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Published on Web 01/04/2003

Formation of Pyrene Dimer Radical Cation in DNA Reflecting DNA Dynamics in the Time Range of 1 μs to 1 ms

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Abstract: Doubly pyrene (Py)-conjugated oligodeoxynucleotides (ODNs) were synthesized and used for measurement of the formation rates of Py dimer radical cation ($Py_2^{\bullet+}$) upon one-electron oxidation during the pulse radiolyses. Formation of Py radical cation ($Py^{\bullet+}$) in the time scale of less than 5 μs was monitored at 470 nm after an electron pulse during pulse radiolysis of D_2O solution of doubly Py-conjugated ODN in the presence of $K_2S_2O_8$. Concomitant with the decay of $Py^{\bullet+}$, formation of $Py_2^{\bullet+}$ with an absorption peak at 1500 nm (charge resonance band) was observed in the time range of ~100 μs. The formation rate of $Py_2^{\bullet+}$ in DNA reflected the dynamics of DNA which allows the interaction between $Py^{\bullet+}$ and Py, since transiently formed DNA structure is trapped by the attractive charge resonance (CR) interaction to give $Py_2^{\bullet+}$. The formation rate of $Py_2^{\bullet+}$ with a characteristic CR absorption band in the near-infrared (near-IR) region was demonstrated to be useful to obtain the structural and dynamical information of transiently formed DNA in the time range of 1 μs to 1 ms.

Introduction

The DNA double helix is a dynamic structure with several transient structures appearing in a short period of time. The structure and dynamics of such locally formed DNA transient structure have been investigated by incorporating two fluorescent probes at the defined sites of DNA. The fluorescence resonance energy transfer (FRET) has been used to measure the relative spatial position between two fluorescent probes.[1−3] In particular, excimer, which requires the close cofacial contact between the excited fluorescent probe and its neutral counterpart, provides the direct structural information through the excimer fluorescence.[4−6] However, since lifetimes of the fluorophores in the singlet excited state are typically in the time range of several nanoseconds or less, it is difficult to obtain the information of the dynamics of DNA in the time range of microseconds to milliseconds by transient fluorescence spectroscopy. On the other hand, some radical cations of aromatic molecules have much longer lifetimes up to 1 min in aqueous solution.[7] The close interaction between radical cation and its neutral counterpart leads to formation of the dimer radical cation, which has a structure similar to that of the excimer.[8−10] The dimer radical cation is stabilized by charge resonance (CR) between the two aromatics which can be monitored as a CR transition band in the absorption spectrum. As for Py, $Py_2^{\bullet+}$ shows a clear CR absorption band in the near-infrared (near-IR) region around 1500 nm together with absorption peak at 380 nm.[9] Therefore, $Py_2^{\bullet+}$ can be readily distinguished from its monomer radical cation ($Py^{\bullet+}$) with an absorption peak at 470 nm.

We have recently reported pulse radiolysis of Py-conjugated oligodeoxynucleotide (ODN) where the $Py^{\bullet+}$ generated can be monitored by the transient absorption.[11] Since the hole is irreversibly generated in DNA free from charge recombination, the long lifetime of $Py^{\bullet+}$ enables measurements of processes in the time range from microseconds to milliseconds. In the present study, pulse radiolyses of doubly Py-conjugated ODNs were performed. The formation rate of the intramolecular $Py_2^{\bullet+}$ was discussed on the basis of the DNA dynamics which allows the interaction between $Py^{\bullet+}$ and Py.

