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Pyrene as a fluorescent probe for DNA base radicals

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Received (in Cambridge, UK) 23rd May 2003, Accepted 11th June 2003
First published as an Advance Article on the web 26th June 2003

The steady-state emission spectra of 5-(1-pyrenyl)-modified pyrimidine and 8-(1-pyrenyl)-modified purine nucleosides in water at different pH values provide important information about the acidity or basicity of photochemically generated DNA base radicals which are key intermediates in DNA-mediated charge transport processes.

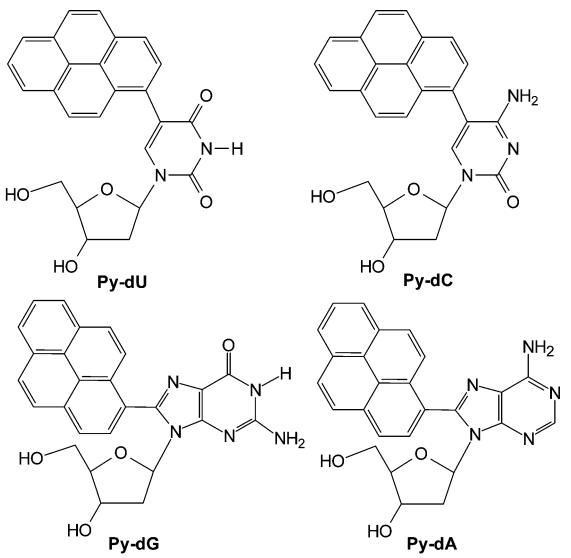
With respect to the biological relevance of DNA-mediated charge transport phenomena, most of the past experiments have focused on oxidative hole transport.[1,2] On the other hand, reductive electron transport has been applied in DNA chip technology[3] and in DNA nanotechnology.[4] It is now well-established that DNA-mediated long-range hole and electron transport occur *via* a diffusive hopping mechanism.[5] During hole hopping, the guanine radical cation has been identified as the intermediate charge carrier.[5,6] With respect to the reduction potentials of the DNA bases,[7,8] it has been proposed that the cytosine and thymine radical anions could act as intermediate charge carriers during electron hopping through DNA. The one-electron oxidation or reduction of DNA bases has drastic effects on their acidity or basicity, respectively. Thus, proton transfer processes can dramatically influence charge transport efficiency due to the separation of spin and charge. So far, most knowledge about the acidity and basicity of DNA base radicals stems from γ-radiolysis studies.[9]

Recently, we prepared 5-(1-pyrenyl)-2′-deoxyuridine (Py-dU),[10] 5-(1-pyrenyl)-2′-deoxycytidine (Py-dC),[11] 8-(1-pyrenyl)-2′-deoxyguanosine (Py-dG),[10,11] and 8-(1-pyrenyl)-2′-deoxyadenosine (Py-dA)[11] as nucleoside models for DNA-mediated charge transport (Scheme 1). Here, we want to report the fluorescence properties of these nucleosides in water at different pH values reflecting important information about the acid–base properties of the generated DNA base radicals.

In the case of the pyrimidine derivatives Py-dU and Py-dC, excitation of the pyrene moiety leads to an intramolecular electron transfer yielding the pyrene radical cation and the corresponding pyrimidine radical anions ($Py^{\cdot+}$-$dX^{\cdot-}$). In the case of Py-dU, this charge transfer assignment has been proven previously.[12,13] Based on the reduction potential for $Py^{\cdot+}$/Py of 1.52 V (*vs.* NHE)[14] and E_{00} = 3.25 eV,[14] the electron transfer process should be exergonic, based on redox potential values in the range of −1.1 to −1.2 V for the $dT/dT^{\cdot-}$ and $dC/dC^{\cdot-}$ couples.[7]

