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Photochemical cleavage of single- and double-stranded oligonucleotides by 3-(*p*-tolylamino)-1,5-azulenequinone

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Abstract—Irradiation, with 350 nm UV light, of specially designed and synthesized single- and double-stranded oligodeoxyribonucleotides in the presence of 3-(*p*-tolylamino)-1,5-azulenequinone produced fragments resulting from the cleavage at the deoxyguanosine residue only. The cleaving efficiency was greater for a single strand than a double helix. The efficiency was increased for a less stable double helix, or while the deoxyguanosine residue therein was located at a bulge, at a hairpin loop, or towards the end of the helix. © 2001 Elsevier Science Ltd. All rights reserved.

Most quinones are benzoquinones or polybenzenoid hydrocarbons.[1] Azulenequinones are non-benzenoid; some of these cytotoxic compounds have been tested against P-388 leukemia in mice.[1] Upon activation by UV light,[2] many azulenequinones exhibit DNA-cleaving activity under controlled conditions.[3] 3-(*p*-Tolylamino)-1,5-azulenequinone **1** represents a prominent example; it exhibits great potency and site-specificity for the deoxyguanosine residue.[3,4] We planned to study the interactions between this organic compound and various oligodeoxyribonucleotides. The results would provide valuable information about the influences resulting from secondary structure on the reaction between deoxyguanosine and azulenequinones during DNA cleavage.

A series of duplexes with different features were designed as listed in Table 1. Each duplex in entries 1, 2 and 4 contains 15 nucleotidyl units, among which there are 14 adenine:thymine (A:T) base pairs. The S1 and S2 oligodeoxyribonucleotides possess only one guanine (G) nucleoside, which is located either in the middle (i.e. S1) or near the 3′-end (i.e. S2). Furthermore, we designed their complementary sequences in the C-series by forming the Watson–Crick base pairs. In entry 3, A15 contains 15-adenyl units, whereas its

Table 1. The sequences of oligodeoxyribonucleotides containing a deoxyguanosine, their complementary oligomers, and the melting temperature (T_m) of the corresponding duplexes (20 mM) in a tris buffer solution with pH 7.0 containing 0.15 M NaCl and 20 mM $MgCl_2$

Entry	Oligonucleotide	Complementary oligomer	Description	T_m (°C)
1	5′-T_7**G**T_7 (S1)	5′-A_7**C**A_7 (C1)	Watson–Crick	48
2	5′-T_{13}**GT** (S2)	5′-**AC**A_{13} (C2)	Watson–Crick	48
3	5′-T_7**G**T_8 (S1T)	5′-A_{15} (A15)	Bulge	39
4	5′-T_7**G**T_7 (S1)	5′-A_{15} (A15)	One mismatch	39
5	5′-$(AT)_4$**TGT**$(AT)_4$ (H3)		Hairpin	51
6	5′-$(AT)_4$**TTGTT**$(AT)_4$ (H5)		Hairpin	49

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complementary sequence S1T contains one more thymidylate unit (T) in comparison with S1. Thus, these two oligodeoxyribonucleotides were designed to form a duplex with a bulge G. Furthermore, we considered the possibility for the formation of a duplex by interaction between A15 and S1 as shown in entry 4. Accordingly, it would generate one G:A mismatch. In addition, we designed two hairpins H3 and H5 (see entries 5 and 6 in Table 1), which possess an $[(AT)_4]_2$ stem and one deoxyguanosine nucleotide in the middle of the loop of oligodeoxyribonucleotides. They differ from each other by having a loop size of three (i.e. TGT) nucleotidyl residues in H3 and five (i.e. TTGTT) in H5.

We prepared four double-stranded helices by annealing the oligodeoxyribonucleotides with their complementary strands listed in Table 1, entries 1–4. Their melting temperatures (T_m) were measured, which indicate their relative stability. The T_m values for duplex S1T–A15 (entry 3) with a bulge or duplex S1–A15 (entry 4) with a mismatch were lower than those in entries 1 and 2. The oligodeoxyribonucleotides H3 and H5 synthesized melted at 51 and 49°C, respectively (see Table 1). These two oligomers did not show T_m changes upon variations in concentration. These results indicate the formation of hairpin duplexes.