Experimental Section

Pulse Radiolysis. Py-conjugated ODNs at the 5′-end and 2′ sugar position were synthesized according to the reported procedure.[12,13] Positive charge was generated in Py-conjugated ODN by the reaction with $SO_4^{\bullet-}$, which was generated during pulse radiolysis (28 MeV, 8 ns) of Ar-saturated D_2O solution containing 10 mM $K_2S_2O_8$, 100 mM *t*-BuOH, 20 mM sodium phosphate buffer (pH 7.0), and 0.1 mM (strand

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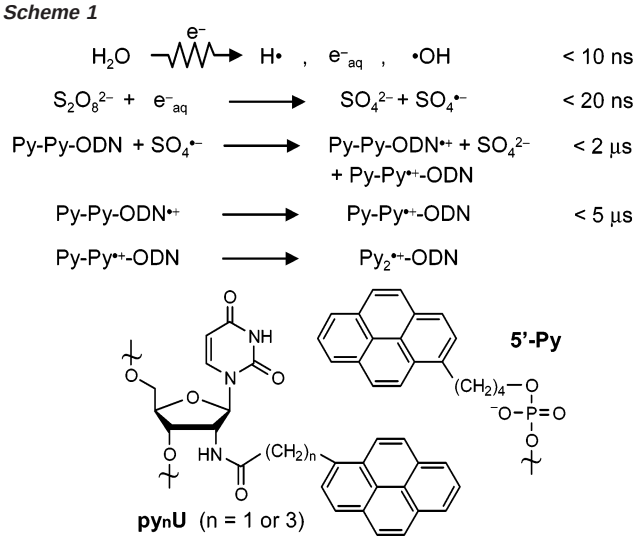
10.1021/ja026754q CCC: $25.00

Scheme 1



concentration) ODN (Scheme 1).[11] A xenon flash lamp (Osram, XBO-450), which was synchronized with the electron pulse, was focused through the sample as a probe light for the transient absorption measurement. Time profiles of the transient absorption at the UV−visible region were measured with a monochromator (CVI, DK-240) equipped with a photomultiplier (Hamamatsu Photonics, R928) and digital oscilloscope (Tektronix, TDS 580D). The monitor light for the measurement of time profiles of the transient absorption in the near-IR region was passed through an interference filter (CVI, transmittance of 40%, bandwidth of 10 nm), and its intensity was monitored with a fast InGaAs PIN photodiode equipped with an amplifier (Thorlabs, PDA255) and digital oscilloscope. For the time-resolved transient absorption spectral measurement, the monitor light was focused into a quartz optical fiber, which transported the electron pulse induced transmittance changes to a gated-multichannel spectrometer (Unisoku, TSP-601−02).

Fluorescence Spectra. Fluorescence spectra of Py-conjugated ODNs were measured in Ar-saturated aqueous solution in the presence of 20 mM sodium phosphate buffer (pH 7.0) at a total strand concentration of 8 μM at 20 °C on a Hitachi 850 spectrofluorometer.

Melting Temperature. The thermal denaturation profile was recorded on a JASCO V-530 spectrometer equipped with a Peltier temperature controller (ETC-505T). The absorbance of the sample was monitored at 260 nm from 10 to 70 °C with a heating rate of 1 °C/min. The T_m value was determined as the maximum in a plot of $\Delta A_{260}/\Delta T$ versus temperature.

Results and Discussion

To introduce two Pys at the defined site in DNA, Py was conjugated at the 5′-end and 2′ sugar position of ODN (Scheme 1). For the singly Py-conjugated ODNs, $Py^{\bullet+}$ absorption band at 470 nm with a lifetime more than 100 μs was observed upon oxidation during the pulse radiolysis (Figure 1A). There are two processes for the formation of $Py^{\bullet+}$: the direct oxidation of Py by $SO_4^{\bullet-}$ and the hole transfer from $G^{\bullet+}$ generated in DNA to Py. In the present study, short ODNs with minimum separation between **pyU** and Gs were used to accomplish the hole-transfer process within 5 μs. Thus, the similar formation rate of $Py^{\bullet+}$ was observed for all six singly **pyU**-conjugated ODNs.[14] Interestingly, in the case of doubly Py-conjugated ODN, formation of the transient absorption peaks at 380 nm (Figure 1B) and 1500 nm (Figures 1C and 2B) was observed concomitant with the decay of $Py^{\bullet+}$ absorption (Figure 2A). Also shown

(14) See Supporting Information.

