# Cleavage of DNA by Irradiation of Substituted Anthraquinones: Intercalation Promotes Electron Transfer and Efficient Reaction at GG Steps

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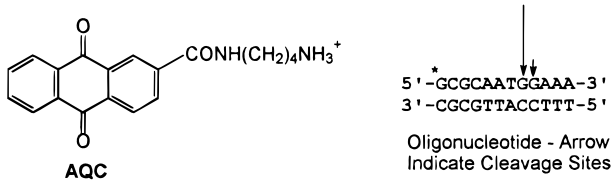
There is widespread interest in compounds that cleave DNA in unique and controllable ways.[1] One focus of this field is the discovery of nucleases that are activated by visible or near-UV light.[2] Photonucleases offer special advantages as footprinting reagents and for examination of processes such as transcription.[3] Intensive investigation has revealed compounds operating by diverse mechanistic paths that cleave DNA when irradiated. Of special relevance to our work are recent reports that irradiation of simple naphthalimide[4a,b] derivatives or riboflavin[4c] causes piperidine-requiring, selective cleavage at the 5′-G of GG steps in duplex DNA. Investigation of this reaction mechanism led to the conclusion that it proceeds by direct electron transfer from a guanine at the GG step to the triplet state of the naphthalimide or riboflavin.[4]

We recently reported that irradiation of certain anthraquinone derivatives leads to piperidine-requiring, GG selective cleavage of duplex DNA.[5] Our investigation of this reaction showed that these anthraquinones bind to DNA by intercalation. We report herein analyses of the products formed from the irradiation of AQC intercalated in DNA and examination of the reaction mechanism. Cleavage of DNA is observed at the 5′-G for each GG step in a 248 base pair restriction fragment. Surprisingly, the cleavage efficiency depends on the sequence flanking the GG step. Investigation of a duplex dodecanucleotide designed to mimic the most efficiently cleaved GG step in the restriction fragment reveals formation of 7,8-dihydro-8-oxoguanine (8-OxoG). These findings, and time-resolved laser spectroscopic experiments, indicate that cleavage of the 5′-G of GG steps is initiated by electron transfer to an excited AQC intercalated in DNA. Subsequent formation of a stabilized G radical cation leads to its damage and then to strand scission.[6]

The ability of AQC to cleave DNA was examined by polyacrylamide gel electrophoresis of a radiolabeled 248 base pair restriction fragment.[7] Air-saturated phosphate buffered solutions of the intercalated quinone[8] were irradiated at 350 nm. These samples were treated with hot piperidine, to visualize alkaline labile damage, and analyzed by gel electrophoresis[9] which reveals cleavage at the 5′-G of each GG step of the restriction fragment. However, the efficiency of reaction at the various GG steps varies significantly. The 5′-G's of the sequences 5′-GGAAA-3′, and 5′-GGAAT-3′ are cleaved more frequently than other GG steps. To assess this, we examined the photochemistry and photophysics of AQC with the duplex dodecanucleotide shown in Chart 1.

**Chart 1**




AQC

AQC was irradiated in the presence of 40 $\mu$M of the duplex dodecanucleotide ($^{32}$P at the 5′-end of the GG-containing strand) and 80 $\mu$M of the quinone (a 6:1 ratio of base pairs to quinone) in 10 mM sodium phosphate buffer.[10] Following piperidine treatment, analysis of the reaction mixture by polyacrylamide gel electrophoresis[9] shows selective cleavage of the labeled strand at the GG step. The products of this reaction were examined by HPLC and mass spectrometry to quantitate the efficiency of cleavage.

Irradiated samples of AQC and the duplex dodecanucleotide were treated with piperidine and then 5′-dephosphorylated with bacterial alkaline phosphatase. The oligonucleotide products were separated by HPLC[11] and identified by comparison with authentic samples. Five products are detected. The major products are 5′-GCGCAAT-3′ and 5′-GAAA-3′ from 5′-G cleavage, lesser amounts of 5′-GCGCAATG-3′ and 5′-AAA-3′ from 3′-G cleavage, and the 5′-TTTCCATTGCGC-3′ strand detected intact. This confirms the GG selective cleavage observed by gel electrophoresis. The quantum yields for these reactions were determined using an anthraquinone-2,6-disulfonate actinometer[12] to be $1.3 \times 10^{-2}$ and $1.1 \times 10^{-3}$ for cleavage at 5′-G and 3′-G, respectively (5′-G:3′-G cleavage 9:1).[13] These results are similar to those of Saito and co-workers for the GG-selective cleavage for naphthalimide where the ratio is reported to be 84:16, but the "limiting" quantum yield is much lower ($3.0 \times 10^{-4}$) than for AQC. In the absence of $O_2$, the quantum yield for reaction initiated by AQC is reduced more than 10-fold. Clearly, $O_2$ is required for efficient cleavage of

