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Efficient photocleavage of DNA utilizing water soluble riboflavin/naphthaleneacetate substituted fullerene complex

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ABSTRACT

Riboflavin (RF), a metabolizable endogenous pigment, can significantly enhance the water solubility of naphthaleneacetate modified fullerene C_{60} (NP-C_{60}) upon formation of a supramolecular complex (RF/NP-C_{60}). NP-C_{60} can quench the fluorescence of RF efficiently through static quenching mechanism. The resulting complex exhibits strong affinity to calf thymus DNA, as indicated by the hypochromism of its absorption spectra. Under anaerobic conditions, RF/NP-C_{60} complex displays much more efficient photocleavage of the DNA than RF itself due to the occurrence of electron transfer from DNA to NP-C_{60}.

1. Introduction

Fullerene derivatives are generally considered as powerful building blocks in material sciences and medical chemistry, owing to their unique photophysical and photochemical behaviors [1,2]. DNA photocleavage is one of the biological activities of fullerene derivatives, which is involved in the potential application of fullerene derivatives in biological systems and pharmaceutical fields [3–7]. Coupling of nucleic acid specific agents to fullerenes can lead to higher affinity of fullerene derivatives towards target DNA [8–10].

However, the well-known lack of solubility of fullerenes in water is a negative aspect for their applications. Among the various approaches to solve this problem, supramolecular method, such as mixing with water soluble polymers [11,12] or lipid membrane [13,14], inclusion into cyclodextrins [15–17] or calixarenes [18,19] to form host–guest complexes, construction of supramolecular assemblies with aromatic component through π–π stacking interactions [20,21], is of great interests.

Riboflavin (RF, vitamins B_2, Fig. 1), an important metabolizable endogenous photosensitizer, is widely distributed in human tissues in free and conjugated forms [22,23]. It participates in various biological reactions such as causing damage of DNA and other cell matrix components [24–26]. It also has been reported that flavin moiety can be attached to single-walled carbon nanotube (SWCNT) through π–π stacking interactions [27–29]. Recently, riboflavin has attracted attention because it can improve the solubility of SWCNT in water [30]. Inspired by these studies, we investigated the interaction between riboflavin and fullerene derivatives.

In the current work, NP-C_{60} is synthesized in which naphthaleneacetate group is introduced to fullerene core to increase the affinity of fullerene to DNA [31,32]. Riboflavin can interact with NP-C_{60} and construct a complex in water. RF/NP-C_{60} complex shows high affinity towards calf thymus DNA and could cause efficient photocleavage of DNA under anaerobic condition. These results indicate that riboflavin can act as a metabolizable solubilizer for fullerene derivatives and the RF/NP-C_{60} complex has potentials to be applied in the medical fields.

2. Experimental

2.1. Chemicals

Malonic acid, 4-dimethylaminopyridine (DMAP), 1-naphthaleneacetic acid, tetrahydrofuran (THF), N,N′-dicyclohexyl carbodiimide (DCC), diethylene glycol, n-tetrabutylammonium perchlorate were purchased from Acros Organics. Fullerene C_{60}, riboflavin, calf thymus DNA (CT DNA) and pBR322 plasmid DNA were purchased

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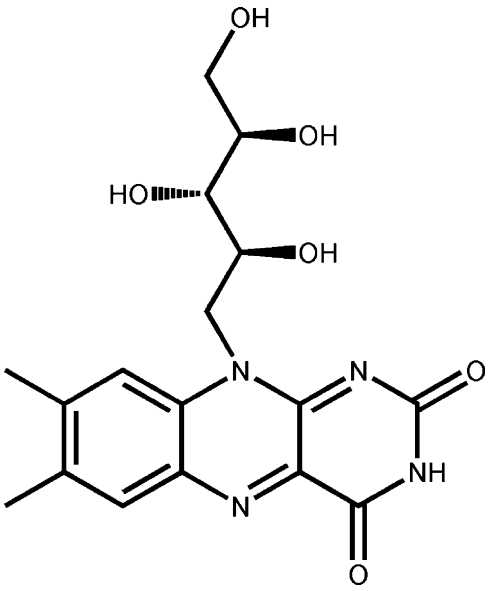

Fig. 1. Chemical structure of riboflavin (RF).

from Sigma Chemical Company. Other chemicals of analytical grade were obtained from Beijing Chemical Plant. The solvents were distilled before use. Water was freshly distilled twice before use.

