A Water-Soluble C_{60}-Porphyrin Compound for Highly Efficient DNA Photocleavage

Cuisong Zhou[1*], Qiaolin Liu[2], Wei Xu[2], Chunru Wang[2] and Xiaohong Fang[2*]

1 College of Chemistry, Sichuan University, Chengdu 610064, PR China

2 Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, PR China

Supporting information

Experimental

Chemicals

pBR332 plasmid DNA and the 30 bp oligonucleotides were purchased from Takara biotechnology Co., Ltd. (Dalian, China). The 30 bp dsDNA was formed by the following two pieces of ssDNA: 5' GTG TGT GTG TGT GTG TGT GTG TGT GTG TGT 3', and 5' ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC 3'. The physiological buffer used in the experiment was a phosphate buffer solution (PBS, 0.20 M, pH 7.5). Milli-Q purified water (18.2 MΩ) was used to prepare all of the solutions.

C_{60}Por was synthesized by covalently coupling trismethylpyridylporphyrin to C_{60}.[18] First, we synthesized trispyridylporphyrin-C_{60}.[19] The characterization results were: MALDI-TOF MS *m/z*: 1407. ^{1}H NMR ($CDCl_3/CS_2$) δ: -3.00 (2H, s, internal pyrrole), 1.71 (3H, t, N-$CH_2C\boldsymbol{H}_3$), 2.91 (1H, q, N-$CH_2C\boldsymbol{H}_3$), 3.70 (1H, q, N-$C\boldsymbol{H}_2CH_3$), 4.30 (1H, d), 5.2 (1H, d), 5.4 (1H, s), 8.10 (10H, m, phenyl and 3,5-pyridyl), 8.80 (8H, m, pyrrole β), 8.99 (6H, br, 2,6-pyridyl).[19] Second, the trispyridylporphyrin-C_{60} was methylated completely using a simple reported experiment.[18] In brief: Methyl p-Toluenesulfonate (0.2 mL) was added to a solution of trispyridylporphyrin-C_{60} (1.8 mg) in 1,2-dichlorobenzene (16 mL). The reaction mixture was refluxed in an N_2 atmosphere for 1.0 h and then evaporated under reduced pressure. The crude tosylate salt of trismethylpyridylporphyrin-C_{60} (C_{60}Por) was passed through an anion-exchange resin (Amberlite IRA-40(CI), Alfa Aesar) repeatedly to yield C_{60}Por as a chloride salt. The Zeta Potential of C_{60}Por is 45.2 mV.

Instrumentations

The Zeta Potentials was measured on a zetapotential analyzer (Malvern Instruments Ltd, Germany). Luminescence measurements were performed on a Fluorolog-3 spectrofluorometer (Jobin Yvon Inc., Edison, NJ). The sample cell was a 1-mL quartz cuvette. The slits for both excitation and emission were set at 10 nm. All experiments were carried out at room temperature. The excitation and emission wavelengths for porphyrin and porphyrin/DNA solution were 420 nm and 665 nm, respectively. The excitation and emission wavelengths for C_{60}Por and C_{60}Por/DNA solution were 435 nm and 632 nm, respectively. The results reported were mean values from triplicate measurements and the standard deviation was less than 2 %.

The calculated binding constant *K*

The calculated binding constant *K* was calculated from the luminescence data with eqs 1 and 2:[20,21]

$$\frac{(I_a - I_f)}{(I_b - I_f)} = \frac{b - (b^2 - 2K^2C[\mathrm{DNA}]/s)^{1/2}}{2KC} \qquad (1)$$

$$b = 1 + KC + K[\mathrm{DNA}]/2s \qquad (2)$$

Where *C* is the constant total concentration of C_{60}Por (14 μM). [DNA], the total concentration of the added 30 bp oligonucleotides, is 30 times the concentration of dsDNA used in Figure S2. I_a is the apparent luminescence of C_{60}Por in the presence of DNA. I_f is the luminescence of the free C_{60}Por. I_b is the extinction luminescence of the DNA-bound C_{60}Por. The value of I_b is obtained from the saturated DNA-bound C_{60}Por where the concentration of DNA is 0.45 μM.

