Photoinduced electron transfer in a Watson–Crick base-paired, 2-aminopurine:uracil-C_{60} hydrogen bonding conjugate†

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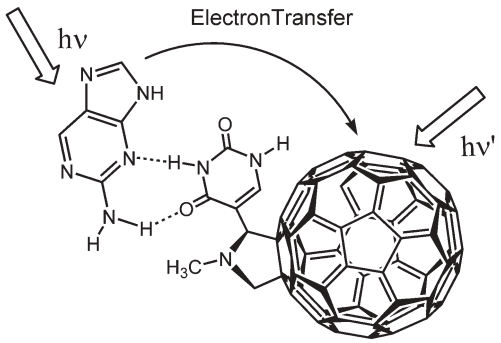
A fluorescent reporter molecule, 2-aminopurine was self-assembled *via* Watson–Crick base-pairing to a uracil appended fullerene to form a donor–acceptor conjugate; efficient photo-induced charge separation was confirmed by time-resolved emission and transient absorption spectral studies.

Electron transfer (ET) processes in DNA have attracted much interest because of their long range ET and photo-therapy applications.[1] To probe the structures and dynamics of nucleic acids, the fluorescent adenine isomer, 2-aminopurine (2-AP) is a widely employed reporter molecule because of its ability to form Watson–Crick base pairs with the appropriate nucleic bases.[2] Additionally, 2-AP exhibits a red shifted absorption maximum, which allows for selective excitation, and its fluorescence is strongly quenched in both single- and double-stranded DNA.[2] Thus, this property has been widely exploited to probe the dynamics of melting, abasic sites, mismatched base pairs, metal ion binding, and thermodynamics and kinetics of protein-induced DNA conformational transitions.[2]

Fluorescence quenching of 2-AP may involve ET processes,[3] although the short lived charge-separation products were difficult to spectrally characterize primarily due to the low absorptivity of the radical ions. Establishing the mechanism of the photoinduced events is important for structure and dynamics evaluation when this probe molecule interacts with biomolecules such as DNA.[4] One way of establishing the ET process is to form a hydrogen bonded conjugate between 2-AP and a well-known acceptor molecule and pursue fluorescence quenching of the constituents followed by appearance of transient peaks diagnostic of cation and anion radical species. Here, we have utilized this strategy by base-pairing the target probe to a uracil functionalized electron acceptor, fullerene[5] (Scheme 1). The employed fullerene is a superior electron acceptor due to the low reorganization energy that results in rapid charge separation and rather slow charge recombination as a consequence of accessing the Marcus inverted region.[6] Importantly, the electron transfer product, fullerene anion radical, exhibits a characteristic peak in the near-IR region around 1020 nm, thus serving as a diagnostic proof of electron transfer.[6]

In the present study, the experiments were performed in CH_2Cl_2–CH_3OH (1 : 1) mixture for better solubility of the donor and acceptor entities. The absorption spectrum of 2-AP revealed a band at 310 nm while the spectrum of uracil-C_{60} was typical of that of fulleropyrrolidine with absorption in the 200–600 nm region accompanied by a sharp band at 430 nm. As shown in Fig. 1a, 2-AP revealed a strong emission at 370 nm. Addition of uracil-C_{60}[7] quenched the intensity of the 370 nm emission band over 80% of its original intensity. The formation constant for the 1:1 conjugate (2-AP:uracil-C_{60}) calculated by constructing a Benesi–Hildebrand plot (see ESI for the plots)[8] was found to be 3.3×10^4 M^{-1}; this value implies *ca.* 80% of the conjugate fraction under 0.1 mM concentrations of both entities in the weakly hydrogen bonding CH_3OH containing solvent system.



Scheme 1 Watson–Crick type hydrogen bonded 2-aminopurine:uracil-C_{60} conjugate showing different routes of light induced electron transfer.

DFT molecular orbital calculations at the B3LYP/3-21G(*) level[9] confirmed a low energy Watson–Crick type base-paired

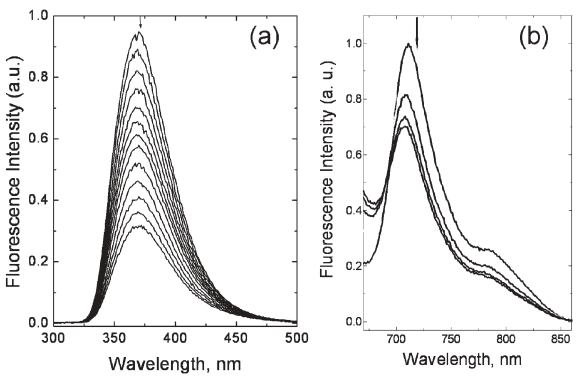


Fig. 1 Fluorescence spectra of (a) 2-AP in the presence of increasing amounts of uracil-C_{60} (1.4 μM each addition, λ_{ex} = 270 nm), and (b) uracil-C_{60} (0.05 mM) in the presence of 2-AP (50 μM each addition); λ_{ex} = 400 nm.

