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Kinetic studies of emissive guanine derivatives bearing anthracene moiety

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ABSTRACT

Photoirradiation of methylene-linked anthracene-guanine compounds, which are substituted at the 2 and 9 positions of the anthracene moiety, showed locally excited emission (LE) as well as intramolecular exciplex emission as a result of quenching of the excited singlet state of the anthracene moiety by the guanine moiety. This was confirmed by time-resolved fluorescence as well as steady-state spectroscopy and provides another approach for detecting base-pair formation. Quenching processes by the guanine moiety were investigated by the assumption of equilibrium in the excited state leading to a consistent explanation of the quenching ratio of LE. The quenching rate constant of the excited anthracene moiety by the guanine moiety mainly depends on the intrinsic reduction potential of the anthracene moiety.

1. Introduction

Recently there has been considerable fundamental and practical interest in the photoreaction of DNA like an appropriate photocleavage agent for initiating cleavage of the target nucleic acid [1,2]. Much effort was devoted to elucidate the mechanism of oxidative reaction of nucleobases with photosensitizers [3–8]. Guanine is well known to undergo photodamage by singlet oxygen or photoadduct formation through the excited state of a photosensitizer [9–11]. Some resulting holes in DNA strands are allowed to hop via a guanine moiety and get trapped in cytosine or thymine sites followed by the formation of oxidized products.

The aim of the present work is to explore the dynamics of the excited state associated with a guanine derivative including time constants involved. However, guanine, the most electron-donating of the nucleobases, exhibits a very short excited singlet state leading to very few emissions in the ultraviolet region as well as difficulty in detection by fluorescence measurements [12–14]. The development of emissive species associated with guanine provides another approach to detect base-pair formation. Subsequent utilization of well-developed techniques including steady state and transient measurements may allow the investigation of dynamic motions of targeted proteins [1,15].

Guanine linked to pyrene, **PyG**, has been reported to show new emission as well as locally excited emission associated with the pyrene moiety [16,17]. This new emission is attributed to exciplex emission preceded by the quenching of the excited singlet state of the pyrene moiety by the guanine moiety. Although the qualitative properties of guanine–pyrene compounds have been investigated in earlier reports, the details of kinetics in the excited state still are unknown. Since pyrene is known to form an excimer, which causes difficulty in investigations of dynamics, anthracene was chosen as a sensitizer due to the rarity of its excimer formation. Time-resolved fluorescence measurements, which allow the estimation of rate constants involved in the excited state, were performed to investigate the formation and relaxation processes of exciplex and locally excited state of the anthracene moiety (LE). Two different guanine derivatives connected to 2 and 9 positions of anthracene, hereafter referred to as **An-2-G** and **An-9-G**, respectively, were prepared to study free energy changes involved in the electron transfer reaction. Both **An-2-G** and **An-9-G** showed exciplex emission in toluene solution containing 10 vol.% of *N*,*N*-dimethylformamide (DMF), while no exciplex emission was observed in DMF due to the promotion of subsequent reactions such as charge separation [18–32]. In general, a fixed geometry of the donor–acceptor system is required to investigate the kinetics of photoreaction in the excited state due to easy estimation of geometric parameters such as distance and orientation involved in the donor–acceptor reaction. However, a flexible linker system would offer advantages for potential various geometries, which allow more conformation and a wider range of environments to be detected in comparison with rigid donor–acceptor systems [33,34]. For this reason, a methylene chain was chosen as the linker, although rigid donor–acceptor systems should not be eliminated as sensors.

As found in pyrene excimer kinetics studied by Birks, it was assumed that there was an equilibrium between the LE and exciplex

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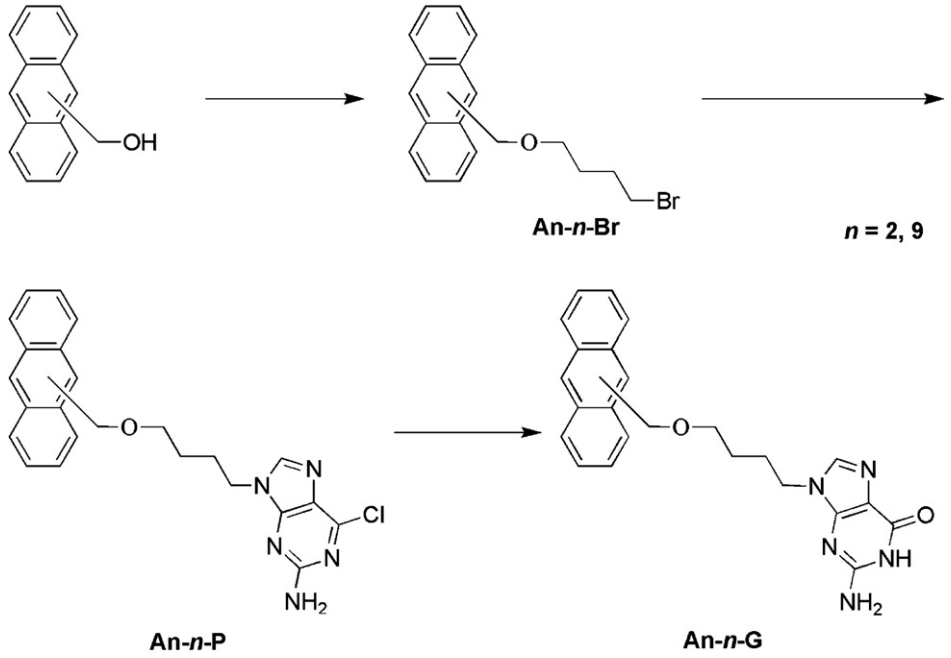

Scheme 1.

since the decay curves exhibited a bi-exponential profile. Longer lifetimes of LE and exciplex were in good agreement with each other [35]. Theoretical population decay according to Birks enabled the determination of forward and backward electron transfer rate constants, k_{CS} and k_{-CS}, between LE and exciplex. It was found that k_{CS} is mainly dependent on the free energy change between the initial and final states in the photoinduced electron transfer reaction.