The fluorescence intensity of Py-dU and Py-dC in water was measured using an equal optical density of the nucleosides at the excitation wavelength 340 nm (Fig. 1). The pH dependence of the emission of Py-dU shows a typical sigmoidal curve representing a pK_a value of ~5 for the protonated biradical $Py^{\cdot+}$-dU(H)$^{\cdot}$.[13] This assignment is supported by recent femtosecond transient absorption experiments showing that the locally excited state (Py*) is quenched as a result of a proton-coupled charge-separation (Scheme 2).[13]

Similar experiments using Py-dC in water showed quite different results. At equal optical density, Py-dC exhibits fluorescence quenching over almost the entire pH range (1.5–12.5). Steady-state fluorescence spectra of Py-dC in MeCN showed a quantum yield of Py-dC which is significantly higher than in water (data not shown). Time-resolved transient absorption spectroscopy indicated that an intramolecular electron transfer does not occur upon photoexcitation of Py-dC in MeCN due to the absence of protons.[13] As in the case of Py-dU,



Scheme 1

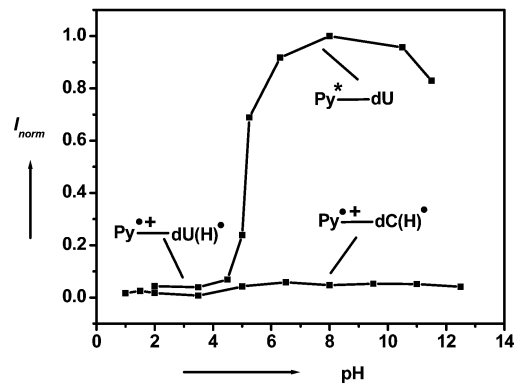
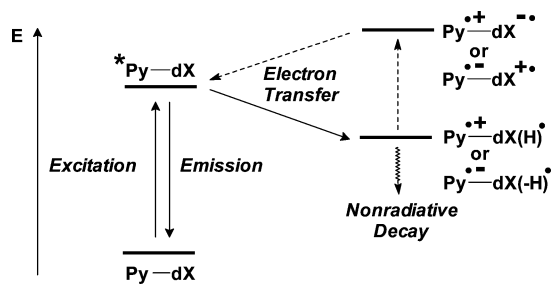


Fig. 1 pH dependence of the fluorescence intensity of Py-dU and Py-dC in water at equal optical density (o.d. = 0.15 at λ_{exc} = 340 nm).



Scheme 2 Photoinduced charge transfer and proton transfer in the pyrene-modified nucleosides Py-dX (X = U, C, G, A).

DOI: 10.1039/b305732a

the fluorescence quenching of Py-dC in water is the result of a proton-coupled electron transport. However, in contrast to Py-dU, the pK_a value for the protonated biradical Py$^{\cdot+}$-dC(H)$^{\cdot}$ must be larger than 12 and therefore the unprotonated charge-separated species Py$^{\cdot+}$-dC$^{\cdot-}$ can not be produced in water.

The remarkable differences in the basicities of the generated pyrimidine radical anions imply an important significance for the mechanism of electron migration in DNA. Based on the reduction potentials, it was proposed that both dC$^{\cdot-}$ and dT$^{\cdot-}$ (structurally very similar to dU$^{\cdot-}$) could act as potential intermediate electron carriers.[5] Our results clearly indicate that the proton transfer does not limit electron hopping *via* dT$^{\cdot-}$-dA base pairs but significantly interferes with electron transport through dC$^{\cdot-}$-dG base pairs.

The modified nucleoside Py-dG represents a model for hole transport in DNA. Photoexcitation of Py-dG initiates the ultrafast formation of the charge-separated state Py$^{\cdot-}$-dG$^{\cdot+}$. A crude estimation of the driving force for this process (using E_{00} = 3.25 eV for Py* and E_{red} = −1.9 V (*vs*. NHE) for Py/Py$^{\cdot-}$ [14] and E_{ox} = +1.3 V for dG$^{\cdot+}$/dG)[15] reveals a ΔG value of −0.05 eV. Upon excitation at 350 nm, the steady-state fluorescence of Py-dG in water shows the strongest intensity at low pH values (< 6) and a significant quenching at high pH values (> 6) (Fig. 2). Hence, the fluorescence exhibits an inverted pH dependence to the one observed for Py-dU. It is important to note that this pH/emission profile is contrasting to Py-dU and clearly demonstrates that the electron transfer processes in Py-dG and Py-dU occurs in opposite directions: The intramolecular charge transfer in Py-dG generates a cationic nucleoside species (Py$^{\cdot-}$-dG$^{\cdot+}$) which deprotonates at higher pH values, whereas the charge transfer in Py-dU yields an anionic DNA base radical (Py$^{\cdot+}$-dU$^{\cdot-}$) which is being protonated at high pH values (> 5).