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(7) (a) A $^{32}$P restriction fragment generated by digestion of the pUC19-derived plasmid P8G2 *Eco*RI and *Pvu*II was labeled on the 3′-OH of the *Eco*RI-generated terminus using [α-$^{32}$P]-dATP and the Klenow fragment of DNA polymerase I and purified by electrophoresis through a 5% nondenaturing polyacrylamide gel.[7b] (b) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning. A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1989.

(8) The association constant of the quinone with calf-thymus DNA is $1 \times 10^6$ M$^{-1}$. Under the conditions of the experiments reported here, essentially all of the quinone is bound to DNA.

(9) The autoradiogram of this gel is included as supporting information to this paper.

(10) The dodecanucleotide was labeled with [$\gamma$-$^{32}$P]-ATP using T4-polynucleotide kinase following the standard procedure.[7b] Cleavage was visualized by a high-resolution denaturing polyacrylamide gel (20%), electrophoresis, and autoradiography.

(11) The samples were separated on Vydac Protein & Peptide C18 reverse-phase columns with water, acetonitrile, triethylammonium acetate (0.1 M), and NaCl (0.5 M) at 1 mL/min. Monitoring the eluent revealed peaks at 61 and 66 min, corresponding to 5′-AAA-3′ and 5′-GAAA-3′, respectively.

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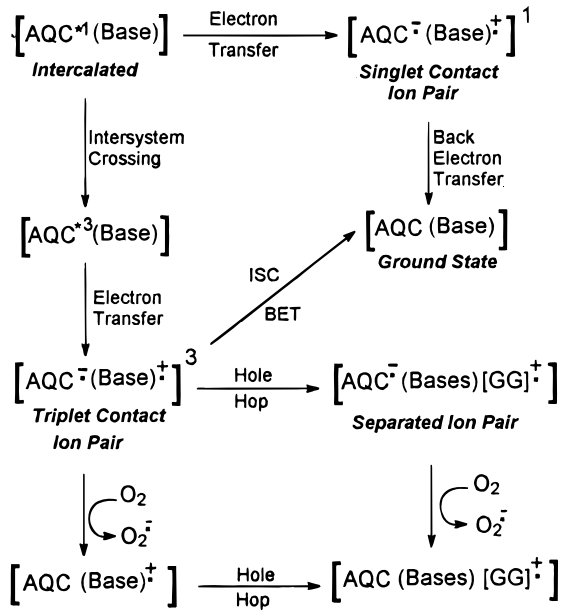
(13) Standard errors in the quantum yields were determined to be 10% of the reported value.

S0002-7863(96)01578-8 CCC: $12.00 

**Scheme 1**




DNA.[14] Significantly, the DNA cleavage efficiency for AQC increases ca. 70% to 2.4 × $10^{-2}$ when superoxide dismutase (SOD, 15 U/mL) is included in the reaction mixture.

The chemical processes that result in reaction of the dodecanucleotide are partly revealed by further examination of the reaction products. An irradiated sample was hydrolyzed with acid, silanized, and analyzed by the GC/MS single-ion monitoring technique developed by von Sonntag and co-workers.[15] This procedure reveals the presence of 8-OxoG and confirms that the cleavage of the DNA proceeds with the formation of an oxidized guanine.[16]

The reactions of AQC intercalated in the duplex dodecamer and in duplex $(dGdC)_4$, which does not have a GG step, were examined by time-resolved laser spectroscopy. Irradiation of intercalated AQC in either oligonucleotide with a 20 ps laser pulse immediately generates equal amounts of an intermediate with $\lambda_{max} \cong 540$ nm assigned to the quinone radical anion.[17,18] The absorption of the radical anion decreases to ca. 70% of its initial value within 200 ps of the laser pulse for both oligonucleotides[19] and then is essentially unchanged for 10 ns. We showed previously that for $N_2$-saturated solutions of calf-thymus DNA this residual absorption of the quinone radical anion decays with (at least) two time constants over a period of several milliseconds.[5] The kinetics of the quinone radical anion decay reveal a complex reaction path.