All experiments involving DNA were performed in 10 mM Tris–HCl buffer (pH 7.4), unless otherwise noted. CT DNA solutions were prepared according to the literature [33]. The concentration of CT DNA was expressed as the concentration of nucleotide and calculated using an average molecular weight of 338 for a nucleotide and an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm [34].

2.2. Synthesis

Compound ***1***. A solution of 1-naphthaleneacetic acid (1.5 g, 8.06 mmol) and DMAP (0.1 g, 0.82 mmol) and diethylene glycol (30 g, 283 mmol) in dry CH_2Cl_2 (120 mL) was cooled to −5 °C and stirred for 1 h. To this solution, DCC (2.6 g, 12.6 mmol) in 20 mL dry CH_2Cl_2 was added dropwise. After 10 h at −5 °C, the mixture was allowed to warm to room temperature. Water (200 mL) was added, and the resulting solution was extracted with $CHCl_3$ (3 mL × 50 mL). The combined organic extracts were washed with water solution of $NaHCO_3$. After solvent evaporation, the crude product was purified by flash chromatography (SiO_2, ethyl acetate/hexanes, 1/1) to yield **1** as colorless oil (1.5 g, 70% yield). ^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.01 (d, J = 7.6 Hz, 1H), 7.99 (d, J = 7.6 Hz, 1H), 7.78–7.85 (m, 1H), 7.51–7.55 (m, 2H), 7.42–7.49 (m, 2H), 4.25 (m, 2H), 4.07 (s, 2H), 3.58–3.64 (m, 4H), 3.41 (t, J = 4.0 Hz, 2H), 1.63 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 133.6, 131.9, 130.2, 128.5, 127.9, 127.8, 126.2, 125.6, 125.3, 123.6, 72.2, 68.6, 63.8, 61.2, 38.8. MS (FAB^+), 296.7 [$(M+Na)^+$], calcd for $C_{16}H_{18}O_4Na$ 297.1.

Compound ***2***. To a solution of malonic acid (0.2 g, 1.9 mmol) and **1** (1.16 g, 4.2 mmol) in dry THF (40 mL) was added into a solution of DMAP (0.1 g, 0.82 mmol), DCC (0.87 g, 4.2 mmol) in dry THF (10 mL) dropwise over 30 min at room temperature. The reaction mixture was stirred for additional 20 h during which time a white precipitation was formed. The precipitation was filtered and washed with CH_2Cl_2 (3 mL × 20 mL), and combined organic fractions were evaporated. The crude product was purified with flash chromatography (SiO_2, ethyl acetate/hexanes, 2/1) to yield **2** as light yellow oil (1.2 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.98 (d, J = 8.0 Hz, 2H), 7.85 (d, J = 8.4 Hz, 2H), 7.78–7.80 (m, 2H), 7.47–7.51 (m, 4H), 7.40–7.51 (m, 4H), 4.23 (t, J = 4.7 Hz, 4H), 4.16 (t, J = 4.7 Hz, 4H), 4.09 (s, 4H), 3.60 (t, J = 4.8 Hz, 4H), 3.48 (t, J = 4.8 Hz, 4H), 3.39 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 166.3, 133.8, 132.1, 130.4, 128.7, 128.1, 128.0, 126.3, 125.8, 125.5, 123.8, 68.9, 68.7, 64.4, 64.0, 41.2, 39.0. MS (FAB^+), 639.6 [$(M+Na)^+$], calcd for $C_{35}H_{36}O_{10}Na$ 639.22.