The question of proton transfer in dG$^{\cdot+}$-dC base pairs is crucial for the understanding of hole hopping in DNA. Based on our results, the pK_a value of dG$^{\cdot+}$ is ~4. The pK_a value of the complementary DNA base cytosine (C) is very similar (4.5).[9] Hence, there is likely a protonation equilibrium in a one-electron oxidized dG$^{\cdot+}$-dC base pair which could interfere with the hole transport and potentially interrupt hole hopping in DNA. In fact, measurements of the kinetic isotope effect of hole transport in DNA have been performed by Giese and Wessely and provide some evidence for a coupling between hole hopping and proton transfer processes.[16]

In Py-dA, a prediction of the charge transfer direction based on the comparison of redox potentials seems to be rather difficult. Based on the potential of the dA$^{\cdot+}$/dA couple,[8] which is 0.1–0.2 V higher than that of the dG$^{\cdot+}$/dG couple, the photoinduced oxidation of dA by the pyrene moiety is slightly endergonic. On the other hand, the photoinduced reduction of dA could be exergonic since the potential for the dA/dA$^{\cdot-}$ couple is ~0.3–0.4 V higher than that of the dU/dU$^{\cdot-}$ couple. It is remarkable that the emission profile of Py-dA in water at different pH values (Fig. 2) gives a clear answer about the charge transfer direction. Comparing the pH-dependent emission of Py-dA with that of Py-dU *vs*. Py-dG, it becomes clear that a photoinduced intramolecular electron transfer takes place in Py-dA resulting in the reduction of the dA moiety. The sigmodial curve of Py-dA would then represent a pK_a value of ~4 for the protonated biradical Py$^{\cdot+}$-dA(H)$^{\cdot}$. Such a low pK_a value appears to be rather unlikely with respect to the 6-aminopurine substructure as part of Py-dA. Interestingly, Steenken reports a pK_a value greater than 13 for dA(H)$^{\cdot}$.[9] Hence, we attribute the fluorescence quenching of Py-dA at pH < 3 to the presence of its ground-state protonated form Py-dA(H)$^+$, which predominantly relaxes non-radiatively after excitation. This interpretation is supported by small changes in emission maxima of Py-dA at pH < 3 (data not shown).

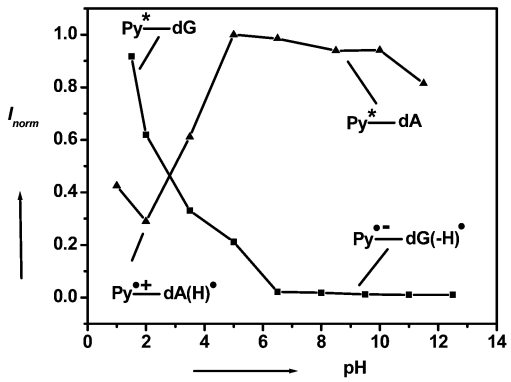


Fig. 2 pH dependence of the fluorescence intensity of Py-dG and Py-dA in water (o.d. = 0.15 at λ_{exc} = 340 nm).

These investigations emphasize the relevance of DNA base radical acidities and basicities for the understanding of charge transport mechanisms in DNA and the formation of DNA damages.

We gratefully acknowledge financial support by the Deutsche Forschungsgemeinschaft, the Volkswagen Foundation and by the Fonds der Chemischen Industrie.

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