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structure for the conjugate as shown in Fig. 2, in which two hydrogen bonds were observed. The HOMO and LUMO were localized on 2-AP and C_{60}, respectively, predicting 2-AP$^{\bullet+}$:uracil-$C_{60}^{\bullet-}$ as charge-separated state upon photo-excitation (Fig. 2). It may be mentioned here that the HOMO of earlier investigated adenine:uracil-C_{60} was largely localized on the uracil moiety,[5a] suggesting better electron donor capability of the present 2-AP than adenine toward uracil-C_{60}. Interestingly, the calculated K value for 2-AP:uracil-C_{60} is close to that reported by Sessler *et al.* for a G:C type base-paired conjugate which involves three H-bonds.[5b] This suggests additional H-bonding interactions or stacking of the aminopurine–uracil entities[3d] in the present conjugate. Indeed, B3LYP/3-21G(*) computations indicate that in contrast to adenine,[5a] 2-aminopurine is capable of a non Watson–Crick structure with three hydrogen bonds with comparable energy.

A Stern–Volmer plot was constructed to evaluate the quenching constant, K_{SV} with uracil-C_{60}, where no appreciable quenching of 2-AP was observed when only uracil was added. The K_{SV} value was found to be 1.1×10^5 M^{-1}, the magnitude of which suggests occurrence of an intramolecular quenching process in the hydrogen bonded conjugate. In a separate experiment, the emission of uracil-C_{60} at 715 nm was monitored in the presence of 2-AP. Quenching of fullerene emission was also observed (Fig. 1b), indicating charge separation *via* the excited singlet state of the C_{60} moiety ($^1C_{60}$*) since energy transfer from the $^1C_{60}$* to 2-AP is not possible based on energy considerations.

Cyclic voltammograms were recorded to evaluate the redox potentials and to estimate the free-energy changes of ET for the 2-AP:uracil-C_{60} conjugate (Fig. 3). 2-AP revealed a reversible oxidation couple located at 260 mV *vs.* Fc/Fc$^+$, indicating it to be a good electron donor. The first reduction of C_{60}-uracil was found to be quasi-reversible and was located at −1.05 V *vs.* Fc/Fc$^+$ which is in good agreement with that of fulleropyrrolidines.[3b] Spectroelectrochemical studies were performed to characterize the 2-AP cation and the uracil-C_{60} anion radicals, which showed bands at 315 and 390 nm (see ESI), and at 1020 nm, respectively. The driving forces for charge separation ($-\Delta G_{CS}$) *via* the excited singlet states of 2-AP and uracil-C_{60} calculated according to the Rehm–Weller equation[10] were found to be 2.0 and 0.4 eV, respectively. In these calculations, $E_{0,0}$ values of 3.35 eV and 1.74 eV, respectively, for 2-AP and C_{60}-uracil were employed. These results indicate the occurrence of an exothermic charge-separation process in the supramolecular dyad either from the singlet excited states of 2-AP or uracil-C_{60} (see ESI for energy level diagram). The free-energy change for charge recombination ($-\Delta G_{CR}$) of 2-AP$^{\bullet+}$:uracil-$C_{60}^{\bullet-}$ was found to be 1.3 eV. Charge-recombination in 2-AP$^{\bullet+}$:uracil-$C_{60}^{\bullet-}$ is predicted to be a slower process since the highly exothermic ΔG_{CR} value lies in the inverted region of the Marcus parabola.[6]

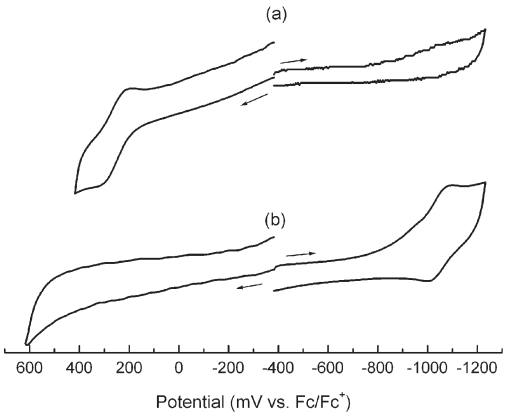

Fig. 3 Cyclic voltammograms of (a) 2-aminopurine and (b) uracil-C_{60} in CH_2Cl_2–CH_3OH (1 : 1) mixture containing 0.1 M $(n\text{-}C_4H_9)_4NClO_4$. Scan rate = 100 mV s^{-1}.

Time-resolved emission[11] and nanosecond transient absorption[12] spectral studies were performed to evaluate the rates of charge separation and charge recombination and to characterize the charge-separated products. Addition of uracil-C_{60} to a solution of 2-AP accelerated the fluorescence decay rate, giving a short major fluorescence lifetime (τ_f) of 116 ps. Fluorescence decay of uracil-C_{60} was also accelerated by the addition of 2-AP, giving a short fluorescence lifetime of 225 ps, which was considerably shorter than that of pristine uracil-C_{60} (τ_{f0} = 1.3 ns). From these data, the charge-separation rate (k_{CS})[13] and quantum yield (Φ_{CS}) *via* the excited singlet state of uracil-C_{60} by 2-AP were evaluated to be 3.5×10^9 s^{-1} and 0.81, respectively. The k_q and Φ_q calculated from the quenching of singlet excited 2-AP by uracil-C_{60} were found to be 8.5×10^9 s^{-1} and 0.99, respectively.

