Preferential Binding of 3,3′-Diethyloxadicarbocyanine to Triplex DNA

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Targeting multistranded nucleic acid structures (triplexes, tetraplexes) with small organic molecules is an active area of research of intense interest.[1−3] Both triplex and tetraplex DNA forms are likely to exist in vivo, and are likely to participate in key biological functions.[4,5] Small molecules designed to selectively bind to triplex or tetraplex regions of the genome might be useful as drugs.[3] Telomeres, for example, are thought to contain tetraplex DNA, which must be replicated separately from the rest of the genome by telomerase enzymes.[6] Small molecules that bind to the tetraplex substrate could selectively inhibit telomerases by preventing the DNA from opening to allow normal initiation of replication. Since maintenance of telomere length is crucial for cell survival, such inhibition might be of therapeutic value.[3]

A number of small molecules that bind to tetraplex DNA have been identified.[7−15] One of the most interesting of these is the carbocyanine dye 3,3′-diethyloxadicarbocyanine (DODC, Figure 1). DODC was identified as a potentially tetraplex selective binding agent through a database searching procedure using the DOCK algorithm.[15] Subsequent experimental studies using a battery of spectroscopic techniques verified that DODC did indeed interact in a unique manner with a DNA hairpin dimer containing an antiparallel tetraplex stack.[15]

We report here a new investigation of the structural selectivity of DODC that uses a novel competition dialysis method that was recently developed in this laboratory.[16] This thermodynamically rigorous method monitors comparative ligand binding to 19 different nucleic acid structures, including single-stranded, duplex, triplex, and tetraplex forms. Both RNA and DNA forms are represented, as are left-handed Z DNA and a DNA:RNA hybrid. Full details of the assay are included as Supporting Information. To our surprise, we found that DODC binds with greater selectivity to triplex DNA than to any of the tetraplex forms included in the assay.

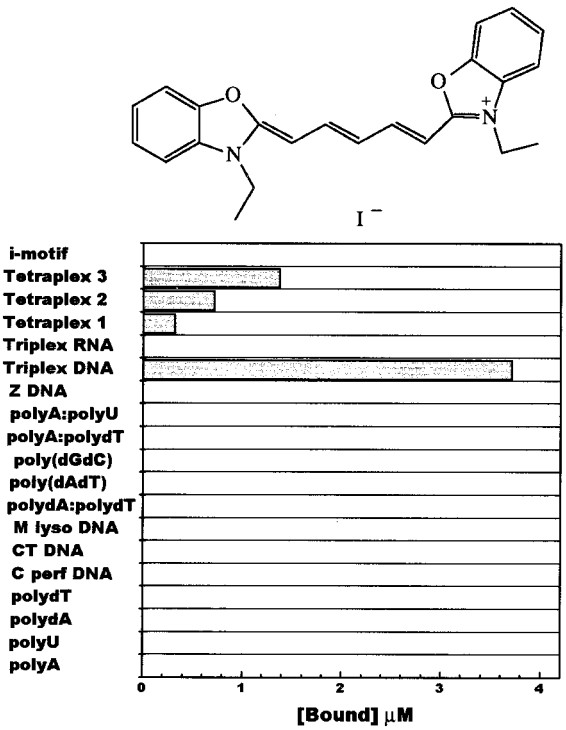

Figure 1. Top: Structure of 3,3′-diethyloxadicarbocyanine (DODC). Bottom: Results of competition dialysis experiment. A 1 μM solution of DODC was dialyzed against 19 different nucleic acid structures (75 μM) for 24 h. The amount of DODC bound to each structure is plotted as a bar graph. Full experimental details are provided as Supporting Information and in ref 16.

Figure 1 shows the results from the competition dialysis assay. Data are shown as a bar graph in which the amount of ligand bound is plotted for each nucleic acid form studied. Table S1 (Supporting Information) describes each nucleic acid form. We emphasize that in the competition dialysis method, the various nucleic acid structures are dialyzed simultaneously against a common free ligand solution. The amount of ligand bound is directly proportional to the binding constant for each conformational form.

