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The spectroscopy, dynamics, and electronic structure of pyrenyl–dU nucleosides: $P^{\bullet+}/dU^{\bullet-}$ charge transfer state photophysics

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Abstract—Various spectroscopies including UV–vis absorbance, emission, and emission quantum yield are combined with a variety of kinetics measurements including time resolved emission and nanosecond, picosecond, and femtosecond transient absorbance (TA) to systematize the $P^{\bullet+}/dU^{\bullet-}$ charge transfer (CT) state dynamics of a variety of pyrenyl–dU nucleoside conjugates in several solvents of varying polarity. These results are then analyzed further by means of electronic structure computations in vacuum and using two different solvent models. Finally, the excess electron dynamics of a number of DNA duplex structures substituted with two different pyrenyl–dU nucleosides and 5-XdU, where X=Br or F, electron traps are discussed in terms of achieving high yields of long-lived photoinduced CT products in DNA.
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1. Introduction

One of the most exciting aspects of the study of covalently labeled DNA nucleosides, duplexes, and hairpins is the increasing use of electronic structure and molecular dynamics (MD) calculations, including their combined use, to provide detailed insights into the excited state structures and dynamics of these systems.[1–5] Control of the emission and photoinduced charge transfer (CT) state lifetimes of these labeled DNA assemblies is of significant practical interest, and increasingly computations are proving useful in understanding how to do this. However, my collaborators and I began synthesizing covalently labeled uridine and cytidine nucleosides in the late 1980s before such computations became as powerful and accessible as they are today.[6] Prior to our entry into this field, Dreyer and Dervan had attached a seven-atom linker terminating in a primary amine to C5 of uracil.[7] This modified uridine was then reacted with Fe–EDTA for DNA footprinting studies. Much of our work in the intervening years has continued to attach linkers to this same C5 site. However, as time progressed we concentrated our work on short, one-to-three atom length linkers to control the location of attached labels in the major groove of the labeled DNA duplex. Also prior to our entry into this field, Gillam and Tener employed a transamination reaction between cytidine and 1,6-diaminohexane to create a labeled cytidine with an eight-atom linker attached to N^4.[8] They then biotinylated this nucleoside conjugate for use in enzymatic detection assays.

In our initial work the octameric base sequence 5′-GCACT CAG-3′ was studied and either the central C or T site was modified with a linker terminating in a primary amine. This amine was in turn reacted with a variety of labels: pyrenesulfonyl, pyrenebutyrate, biotin, and fluorescein.[6] Duplexes formed from oligomers with a labeled U (in place of T) showed normal melting behavior while those formed with a labeled C did not. In the U-labeled duplexes, fluorescein emission was quenched a factor of six relative to the label itself; pyrenesulfonyl emission was quenched a factor of 12; and pyrenebutyrate emission was quenched a factor of 500. This work showed that chemically modified bases and standard solid state synthesis protocols could be used to make DNA oligomers and duplexes selectively labeled at internal sites. While it was speculated in this work that intramolecular CT between DNA bases and the photoexcited labels may have been responsible for the extensive emission quenching other studies were needed to confirm this possibility.

Generally organic labels were attached to DNA oligomers. One of the first studies to attach metalloporphyrins to oligomers was carried out by Helene and co-workers.[9] The attachment points in this work and in many others were at the 3′ or 5′ terminal phosphates. As note above Dreyer and Dervan had attached an inorganic coordination complex, Fe–EDTA,

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to a modified internal base in an oligomer.[7] We extended this latter work by incorporating a fluorescent as well as redox-active inorganic label to an internal base; the label was a derivative of tris(2,2′-bipyridine)ruthenium(II) ($Ru(bpy)_3^{2+}$).[10] Again either a central C or T site was modified in octamers with the same 5′-GCACTCAG-3′ sequence. The linker was eight atoms long for addition of bipyridine to C5 of uracil and six atoms long for addition to N^4 of the cytosine. In contrast to the fluorescence behavior of the above organic fluorophores, ruthenium emission yields from covalently attached $Ru(bpy)_3^{2+}$ labels, in short DNA duplexes were identical to that of $Ru(bpy)_3^{2+}$ itself in the same buffer solution. The fact that covalent attachment of $Ru(bpy)_3^{2+}$ to DNA duplexes caused neither emission quenching nor enhancement for either U or C modes of attachment was consistent with an earlier report that exogenous $Ru(bpy)_3^{2+}$ did not associate with duplex DNA.[11] Neither mode of Ru-labeling caused appreciable duplex destabilization relative to the corresponding unmodified duplex.

