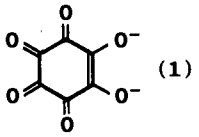
arises. What is really oxidized in the one-electron reduction of Q in the presence of OH^- in MeCN? Scrutiny of the detailed stoichiometry of the reaction revealed that a slight excess of OH^- is required to complete the reaction when a part of *p*-benzoquinone (ca. 10%) is found to be oxidized to rhodizonate dianion (**1**: λ_{max} = 483 nm),[15,16] which corresponds to the 10-electron-oxidized species of *p*-benzoquinone.[17] Under such conditions, the stoichiometry of the reaction is given by eq 1, where the one-electron reduction of 10 Q is accompanied by the 10-electron oxidation of one Q.

$$11Q + 12OH^- \longrightarrow 10Q^- + \underline{1} + 8H_2O \quad (1)$$



(**1**)

Rates of the formation of $Q^{\bullet-}$ obeyed pseudo-first-order kinetics under conditions wherein the quinone concentrations [Q] were maintained at more than a 10-fold excess of OH^-. The observed pseudo-first-order rate constants k_{obsd} of most quinones were independent of [Q]. In the case of 2,5-dimethyl-*p*-benzoquinone (Me_2Q) the k_{obsd} value increased with an increase in [Me_2Q] to reach a plateau value. Such a saturated dependence of k_{obsd} on the quinone concentration indicates that the formation of $Q^{\bullet-}$ may proceed via the initial formation of the OH^- adduct of Q, followed by the rate-determining unimolecular step.

There are three possible forms of the OH^- adduct of Q. The first is the OH^- adduct on the carbonyl carbon of Q (**2**); the second is that on the carbonyl oxygen (**3**); and the last one is that on the carbon next to the carbonyl group (**4**). The first one has so far been simply assumed to be formed.[1,18] Our MNDO calculation[19] revealed that OH^- adduct **4** is most stable, as shown in Scheme I.[20-22] Deprotonation of OH^- adduct **4** produces the corresponding hydroxyhydroquinone dianion (OHQ^{2-}), which is a stronger reductant than $Q^{\bullet-}$ and therefore can reduce two Q to two $Q^{\bullet-}$ to yield OHQ (Scheme I). Thus, substitution of one hydrogen of Q by OH, which corresponds to the two-electron oxidation of Q, results in the one-electron reduction of two Q to yield two $Q^{\bullet-}$. Consequently, successive substitutions by OH finally result in the formation of 10-electron-oxidized species **1**, accompanied by the one-electron reduction of 10 Q to yield $10Q^{\bullet-}$ (Scheme I). Such novel disproportionation of Q is responsible for the apparently quantitative formation of $Q^{\bullet-}$ (eq 1). The rate-determining step may be the deprotonation of **4**. In such a case, k_{obsd} may be independent of the quinone concentration when the formation constant of the OH^- adduct is large enough, in agreement with the experimental results.

Acknowledgment. This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture, Japan.

Supplementary Material Available: A table showing the yields of semiquinone radical anions derived from various quinones and a figure exhibiting relations of k_{obsd} vs [Q] (2 pages). Ordering information is given on any current masthead page.

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(14) No succinonitrile has been detected by the GLC analysis of the product mixture. The ^{13}C NMR spectrum of the product mixture was also measured by using a Bruker AM-600 spectrometer (600 MHz), indicating that CD_3CN remains unchanged by the reaction.

(15) (a) Patton, E.; West, R. *J. Phys. Chem.* **1970**, *74*, 2512. (b) Zhao, B.; Back, M. H. *Can. J. Chem.* **1990**, *69*, 528. (c) Endo, T.; Takada, T.; Okawara, M. *Tetrahedron Lett.* **1986**, *27*, 615.

(16) The yield of **1** was determined by comparing the electronic spectrum in a diluted deaerated aqueous solution (×10) of the product mixture with the characteristic spectrum of the authentic sample under the same conditions (λ_{max} = 483 nm, $\epsilon_{max} = 1.5 \times 10^4$ M^{-1} cm^{-1}).[15] The dilution with deaerated H_2O resulted in the disappearance of the semiquinone radical anion and thereby no interruption in determining the yield of **1**.

(17) It was confirmed that **1** is converted to rhodizonic acid (λ_{max} 360 nm) in the presence of $HClO_4$.[15]

(18) (a) Bishop, C. A.; Tong, L. K. J. *Tetrahedron Lett.* **1964**, 3043. (b) Bishop, C. A.; Tong, L. K. J. *J. Am. Chem. Soc.* **1965**, *87*, 501.

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(20) Essentially the same results were obtained for methyl-*p*-benzoquinone (ΔH_f = -109, -77, and -127 kcal mol^{-1} for the **2**, **3**, and **4** type adducts, respectively) and chloro-*p*-benzoquinone (ΔH_f = -115, -86, and -134 kcal mol^{-1} for the **2**, **3**, and **4** type adducts, respectively).