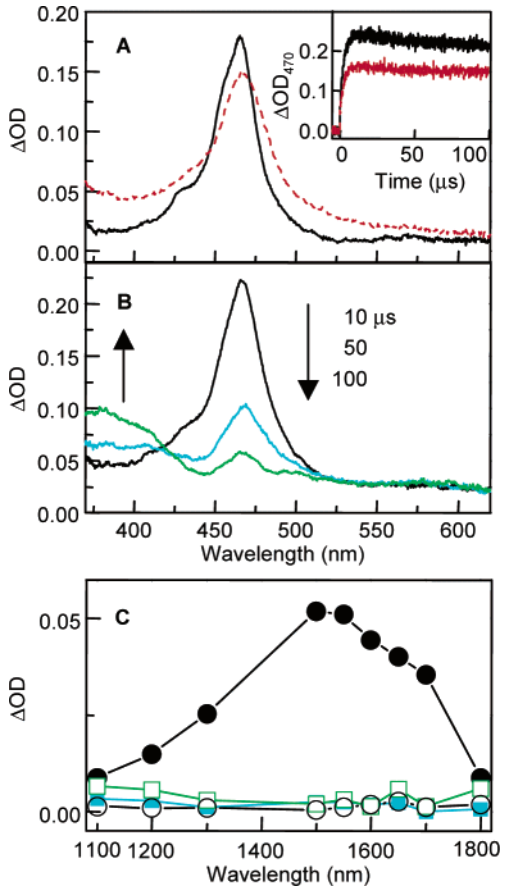


Figure 1. Transient absorption spectra observed at 100 μs (A) and at 10, 50, and 100 μs (B) after the electron pulse during pulse radiolysis of Iapy/Ib (A, solid line), Ia/Ibpy$_1$U (A, dashed line), and Iapy/Ibpy$_1$U (B) in Ar-saturated D_2O solution containing 0.1 mM ODN. The inset shows the time profiles of the transient absorption of $Py^{\bullet+}$ for Iapy/Ib (with large ΔOD) and Ia/Ibpy$_1$U (with small ΔOD) monitored at 470 nm (A). Transient absorption spectra observed at 50 μs after the electron pulse during pulse radiolysis of Iapy/Ibpy$_1$U (solid circle), Ia/Ibpy$_1$U (open square), Iapy/Ib (solid square), and Ia/Ib (open circle) at the near-IR region (C).

in Figure 1C, the control experiments were carried out for the ODNs lacking 5′-Py (Ia/Ibpy$_1$U) or **py$_1$U** (Iapy/Ib), and ODN without the Py moiety (Ia/Ib), where no distinct absorption was evident. Hence, these characteristic absorption bands are ascribed to $Py_2^{\bullet+}$. The 1500 nm band in the near-IR region is assigned to the CR band. $Py_2^{\bullet+}$ is expected to have a sandwich structure similar to that of the excimer.[15] Since only weak fluorescence of the Py excimer was observed for Iapy/Ibpy$_1$U, as shown in Figure 3, population of the sandwich structure between two Pys is low in the non-oxidized state. Therefore, the formation of $Py_2^{\bullet+}$ clearly demonstrates trapping of the transiently formed DNA structure by the attractive CR interaction between $Py^{\bullet+}$ and Py in the time range up to 100 μs.[16]

Almost absence of the excimer fluorescence and the slow formation rate of $Py_2^{\bullet+}$ in doubly Py-conjugated ODN compared to the formation rate of $Py^{\bullet+}$ demonstrate that the dynamics of the DNA will infrequently lead to the conformation where two Pys come in contact. In order to elucidate the dynamics of such

(15) Although we cannot access the detail structure of $Py_2^{\bullet+}$ in DNA, $Py_2^{\bullet+}$ is likely to be formed at the minor groove where such $\pi-\pi$ dimer formation is possible.[6] For the molecular model of doubly Py-labeled ODN see Supporting Information.

