

## On the Mechanism of DNA Cleavage by Fullerenes Investigated in Model Systems: Electron Transfer from Guanosine and 8-Oxo-Guanosine Derivatives to C<sub>60</sub>

Robert Bernstein, Ferran Prat, and Christopher S. Foote\*

Department of Chemistry & Biochemistry  
University of California, Los Angeles, California 90095-1569

Received September 18, 1998

Selective DNA photocleavage is a very active field of research, and a number of artificial photonucleases have been reported.<sup>1–3</sup> Fullerenes and dihydrofullerenes are electron-poor photosensitizers, and DNA photocleavage (selective for G) mediated by these compounds has been reported by several groups.<sup>4–7</sup> Two mechanisms are possible, type I, involving electron transfer from G to fullerene, and type II, in which singlet oxygen (<sup>1</sup>O<sub>2</sub>) generated by the fullerene is the active oxidant (Scheme 1).

The photophysical and electrochemical properties of fullerenes and dihydrofullerenes make both type I and type II pathways possible. The reduction potential of the excited triplet state of C<sub>60</sub>, E<sup>0</sup>(<sup>3</sup>C<sub>60</sub>/C<sub>60</sub><sup>•-</sup>), is +1.14 V vs SCE in benzonitrile,<sup>8</sup> whereas that of Guo in DMF, E<sup>0</sup>(Guo<sup>•+</sup>/Guo), is +1.26 V.<sup>9</sup> Therefore, slightly endergonic electronic transfer could occur from Guo to <sup>3</sup>C<sub>60</sub>. C<sub>60</sub> is also an excellent singlet oxygen sensitizer ( $\phi_{\Delta} = 1$ ),<sup>10</sup> and its triplet state reacts with oxygen rapidly ( $k_q = 1.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ).<sup>11</sup> Singlet oxygen is quenched by Guo with a rate constant of  $\sim 6 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  in water and polar solvents, but the chemical reaction rate constant is only  $\sim 1 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>9,12</sup> The type II mechanism has been assumed by a majority of authors. However, a report by An *et al.* showed that, in at least one case, the mechanism of G oxidation in an oligonucleotide bound to a complementary strand bearing a dihydrofullerene sensitizer was probably type I.<sup>5</sup>

Two additional features of DNA oxidation further complicate the situation. First, guanosine stacks are more prone to oxidation than Guo itself.<sup>13</sup> Calculations suggest that a GG stack in a B-DNA conformation can be as much as 0.4 V (equivalent to 9.2 kcal/mol) more easily oxidized than an isolated Guo, and that the 5' G should be preferentially attacked.<sup>14,15</sup> Therefore, DNA strands containing GG stacks might well favor type I oxidation over strands without this particular arrangement.

(1) Murphy, C. J.; Arkin, M. R.; Jenkins, Y.; Ghatlia, N. D.; Bossmann, S. H.; Turro, N. J.; Barton, J. K. *Science* **1993**, *262*, 1025–1029.  
(2) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1351–1353.

(3) Armitage, B.; Yu, C.; Devadoss, C.; Schuster, G. B. *J. Am. Chem. Soc.* **1994**, *116*, 9847–9859.

(4) Boutorine, A. S.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Helene, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2462–2465.

(5) An, Y.-Z.; Chen, C.-H. B.; Anderson, J. L.; Sigman, D. S.; Foote, C. S.; Rubin, Y. *Tetrahedron* **1996**, *52*, 5179–5189.

(6) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918–7919.

(7) Yamakoshi, Y. N.; Yagami, T.; Sueyoshi, S.; Miyata, N. *J. Org. Chem.* **1996**, *61*, 7236–7237.

(8) Arbogast, J. W.; Foote, C. S.; Kao, M. *J. Am. Chem. Soc.* **1992**, *114*, 2277–2279.

(9) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 6439–6442.

(10) Terazima, M.; Hirota, N.; Shinohara, H.; Saito, Y. *J. Phys. Chem.* **1991**, *95*, 6490–6495.

(11) Arbogast, J. W.; Darmanyan, A. P.; Foote, C. S.; Rubin, Y.; Diederich, F. N.; Alvarez, M. M.; Anz, S. J.; Whetten, R. L. *J. Phys. Chem.* **1991**, *95*, 11–12.