There is a wealth of information in Figure 1. DODC binds to only a limited number of nucleic acid structures under these conditions, indicating that it is indeed a structurally selective ligand. Triplex DNA (poly dA:[poly dT]$_2$) binds the most DODC. The three G-tetraplex forms bind DODC to varying extents, but none of them bind as well as the triplex. Binding to all other nucleic acid forms is negligible under the ionic conditions of this assay. Even though DODC was selected by DOCK as tetraplex selective ligand, these results indicate that it preferentially binds to triplex DNA over all nucleic acid conformation included in the assay.

The preferential binding of DODC to triplex DNA was verified by thermal denaturation studies using poly dA:[poly dT]$_2$ (Figure 2). Under these ionic conditions, the third strand dissociates at 42.5 °C and the remaining duplex melts at 75.2 °C. Figure 2 shows that upon addition of increasing molar ratios of DODC the triplex form is selectively stabilized. DODC has little effect on duplex melting. The results shown in Figure 2 are fully consistent with the preferential binding of DODC, observed by the competition dialysis method. Since DODC appears not to interact with duplex DNA at all under the conditions of this assay, its triplex selectivity is more stringent than that of other triplex binders such as BePI[17] and coralyne.[18]

Figure 3 shows a binding isotherm for the interaction of DODC with the triplex DNA, obtained by fluorescence intensity mea-

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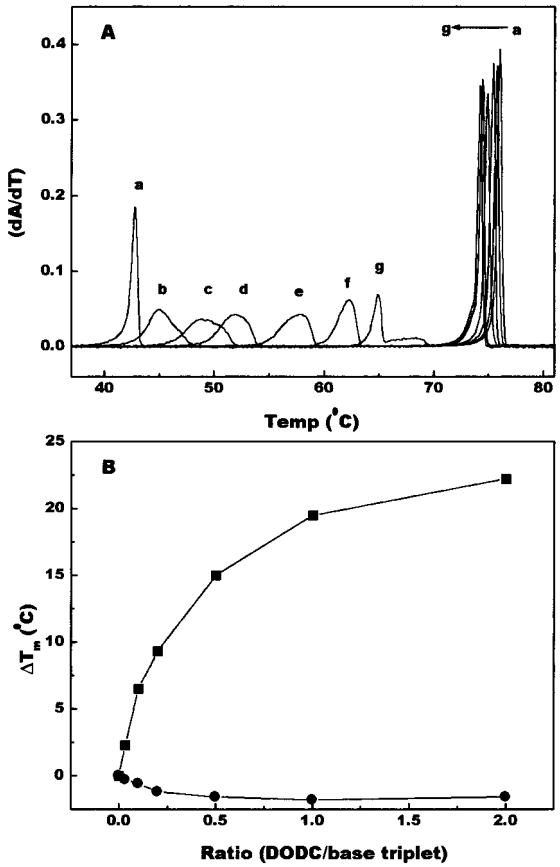

Figure 2. Results of thermal denaturation studies of triplex DNA (poly dA:[poly dT]$_2$) in the presence of added DODC. (A) Derivative melting curves obtained with increasing molar ratios of added DODC. (B) Plot of ΔT_m (the difference in transition midpoint in the presence of DODC relative to melting of poly dA:[poly dT]$_2$ alone) as a function of the molar ratio of added DODC. Filled squares are for the transition for dissociation of the third strand. Filled circles are for the duplex melting transition.

surements.[19] Fluorescence emission of DODC is strongly quenched upon binding. An association constant of 1.3 ($\pm$0.2) $\times$ 10^5 M^{-1} was determined from the titration data in a buffer containing 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, and 185 mM NaCl (pH 7.0). The dialysis results (Figure 1) are in good agreement with this value and yield an apparent binding constant of 1.0 $\times$ 10^5 M^{-1}. Upon binding to triplex DNA, the absorbance spectrum of DODC is red-shifted, and there is an observed nonconservative induced CD spectrum centered at 610 nm (see figure S21 in Supporting Information).