Compound ***3*** (*NP-C_{60}*). C_{60} (50 mg, 0.069 mmol) and CBr_4 (34.8 mg, 0.106 mmol) were dissolved in anhydrous toluene (100 mL) with vigorous stirring. After degassing for 45 min, compound **2** (65.4 mg, 0.106 mmol) and DBU (20 μl, 0.16 mmol) were added and the mixture color changed gradually from purple to dark red. After stirring for 12 h, the solvent was removed under reduced pressure. The residue was purified with flash chromatography (SiO_2, toluene/ethyl acetate, 10/1) to yield **3** as brown solid (38.7%). ^{1}H NMR: (400 MHz, $CDCl_3$) 7.98 (d, J = 8.4 Hz, 2H), 7.77–7.84 (m, 4H), 7.41–7.53 (m, 8H), 4.48 (t, J = 4.5 Hz, 4H), 4.31 (t, J = 4.5 Hz, 4H), 4.10 (s, 4H), 3.61–3.63 (m, 8H). ^{13}C NMR: (100 MHz, $CDCl_3$) 171.39, 163.45, 145.28, 145.20, 145.18, 145.11, 144.92, 144.69, 144.64, 144.58, 143.89, 143.11, 143.03, 142.98, 142.20, 141.82, 140.94, 139.05, 133.83, 132.10, 130.44, 128.77, 128.13, 128.02, 126.40, 125.85, 125.52, 123.80, 71.39, 69.04, 68.60, 66.02, 64.08, 52.04, 39.08. MS (MALDI-TOF): m/z:1374.1 [$(M+K)^+$], calcd for $C_{95}H_{34}O_{10}K$ 1374.4. Elemental analysis, calcd for $C_{95}H_{34}O_{10}$: C, 85.45; H, 2.57. Found: C, 85.28; H, 2.64.

Preparation of RF/NP-C_{60} complex. Aqueous solution of NP-C_{60} was obtained according to the literature method [35], using RF as solubilizer. In detail, different amount of NP-C_{60} (0.1–2.7 mg) was added to 10 mL RF (0.2 mM) aqueous solution. The suspension was kept in a temperature-controlled (25 °C) sonicater (200 W) for 1.5 h, and then stirred at room temperature in the dark for 48 h. The insoluble NP-C_{60} was removed by centrifugation at 6000 rpm for 15 min. UV–vis spectrum was used to monitor whether NP-C_{60} was dissolved in water solution by examining the characteristic absorption maximum of precipitated NP-C_{60} in toluene. The obtained RF/NP-C_{60} solution was used for fluorescence and UV–vis spectra, and DNA cleavage experiment.

2.3. Measurements of spectral and electrochemical properties

The UV–vis absorption spectra and fluorescence emission spectra were recorded on a Hitachi U-3010 spectrophotometer and a Hitachi F-4500 fluorescence spectrometer, respectively.

Fluorescence lifetimes were measured by time-correlated single-photon counting technique with Edinburgh FL-900 spectrophotometer upon 400 nm laser pulse irradiation.

EPR spectra were obtained using a Bruker ESP-300E spectrometer operating at room temperature, and the operating conditions were as following: microwave bridge, X-band with 100 Hz field modulation; sweep width, 100 G; receiver gain, 1×10^5; microwave power, 5 mW. Samples were injected into the specially made quartz capillaries for analysis, purged with argon for 30 min in the dark, and illuminated directly in the cavity of the spectrometer with a Nd:YAG laser (355 nm, 5–6 ns pulse width, 10 mJ/pulse energy).

Cyclic voltammetry (CV) experiments were performed on a potentiostat/galvanostat Model 283A (EG&G Princeton Applied Research) in DMSO–toluene (9/1, v/v) solution, using two platinum wires as the working and counter electrodes, respectively, and a saturated calomel electrode (SCE) as reference electrode in the presence of 1 mM n-tetrabutylammonium perchlorate as the supporting electrolyte.

2.4. Melting temperature (T_m) determination of DNA samples

The absorption versus temperature was monitored at 260 nm using a U-3010 spectrometer with a temperature-controlled cell, and experiments were run in steps of 0.5 °C min^{-1} from 35 to 89 °C. The UV absorption curves were normalized from 0 (the absorbance at 260 nm of base line before the transition) to 1 (the absorbance at