Our findings show that the GG-selective cleavage of DNA is initiated by irradiation of intercalated AQC. The product studies and laser spectroscopy support the mechanism shown in Scheme 1. Calculations indicate that electron transfer from any of the DNA bases to the excited singlet or triplet state of AQC is exothermic. In this circumstance the rate of electron transfer to give a quinone−base contact ion pair should be rapid. Experimentally, we observe this reaction to be complete in less than 20 ps.[20] Intersystem crossing of anthraquinone is reported to occur in less than 10 ps.[17] Our experiments indicate that both singlet and triplet contact ion pairs are formed.

We postulate three general reaction pathways for the ion pair: (i) back-electron transfer to regenerate ground states; (ii) migration of the base radical cation by "hopping" through the bases of the DNA helix; (iii) reaction with water or $O_2$. According to this hypothesis, the rapid (200 ps) initial decay of the quinone radical anion occurs from the singlet contact ion pair. This reaction will be in the Marcus inverted region[21] since $\Delta G_{bet} \cong -0.98$ eV, if a guanine has been oxidized,[22] and $\lambda$ is estimated to be 0.6 eV.[23] Because of this short lifetime, efficient cleavage of DNA will not result from the formation of singlet ion pairs.

We associate the long-lived quinone radical anions and DNA cleavage reaction with triplet contact ion pairs. The increased ion pair lifetime may permit the radical cation ("hole") to migrate (hop) if a suitable path in the DNA exists. If the hole hops from A to G, $\Delta G_{hop} = -0.1$ eV, and with $\lambda = 0.6$ eV this reaction will occur on the microsecond time scale.[21] The base sequence thus determines $\Delta G_{hop}$ and whether a path is suitable for hopping. The increased cleavage efficiency seen for certain GG-containing sequences in the restriction fragment may be due to their ability to support hole hopping. Similarly, migration of the hole out of a GG trap will be slow, because of its low oxidation potential,[4] thus fixing the hole for reaction with $H_2O$ or $O_2$ and subsequent cleavage of the DNA. Also, the increased lifetime of the triplet ion pair will permit reaction of the quinone radical anion with $O_2$ and similarly provides time for the hole to hop. The reduction in the quantum yield for cleavage in $O_2$-free solution is consistent with this central role for $O_2$ as is the increase in quantum yield seen in the presence of SOD. We have shown that superoxide is generated by irradiation of intercalated quinone in air-saturated solutions.[5] Superoxide will quench the base radical cation in competition with its reactions that lead to DNA cleavage.

In summary, we postulate that GG selective DNA cleavage is initiated by electron transfer from a base to the triplet state of an intercalated quinone. The base radical cation of the triplet contact ion pair can hop away from the intercalation site if the base sequence is suitable. The quinone radical anion is trapped by $O_2$ to form superoxide. The hole will be trapped at GG steps where damage caused by reaction with water or $O_2$ is revealed by treatment with piperidine.

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**Supporting Information Available:** Autoradiograms demonstrating GG selective cleavage in the dodecamer and the restriction fragment (3 pages). See any current masthead page for ordering and Internet access instructions.

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(14) The effect of $[O_2]$ is complex, since the cleavage quantum yield is reduced in $O_2$-saturated solutions.

(15) von Sonntag, C.; Schuchman, H.-P. *Methods Enzymol.* **1990**, *186*, 511.

(16) Although the formation of 8-OxoG is often implicated in the cleavage of DNA, recent results[6] indicate that other pathways may also operate.

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(18) Moore, J. N.; Phillips, D.; Nakashima, N.; Yoshihara, K. *J. Chem. Soc., Faraday Trans. 2* **1987**, *83*, 1487.

(19) The rapid recombination of a singlet ion pair in DNA has been reported: Brun, A. Harriman, A. *J. Am. Chem. Soc.* **1991**, *113*, 8153.

(20) A related event is reported to occur on a ca. 10 $\mu$s time scale from the naphthalimide;[4a] however, it is not clear that this process initiates the observed DNA cleavage.

(21) Siders, P.; Marcus, R. *J. Am. Chem. Soc.* **1981**, *103*, 741.

(22) Jovanovic, S. V.; Simic, M. G. *J. Phys. Chem.* **1986**, *90*, 974.

(23) The value of $\lambda$ is uncertain. The dielectric constant of the "medium" is unknown, and $\lambda_i$ must be approximated. Assuming a quinoline-like medium, a "van der Waals radius" of the base and quinone equals 1.5 Å, and the distance between them equals 3.5 Å, $\lambda_s \cong 0.5$ eV. Since the quinone and the DNA base have $\sigma$-bonded frameworks that will not undergo significant distortion on oxidation, $\lambda_i$, = 0.1 eV.