Examination of DNA-cleaving activity

Cleaved DNA products were detected by agarose gel electrophoresis using pBR332 DNA (38.3 μg/mL) as a substrate. A serial of ten sample solutions in Figure 3 were prepared (Table S1). The sample solutions were or were not vacuumed for 10 min by

a vacuum pump to remove the air. Subsequently, the samples were incubated in the dark or under radiation for 30 min. The radiation was applied by a 16-W photoreflector lamp (365 nm) (Kanghua Inc., Shanghai, China). The radiation distance between the lamp and the sample solution was 20 cm. After radiation, 2 μL sample solution was mixed with 10 μL loading buffer, and then 10 μL mixture was loaded onto a 1 % agarose gel. The gels were applied by a constant voltage of 120 V for 30 min in TAE (Tris-acetate-EDTA) buffer, stained with ethidium bromide, visualized under a UV transilluminator, and then photographed by a digital camera.

The DNA-cleaving activity is defined as the following equation:

$$\text{The cleaving activity (\%)} = \frac{F_2}{F_1 + F_2}$$

Where the F_1 and F_2 are the intensity of Form Ⅰ (supercoiled) and Form Ⅱ (nicked), respectively.

Table S1

Compositions of the ten aqueous solutions for the examination of C_{60}Por's DNA-cleaving activity. Chloroform-H_2O solution (1 : 1, v/v) was used to prepare the C_{60} solution. PBS buffer was used for all other solutions.

Solution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
pBR322 DNA (μg/mL)	38.3	38.3	38.3	38.3	38.3	38.3	38.3	38.3	38.3	38.3
C_{60}Por (μM)	13	6.5	3.3	1.6	0.8	0.4	0.2	0	0	0
Prophyrin (μM)	0	0	0	0	0	0	0	0	0	13
C_{60} (μM)	0	0	0	0	0	0	0	0	13	0

Figures for supporting information

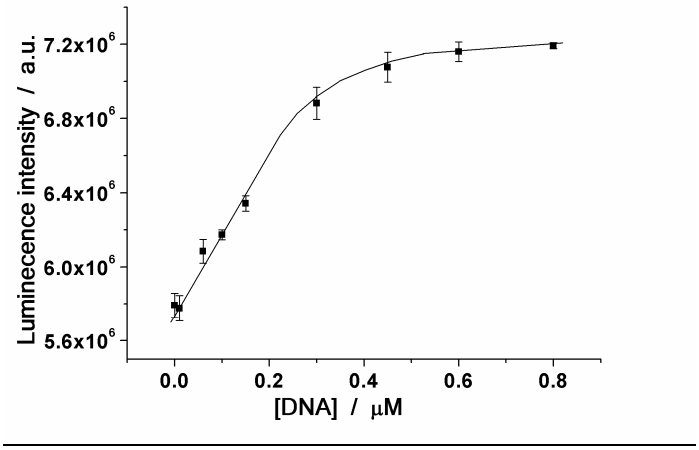

Figure S1. Luminescence intensity of porphyrin in the presence of DNA ranging from 0 to 0.80 μM. Total concentration of porphyrin is 14 μM.

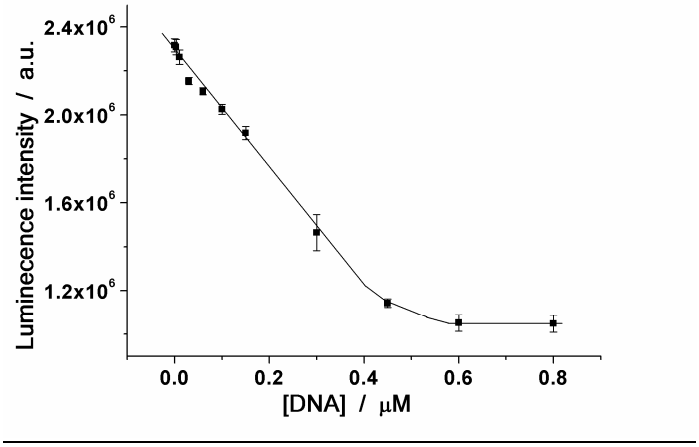

Figure S2. Titration of C_{60}Por with dsDNA in the concentration range of 0–0.8 μM. The concentration of C_{60}Por is 14 μM.