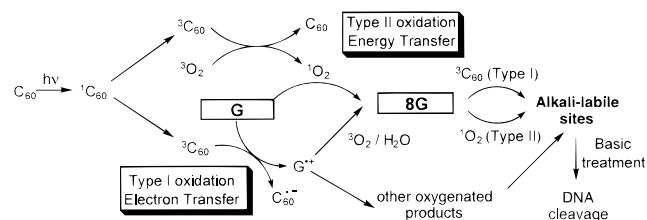
(12) Prat, F.; Hou, C. C.; Foote, C. S. *J. Am. Chem. Soc.* **1997**, *119*, 5051–5052.

(13) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406–6407.

(14) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063–7068.

(15) Prat, F.; Houk, K. N.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845–846.

Scheme 1



Second, the role of 8-Oxo-guanine (8G) is important in DNA oxidation. Both type I and type II oxidations transform G into 8G and other oxygenated products.<sup>16–18</sup> Type I mechanisms should favor oxidation of the G in the 5' position in GG stacks, at least in double-stranded DNA. The type II mechanism should generate 8G randomly along the strand.<sup>19</sup> However, 8G is easily oxidized by both electron-transfer and singlet-oxygen mechanisms ( $k_q(^1\text{O}_2) = 5.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$  in acetone, E<sup>0</sup>(8Guo<sup>•+</sup>/8Guo) = +0.85 V vs AgCl in DMF).<sup>9,20</sup> Further oxidation of 8G will generate an alkali-labile site, which can lead to strand breaks.<sup>20–22</sup> Direct  $\gamma$  radiation of 8G-containing oligomers also leads to strand breaks at the 8G position after piperidine treatment.<sup>23</sup> This modified base is the most likely intermediate in a wide variety of oxidations.

In this paper, we report detection of electron transfer from a guanosine derivative to C<sub>60</sub> for the first time and show that an 8-oxo-guanosine derivative is far more reactive under the same conditions. All experiments were done in argon-saturated benzonitrile, the only polar solvent in which C<sub>60</sub> is modestly soluble.<sup>8,24</sup> Since Guo and 8Guo are not soluble in this solvent, the 2',3',5'-tri-TBDMS (*t*-butyldimethylsilyl) derivatives of both compounds (*t*-Guo and *t*-8Guo) were synthesized as previously described.<sup>9</sup> Transient species following irradiation by 9-ns laser pulses at 532 nm were detected in the near-IR region by laser flash photolysis using a sensitive liquid nitrogen-cooled germanium photodiode.<sup>9</sup>

The electron-transfer reaction between the redox pairs *t*-Guo/C<sub>60</sub> and *t*-8Guo/C<sub>60</sub> were monitored by the characteristic near-IR band of C<sub>60</sub><sup>•-</sup> at 1070 nm<sup>8,25</sup> (Figure 1). Recombination was slow (on the order of milliseconds) in both cases. Identification of *t*-Guo<sup>•+</sup> absorption was attempted without success.

The quenching rate constants of <sup>3</sup>C<sub>60</sub> by *t*-Guo and *t*-8Guo were determined from the decay kinetics of transient <sup>3</sup>C<sub>60</sub> triplet–triplet absorption at 720 nm. The plot of  $k_{\text{obsd}}$  vs [Q] yielded values of  $k_q = 3.3 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$  for *t*-Guo and  $1.1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  for *t*-8Guo. Similar values were obtained from measurements of the appearance of C<sub>60</sub><sup>•-</sup>.

In our study of DNA cutting of a hybridized deoxynucleotide, a dihydrofullerene, (DHF, a fullerene with two sp<sup>3</sup> carbons) was necessarily used. For this reason, the reactivity of a DHF was

(16) Floyd, R. A.; West, M. S.; Eneff, K. L.; Schneider, J. E. *Arch. Biochem. Biophys.* **1989**, *273*, 106–111.

(17) Kasai, H.; Yamaizumi, Z.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1992**, *114*, 9692–9694.

(18) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283–6289.

(19) Burrows, C. J., personal communication.

(20) Gasper, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762–12771.

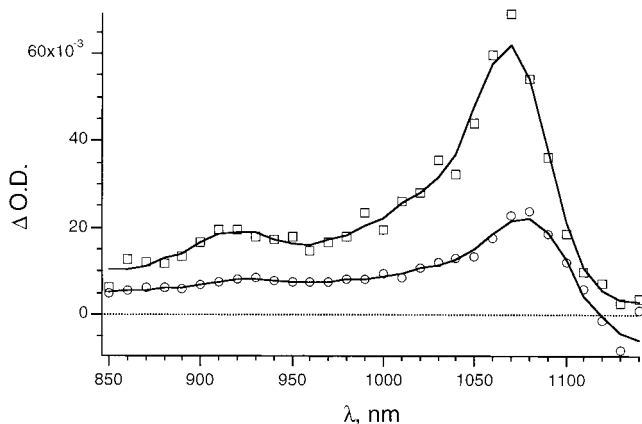
(21) Koizume, S.; Inoue, H.; Kamiya, H.; Ohtsuka, E. *Chem. Commun.* **1996**, 265–266.

