

## Near-UV Induced Interstrand Cross-Links in Anthraquinone–DNA Duplexes

François Bergeron, Vandana K. Nair, and J. Richard Wagner\*

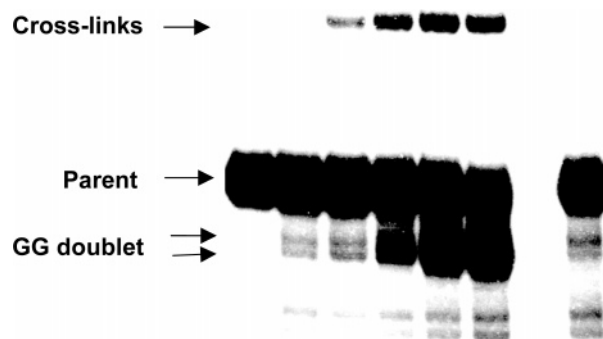
Group in the Radiation Sciences, Faculté de Médecine et des Sciences de la Santé, Université de Sherbrooke, Quebec, Canada J1H 5N4

Received June 29, 2006; E-mail: richard.wagner@usherbrooke.ca

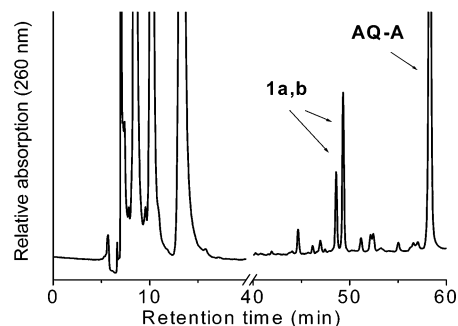
The ability of DNA to facilitate charge transfer has largely been examined using photosensitizers to generate base radical cations at specific sites in oligonucleotides.<sup>1</sup> In particular, anthraquinone (AQ) has been extensively used as a photosensitizer to study charge transfer in DNA.<sup>2</sup> Despite the complexity of model systems, the bulk of evidence supporting charge transfer in DNA is based on the formation of alkaline labile strand breaks at GG doublets and other sites of low oxidation potential.<sup>3</sup> In contrast, there is a serious lack of information about the distribution of damage in photochemical systems. Recently, we showed that 2-methyl-1,4-naphthoquinone (menadione) tethered DNA induced a variety of damage, including alkaline labile breaks, interstrand cross-links, and damage at all four DNA bases.<sup>4</sup> These and other examples<sup>5</sup> demonstrate the surprising reactivity of base radicals imbedded in DNA duplexes. Here, we report the formation of novel interstrand cross-links between AQ and T in DNA.

Anthraquinone (AQ) tethered oligonucleotides (ODN) (Chart 1) were prepared by conjugation of AQ succinyl-ester with ODN containing an amino linker (see Supporting Information). Near-UV photolysis of AQ–ODN duplexes led to the formation of novel products that migrated slower than the parent strand on denaturing gel electrophoresis (Figure 1). Hydrolysis of irradiated AQ–ODN duplexes with *P1* nuclease and alkaline phosphatase depicted the four early eluting nonmodified nucleosides of DNA followed by several late-eluting peaks (Figure 2). The peak at 58 min, which was the only late-eluting peak observed in nonirradiated samples, may be attributed to AQ linker attached to nonmodified A (AQ–A;  $m/z$  739 ( $M + 2Na^+$ )). Because AQ–A was observed upon digestion of both ODN1 and ODN3, we conclude that the phosphodiester bond on the 3'-side of AQ was not hydrolyzed by *P1* nuclease. This agrees with the sequence specificity of *P1* nuclease toward certain base damage.<sup>6</sup> In contrast, AQ–A was hydrolyzed to AQ linker ( $m/z$  404 ( $M + Na^+$ )) and A when treated with snake venom (SV) phosphodiesterase.