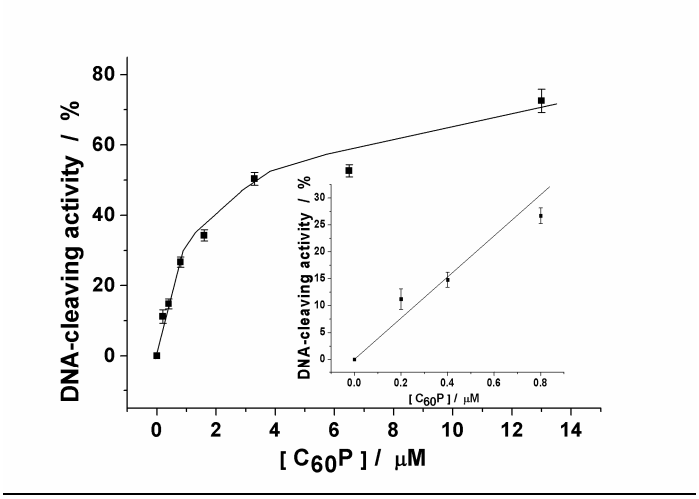

Figure S3. The relationship between the cleaving activity and the C_{60}Por concentration ranging from 0 to 13 μM (the data is from figure 3).

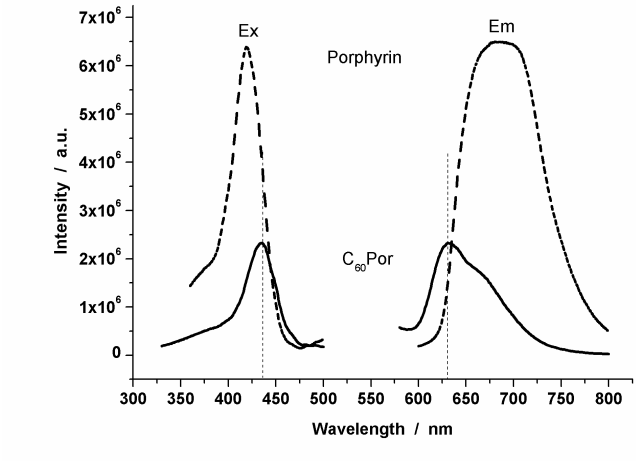

Figure S4. Luminescence spectra of porphyrin (point segment) and C_{60}Por (solid line). The concentration of porphyrin or C_{60}Por was 14 μM.

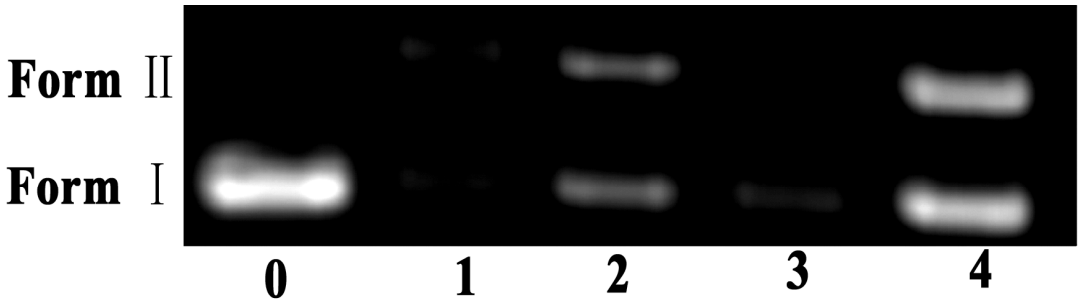

Figure S5. Photocleavage of pBR322 DNA (38.3 μg/mL) in the presence of C_{60}Por and different ROS scavengers under non-vacuum and radiation. Lane 0: pBR322 DNA only; Lane 1: DNA and C_{60}Por, no scavenger; Lane 2–6: DNA and C_{60}Por, with different scavengers, (2) NaN_3 (8 mM); (3) SOD (0.13 units/mL) ; and (4) DMSO (8 mM). The concentration of C_{60}Por was 13 μM.