(16) The stabilization energies of $Py_2^{\bullet+}$ can be estimated as half the energy of the observed transition, and the CR band with the peak at 1500 nm corresponds to the stabilization energy of 9.5 kcal mol^{-1}.[8]

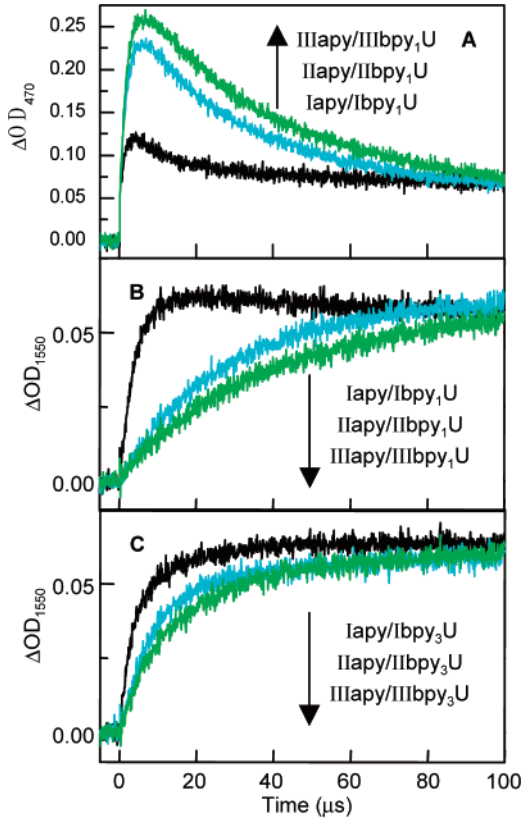


Figure 2. Time profiles of the transient absorptions assigned to $Py^{\bullet+}$ and $Py_2^{\bullet+}$ monitored at 470 (A) and 1550 nm (B), respectively, with the $n = 1$ methylene linker for Py attached at the 2′-position of uridine and 1550 nm (C) with the $n = 3$ methylene linker for Py attached at the 2′-position of uridine during pulse radiolysis of doubly Py-conjugated ODNs.

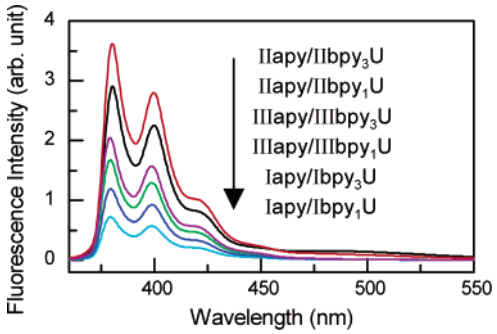


Figure 3. Fluorescence spectra ($\lambda_{ex} = 346$ nm) for doubly Py-conjugated ODNs in Ar-saturated aqueous solution in the presence of 20 mM sodium phosphate buffer (pH 7.0) at a total strand concentration of 8 μM.

DNA local fluctuations, we next studied the $Py_2^{\bullet+}$ formation process for eight ODNs (Table 1). A slower formation rate of $Py_2^{\bullet+}$ was observed for IIapy/IIbpy$_1$U with longer distance separation between two Pys compared to that for Iapy/Ibpy$_1$U (Figure 2B). The change of the A−T base pair to the G−C base pair between two Pys (IIIapy/IIIbpy$_1$U) led to a further decrease in the formation rate of $Py_2^{\bullet+}$. Since the G−C base pair is presumed to have more rigid structure, as can be seen from the higher T_m value, the slower formation rate of $Py_2^{\bullet+}$ can be interpreted in terms of the reduced flexibility of DNA. The formation rate of $Py_2^{\bullet+}$ agreed well with the decay of $Py^{\bullet+}$ monitored at 470 nm, as shown in Figure 2A. As for ODNs Iapy/Ibpy$_3$U, IIapy/IIbpy$_3$U, and IIIapy/IIIbpy$_3$U where a longer trimethylene linker ($n = 3$) was used for **pyU** (**py$_3$U**), a similar sequence dependency for the faster rate was observed due to the increased flexibility for Py attached at the 2′-position (Figure 2C). Since similar results were also observed when the concentration of DNA was decreased by half, the observed results are accounted for by intramolecular processes (data not shown). Consequently, the formation rate of $Py_2^{\bullet+}$ clearly reflected the dynamic interaction between $Py^{\bullet+}$ and Py in DNA.

