Affinity Cross-Linking of Duplex DNA by a Pyrrole–Oligopeptide Conjugate

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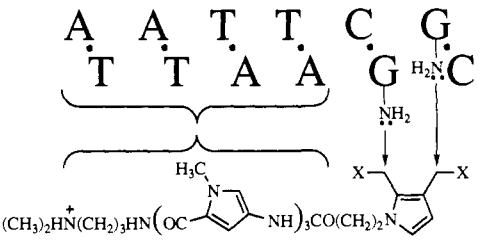
DNA–DNA cross-linking is widely held to be the primary cytotoxic mechanism of several clinically useful antitumor substances (e.g., cisplatin and mitomycin C).[1] The selective toxicity toward tumor cells must derive from mechanisms other than DNA sequence recognition alone, because the short sequences recognized[2] (two to four nucleotides) presumably occur frequently in all genomes. Increasing this selectivity by targeting lower frequency sites[3] requires the development of affinity[4] cross-linking agents which selectively cross-link longer DNA sequences.[5,6]

We document herein the synthesis and reactions with DNA of the affinity cross-linking agent **1**, composed of the minor groove sequence-recognizing elements of the oligopeptide distamycin[7,8] joined to a pyrrole-derived, minor groove cross-linking agent.[9,10] This conjugate is a highly efficient DNA–DNA cross-linking agent in both a linearized plasmid and synthetic DNA. We demonstrate that **1** efficiently cross-links deoxyguanosyl residues in synthetic duplex DNA at the sequences 5′-d(CGAATT) (interstrand) and 5′-d(GGAATT) (intrastrand) (see Figure 1).

The pyrrole–oligopeptide conjugate **1** was synthesized as shown in Figure 2.[11,12] The interstrand cross-linking activity of **1** was determined in a linearized plasmid (Figure 3) containing 27 occurrences of four sequential A or T residues, potential binding sites for the oligopeptide, adjacent to the sequence 5′-d(CG), the preferred site for interstrand cross-linking by related pyrroles.[9] Despite a denaturation step, this assay returns interstrand cross-linked DNA as a duplex, due to rapid renaturation initiated at

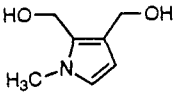


1 X = OH

2 X = N^2- (2′-deoxyguanosyl)

Figure 1. The pyrrole–oligopeptide conjugate **1** cross-links deoxyguanosine residues at the illustrated sequence.

site(s) of cross-linking.[13] Conjugate **1** is a highly efficient interstrand cross-linking agent at concentrations as low as 10 nM (**1**:bp ratio of 0.03). The oligopeptide is important: 2,3-bis-(hydroxymethyl)-1-methylpyrrole (**5**) is 1000-fold less active.



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Reactions of **1** were studied at higher resolution in several synthetic DNAs (Figure 4). Of these, only the DNA containing the central sequence N_8 = CGAATTCG was efficiently interstrand cross-linked, and in a remarkable 67% yield.[14] This DNA joins a distamycin binding site (AATT) to the consensus sequence interstrand cross-linked by the pyrrole family (CG).[9] The importance of the presence of *both* of these sites is underscored by the failure of DNAs lacking *either* to form interstrand cross-links. Several of the DNAs returned products intermediate in mobility between single strands and interstrand cross-links which we assign as conjugates of **1** with a single strand of DNA. The DNA containing the sequence N_8 = GGAATTCC formed this product especially efficiently; this product (see below) is an intrastrand cross-link.[15]

The covalent structures of the major lesions follow from several observations. The major products with DNAs N_8 = CGAATTCG and GGAATTCC (isolated from DPAGE) were pyrrole-derived interstrand and intrastrand cross-links, respectively, with deoxyguanosine residues as the site of alkylation, on the basis of (a) the presence in these samples of a UV chromophore unique to the oligopeptide; (b) the failure to interstrand cross-link of the DNAs N_8 = CIAATTCG and CGAATTCI (see Figure 4), which both lack one N2 amino at each of what would otherwise be the two CG sites; (c) HPLC quantitation of the released deoxyribonucleotides following enzymatic digestion (DNase I, DNase II, snake venom phosphodiesterase, and alkaline phosphatase; HPLC analysis; 3:2:4:4 ratio of dC:dG:dT:dA from the interstrand linkage; 3:1:4:4 from the intrastrand); and (d) recovery of the *same lesion* from these two hydrolysates (coelution on HPLC, identical UV and mass spectra) with a molecular weight (electrospray MS) of the sum of the masses of **1** and two dG residues less two water molecules ($M + H^+$, m/e 1148.4). The observation that efficient cross-linking requires both N2 amino groups of deoxyguanosine residues at the sequence CG strongly

