Formation of Pyrene Dimer Radical Cation at the Internal Site of Oligodeoxynucleotides

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Received: February 2, 2004; In Final Form: April 19, 2004

Doubly internally pyrene (Py) modified oligodeoxynucleotides (ODNs) were synthesized, and the formation rates of the Py dimer radical cation ($Py_2^{\bullet+}$) were measured upon one-electron oxidation during pulse radiolysis. The formation of $Py_2^{\bullet+}$ with an optical absorption at 1550 nm (charge resonance band) was observed in the time range of 1 μs to 1 ms after an electron pulse during the pulse radiolysis of a D_2O solution of the doubly internally Py modified ODN in the presence of $K_2S_2O_8$. The formation rate of $Py_2^{\bullet+}$ in DNA reflected the dynamics of the DNA, which allows the interaction between $Py^{\bullet+}$ and Py, and was affected by the distance between the two Py's and the local environment of each Py. The trapping of the transiently formed DNA conformation by the attractive charge resonance interaction was demonstrated to be useful to obtain structural and dynamic information of the fluctuating DNA in the time range of 1 μs to 1 ms.

Introduction

Biopolymers including peptides, DNA, and RNA are dynamic structures with several transient conformations appearing in a short period of time. Such a transiently existing conformation is thought to play a variety of important biological roles in processes such as enzymatic activity, protein−protein interactions, and DNA/RNA−protein interactions.[1,2] However, the low population and the short lifetime of these transient conformations make their characterization very difficult.

Fluorescence resonance energy transfer (FRET) has been widely used for analyzing the structure and dynamics of biomolecules.[3−5] However, since FRET occurs regardless of the direct contact between two fluorophores, it reports the relative spatial position between two fluorescent probes within biopolymers, and it is not an adequate method to measure the kinetics of short-lived transiently appearing conformations. In this instance, the probe pair should be designed in such a way that direct molecular contact is necessary for the following kinetic events, which reflects the kinetics of formation of the transient conformation.[6] Nau et al. used fluoroazophore as a fluorescent probe and tryptophan as a quencher, where quenching of fluoroazophore occurs through an exciplex intermediate with close contact. By using the polypeptide labeled with a fluoroazophore at one end and tryptophan at the other end, they have measured the end-to-end collision rate in the time range of nanoseconds to microseconds, which clearly reflected the flexibility of the polypeptide.[7−9]

One way to identify much less frequent motions of biomolecules occurring on a longer time scale is to chemically trap such transient conformations.[10] Cohen et al. incorporated two thiol groups at various positions within a large catalytic RNA molecule (ribozyme). They measured the rate of formation of disulfide cross-links, which occurs in the time range of minutes to hours, and revealed unexpectedly large thermal motions between the domains of a compact folded ribozyme.[11]

On the basis of the concept of the transient trapping of thermal motion, we have recently reported the use of the pyrene (Py) dimer radical cation ($Py_2^{\bullet+}$), which is stabilized by the charge resonance (CR) between the two aromatics.[12−14] Two Py's were introduced at one end of the oligodeoxynucleotides (ODNs), and the formation rates of $Py_2^{\bullet+}$ upon one-electron oxidation during the pulse radiolysis were measured, where a transiently formed DNA conformation is trapped by the attractive CR interaction.[15] The rate of formation of $Py_2^{\bullet+}$ reflects the frequency of occurrence of the DNA conformation in which $Py^{\bullet+}$ and Py can associate. The formation rate of $Py_2^{\bullet+}$ in the time range of ∼100 μs was attributed to the rate of the end fraying of DNA, which allows the intramolecular contact between $Py^{\bullet+}$ and Py in DNA. In this method, the long lifetimes of $Py^{\bullet+}$ and $Py_2^{\bullet+}$ allow us to measure the DNA dynamics in the time range from 1 μs to 1 ms. Here, to investigate the transient motion of DNA at the internal site, we synthesized the doubly internally Py modified ODNs. The formation of $Py_2^{\bullet+}$ in DNA was measured during the pulse radiolysis of the doubly internally Py modified ODNs, and 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

DNA Synthesis. 2′ Py modified ODNs were synthesized according to the reported procedure.[15−20] In brief, a nucleoside derivative containing a Py group (**U**) was synthesized by the reaction of 2′-amino-2′-deoxyuridine with 1-pyreneacetic acid. **U** was converted to the phosphoroamidite derivative, and **U**-containing ODNs used in this study were synthesized with Expedite 8909 DNA synthesizer (Applied Biosystems). Crude ODN was purified by reversed-phase HPLC and lyophilized. The purity and concentration of all ODNs studied here were determined by complete digestion with sv PDE, nuclease P1, and AP to 2′-deoxyribonucleosides.