Cleavage of single- and double-stranded oligodeoxyribonucleotides by azulenequinone **1** upon photolysis with 350 nm UV light generated patterns shown on autoradiograms of a 20% polyacrylamide/8.0 M urea gel (see Fig. 1A–C). Our results indicate that **1** can cleave S1 and S1–C1 at the deoxyguanosine unit (see Fig. 1A). The ratio of the intensities was about 1.9:1 for the two lower bands in Lanes 3 and 4. Thus, the efficiency of **1** in cleaving single-stranded oligomers was about twice that of the double-stranded helices.[5–7]

For the duplex S2–C2 with a guanine base towards the end (see Table 1), the results of their cleavage by agent **1** are shown in Lane 6 of Fig. 1B, in which a band appeared at the position for a 13-nucleotidyl unit as indicated by an arrow. The efficiency of agent **1** to cleave duplex S2–C2 at the deoxyguanosine residue was much higher (188%) than that for the duplex S1–C1 (100%, see Lane 3 of Fig. 1B). For duplexes S1T–A15 and S1–A15 with lesions, we found that agent **1** also cleaved the S1T and S1 strands in these duplexes at the deoxyguanosine site, exclusively (see Lanes 4 and 5). In comparison with the Watson–Crick duplex S1–C1, the efficiency of agent **1** in cleaving duplexes with lesions was 1.7–1.9 times higher.

For duplexes H3 and H5 with a hairpin loop (see Table 1), the results from their UV-initiated cleavages are shown in Fig. 1C, Lanes 3 and 6, respectively. The intensity of the fragment in Lane 6 resulting from the cleaved fragment of H5 was slightly stronger (110 versus 100%) than that in Lane 3 resulting from H3. Because H3 was two units shorter than H5 in sequence, H3 (Lanes 1–3) migrated faster than H5 (Lanes 4–6) in polyacrylamide gel during electrophoresis. Consequently, the fragment of H3 with 9-nucleotidyl units in sequence also migrated faster than that of H5 with 10-nucleotidyl units.[8–11]

We carried out graphic molecular modeling by assigning oligodeoxyribonucleotides as the host, which was accompanied by agent **1** as the guest. Our results show that the agent **1** can bind the guanine residue in the duplex S1–C1 as shown in Scheme 1A. In this complex, two hydrogen bonds linked the agent **1** to the duplex S1–C1 through the minor grove with bond distances 1.99 Å for the N–H···N= and 1.95 Å for the N–

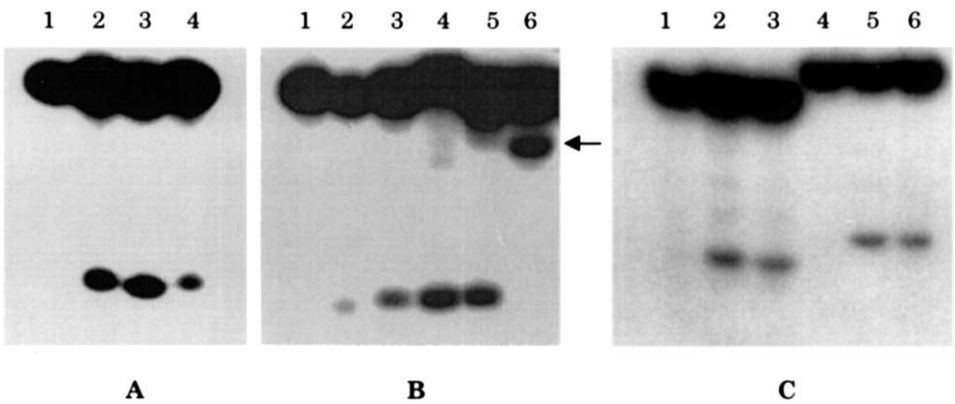
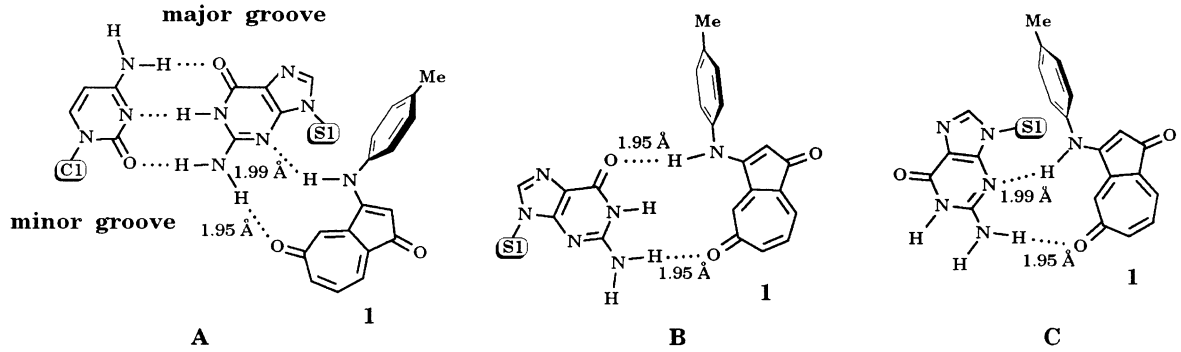