The base pair opening-closing kinetics has been well-studied by imino proton exchange experiments in ^{1}H NMR. The transient opening of an individual base pair occurs in the millisecond time scale for the central base pairs, and the faster rate has been suggested for the terminal base pairs.[17,18] Therefore, the forma-

Table 1. Melting Temperature and Formation Rate Constants (k_{dimer}) of Pyrene Dimer Radial Cation of Doubly Py-Conjugated ODNs

ODN	sequence[a]	T_m[b](°C)	k_{dimer}[c](10^4 s^{-1})
Ia	5′-AAGCGCGA	36	
Ib	3′-TTCGCGCT		
IIa	5′-AAAGCGCGA	36	
IIb	3′-TTTCGCGCT		
IIIa	5′-AGAGCGCGA	39	
IIIb	3′-TCTCGCGCT		
Iapy	**5′-Py**-AAGCGCGA	48	27 ± 0.4
Ibpy$_1$U	3′-T**py$_1$U**CGCGCT		
IIapy	5′-Py-AA-AGCGCGA	43	3.7 ± 0.02
IIbpy$_1$U	3′-TT**py$_1$U**CGCGCT		
IIIapy	**5′-Py**-AG-AGCGCGA	46	2.7 ± 0.02
IIbpy$_1$U	3′-TC**py$_1$U**CGCGCT		
Iapy	**5′-Py**-A-AGCGCGA	46	22 ± 0.4
Ibpy$_3$U	3′-T**py$_3$U**CGCGCT		
IIIapy	**5′-Py**-AAAGCGCGA	42	12 ± 0.2
IIIbpy$_3$U	3′-TT**py$_3$U**CGCGCT		
IIIapy	**5′-Py**-AGAGCGCGA	45	9.1 ± 0.2
IIIbpy$_3$U	3′-TC**py$_3$U**CGCGCT		
IV	5′-GCG**py$_1$U**TAGCG	19	37 ± 2.5
	3′-CGCAA**py$_1$U**CGC		
V	5′-**py$_1$U**TAGCGCGA	46	3.0 ± 0.02
	3′-AA**py$_1$U**CGCGCT		

[a] See Scheme 1 for **5′-Py**, **py$_1$U**, and **py$_3$U**. [b] UV-melting measurements were carried out in the presence of 20 mM sodium phosphate buffer (pH 7.0) at a total strand concentration of 10 μM. [c] Determined from the time profiles of transient absorption in Figure 2.

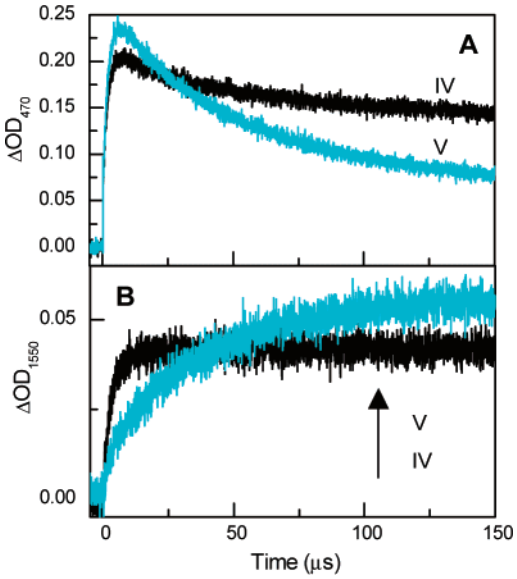


Figure 4. Time profiles of the transient absorptions assigned to $Py^{\bullet+}$ and $Py_2^{\bullet+}$ monitored at 470 (A) and 1550 nm (B), respectively, during pulse radiolyses of doubly Py-conjugated ODNs IV and V.