Nanosecond transient absorption spectra recorded after the 355 or 532 nm laser light irradiation unambiguously proved the formation of a 2-AP$^{\bullet+}$:uracil-$C_{60}^{\bullet-}$ charge-separated state. As

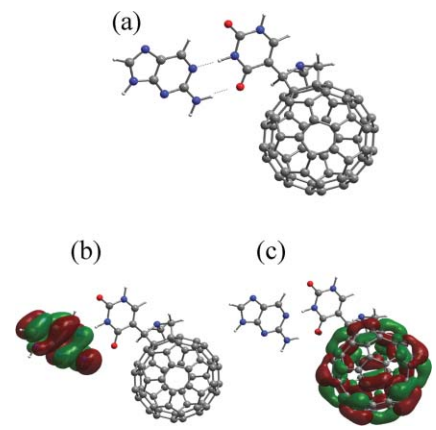

Fig. 2 (a) B3LYP/3-21G(*) optimized structure of 2-AP:uracil-C_{60} conjugate (red = O, blue = N, gray = C, and white = H). The HOMO and LUMO are shown in (b) and (c), respectively.

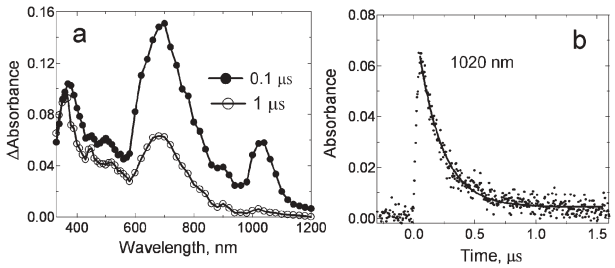

Fig. 4 (a) Transient absorption spectra observed by the excitation of 2-AP:uracil-C_{60} conjugate with 355 nm laser light in CH_2Cl_2–CH_3OH (6 : 4) mixture. (b) Absorption time profile of the 1020 nm band.

shown in Fig. 4, the spectra revealed an absorption peak around 390 nm corresponding to the formation of 2-AP$^{\bullet+}$ (see ESI)[14] and one at 1020 nm corresponding to uracil-$C_{60}^{\bullet-}$,[6] although the decay profile of the 390 nm band is not the same as that of the 1020 nm band, probably because of the overlapping triplet states of the donor and acceptor entities in the short wavelength region. The time profile of the 1020 nm band revealed a quick rise after 6 ns laser excitation, confirming charge separation *via* the singlet excited state, but not *via* the triplet state. Since the 355-nm light predominantly excites the C_{60} moiety, the charge separation *via* $^1C_{60}$* was confirmed. From the excited singlet state of 2-AP, both the charge separation and energy transfer could occur competitively. The band around 700 nm, ascribed to the triplet state of the C_{60} moiety,[15] may be produced *via* intersystem crossing ($1 - \Phi_{CS} = 0.19$) by the direct excitation of the uracil-C_{60} moiety with 355 nm light.

From the decay of the 1020 nm band obeying first-order kinetics, the charge recombination rate (k_{CR}) was evaluated to be 6×10^6 s^{-1}, which was three orders of magnitude smaller than k_{CS}. Such a slow k_{CR} value can be rationalized by the large ΔG_{CR} (-1.3 eV) and small reorganization energy characteristics of the spherical C_{60} moiety.[6]

In summary, photoinduced charge separation involving the widely used reporter molecule in biochemistry for DNA structure and dynamics, 2-aminopurine, was unraveled by forming a base-paired conjugate with a uracil-C_{60} acceptor molecule. The present adopted strategy and the choice of the electron acceptor, and time-resolved emission and nanosecond transient absorption spectral techniques covering the wide UV–visible–near IR region unanimously proved the electron donor ability of 2-AP in a base-paired donor–acceptor conjugate. That is, the electron transfer products of 2-AP$^{\bullet+}$ and uracil-$C_{60}^{\bullet-}$ in the hydrogen-bonded base-paired conjugate were spectrally characterized. The present study suggests that ET reactions can occur in DNA related base-paired systems where fluorescence quenching of the probe is observed. Further studies along this line are in progress.

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12 Nanosecond transient absorption measurements were carried out using THG (355 nm) of a Nd:YAG laser (Spectra-Physics, Quanta-Ray GCR-130, fwhm = 6 ns) as an excitation source. For transient absorption spectra in the near-IR region (600–1600 nm), monitoring light from a pulsed Xe lamp was detected with a Ge-avalanche photodiode. For transient absorption spectra in the UV–Vis region (350–600 nm), a Si-PIN photodiode was used as a detector. All the samples in a quartz cell were deaerated by bubbling argon through the solution for 15 min.

13 The k_{CS} and Φ_{CS} were calculated using: $k_{CS} = (1/\tau_f)_{complex} - (1/\tau_{f0})_{free}$, $\Phi_{CS} = [(1/\tau_f)_{complex} - (1/\tau_{f0})_{free}]/(1/\tau_f)_{complex}$.

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