DODC binds 2−3 times better to the triplex than to any of the tetraplex forms used here. In the original report of the DODC−tetraplex interaction, the hairpin dimer [d($G_4T_4G_4$)]$_2$ was found provide the optimal ligand binding site.[15] The exact binding mode of DODC was unclear, although the combined experimental results were most consistent with a binding mode in which part of the dye bound to the tetraplex groove, but with another portion the molecule stacked between the terminal G-tetrad and the thymine loops. A linear, parallel-stranded tetraplex [d(TG_4T)]$_4$ was reported to be a poor receptor for DODC. Our assay does not include either of these exact forms because of our need to use larger molecules that can be retained by our dialysis tubing. Tetraplex **2**, however, is a 22 nt oligonucleotide with the sequence d($AG_3[T_2AG_3]_3$) which adopts a folded, antiparallel structure in solution[8] that would be structurally similar to the hairpin dimer

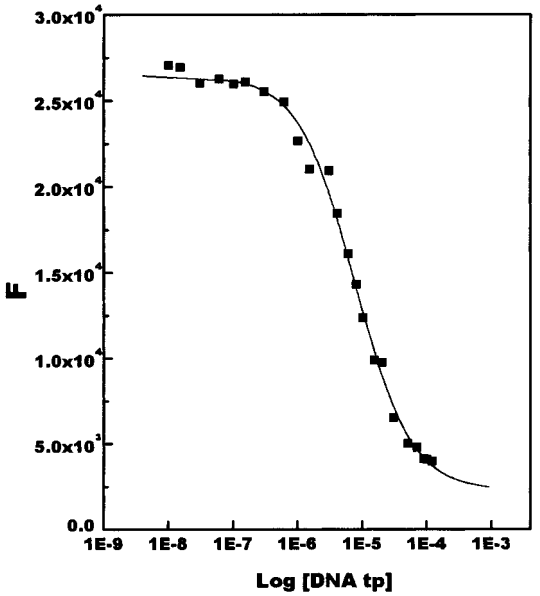

Figure 3. Binding isotherm for the interaction of DODC with the triplex poly dA:[poly dT]$_2$. A fixed concentration (1 μM) of DODC was titrated with increasing concentrations of triplex DNA while monitoring fluorescence emission intensity. The points are the experimental data. The solid line is a nonlinear least-squares fit of the data yielding the binding parameters K = 1.3 ($\pm$0.2) $\times$ 10^5 M^{-1}, F_0 = 26460 ($\pm$369), F_b = 2274 ($\pm$223). K is the association constant for the interaction of DODC with a triplet, and F_0 and F_b are the fluorescence emission intensity of the free and bound forms, respectively. Experimental details of the titration method are described in ref 19.

[d($G_4T_4G_4$)]$_2$ and which would contain the tetrad stem and thymine loop elements thought to be important for DODC binding. DODC binds better to tetraplex **2** than to tetraplex **1** ([d($T_2G_{20}T_2$)]$_4$), which is a parallel 4-stranded structure, a finding consistent with the original report.[15] Of the three tetraplex forms represented, DODC apparently binds best to tetraplex **3** (d($G_{10}T_4G_{10}$)) which appears to form a "G-wire" under the conditions of this assay.[20−23]

The competition dialysis technique used in this study provides a rapid, high-throughput, thermodynamically sound method for evaluating structural selectivity. Unlike the more laborious thermal denaturation and titration binding studies normally used to investigate structural selectivity, competition dialysis allows for a comparison of binding to multiple nucleic acid conformations simultaneously. In the case of DODC, the method reveals an unexpected selectivity for triplex DNA over tetraplex forms, even though a tetraplex receptor model was used to select the ligand by the DOCK algorithm. This finding suggests that appropriate caution should be used when applying computer-based drug discovery algorithms, since receptors with unanticipated higher ligand affinity might well exist that were not part of the selection strategy and which might compete effectively for ligand binding in an environment in which many nucleic acid conformations are present. Rigorous experimental verification of ligand binding preference and affinity is clearly still essential. The competition dialysis method provides a powerful new tool for verifying the structural selectivity of nucleic acid binding agents.

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Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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