2. Experimental

2.1. Instruments

Absorption and fluorescence spectra were measured using Shimadzu UV-1600 and on Hitachi F-4500 fluorescence spectrometers, respectively. Fluorescence decay measurement was performed by using the time-correlated single-photon counting method with excitation of 375 nm, which was achieved by using a diode laser (PicoQuant, LDH-P-C-375) with a power control unit (PicoQuant, PDL 800-B) in a repetition rate of 2.5 MHz [36,37]. Differential pulse voltammetric measurement was carried out by CV-50W Voltammetric analyzer (BAS) with an Ag/AgCl reference electrode. ^{1}H and ^{13}C NMR spectra were measured by using a 400-MHz NMR spectrometer (ARX-400, Bruker). ESI-mass spectra were acquired by API QSTAR pulsar i (Applied Biosystems/MDS SCIEX). Solvent used in spectroscopic measurement was toluene containing 10 vol.% of *N*,*N*-dimethylformamide (DMF) due to the limited solubility of samples in toluene. Fluorescence quantum yields were estimated by using anthracene as a reference compound (Φ_F = 0.27 in ethanol).

3. Materials

Samples were prepared as shown in Scheme 1. See also Supporting Information (Figs. S7–S15).

Synthesis of An-9-Br. Anthracene-9-methanol (1.11 g, 5.35 mmol) and 1,4-dibromobutane (2.46 g, 11.4 mmol) were stirred in dry THF (20 ml) at 70 °C. After adding NaH (50%, 0.58 g, 12 mmol) to this mixture, the mixture was refluxed for 4 h at 100 °C under N_2.

The crude product was dissolved in water (30 ml) and extracted with diethylether (50 ml, 3×). The organic phase was dried over anhydrous $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography (eluent:hexane-EtOAc = 10:1) to yield **An-9-Br** (641 mg, 1.87 mmol, 34.9% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.41 (s, 1H, An-10), 8.33 (d, J = 8.8 Hz, 2H, An-1,8 or An-4,5), 7.97 (d, J = 8.4 Hz, 2H, An-4,5 or An-1,8), 7.51 (dd, J = 8.8, 6.6 Hz, 2H, An-2,7 or An-3,6), 7.44 (dd, J = 8.4, 6.6 Hz, 2H, An-3,6 or An-2,7), 5.41 (s, 2H, An–CH_2), 3.63 (t, J = 6.2 Hz, 2H, O–CH_2– or –CH_2–Br), 3.31 (t, J = 6.6 Hz, 2H, –CH_2–Br or O–CH_2–), 1.88 (tt, J = 7.4, 6.6 Hz, 2H, –CH_2–CH_2Br or OCH_2–CH_2–), 1.73 (tt, J = 7.4, 6.2 Hz, 2H, OCH_2–CH_2– or –CH_2–CH_2Br); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 131.4, 130.9, 129.0, 128.7, 128.3, 126.1, 124.9, 124.2, 69.3, 64.9, 33.7, 29.6, 28.4.

Synthesis of An-9-P. A mixture of **An-9-Br** (640 mg, 1.86 mmol), K_2CO_3 (439 mg, 3.18 mmol), TBAI (69.0 mg, 0.187 mmol), and 2-amino-6-chloropurine (268 mg, 1.58 mmol) in DMF (40 ml) was stirred for 15 h at 80 °C under N_2. After adding CH_2Cl_2 (60 ml) to this mixture, the mixture was washed with water (30 ml, 10×). The organic phase was dried over anhydrous $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography (eluent:CH_3Cl–EtOAc = 2:1) to yield **An-9-P** (336 mg, 0.778 mmol, 49.2% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.46 (s, 1H, An-10), 8.36 (d, J = 8.8 Hz, 2H, An-1,8 or An-4,5), 8.01 (d, J = 8.4 Hz, 2H, An-4,5 or An-1,8), 7.53 (dd, J = 8.8, 6.5 Hz, 2H, An-2,7 or An-3,6), 7.48 (s, 1H, Purine-8), 7.47 (dd, J = 8.5, 6.5 Hz, 2H, An-3,6 or An-2,7), 5.48 (s, 2H, An–CH_2), 5.04 (s, 2H, Purine–NH_2), 3.90 (t, J = 7.4 Hz, 2H, –CH_2–N or O–CH_2–), 3.68 (t, J = 5.9 Hz, 2H, O–CH_2– or –CH_2–N), 1.86 (tt, J = 7.4, 7.3 Hz, 2H, –CH_2–CH_2N or OCH_2–CH_2–), 1.60 (tt, J = 7.3, 5.9 Hz, 2H, OCH_2–CH_2– or –CH_2–CH_2N); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 158.9, 153.7, 151.1, 142.4, 131.4, 130.9, 129.1, 128.7, 128.4, 126.2, 125.3, 125.0, 124.1, 69.4, 65.0, 43.4, 26.8, 26.7.

Synthesis of An-9-G. Aqueous 0.33 N NaOH (15 ml) was added to a stirring solution of **An-9-P** (336 mg, 0.778 mmol) in 1,4-dioxane (30 ml) and refluxed for 3 h at 120 °C under N_2. This mixture was cooled to room temperature and acidified to pH 4 with 1 N HCl. After adding water (300 ml) to this mixture, the organic phase was evaporated under reduced pressure. The residue was puri-