(22) Muller, J. G.; Duarte, V.; Hickerson, R. P.; Burrows, C. J. *Nucleic Acids Res.* **1998**, *26*, 2247–2249.

(23) Doddridge, Z. A.; Cullis, P. M.; Jones, G. D. D.; Malone, M. E. *J. Am. Chem. Soc.* **1998**, *120*, 10998–10999.

(24) Ruoff, R. S.; Tse, D. S.; Malhotra, R.; Lorents, D. C. *J. Phys. Chem.* **1993**, *97*, 3379–3383.

(25) Kato, T.; Kodama, T.; Shida, T.; Nakagawa, T.; Matsui, Y.; Suzuki, S.; Shiromaru, H.; Yamauchi, K.; Achiba, Y. *Chem. Phys. Lett.* **1991**, *180*, 446–450.



**Figure 1.** Transient absorption spectrum of  $C_{60}^{*-}$  generated from an Ar-saturated 1.4 mM benzonitrile solution of  $t$ -Guo (O) and from a 5.2 mM solution of  $t$ -8Guo (□).

also studied. For a typical DHF, the singlet oxygen quantum yield decreases by ca. 10–25%, and the reduction potential is  $\sim 0.24$  V lower than that for  $C_{60}$ .<sup>26</sup> Electron transfer from  $t$ -Guo to DHF was not observed by transient absorption spectroscopy ( $k < 5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ ), but  $t$ -8Guo reacted at a rate of  $k = 1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ .

Transient absorption spectra of a series of six amines with various oxidation potentials were investigated. All amines reacted with the triplet excited state of  $C_{60}$  by electron transfer to generate the radical cation of the amine and the radical anion of  $C_{60}$ . In every case, the transient absorption spectrum in the visible range matched the spectrum of the amine radical cation,<sup>8</sup> whereas the near-IR region showed the characteristic band of  $C_{60}^{*-}$  at 1070 nm. The quenching rate constants were determined from the decay kinetics of transient  ${}^3C_{60}$  triplet–triplet absorption at 720 nm.

The free energy difference for electron transfer to  ${}^3C_{60}$  ( $\Delta G_{\text{et}}$ ) from the semiempirical Rehm–Weller equation<sup>27</sup> (1) is:

$$\Delta G_{\text{et}} = 23.06[E(D^+/D) - E(A^-/A) - \Delta E_{0,0} - e_0^2/a\epsilon] \quad (1)$$

where  $E(D^+/D)$  is the oxidation potential of the donor,<sup>28</sup>  $E(A^-/A)$  is the reduction potential of the acceptor ( $-0.42$  V for  $C_{60}$ ),<sup>8</sup>  $\Delta E_{0,0}$  is the excitation energy of  ${}^3C_{60}$  (1.56 eV)<sup>10</sup> and  $e_0^2/a\epsilon$  is the energy gained in bringing the two radical ions to the encounter distance ( $a$ ) in a solvent of dielectric constant  $\epsilon$ , where  $e_0$  is the electronic charge ( $e_0^2/a\epsilon = 0.126$  in benzonitrile).<sup>29</sup>

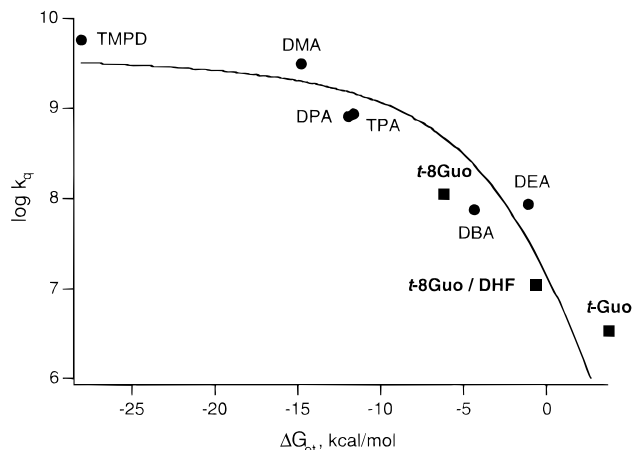
A plot of  $\log k_q$  vs  $\Delta G_{\text{et}}$  (Figure 2) shows an excellent correlation with the rate constants calculated for electron transfer by eq 1, using the diffusion rate in benzonitrile calculated from the Stokes–Einstein equation ( $k_{\text{dif}} = 5.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ).<sup>8</sup> The Rehm–Weller equation depends only on  $\Delta G_{\text{et}}^\ddagger(0)$ , the free energy of activation for electron transfer at  $\Delta G_{\text{et}} = 0$ . A single-variable

(26) Anderson, J. L.; An, Y.-Z.; Rubin, Y.; Foote, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 9763–9764.