Two major photoproducts were observed by HPLC analysis of irradiated AQ–ODN duplexes (**1a,b**; Figure 2; Chart 2). These products gave the same molecular ions ( $m/z$  979 ( $M + 2Na^+$ )), which included the mass of AQ–A ( $m/z$  739) plus T ( $m/z$  242) minus two H-atoms. Upon treatment with SV phosphodiesterase, **1a,b** converted quantitatively to two fragments (**2a,b**) with a longer retention time on HPLC (~54 min) and a lighter molecular ion ( $m/z$  644 ( $M + Na^+$ ); Figures S1, S2). In addition, hydrolysis of **1a,b** led to the release of nonmodified dAdo as observed by HPLC. Thus, both cross-links (**1a,b** and **2a,b**) are composed of T and AQ moieties. Furthermore, formation of a bond between T and AQ did not significantly modify the structure of each moiety as inferred by the UV absorption of **1a,b** (260 nm/340 nm = 10) and **2a,b** (= 9) compared to AQ–A (= 11). The site of attachment of T was determined by examining the effect of replacing T for U in AQ–DNA duplexes (Chart 1, ODN3/5 and ODN3/6). Substitution of T on the 5'-side (ODN6) had no effect on the formation of **1a,b**.



**Figure 1.** Analysis of DNA damage by PAGE; near-UV photolysis of ODN1/2 duplex in O<sub>2</sub>. Left to right shows dark control, 15, 30, 60, 120, 180 min of photolysis, and G + A sequence ladder. All samples were treated with hot piperidine before analysis.



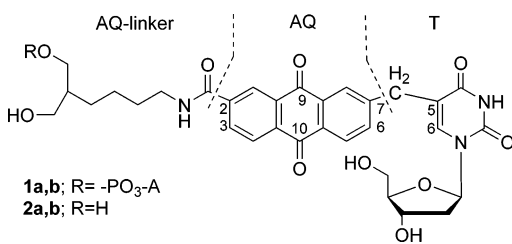
**Figure 2.** Analysis of DNA damage by HPLC. ODN3/4 duplex was irradiated in O<sub>2</sub> for 60 min and enzymatically digested to its components with *P1* nuclease and alkaline phosphatase.

### Chart 1. Sequences of AQ–ODN Duplexes

- 1: 5'-XATACCATACCATACCATACCATAGCG  
2: 3'-TATGGTATGGTATGGTATGGTATCGC

- 3: 5'-GCGATACCATACCATAXATACCATACCATAGCG  
4: 3'-CGCTATGGTATGGTATATATGGTATGGTATCGC  
5: 3'-..... UAT.....  
6: 3'-..... TAU.....  
X = AQ–amino linker (see structure, Chart 2).

In contrast, substitution of T on the 3'-side (ODN5) completely blocked **1a,b** with no indication of new cross-links involving U. Thus, the formation of **1a,b** takes place with T on the 3'-side and moreover involves the methyl group of T as the site of attachment between T and AQ. The possibility that T was attached to AQ via a position with an exchangeable proton, that is, the N3 position of T, was ruled out by MS analysis of deuterated compounds (Figures S1–S4). Last, the position of attachment of T to AQ was investigated by <sup>1</sup>H-NMR analysis. The spectrum of **2b** showed a similar splitting pattern of aromatic protons for the ring containing AQ-linker and for the ring containing T (Figure S5). This is

**Chart 2.** Proposed Structure of AQ–T Cross-Links (1,2)**Table 1.** Formation of Damage and Consumption of AQ–A<sup>a</sup>