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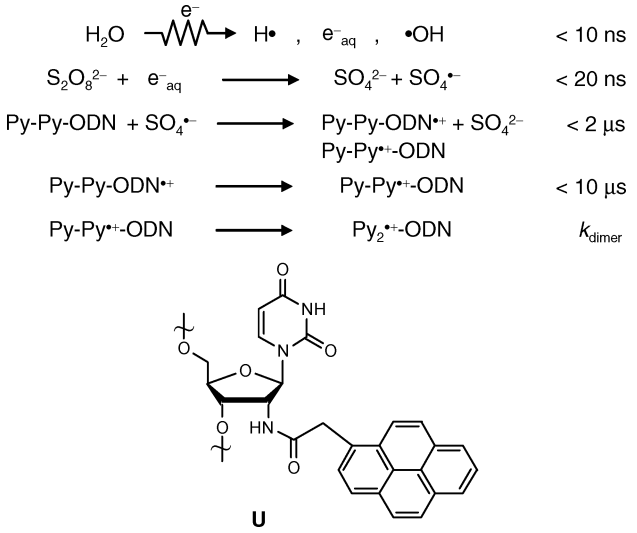
10.1021/jp049543b CCC: $27.50
Published on Web 08/11/2004

SCHEME 1



Pulse Radiolysis. $Py^{\bullet+}$ was formed 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 concentration) ODN (Scheme 1).[15,21,22] 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. 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 (Tektronix, TDS 580D).

Fluorescence Spectra. Fluorescence spectra of Py modifed ODNs were measured in 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

For incorporation of two Py's at the desired position inside the DNA, Py was conjugated at the 2′ sugar position of uridine as previously reported,[16,17] and several ODNs with a different number (*n*) of intervening A−T base pairs between the two Py's were synthesized as shown in Scheme 1 and Table 1. First, to check the duplex stability of the Py modified ODNs, the T_m values were measured for the representative ODNs studied here. Singly Py modified ODN (**a**/**b**′, T_m = 36 °C) showed a similar duplex stability compared to the corresponding unmodified ODN (**a**/**a**′, T_m = 36 °C). Doubly Py modified ODNs resulted in only a slight destabilization of the ODN compared to that of **a**/**a**′ (**b**/**c**′, T_m = 30 °C; **c**/**c**′, T_m = 31 °C; **d**/**c**′, T_m = 28 °C). Thus, two Py's were incorporated into DNA without a large disturbance in the duplex stability.

To identify the population of the DNA conformation in which two Py's associate close to each other at the nonoxidized state, the fluorescence spectra of the doubly Py modified ODNs were measured. Since the formation of the Py excimer requires the close cofacial contact between the excited fluorescent probe (^{1}Py*) and its neutral counterpart (Py) within the short lifetime of ^{1}Py* in the DNA conformations,[23−26] Py excimer emission provides structural information about the two Py's. Py excimer emission was observed for ODNs **b**/**c**′ and **c**/**b**′ as shown in Figure 1. Therefore, in the case of ODNs with one intervening A−T base pair between two Py's (n = 1), a high population of DNA conformations has a cofacial dimer structure. For other ODNs, no excimer emission was observed, demonstrating that the population of the sandwich conformation between two Py's is low in the nonoxidized state.

Py modified ODNs were oxidized by $SO_4^{\bullet-}$, which was generated during the pulse radiolysis of an aqueous solution in

TABLE 1: Formation Rate Constants (k_{dimer}) of Pyrene Dimer Radical Cation of Doubly Py Modified ODNs

ODNs	n[a]	Sequence[b]	k_{dimer}[c] (10^4 s^{-1})
a/**a'**	–	5′CGCTTTAAACGC 3′GCGAAATTTGCG	–
a/**b'**	–	5′CGCTTTAAACGC 3′GCGAAA**U**TTGCG	–
b/**b'**	0	5′CGCTT**U**AAACGC 3′GCGAAA**U**TTGCG	0.43 ± 0.06
b/**c'**	1	5′CGCTT**U**AAACGC 3′GCGAAAT**U**TGCG	11 ± 1.9
b/**d'**	2	5′CGCTT**U**AAACGC 3′GCGAAATT**U**GCG	nd[d]
c/**b'**	1	5′CGCT**U**TAAACGC 3′GCGAAA**U**TTGCG	11 ± 3.2
c/**c'**	2	5′CGCT**U**TAAACGC 3′GCGAAAT**U**TGCG	0.42 ± 0.02
c/**d'**	3	5′CGCT**U**TAAACGC 3′GCGAAATT**U**GCG	nd
d/**b'**	2	5′CGC**U**TTAAACGC 3′GCGAAA**U**TTGCG	nd
d/**c'**	3	5′CGC**U**TTAAACGC 3′GCGAAAT**U**TGCG	nd
d/**d'**	4	5′CGC**U**TTAAACGC 3′GCGAAATT**U**GCG	nd