An interesting attempt to label doubly DNA duplexes and thereby to induce label/label interactions, either excimer formation or CT emission quenching, in preference to label/duplex interactions involved the use of pairs of pyrenebutyrate and pyrenesulfonate and mixed pairs of pyrene/anthraquinone labels attached via eight-atom linkers to a central T site (i.e., at C5 uracil) in each strand of a duplex based on the 5′-GCACTCAG-3′ sequence and its complement.[12] DNA melting studies of these duplexes showed that both the pyrenebutyrate and anthraquinone labels stabilized duplexes by 1.5 kcal/mol per label relative to the corresponding duplexes modified only with terminal amines (i.e., lacking pyrenyl or anthraquinone labels). In contrast a single pyrenesulfonate label had the same free energy of duplex formation as the corresponding duplex with only a terminal amine linker. Not surprisingly under these circumstances label/duplex interactions dominated label/label interactions for duplexes with pyrenebutyrate, mixed pyrenebutyrate/anthraquinone, and mixed pyrenesulfonate/anthraquinone pairs of ligands. The single instance of label/label interactions dominating label/duplex ones occurred for the duplex with two pyrenesulfonate labels. In this case, the emission quantum yield was one-third lower for the latter duplex compared to one with a single pyrenesulfonate label. Additionally, each of the three emission lifetime components measured was significantly shortened in the case of two labels compared to only one label. Excimer emission was not seen from the duplex with two pyrenesulfonate labels, however, most likely due to CT quenching of excimers by neighboring DNA bases. The reactivity of a 5′-phosphate linked pyrenyl label toward bases in the attached duplex was confirmed subsequently.[13] In that work 96% of the pyrenyl label's emission was quenched compared to an identical concentration of free 4-(1-pyrenyl)butanol. Clearly, DNA bases are too reactive toward pyrenyl excited singlet states to use long flexible linkers if the goal is to direct excited state reactivity along specific channels. Ways of reducing label/duplex interactions include using shorter linkers to join bases and labels and also introducing redox-inactive 'blocking' groups on neighboring nucleotides to prevent excited labels from contacting DNA bases. The remainder of this paper will explore these ideas in greater detail.

2. Results and discussion

2.1. Demonstrations of CT quenching of photoexcited pyrene by DNA bases

2.1.1. Pyrenyl-dG. Geacintov and co-workers[14–17] examined photoinduced CT mechanisms in the covalently linked pyrenyl-dG adduct a shown in Figure 1 and in mixtures of 7,8,9,10-tetrahydroxytetrahydrobenzo{*a*}pyrene (BPT) with dG.[15] In polar organic solvents, pyrenyl radical anions ($BPT^{\bullet-}$) were observed in solutions of BPT and dG (0.1 M). Radical ions were not seen in either the covalent adduct (+)-*trans*-BPDE-N^2-dG or in aqueous solutions of BPT and dG on timescales >10 ns. However, in these latter systems the primary products of fluorescence quenching were pyrenyl triplet excited states with greatly enhanced yields (3–10 fold larger than expected from normal intersystem crossing). The mechanism of enhanced triplet yield involves photoinduced CT from dG to pyrenyl residues, followed by rapid and efficient charge recombination to form pyrenyl triplet excited states. A follow-on study with higher time resolution showed that the rate of charge separation within (+)-*trans*-BPDE-N^2-dG in *N*,*N*′-dimethyl formamide (DMF) was 110 ps and the rate of charge recombination was 560 ps resulting in a CT efficiency of 0.8±0.2. In hydrogen bonding solvents such as *N*-methylformamide (NMF) and formamide (FA) the rates of CT from dG to BPDE decreased, while the rates of charge recombination increased. The overall result was that the yields of radical ions were less than 0.15 in the hydrogen bonding solvents. In aqueous solutions of the adduct, the rate of CT slowed even further, and radical ion products were not observed, presumably due to very rapid charge recombination. Interestingly, a kinetic isotope solvent effect (k_H/k_D) of 1.5 suggests that solvent H-bonds influence the intramolecular CT rates in (+)-*trans*-BPDE-N^2-dG.[17] Relatedly, Wagenknecht and co-workers have recently reported studying the fluorescence properties of the $P^{\bullet-}/dG^{\bullet+}$ CT state in DNA single strands and duplexes

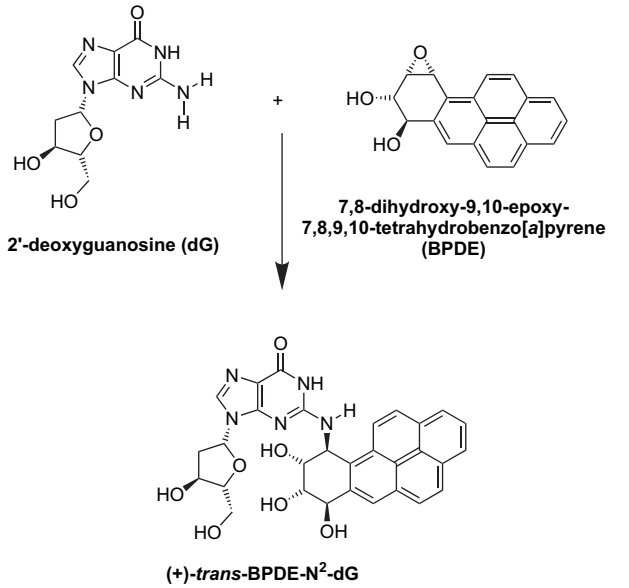

Figure 1. Reaction of carcinogenic and mutagenic BPDE with the exocyclic amino group of dG to form the (+)-*trans*-BPDE-N^2-dG adduct.

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