(27) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259–271.

(28) The values for the redox potential for the donors were corrected from acetonitrile to benzonitrile using the same correction as Arbogast et al.<sup>8</sup> which was +0.09 V. A similar correction was applied on the purine values, which were obtained in DMF. This correction is  $-0.14$  V, as observed in the 1,4-benzoquinone system (*Electrochemical Data, Part I*; John Wiley and Sons: New York, 1974; pp 404–405).

(29) The value for this constant in acetonitrile (0.085) was corrected by a factor of 1.48 due to the different dielectric constant of the two solvents ( $\text{CH}_3\text{CN}/\text{PHCN}$ , 37.5:25.2).



**Figure 2.** Plot of  $\log k_q$  vs  $\Delta G_{\text{et}}$  for different amines (TMPD, tetramethylphenylene diamine; DMA, *N,N*-dimethylaniline; DPA, diphenylamine; TPA, triphenylamine; DEA, diethylamine; DBA, dibutylamine) and the two examined purines in benzonitrile. The solid line was calculated from the Rehm–Weller equation.

fit gave a value of 4.3 kcal/mole for  $\Delta G_{\text{et}}^\ddagger(0)$  in benzonitrile, considerably higher than that for aromatic hydrocarbons in acetonitrile ( $\Delta G_{\text{et}}^\ddagger(0) = 2.4$  kcal/mol)<sup>27</sup>, but lower than the value reported for GMP quenching of ruthenium complexes in water solution ( $\Delta G_{\text{et}}^\ddagger(0) = 7.5$  kcal/mol).<sup>30</sup> The generation of the fullerene radical anion, and the fact that both  $t$ -8Guo and  $t$ -Guo rate constants fit the Rehm–Weller equation, allow the conclusion that the reaction between excited fullerene and the modified nucleobases in benzonitrile proceeds through a single electron transfer. Electron transfer for  $t$ -Guo is slightly endergonic ( $\Delta G_{\text{et}} = 3.8$  kcal/mol), while for  $t$ -8Guo it is substantially exergonic ( $\Delta G_{\text{et}} = -6.1$  kcal/mol).

The quenching rate constants of  ${}^3C_{60}$  and  ${}^1O_2$  by  $t$ -Guo are in the range  $10^6 \text{ M}^{-1} \text{ s}^{-1}$ , but the chemical oxidation rate constant of  $t$ -Guo by  ${}^1O_2$  is  $10^5 \text{ M}^{-1} \text{ s}^{-1}$ .<sup>9</sup> For an isolated G in a DNA strand, type II is the oxidation mechanism expected since the  $\Delta G$  of the electron transfer is slightly endergonic to  $C_{60}$  and more so to DHF. For GG stacks, which are ca. 0.4 V more easily oxidizable than G, the electron transfer is exergonic ( $\Delta G_{\text{et}} = 3.8 - 9.2$  kcal/mol =  $-5.3$  kcal/mol), and the rate constant predicted by the Rehm–Weller equation is ca.  $4 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ . Thus, the most likely mechanism is type I. Both mechanisms will primarily yield 8G, along with other derivatized nucleobases. Once 8G is formed, electron transfer to the excited state of  $C_{60}$  or DHF should occur readily, since 8G quenches  ${}^3C_{60}$  faster than  ${}^1O_2$ .<sup>9</sup> These are model studies of modified bases in benzonitrile. The behavior of oligonucleotides in aqueous solution might differ. However, we note that the interior of double helical DNA is not an aqueous environment.

In the special case of the DHF-deoxynucleotide studied previously,<sup>5</sup> the results of this study support our suggestion that the reaction occurs by a type I mechanism. The high local concentration of the DHF bound near the GG stack provides a driving force for even endergonic oxidation of G and should give exergonic oxidation of the GG stack in this particular nucleotide.

**Acknowledgment.** Supported by the National Science Foundation (CHE 94-23027 and CHE 94-730386).

JA983335D

(30) Lecomte, J. P.; Kirschdemesmaeker, A.; Feeney, M. M.; Kelly, J. M. *Inorg. Chem.* **1995**, *34*, 6481–6491.