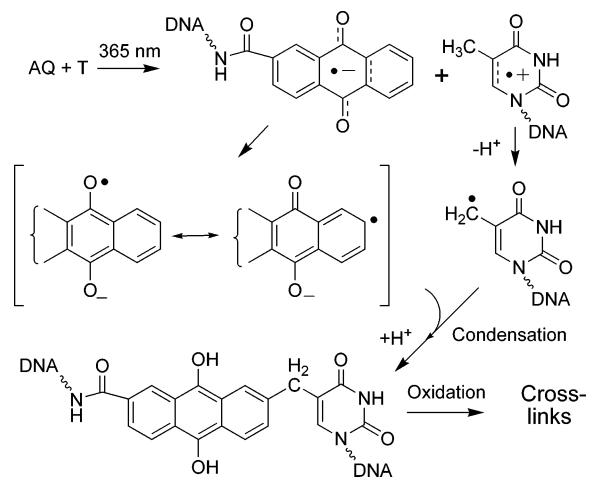
	CL(-P)	CL(+P)	GG(+P)	AQ–A	1a,b
ODN1/2-N <sub>2</sub>	2.8	1.4	n.d.	-3.6	1.3
ODN1/2-O <sub>2</sub>	2.5	0.9	22.8	-4.0	0.4
ODN3/4-N <sub>2</sub>	3.7	1.4	n.d.	-3.5	1.1
ODN3/4-O <sub>2</sub>	2.8	0.9	<0.5	-2.7	0.5

<sup>a</sup> Fraction of initial substrate per second ( $\times 10^{-3}$ ). Cross-links (CL) and strand breaks at GG doublets were estimated by PAGE (Figure 1). (-P), untreated; (+P), treated with piperidine for 30 min at 90 °C. AQ–A and CL was estimated by HPLC analysis (Figure 2). Rates were calculated by linear regression of the data ( $n = 5$ ,  $SD \leq 10\%$ ). n.d. = not detected.

consistent with attachment of T at either C6 or C7 of AQ but *not* with that of T at other positions (C1, C3, C4, C5, and C8). Thus, the structure of **2b** involves a cross-link between the methyl group of T and either the C6 position (not shown) or the C7 position of AQ (Chart 2).

Cross-links were observed for both end and centrally tethered AQ–ODN duplexes (Table 1). Upon treatment with hot piperidine, at least half of the cross-links collapsed to fragments with the same mobility as parent strands. The formation of piperidine resistant cross-links (CL(+P)) was comparable to the formation of **1a,b**, suggesting that CL(+P) consists mainly of **1a,b**. The formation of cross-links depended on the presence of O<sub>2</sub>. Although the effect of O<sub>2</sub> on total cross-links was small and variable, there was a clear inhibitory effect toward the formation of both CL(+P) and **1a,b** (Table 1). Interestingly, the formation of breaks at GG doublets was only observed in the presence of O<sub>2</sub> and only in the case of end-tethered AQ–ODN duplexes.

The mechanism of formation of cross-links likely involves initial charge transfer from T to excited AQ giving T radical cations and AQ radical anions (Scheme 1). In view of the relatively low quantum yield of damage ( $10^{-3}$ – $10^{-5}$ ), the majority of radical ions undergoes back transfer to T and AQ.<sup>2b,4</sup> At this point, we propose a deviation from the generally accepted mechanism involving the transport of electron holes and eventual damage at GG doublets. We propose that T radical cations undergo deprotonation to T methyl radicals. This is a major pathway for the radical cations of T and 5-methylcytosine.<sup>7</sup> Recently, similar reactions were implicated in the formation of breaks in AQ– and menadione–DNA duplexes.<sup>8</sup> Thus, it is reasonable to propose that T methyl radicals in AQ–DNA duplexes react with AQ semi-quinone radicals to give cross-links. The resulting hydroquinone then undergoes oxidation to the quinone as the stable product. The formation of isomers (a,b)

**Scheme 1.** Proposed Mechanism of Formation of Cross-Links

may be explained by condensation of AQ radicals at different radical sites (C6 or C7). Last, the inhibitory action of O<sub>2</sub> suggests that O<sub>2</sub> reacts with either AQ radicals (to give AQ) or with T methyl radicals (to give T peroxy radicals), diverting the pathway to other damage.

In summary, we report the formation of novel interstrand cross-links in AQ–ODN duplexes. This damage is comparable in yield to damage at GG doublets, depending on the site of attachment and the reaction conditions.

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**Supporting Information Available:** Experimental details; MALDI-TOF of ODN1–4; ESI-MS/MS of **2a,b**; and <sup>1</sup>H-NMR of **2b**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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