(21) This mechanism is analogous to that suggested for the one-electron reduction of methylviologen.[8a]

(22) This may be the reason why the semiquinone radical anions derived from tetrasubstituted *p*-benzoquinone derivatives are not formed efficiently compared with those that have no site for the OH^- addition.[11] In the case of *p*-chloranil, nucleophilic substitution by OH^- is known to occur to yield chloranilic acid: Hancock, J. W.; Morrell, C. E.; Rhum, D. *Tetrahedron Lett.* **1962**, 987.

Specific, High-Efficiency, Triple-Helix-Mediated Cross-Linking to Duplex DNA

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Received December 12, 1990

The concept of sequence-specific cross-linking of nucleic acids was first suggested over two decades ago.[1] Since that time, numerous demonstrations have appeared.[2] Most of these involve the targeting of a single-stranded DNA or RNA with a deoxyoligonucleotide (DON) bearing a reactive moiety. The concept of sequence-specific double-stranded DNA recognition via triple-helix formation has recently been demonstrated,[3] and several examples of cross-linking to duplex DNA have been reported.[4] We report the high-efficiency cross-linking of DONs containing the modified nucleoside N_4,N_4-ethano-5-methyldeoxycytidine (**1**, Figure 1) to the N_7 of a specific guanine (G) in a double-stranded DNA target.

The deoxycytidine analogue **1** has previously been incorporated into DONs and been shown to specifically cross-link to single-stranded DNA.[5] Triple-helix formation via Hoogsteen base pairing occurs with the third strand binding in the major groove of the duplex.[3a] Substitution of the third-strand C of a C^+:G:C triplet with **1** would allow for the placement of the electrophilic methylene of **1** near the nucleophilic N_7 or O_6 sites of the G in the duplex (Figure 1).

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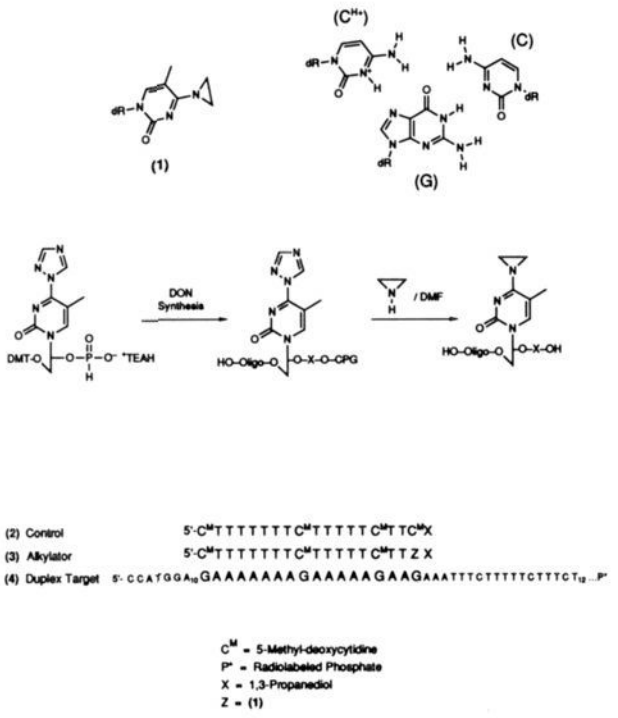

Figure 1. Structure of ethano-5-methyldeoxycytidine, C^+:G:C base triplet, and synthetic scheme for DONs and sequences.

The incorporation of **1** into DONs was accomplished by aziridine displacement of a precursor 4-triazolopyrimidine in a controlled pore glass (CPG) bound DON as previously described.[5] This scheme is shown in Figure 1. The use of an oxalyl ester 3′ linker to the solid support[6] and the 9-fluorenylmethyl carbamate (FMOC) protecting group on 5-methyldeoxycytosine[7] allowed for aziridine to be the reagent for the 3′ hydroxyl and exocyclic amino group deprotection steps and to simultaneously displace the triazole moiety to generate **3**. The DONs synthesized and the sequence of the duplex target are shown in Figure 1. **3** was characterized for the presence of **1** by treatment with ethylenediamine (EDA). The observed electrophoretic retardation upon EDA treatment was consistent with three-membered-ring opening with EDA.[8]

The results of the cross-linking experiment are shown in Figure 2A. DONs **2** and **3** were incubated with radiolabeled duplex target **4** under physiological salt conditions.[9] Pyrrolidine treatment resulted in the depurination of guanosines which have been N_7 alkylated and chain cleavage at the depurinated sites.[10] The reaction with **3** (lane 3) was *greater than 95%* after 16 h.[11] The G-specific dimethyl sulfate sequencing lane (lane 4) correlated the major product to the modified G residue consistent with