Figure 1. Autoradiograms of polyacrylamide gel electrophoresis. (A) Single- versus double-stranded oligodeoxyribonucleotides: The S1 is shown at the top of each lane. The cleaved fragments containing a sequence of 7-nucleotidyl units are presented at the lower band. Lane 1: S1 in the absence of agent **1**. Lane 2: Cleavage of S1 by the Maxam–Gilbert G reaction. Lanes 3 and 4: Cleavages of S1 and S1–C1, respectively, by **1** with UV light. (B) Duplexes with a bulge, a mismatched base pair, or a guanine base towards the end: The identities of the bands are the same except that in Lane 6, which contains a larger fragment with 13-nucleotidyl units as indicated by an arrow. Lane 1: Duplex S1–C1 in the absence of **1**. Lane 2: Cleavage of S1 in duplex S1–C1 by the Maxam–Gilbert G reaction. Lane 3: Cleavage of S1 in duplex S1–C1 by **1** with UV light. Lane 4: Cleavage of S1T in bulge duplex S1T–A15 by **1** with UV light. Lane 5: Cleavage of S1 in S1–A15 containing one G:A mis-pair by **1** with UV light. Lane 6: Cleavage of S2 in duplex S2–C2 by **1** with UV light. (C) Hairpin duplexes: Lanes 1 and 4: H3 and H5, respectively, in the absence of **1**. Lanes 2 and 5: Cleavage of H3 and H5, respectively, by the Maxam–Gilbert G reaction. Lanes 3 and 6: Cleavage of H3 and H5 by **1** with UV light.



Scheme 1. Structures to indicate hydrogen bonds between the agent **1** and the guanine residues in oligodeoxyribonucleotides S1–C1 (see A) and S1 (see B and C), respectively.

H···O=.[12] Because of the lack of a second acidic proton to form Hoogsteen hydrogen bonding with the guanine residue, agent **1** could not bind efficiently to oligomer S1 through the major groove. On the other hand, the complex involving the agent **1** binding with the single-stranded S1 from the 'front' site of the guanine residue (see Scheme 1B) possessed two N–H···O= hydrogen bonds, 1.95 Å in length. Agent **1** could also bind with oligomer S1 from the 'rear' site by an N–H···N= bond and an N–H···O= hydrogen bond as shown in Scheme 1C.

Our computational results indicate that azulenequinone **1** could bind the guanine residue in single-stranded oligodeoxyribonucleotides from two possible sites. The 'Watson–Crick type' base pairs were generated in the resultant stable guest–host complexes, yet it could bind to double-stranded oligodeoxyribonucleotides by two hydrogen bonds from only one site. These phenomena can be used to account for the experimental results that the efficiency of azulenequinone **1** was double for cleaving the single rather than the double-helical oligodeoxyribonucleotides.

Acknowledgements

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References

1. Scott, T. L. In *The Chemistry of Quinonoid Compounds*; Patai, S.; Rappoport, Z., Eds.; Wiley: New York, 1988; Vol. 2, Chapter 24.
2. For a recent review, see: Armitage, B. *Chem. Rev.* **1998**, *98*, 1171–1200.
3. Nozoe, T.; Lin, C. C.; Tsay, S.-C.; Yu, S.-F.; Lin, L. C.; Yang, P. W.; Hwu, J. R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 975–978.
4. For recent, related reports, see: (a) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109–1151; (b) Fernandez–Saiz, M.; Henderson, P. T.; Wilson, W. D.; Schuster, G. B. *Photochem. Photobiol.* **1999**, *70*, 847–852; (c) Stevenson, C.; Davies, R. J. H. *Chem. Res. Toxicol.* **1999**, *12*, 38–45.
5. Burrows, C. J.; Rokita, S. E. *Acc. Chem. Res.* **1994**, *27*, 295–301.
6. Chen, X.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1992**, *114*, 322–325.
7. Muller, J. G.; Chen, X.; Dadiz, A. C.; Rokita, S. E.; Burrows, C. J. *Pure Appl. Chem.* **1993**, *65*, 545–550.
8. Henderson, P. T.; Armitage, B.; Schuster, G. B. *Biochemistry* **1998**, *37*, 2991–3000.
9. Ciftan, S. A.; Thorp, H. H. *J. Am. Chem. Soc.* **1998**, *120*, 9995–10000.
10. Carter, P. J.; Cheng, C.-C.; Thorp, H. H. *Inorg. Chem.* **1996**, *35*, 3348–3354.
11. Carter, P. J.; Breiner, K. M.; Thorp, H. H. *Biochemistry* **1998**, *37*, 13736–13743.
12. Saenger, W. In *Principles of Nucleic Acid Structure*; Cantor, C. R., Ed.; Springer-Verlag: New York, 1984; p. 118.