[a] Number of intervening A−T base pair(s) between two **U**'s. [b] See Scheme 1 for the chemical structure of **U**. [c] Determined from the time profiles of transient absorption in Figure 2. [d] Not detected.

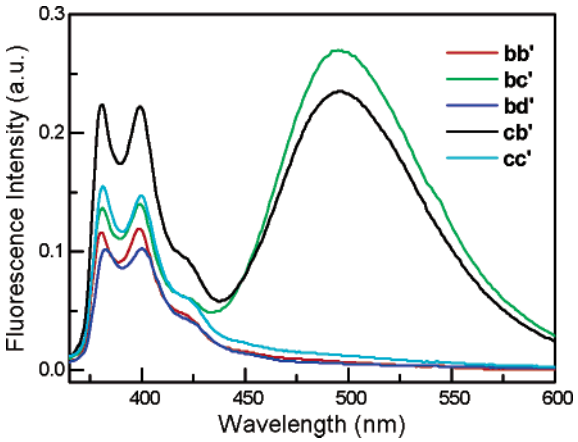


Figure 1. Fluorescence spectra (λ_{ex} = 349 nm) for doubly Py modified ODNs in aqueous solution in the presence of 20 mM sodium phosphate buffer (pH 7.0) at a total strand concentration of 8 μM.

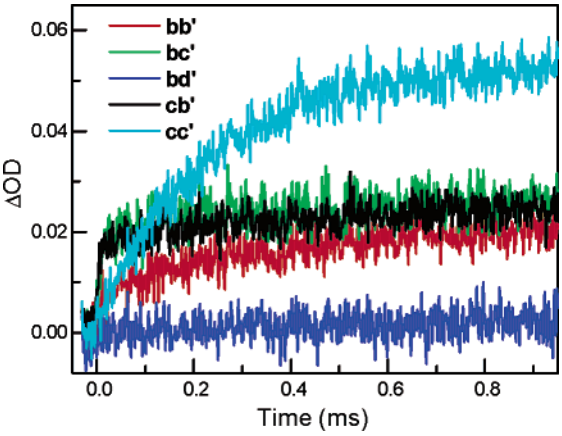


Figure 2. Time profiles of the transient absorption of $Py_2^{\bullet+}$ monitored at 1550 nm during pulse radiolysis of doubly Py conjugated ODNs in Ar-saturated D_2O solution in the presence of 10 mM $K_2S_2O_8$, 100 mM *t*-BuOH, and 20 mM sodium phosphate buffer (pH 7.0), at a total strand concentration of 200 μM.