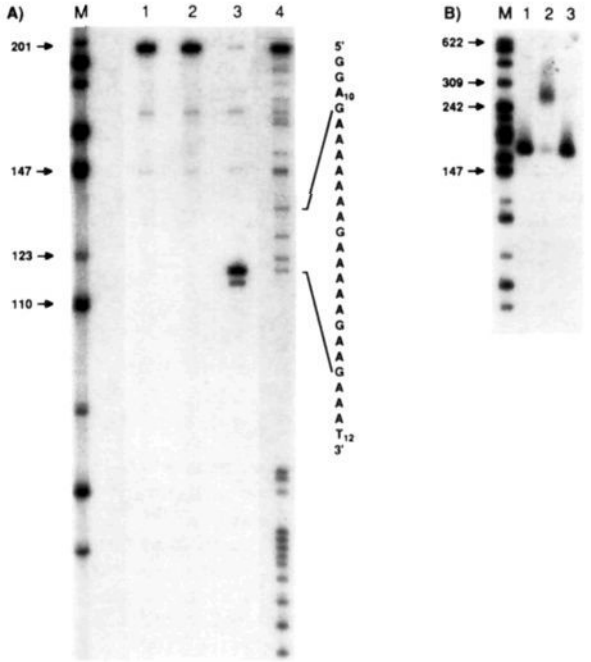

Figure 2. (A) Cross-linking and cleavage experiment: 6% denaturing PAGE performed with 7 M urea and 90 mM TBE at pH 8.3. Lane M: markers derived from digesting pBR322 and *Msp*I (band sizes are shown in base pairs). Lanes 1–4 were pyrrolidine treated for 10 min at 95 °C: lane 1, target **4** only; lane 2, control **2** and **4**; lane 3, **3** and **4**; lane 4, dimethyl sulfate (G only) sequencing ladder performed on target **4**. (B) Cross-linking experiment: PAGE performed as in A; Lane M, same as in A; lane 1, target **4** only; lane 2, target **3** and **4**; lane 3, **2** and **4**.

triple-helix formation of the DONs with the duplex target.

The existence of a stable cross-link was demonstrated by the experiment shown in Figure 2B. Lanes 1–3 resulted from reactions that were not treated with pyrrolidine and merely subjected to denaturing PAGE.[12] A N_7-alkylated G does not depurinate and result in chain cleavage under these conditions of analysis.[10] A band shift to a slower migrating species was observed for **3** and not the control **2**. This shift is consistent with a stable cross-link between the reactive DON and the duplex target. This demonstrates that the cross-link was largely stable under physiological conditions for at least 72 h.

Recent calculations suggest that the major and minor grooves of DNA are acidic environments that enhance the reactivity of acid-catalyzed electrophiles such as epoxides toward DNA.[13] The reaction of **1** with nucleophiles is an acid-catalyzed process[5] and could result in an acidic trigger upon triple-helix formation.

We have shown N_4,N_4-ethano-5-methyldeoxycytosine to be a highly efficient and sequence-specific cross-linking functionality for duplex DNA when substituted for 5-methyldeoxycytidine in a triple-helix-forming DON. The reaction does not require the addition of light or any external reagent and occurs under physiological conditions. These properties make it promising for the in vivo application of triple-helix-mediated, sequence-specific inhibition of RNA transcription and DNA replication.

Acknowledgment. We thank Sarah McCurdy and Terry Terhorst for DON synthesis, Dave Sweedler for the FMOC-5-methyldeoxycytidine synthesis, Professor Bob Letsinger, Dr. Bob Jones, and Dr. Jay Toole for helpful discussions, and Debbie Surdel and Susan Hubbard for manuscript preparation.

Supplementary Material Available: Electrophoretic gel of EDA-treated DONs (1 page). Ordering information is given on any current masthead page.

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(8) Conditions: EDA, 10% solution in H_2O, 10 min, 90 °C. These samples were compared to untreated controls after evaporation, denaturing polyacrylamide gel electrophoresis (PAGE), and visualization (data in supplementary material).

(9) Conditions: 140 mM KCl, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM spermine, 25 mM MOPS, pH 7.2 at 37 °C for 16 h. Target and DON concentrations were 1 nM and 10 μM, respectively. Lanes 1–3 resulted from reactions that were quenched with pyrrolidine, heated at 95 °C for 10 min, evaporated, and dissolved in formamide before denaturing PAGE and visualization by autoradiography.

(10) Maxam, A.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 560.

(11) Quantitation was determined by band excision and scintillation counting and includes the minor product of the reaction on a neighboring adenine. Intermediate time points showed an apparent half-life for this reaction of approximately 1 h (data not shown). The extent of reaction at 16 h was not detectably different when the target concentration was increased to 0.2 μM and the concentration of **3** decreased to 1 μM (data not shown). Detailed kinetic analysis will be complicated by the rate of triple-helix formation and is beyond the scope of this study.

(12) Conditions for the experiment are the same as listed in footnote 9 with the exception that the reaction mixtures were incubated at 37 °C for 72 h and directly subjected to PAGE without pyrrolidine, heat, or formamide.

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