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Exciplex formation between pyrene and guanine in highly polar solvents†

Takahiro Kawai, Masashi Ikegami and Tatsuo Arai*
Department of Chemistry, University of Tsukuba, Tsukuba, Ibaraki, 305-8571, Japan.
E-mail: arai@chem.tsukuba.ac.jp; Fax: 81 29 853 6503; Tel: 81 29 853 4315

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Guanine derivatives connected to pyrene with methylene spacers exhibited exciplex emission in highly polar solvents such as *N*,*N*-dimethylformamide, acetonitrile, and water. The result will open up a novel approach to synthesis of fluorescent nucleic bases.

The one-electron oxidation of DNA has been extensively studied by laser flash photolysis and pulse radiolysis and so on in recent years.[1–6] Especially, the photoinduced electron transfer reaction has received much attention from the viewpoint of the design of artificial photonucleases as therapeutic and diagnostic agents. Guanine has the lowest oxidation potential among the four bases of DNA and the oxidation potential is reported to be dependent on π–π stacking and hydrogen bonding interaction.[5,6] For example, it is reported that the oxidation potential of guanine is affected by base-pair formation with cytosine.[5] Furthermore, the introduction of a methyl or bromo group into the C5 position of cytosine results in acceleration or suppression, respectively, of the one-electron oxidation compared to the normal guanine–cytosine base pair.[5]

As to the exciplex emission between pyrene and nucleotides, the Stern–Volmer analyses of the fluorescence spectra of pyrene in the presence of nucleotides have been reported.[7,8] The possibility of exciplex emission between pyrene and the uracil group in DNA has been suggested without clear experimental results.[7]

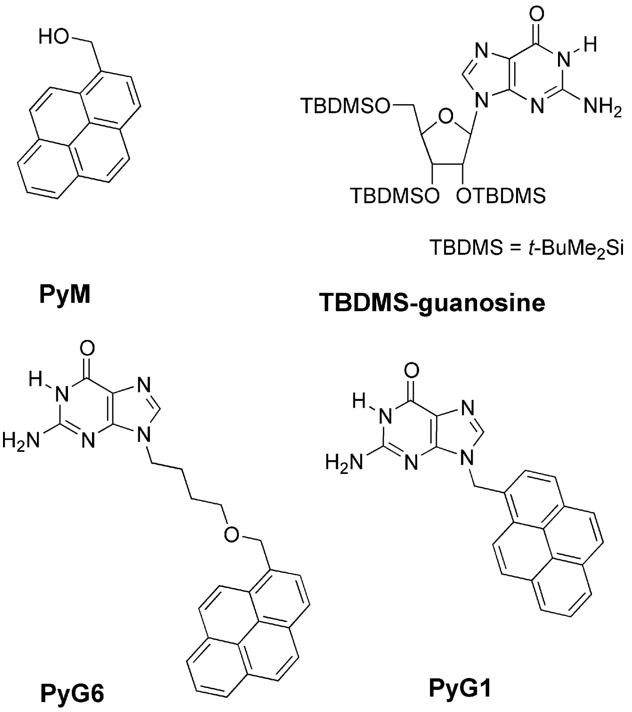
In the course of our studies on the electron or charge transfer reactions of intermolecular hydrogen bonded systems,[9] we have been interested in constructing electron transfer or energy transfer systems connected by intermolecular hydrogen bonding of guanine and cytosine base pairs. Thus, we have prepared guanine derivatives covalently bound to pyrene, **PyG1** and **PyG6** (Scheme 1). Despite extensive interest in the introduction of a pyrene ring into the DNA double helix to study the electron transfer between pyrene and a nucleic base and hole transfer through the DNA double helix,[1–7,10,11] the synthesis and photochemical behavior of pyrene directly attached to the nucleic base by methylene spacer have scarcely been reported.

We wish to report here novel findings on the exciplex emission of **PyG1** and **PyG6** in highly polar solvents.

Fig. 1 shows absorption and fluorescence spectra of **PyG1** and **PyG6** in comparison with those of 1-pyrenemethanol (**PyM**) in *N*,*N*-dimethylformamide (DMF). **PyG1** and **PyG6** exhibited large Stokes shifted emission at 450–600 nm in addition to the monomer emission at 370–430 nm. The ratio of the fluorescence intensity of the monomer and the exciplex emission was the same in the concentration range from 1.0 μM to 0.1 mM in DMF. In addition, the fluorescence excitation spectra monitored at 397 nm and 480 nm were the same in all the solvents examined. These results indicate that the fluorescence at 450–600 nm was not the excimer emission.