the presence of $K_2S_2O_8$. $Py^{\bullet+}$ was formed by two processes as shown in Scheme 1: the direct oxidation of Py by $SO_4^{\bullet-}$ and the hole transfer from $G^{\bullet+}$ generated in DNA to Py. Since the hole is irreversibly generated in DNA free from charge recombination, the long lifetimes of $Py^{\bullet+}$ and $Py_2^{\bullet+}$ enable us to measure the processes in the time range from microseconds to milliseconds. The formation of $Py_2^{\bullet+}$ was monitored by the transient absorption at 1550 nm in the near-IR region, which corresponds to the CR band. To elucidate the dynamics of local fluctuations at the internal site of DNA, we studied the $Py_2^{\bullet+}$ formation process for the nine ODNs shown in Table 1. In the case of ODN **b**/**b′** in which two Py's are located adjacent to each other ($n = 0$), the formation of $Py_2^{\bullet+}$ in the time range of ~1 ms was observed (Figure 2). Since Py excimer emission was not observed for ODN **b**/**b′**, this slow rise of $Py_2^{\bullet+}$ in the time range of 1 ms was explained as a consequence of the DNA dynamics, which allows intramolecular contact between $Py^{\bullet+}$ and Py in DNA. For the $n = 1$ ODNs, the fast formation of $Py_2^{\bullet+}$ within 10 μs was observed. The fast component of $Py_2^{\bullet+}$ formed within 10 μs is due to the population of the conformation in which two Py's locate close to each other at the nonoxidized state. This is consistent with the observed excimer emission in the case of $n = 1$ ODNs (Figure 1). When two Py's are separated by two intervening base pairs ($n = 2$), the formation process of $Py_2^{\bullet+}$ is significantly affected by the local environments of each of the two Py's. In the case of ODN **c**/**c′**, where both of the Py's are surrounded by A−T base pairs, the formation of $Py_2^{\bullet+}$ was observed in the time range of 1 ms. In contrast, the formation of $Py_2^{\bullet+}$ was not observed for the ODNs **b**/**d′** and **d**/**b′** in which one Py is located adjacent to the G−C base pair, and $Py^{\bullet+}$ persisted over 10 ms. The absence of the formation of $Py_2^{\bullet+}$ can be interpreted in terms of the reduced flexibility of DNA around the Py, since the G−C base pair has a more rigid structure. For the ODNs with more than three intervening A−T base pairs between the two Py's, the formation of $Py_2^{\bullet+}$ was not detected, indicating that there is no DNA motion which brings two Py's into contact on this time scale, or the transiently formed $Py_2^{\bullet+}$ is too unstable to be trapped by the 9 kcal mol^{-1} CR energy. Similar results were also observed when the concentration of DNA was decreased by half, and the formation of $Py_2^{\bullet+}$ was not observed for the single-stranded ODNs, demonstrating that the observed results are due to intramolecular processes. Thus, the formation rate of $Py_2^{\bullet+}$ clearly reflects the dynamic interaction between $Py^{\bullet+}$ and Py in DNA.

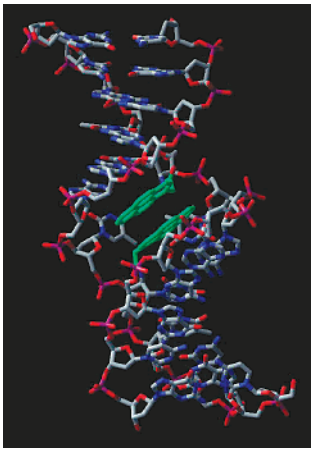


Figure 3. Energy-minimized structure of doubly Py modified ODN **c**/**c′** in which two Py's closely associate in the minor groove. Molecular modeling simulations were carried out with MacroModel (version 8.0) with the Amber* force field. The initial structure of the doubly Py modified ODN was obtained by attaching Py via an amide bond to the B-form duplex DNA constructed by using the builder module in Spartan (02). Energy minimization was done for the initial structure with GBSA treatment of water.

DNA is a dynamic molecule that exhibits different motions on various time scales. Transient opening of an individual base pair occurs on the millisecond time scale for the central base pairs, and it occurs at a faster rate in the terminal bases. When two Py's are introduced at the terminal of ODN, the formation of $Py_2^{\bullet+}$ in the time range of ~100 μs, which corresponds to the end fraying in DNA, was observed as previously reported.[15] In this study, for the ODNs with two Py's at the internal site, the formation of $Py_2^{\bullet+}$ occurred on significantly longer time scales (~1 ms). Usually, this motion is too fast to be accounted for by a base-pair-opening event,[27,28] even though molecular dynamics simulations suggested that an increased flexibility is introduced as a consequence of the Py substitution.[29] Indeed, molecular modeling studies indicate that the formation of $Py_2^{\bullet+}$ in the minor groove is possible with a slight unwinding and bending of DNA as shown in Figure 3. Therefore, the formation of $Py_2^{\bullet+}$ on the time scale of ~1 ms may correspond to the DNA motions such as DNA unwinding and bending which lead to the conformation where Py and $Py^{\bullet+}$ come into contact.

Conclusions

Two Py's were incorporated at the internal site of ODN, and the formation of $Py_2^{\bullet+}$ was monitored by the CR band (1550 nm) in the near-IR region during pulse radiolysis. The formation of $Py_2^{\bullet+}$ was observed in the time ranges of ~10 μs and ~1 ms. The fast component was due to the population of the conformation in which two Py's are located close to each other in the nonoxidized state, while the slow component was attributed to the DNA dynamics such as DNA unwinding and bending, which allows the intramolecular contact between $Py^{\bullet+}$ and Py at the internal site of DNA. The long lifetimes of $Py^{\bullet+}$ and $Py_2^{\bullet+}$ and the attractive CR energy enable the trapping of the transiently formed DNA conformation, providing valuable information about the dynamics of DNA conformational fluctuation in the time range from 1 μs to 1 ms.

