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COMMUNICATIONS

Published on Web 08/31/2002

Long-Range Oxidative Damage to DNA: Protection of Guanines by a Nonspecifically Bound Disulfide

Sriram Kanvah and Gary B. Schuster*

School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

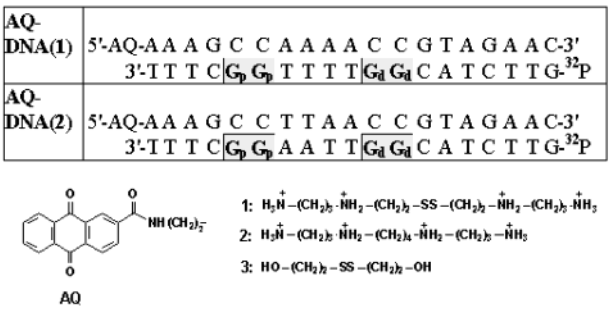
Received June 11, 2002

It is vital that organisms protect their DNA from oxidative reactions, because the resulting damage can lead to disease or death. One-electron oxidation of DNA by ionizing radiation, photosensitization, or natural metabolic processes results in the formation of a base radical cation ("hole") that migrates through duplex DNA and reacts with water primarily at the 5′-terminus of G_n sequences to form 8-oxo-7,8-dihydroguanine and other oxidation products.[1−6] Damage repair mechanisms have evolved as one strategy for protection of DNA.[7] An alternative strategy would be to steer radical cations away from critical coding regions of the genome. In this regard, it has been suggested by analogy with the protection of metals from corrosion by sacrificial anodes that G_n sequences in introns are positioned optimally to absorb holes and thereby protect the bases in adjacent exons from oxidative damage.[8,9] Similarly, it may be possible that a noncovalently bound sacrificial reagent will be able to protect DNA bases from oxidative damage. We considered compounds containing disulfide groups as likely candidates for this role because the oxidation potentials (E_{ox}) of aliphatic disulfides are below that of guanine,[10] which is the most easily oxidized base, and they are common cellular constituents. In particular, experiments reported here show that bis[2-(3-(aminopropyl)amino)ethyl]disulfide (**1**), which has been studied previously as a DNA radioprotectant,[11] stabilizes duplex DNA and suppresses the oxidative damage of guanines.

It has long been recognized that polycations such as spermine (**2**) at pH = 7 associate with and stabilize DNA oligonucleotides.[12−14] Early theoretical studies indicated that the most favorable binding site is the minor groove of $(A/T)_n$ sequences.[15] However, recent molecular dynamics simulations suggest that spermine binds to B-form DNA nonselectively by occupying sites along the backbone and bridging both the major and the minor grooves.[16] We examined the interaction of spermine with the duplex DNA oligonucleotides AQ-DNA(1) and AQ-DNA(2), see Chart 1, to assess its effect on long-range radical cation transport and to compare it with disulfides **1** and **3**.

The addition of spermine to solutions of either AQ-DNA(1, 2) causes the melting temperature (T_m) of the DNA to increase, which indicates that spermine associates with the DNA and stabilizes it. Similarly, addition of spermine disulfide **1** causes the T_m of AQ-DNA(1, 2) to increase, but the magnitude of that increase is less than that for spermine. In contrast, the addition of bis(2-hydroxyethyl)disulfide (**3**), which serves as a control compound for **1**, has no measurable effect on the T_m; these data are reported in Table 1. Spermine is known to facilitate structural change of DNA from B-form to A or Z;[17] however, we observe no meaningful changes to the circular dichroism spectra of AQ-DNA(1, 2) in the presence of **1**, **2**, or **3**. Clearly, polycations **1** and **2** bind to DNA, but disulfide

Chart 1



3, which is uncharged, does not, and, under the conditions examined, these compounds do not alter the structure of DNA significantly.

Irradiation of AQ-linked DNA samples at 350 nm injects a radical cation into the DNA that migrates through the duplex and reacts with water primarily at the 5′-G of GG steps.[3] Samples of the duplexes AQ-DNA(1, 2) containing **1**, **2**, or **3** (0−300 μM) were irradiated under identical conditions (20 min, 5 μM DNA, 10 mM sodium phosphate, pH = 7.0, ca. 30 °C) and then treated with piperidine at 90 °C for 30 min or with formamidopyrimidine-DNA glycosylase (Fpg), which cleaves DNA primarily at oxidized guanines.[7] The irradiated DNA samples were analyzed by electrophoresis on a denaturing polyacrylamide gel (PAGE), visualized by autoradiography, and quantified by phosphorimagery. The results for piperidine treatment of AQ-DNA(2) are shown in Figure 1; similar results are obtained after treatment with Fpg. The results obtained for AQ-DNA(1), which are presented in the Supporting Information, are comparable to those reported here for AQ-DNA-(2). The Supporting Information also contains descriptions of the experimental procedures.[18]