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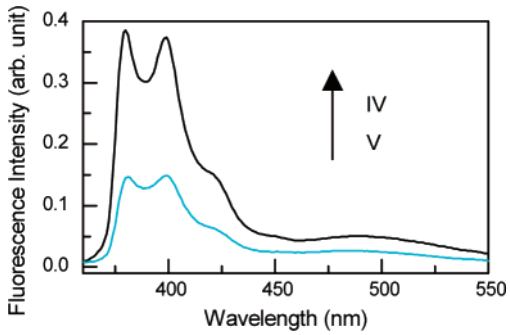

Figure 5. Fluorescence spectra (λ_{ex} = 346 nm) for doubly Py-conjugated ODNs IV and V in Ar-saturated aqueous solution in the presence of 20 mM sodium phosphate buffer (pH 7.0) at a total strand concentration of 8 μM.

tion rate of $Py_2^{\bullet+}$ in DNA may correspond to the end fraying of DNA. To test this hypothesis, we have synthesized two ODNs which have 5′-**py**$_1$**U**TA/AA**py**$_1$**U**-3′ configuration at the internal site (IV) and at the end of duplex (V). Figure 4A shows the formation and decay of $Py^{\bullet+}$ monitored at 470 nm, while Figure 4B displays the formation of the transient absorption of $Py_2^{\bullet+}$ monitored at 1550 nm during pulse radiolyses of ODNs IV and V. In the case of ODN V, concomitant with the decay of $Py^{\bullet+}$, $Py_2^{\bullet+}$ formed in the time scale of ~100 μs, similarly to that observed for IIapy/IIbpy$_1$U, which is explained by the end fraying of DNA. In contrast, only fast formation of $Py_2^{\bullet+}$ within 10 μs was observed for ODN IV and formation yield of $Py_2^{\bullet+}$ (ΔOD_{1550}) was lower compared to other doubly Py-conjugated ODNs studied here. Interestingly, only a little decay of $Py^{\bullet+}$ for ODN IV was found in the time range of 10−100 μs. Thus, some extent of $Py^{\bullet+}$ remained as a monomer without forming $Py_2^{\bullet+}$ in the time scale of ~100 μs for ODN IV. The fast component of $Py_2^{\bullet+}$ formed within 10 μs is due to population of the structure in which two Pys locate close to each other at the non-oxidized state. This is consistent with the weak excimer emission at 490 nm, as can be seen in Figure 5. The absence of the slower component of $Py_2^{\bullet+}$ formed in the time scale longer than 10 μs in ODN IV is attributable to the slower base pair opening kinetics at the internal site of DNA. Therefore, these results demonstrate that formation of $Py_2^{\bullet+}$ in the time range of ~100 μs corresponds to the end fraying in DNA.

Conclusions

The formation of $Py_2^{\bullet+}$ in DNA was observed during pulse radiolyses of doubly Py-conjugated ODNs. The formation rate of $Py_2^{\bullet+}$ was found to reflect the rate of the end fraying of DNA which allows the intramolecular contact between $Py^{\bullet+}$ and Py in DNA. The long lifetimes of $Py^{\bullet+}$ and $Py_2^{\bullet+}$ enable the measurements of the DNA dynamics in the time range from 1 μs to 1 ms.

Acknowledgment. We thank the members of the Radiation Laboratory of SANKEN, Osaka University, for running the linear accelerator. This work has been partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sport and Culture of Japan.

Supporting Information Available: Pulse radiolysis of singly **Upy** labeled ODNs and the molecular model of doubly Py-labeled ODN (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA026754Q