also by low hypochromicity values (Table 1). A second-generation set of decapeptide conjugates were modified to incorporate additional cationic and polar residues that might facilitate DNA binding. These sequence changes resulted in significantly increased quantum yields, as reflected in the values measured for conjugates containing a **K**AQ**S**WG**K**SAK sequence (bolded positions represent altered residues). Thus, the appended peptide appears to significantly alter the DNA-binding properties of a conjugate. A comparison of the properties of TO−K and TO−WK (Table 1) indicates that even more subtle changes in peptide sequence affect the binding mode and photophysical properties of the conjugates. The TO−WK conjugates containing a bulky aromatic residue maintain high DNA affinity but exhibit significantly lower quantum yields.

Interestingly, most of the DNA-bound conjugates based on the TO_Q derivative displayed higher quantum yields than those with the TO_Z scaffold. This trend may signify that the

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Table 1. Photophysical Properties and DNA-Binding Affinities for TO−Peptide Conjugates[a]

compd	Φ_{rel} (DNA)[b]	K_d (μM)	hypochromicity[c] (%)	k_q ($\times 10^{12}$ M^{-1} s^{-1})	displacement (%)
TO_Q−K	0.13 ± 0.01	1.7 ± 0.2	29 ± 2	1.9 ± 0.2	33 ± 2
TO_Z−K	0.043 ± 0.001	0.9 ± 0.1	31 ± 2	4.7 ± 0.1	30 ± 5
TO_Q−WK	0.078 ± 0.003	1.2 ± 0.3	26 ± 1	4.4 ± 0.5	40 ± 6
TO_Z−WK	0.021 ± 0.002	3.1 ± 0.4	37 ± 2	12 ± 2	26 ± 5
TO_Q−GAQVWGFSAK	0.008 ± 0.002	not measured	9 ± 3	30 ± 9	34 ± 4
TO_Z−GAQVWGFSAK	0.012 ± 0.001	not measured	12 ± 1	38 ± 4	36 ± 2
TO_Q−**K**AQ**S**WG**K**SAK	0.13 ± 0.01	0.4 ± 0.1	21 ± 1	1.4 ± 0.1	33 ± 2
TO_Z−**K**AQ**S**WG**K**SAK	0.042 ± 0.002	0.5 ± 0.1	31 ± 2	3.4 ± 0.1	21 ± 1

[a] See the Supporting Information for methods used to obtain values tabulated. Bolded residues in decapeptide conjugates represent positions varied to improve DNA binding. [b] Measured in samples containing 1.5 μM TO−peptide, 45 μM bp CT DNA and reported relative to a fluorescein standard. [c] Measured at λ_{max} (~500 nm).

attachment of the peptide chain to the benzothiazole nitrogen impedes intercalation. Many intercalators bind from the minor groove of DNA;[10] therefore, the addition of substituents to a TO heteroatom that faces into the major groove might interfere with binding or alter the binding mode by requiring threading of the appendage through the DNA helix.

While the peptide conjugates featuring the TO_Z or TO_Q derivatives exhibited comparable DNA-binding affinities, there were significant changes in their photophysical properties (Table 1). To further test the idea that the binding modes of the isomeric derivatives differed, quenching of *TO by $Ru(NH_3)_6^{3+}$ was monitored (Figure 1). The reactivity of this

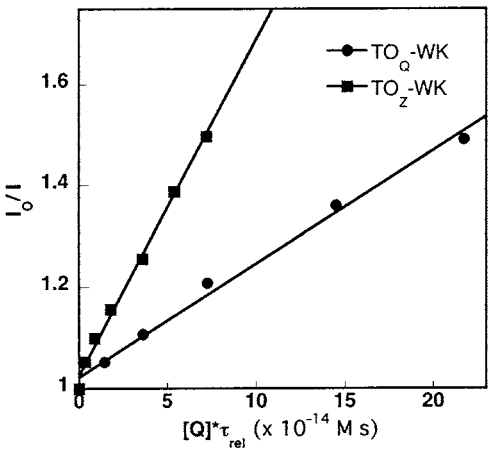

Figure 1. Stern−Volmer analysis of quenching of $*TO_Q$-WK and $*TO_Z$-WK by $Ru(NH_3)_6^{3+}$.

diffusible quencher with the different conjugates provides an estimate of the solution accessibility of TO.[11] For the sets of mono-, di-, and decapeptide conjugates, the TO_Z isomers exhibited higher Stern−Volmer k_q values (Table 1), indicating that the fluorophore is more accessible when the peptide is tethered to the benzothiazole nitrogen. This observation is consistent with the quantum yield values that suggested that the binding mode of the TO_Z derivatives involves a conformation with less stacking and protection than observed for the TO_Q derivatives.

The binding modes of DNA-bound TO−peptide derivatives were also monitored using a distamycin displacement assay (Table 1).[12] Distamycin binds to the minor groove of DNA and can block the binding of ligands that bind to DNA intercalatively, particularly if large functional groups reside in the minor groove. Interestingly, the binding of many of the TO_Q derivatives was more sensitive to the presence of distamycin than the TO_Z counterparts. This trend may suggest that the TO_Z derivatives thread the attached peptide into the major groove, which could permit simultaneous binding of distamycin in the minor groove and the TO_Z−peptide conjugates.

TO−peptide conjugates represent a new class of DNA-binding compounds that may have utility as probes of nucleic acids structure and function, as evidenced by recent studies of DNA cleavage reactions facilitated by these agents.[7] The systematic analysis of a family of TO−peptides, prepared by an efficient and facile solid-phase synthetic approach, revealed that the DNA-binding properties of the conjugates were affected by the position of the appended peptide within the heterocyclic dye structure and the composition of the peptide sequence. The generation of two isomeric TO derivatives as the core for peptide conjugates presents the potential to deliver functional groups to the minor and major grooves of DNA, providing a versatile framework for the development of model systems mimicking protein−DNA complexes.

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Supporting Information Available: Experimental procedures and full characterization of compounds **1**−**6**, **3a**−**d**, and **6a**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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