As expected, irradiation causes strand cleavage at the proximal (G_p) and distal (G_d) GG steps of these oligomers. The ratio of strand cleavage, (5′-G_p)/(5′-G_d), for AQ-DNA(1) is 8.3, and it is not measurably affected by addition of up to 300 μM of **2** or **3**. In contrast, addition of spermine disulfide **1** to the DNA solutions before irradiation inhibits the reaction at G_p and even more so at G_d. For example, in solutions that contain 300 μM of **1**, (5′-G_p)/(5′-G_d) increases to 35. A similar result is observed for AQ-DNA-(2) where the cleavage ratio (5′-G_p)/(5′-G_d) = 12 in the absence of additive or in the presence of **2** or **3**, but increases to 35 when the irradiated solution contains 300 μM of spermine disulfide **1**. Clearly, addition of disulfide **1** to these DNA solutions causes a significant reduction in the amount of strand cleavage detected at the GG steps.

Analysis of the binding of polyamine cations to DNA suggests that these interactions are governed primarily by electrostatic forces.[19] On this basis, and from the T_m experiments, we conclude that the association of disulfide **1** with DNA is similar to that of spermine and that it is bound primarily at sites along the backbone

* To whom correspondence should be addressed. E-mail: gary.schuster@cos.gatech.edu.

10.1021/ja027259x CCC: $22.00

Table 1. Effect of Addition of **1**, **2**, or **3** to AQ-DNA(1, 2) on T_m and Cleavage Ratio

concentration (μM)	AQ-DNA(1)						AQ-DNA(2)					
	1		**2**		**3**		**1**		**2**		**3**	
	T_m[a]	G_p/G_d[b]	T_m	G_p/G_d	T_m	G_p/G_d	T_m	G_p/G_d	T_m	G_p/G_d	T_m	G_p/G_d
0	43.6	8.4	44.0	8.3	43.6	8.0	44.0	13	44	12	43.6	11
2.5	43.6	8.5	44.5	8.3	43.6	7.7	44.5	13	44.5	13	44	13
5.0	45.6	8.2	45.0	8.1	43.6	8.7	45.0	14	45	12	44	12
50.0	47.6	25	58	8.3	44.6	8.2	52	24	58.5	11	44	13
300	57.0	35.6	63	8.5	44.6	8.5	58	35	63	12	44.6	12

[a] The melting temperature of the DNA duplex determined by optical spectroscopy. [b] The ratio of cleavage at the 5′-guanines proximal (G_p) and distal (G_d) determined by phosphorimagery.

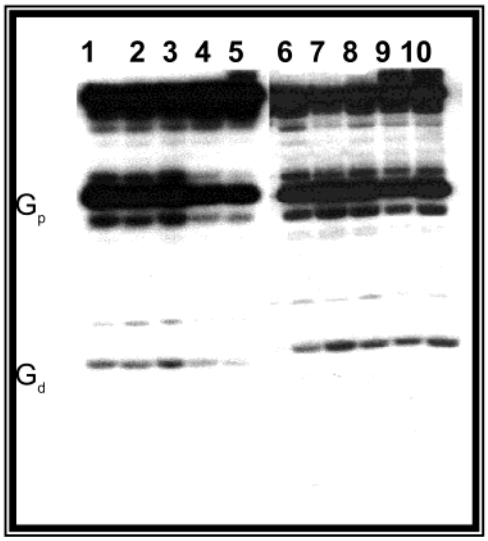


Figure 1. Autoradiograms from the irradiation of AQ-DNA(2). Lanes 1−5 (0, 2.5, 5.0, 50, and 300 μM, respectively) correspond to the increased concentrations of spermine disulfide (**1**), and lanes 6−10 (0, 2.5, 5.0, 50, and 300 μM, respectively) correspond to spermine hydrochloride (**2**). All of the samples were irradiated for 20 min using 8 × 350 nm Rayonet lamps and were worked up by treatment with 1 M piperidine.

and in the major and minor grooves. Because disulfide **3**, which does not bind to DNA, has no effect on the radical cation reaction at the guanines, we conclude that only disulfide **1** that is bound protects DNA from damage. Evidently, transfer of an electron from the bound disulfide group to a base radical cation competes effectively with the migration of the charge and its reaction with water at GG steps. The efficiency or orientation of binding appears to depend only weakly on base sequence because AQ-DNA(1) and AQ-DNA(2) give very similar results.

Disulfide **1** has not been optimized for binding to DNA or for the neutralization of base radical cations; nonetheless, it is effective. It seems likely that evolutionary pressure for the preservation of genomic integrity would generate disulfide-containing compounds optimized to bind to DNA that reduce radical cations and re-form the undamaged base.

Acknowledgment. We thank the National Cancer Institute, Bethesda, MD, for kindly providing spermine disulfide (**1**) and the NSF for financial support.

Supporting Information Available: A brief description of experimental details, autoradiograms for AQ-DNA(1), relative efficiencies of photocleavage of AQ-DNA(1) and AQ-DNA(2) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA027259X