Acknowledgment. We thank the members of the Radiation Laboratory of ISIR (SANKEN), Osaka University, for running the linear accelerator. This work has been partly supported by a Grant-in-Aid for Scientific Research on Priority Area 417,

21st Century COE Research, and others from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of the Japanese Government.

References and Notes

(1) Travers, A. A. *Annu. Rev. Biochem.* **1989**, *58*, 427.
(2) Kitahara, R.; Akasaka, K. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3167.
(3) Lorenz, M.; Hillisch, A.; Payet, D.; Buttinelli, M.; Travers, A.; Diekmann, S. *Biochemistry* **1999**, *38*, 12150.
(4) Toth, K.; Sauermann, V.; Langowski, J. *Biochemistry* **1998**, *37*, 8173.
(5) Akiyama, T.; Hogan, M. E. *Biochemistry* **1997**, *36*, 2307.
(6) Johansson, M. K.; Cook, R. M. *Chem.*−*Eur. J.* **2003**, *9*, 3466.
(7) Nau, W. M.; Wang, X. *ChemPhysChem* **2002**, *3*, 393.
(8) Hudgins, R. R.; Huang, F.; Gramlich, G.; Nau, W. M. *J. Am. Chem. Soc.* **2002**, *124*, 556.
(9) Huang, F.; Nau, W. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 2269.
(10) Spies, M. A.; Schowen, R. L. *J. Am. Chem. Soc.* **2002**, *124*, 14049.
(11) Cohen, S. B.; Cech, T. R. *J. Am. Chem. Soc.* **1997**, *119*, 6259.
(12) Tsuchida, A.; Ikawa, T.; Yamamoto, M.; Ishida, A.; Takamuku, S. *J. Phys. Chem.* **1995**, *99*, 14793.
(13) Tsuchida, A.; Tsujii, Y.; Ohoka, M.; Yamamoto, M. *J. Phys. Chem.* **1991**, *95*, 5797.
(14) Kira, A.; Arai, S.; Imamura, M. *J. Chem. Phys.* **1971**, *54*, 4890.
(15) Kawai, K.; Miyamoto, K.; Tojo, S.; Majima, T. *J. Am. Chem. Soc.* **2003**, *125*, 912.
(16) Takada, T.; Kawai, K.; Tojo, S.; Majima, T. *Tetrahedron Lett.* **2003**, *44*, 3851.
(17) Kawai, K.; Kawabata, K.; Tojo, S.; Majima, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2363.
(18) Mitsui, T.; Nakano, H.; Yamana, K. *Tetrahedron Lett.* **2000**, *41*, 2605.
(19) Yamana, K.; Asazuma, K.; Nakano, H. *Nucleic Acids Res. Symp. Ser.* **1999**, *42*, 113.
(20) Yamana, K.; Mitsui, T.; Nakano, H. *Tetrahedron* **1999**, *55*, 9143.
(21) Kawai, K.; Takada, T.; Tojo, S.; Ichinose, N.; Majima, T. *J. Am. Chem. Soc.* **2001**, *123*, 12688.
(22) Takada, T.; Kawai, K.; Tojo, S.; Majima, T. *J. Phys. Chem. B* **2003**, *107*, 14052.
(23) Netzel, T. L.; Zhao, M.; Nafisi, K.; Headrick, J.; Sigman, M. S.; Eaton, B. E. *J. Am. Chem. Soc.* **1995**, *117*, 9119.
(24) Manoharan, M.; Tivel, K. L.; Zhao, M.; Nafisi, K.; Netzel, T. L. *J. Phys. Chem.* **1995**, *99*, 17461.
(25) Amann, N.; Pandurski, E.; Fiebig, T.; Wagenknecht, H.-A. *Angew. Chem., Int. Ed.* **2002**, *41*, 2978.
(26) Huber, R.; Fiebig, T.; Wagenknecht, H.-A. *Chem. Commun.* **2003**, 1878.
(27) Leijon, M.; Graslund, A. *Nucleic Acids Res.* **1992**, *20*, 5339.
(28) Snoussi, K.; Leroy, J. L. *Biochemistry* **2001**, *40*, 8898.
(29) Cui, G.; Simmerling, C. *J. Am. Chem. Soc.* **2002**, *124*, 12154.