As to the **PyG6**, the fluorescence quantum yield (Φ_f) was determined to be 0.03. The fluorescence lifetime was determined to be τ = 10.6 ns and 49 ns, respectively at a monitoring wavelength of 378 nm and 480 nm.

In order to assign the fluorescence emission observed at 480 nm in **PyG6**, the oxidation potentials of **PyM** and TBDMS-guanosine were measured. Since the solubility of guanine and guanosine was too low to observe the oxidation potential by cyclic voltammetry (CV), the *tert*-butyldimethylsilyl (TBDMS) group was introduced on the ribose unit of guanosine.[5,6] The oxidation potential of **PyM** was estimated to be 1.11, 0.88 and 0.90 V *vs.* Ag/Ag$^+$ in dichloromethane, acetonitrile, and DMF, respectively, and that of TBDMS-guanosine was estimated to be 1.06, 0.88 and 0.93 V *vs.* Ag/Ag$^+$ in dichloromethane, acetonitrile, and DMF, respectively. The observed oxidation potential of TBDMS-guanosine is in good agreement with the reported oxidation potential of guanine.[12]



Scheme 1

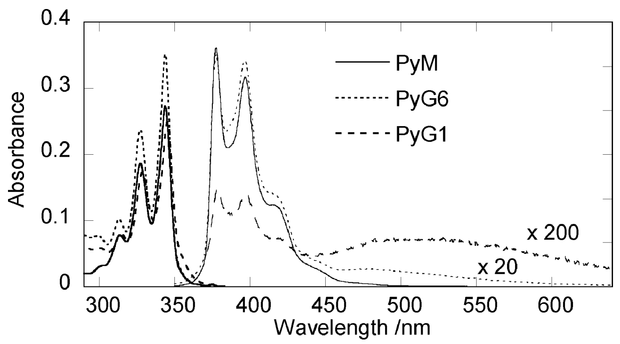


Fig. 1 Absorption and fluorescence spectra of **PyM** (1.0 × 10^{-5} M), **PyG1** (5.0 × 10^{-5} M), and **PyG6** (1.0 × 10^{-5} M) in DMF on excitation at 328 nm under argon atmosphere.

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† Electronic supplementary information (ESI) available: fluorescence and fluorescence excitation spectra of **PyG6**. See http://www.rsc.org/suppdata/cc/b3/b316315f/

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Table 1 Photophysical parameters of **PyM** and **PyG6**

	PyM			**PyG6**							
Solvent	Φ_f	τ_f[b]	k_f[c]	Φ_{total}	$\Phi_{f(mon)}$	$\tau_{f(mon)}$[b]	$k_{f(mon)}$[c]	$\Phi_{f(ex)}$	$\tau_{f(ex)}$[b]	k_{ET}[c]	Φ_{ET}
$CHCl_3$[a]	0.23	103	2.3	0.32	0.31	106	2.9	0.007	—	—	—
CH_2Cl_2[a]	0.31	133	2.4	0.20	0.19	92	2.0	0.010	—	4	0.33
MeOH[a]	0.46	185	2.5	0.11	0.11	39	2.7	0.005	88.9	20	0.78
MeCN[a]	0.51	175	2.9	0.03	0.02	8.9	2.2	0.005	47.0	107	0.96
DMF	0.64	202	3.2	0.03	0.03	10.6	2.6	0.002	48.6	90	0.95
H_2O[a]	0.55	222	2.5	0.03	0.03	9.8	2.9	0.006	31.6	97	0.95

[a] Solvent is used in the presence of 10% v/v of DMF because of low solubility of **PyG6**. [b] In ns. [c] In 10^6 s^{-1}.

According to the results, the oxidation potential of the pyrene ring in **PyG6** was estimated to be slightly higher than that of guanine in DMF and in dichloromethane as well. The reduction potential of **PyM** is estimated to be −2.57 eV *vs.* Ag/Ag^+ in acetonitrile. Therefore, the thermodynamic driving force of the electron transfer (ΔG) was estimated from eqn. (1) to be +0.13 eV, where the electrostatic term in acetonitrile and the singlet energy of **PyM** (E_S(PyM)) were estimated to be 0.06 V[13] and 3.4 eV, respectively. The slightly positive value estimated for ΔG indicates that the rate constant for quenching by electron transfer from guanine to the singlet excited state pyrene should be lower than the diffusion controlled rate constant. Actually, the fluorescence spectrum of **PyM** was quenched by TBDMS-guanosine, and the quenching rate constant was estimated to be 1.6×10^8 M^{-1} s^{-1} and 3.0×10^8 M^{-1} s^{-1} in DMF and acetonitrile, respectively.

$$\Delta G = [E(G^{\cdot+}/G) - E(PyM/PyM^{\cdot-}) - E_S(PyM)] + 0.06 \quad (1)$$

The fluorescence spectrum of **PyG6** was observed in various solvents as shown in Fig. 2. The solvent contained 10% v/v of DMF, because of the low solubility of **PyG6**. The quantum yield of fluorescence and lifetime of **PyM** and **PyG6** are summarized in Table 1. The quantum yield of exciplex emission (Φ_f(ex)) is determined by subtracting the normalized fluorescence spectrum of **PyM** from the observed fluorescence spectrum. The calculated k_f values of monomer emission of **PyM** and **PyG6** were almost the same in all the solvents examined. Therefore, the smaller quantum yield of fluorescence for **PyG6** in polar solvents was explained by exciplex formation with the photoinduced charge transfer reaction. Postulating that the k_f values of **PyM** and **PyG6** are the same, we can estimate the rate constant and the quantum yield of the exciplex formation by excited state charge transfer interaction, k_{ET} and Φ_{ET} (Table 1).

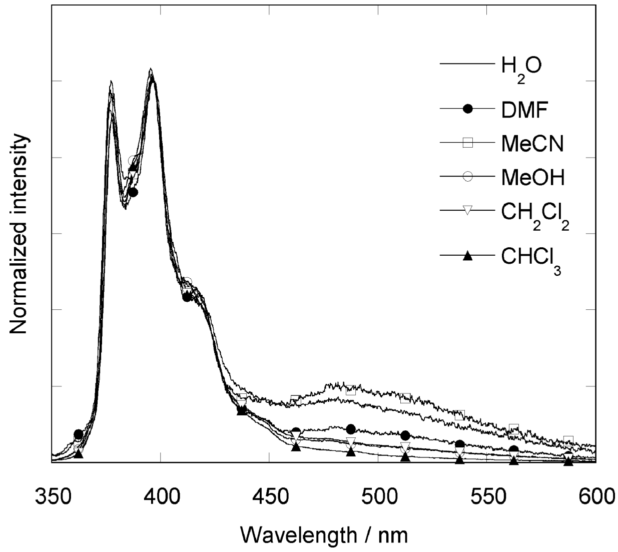

Fig. 2 Normalized fluorescence spectra of **PyG6** in various solvents. The concentrations of **PyG6** were 1.0×10^{-6} M in H_2O and 2.5×10^{-6} M in the other solvents. The excitation wavelengths were 325 nm in acetonitrile and methanol, 328 nm in the other solvents.

As shown in Table 1, the quantum yield of fluorescence of the monomer emission for **PyG6** decreases and the relative quantum yield of the exciplex emission to the monomer emission increases, as the solvent polarity increases. The rate constant of the charge transfer from guanine to the singlet excited state pyrene in polar solvent is higher than that in chloroform and dichloromethane. Although the polarity of methanol is similar to that of acetonitrile and DMF, the rate constant of charge transfer in methanol is smaller than that in acetonitrile. The oxidation potential of guanine might be affected by intermolecular hydrogen bonding interaction with methanol.

In conclusion, we can successfully observe exciplex emission between pyrene and guanine in a highly polar solvent such as DMF or acetonitrile. Therefore, to the best of our knowledge, this is the first clear report to observe fluorescence emission from an exciplex including guanine. Furthermore, the present finding that exciplex emission has been observed even in a highly polar solvent is unusual and opens up a novel approach to use the exciplex emission to study the behavior of a DNA base pair in a hydrophobic (non-polar) environment or hydrophilic (highly polar) environment.

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