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(2) For example: Hopkins, P. B.; et al. *Tetrahedron* **1991**, *47*, 2475.
(3) Dervan, P. B. *Science* **1986**, *232*, 464.
(4) The term "affinity" refers to targeting of an event to a site by virtue of the noncovalent affinity of the attacking agent for the target.
(5) For examples of designed DNA interstrand cross-linking agents which are a tethered pair of DNA monoalkylating agents, see the following. Anthramycin/tomaymycin: (a) Farmer, J. D.; Rudnicki, S. M., Jr.; Suggs, J. W. *Tetrahedron Lett.* **1988**, *29*, 5105. (b) Farmer, J. D., Jr.; Gustafson, G. R.; Conti, A.; Zimmt, M. B.; Suggs, J. W. *Nucleic Acids Res.* **1991**, *19*, 899. (c) Bose, D. S.; et al. *J. Am. Chem. Soc.* **1992**, *114*, 4939. CC-1065: (d) Want, J.-J.; Hill, C.; Hurley, L. H. *J. Med. Chem.* **1992**, *35*, 2995. (e) Mitchell, M. A.; Johnson, P. D.; Williams, M. G.; Aristoff, P. A. *J. Am. Chem. Soc.* **1989**, *111*, 6428. (f) Mitchell, M. A.; et al. *J. Am. Chem. Soc.* **1991**, *113*, 8994. (g) Sun, D.; Hurley, L. H. *J. Am. Chem. Soc.*, in press. For a rationally designed, nondimeric cross-linking agent based upon duocarmycin A, see: Boger, D. L.; Palanki, M. S. S. *J. Am. Chem. Soc.* **1992**, *114*, 9318.
(6) Takasugi, M.; Helene, C.; et al. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 5602.
(7) (a) Coll, M.; Frederick, C. A.; Wang, A. H.-J.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8385. (b) Klevit, R. E.; Wemmer, D. E.; Reid, B. R. *Biochemistry* **1986**, *25*, 3296. (c) Pelton, J. G.; Wemmer, D. E. *J. Am. Chem. Soc.* **1990**, *112*, 1393.
(8) (a) Schultz, P. G.; Dervan, P. B. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1133. (b) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1985**, *107*, 8266.
(9) Woo, J.; Sigurdsson, S. Th.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 3407.
(10) The efficiency of interstrand cross-linking agents which function via the major groove is not greatly enhanced by conjugation to distamycin: (a) Church, K. V.; Wurdeman, R. L.; Zhang, Y.; Chen, F.-X.; Gold, B. *Biochemistry* **1990**, *29*, 6827. (b) Montecucco, A.; Fontana, M.; Focher, F.; Lestingi, M.; Spadari, S.; Ciarrocchi, G. *Nucleic Acids Res.* **1991**, *19*, 1067. (c) Broggini, M.; et al. *Cancer Res.* **1991**, *51*, 199. (d) Lee, M.; Rhodes, A. L.; Wyatt, M. D.; D'Incalci, M.; Forrow, S.; Hartley, J. A. *J. Med. Chem.* **1993**, *36*, 863.
(11) Key intermediates were characterized by ^1H NMR. **1** afforded satisfactory ^1H NMR, UV, and HRMS.
(12) (a) Huisgen, R.; Laschtuvka, E. *Chem. Ber.* **1960**, *93*, 65. (b) Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *27*, 1945.
(13) Hartley, J. A.; Berardini, M. D.; Souhami, R. L. *Anal. Biochem.* **1991**, *193*, 131.
(14) On a per nucleotide (rather than per duplex) basis, many more interstrand cross-links must be formed in short oligonucleotides than in a plasmid to achieve comparable yields of interstrand cross-linked duplex.
(15) An analogous lesion has been observed for mitomycin C-treated DNA: Bizanek, R.; McGuiness, B. F.; Nakanishi, K.; Tomasz, M. *Biochemistry* **1992**, *32*, 3306.

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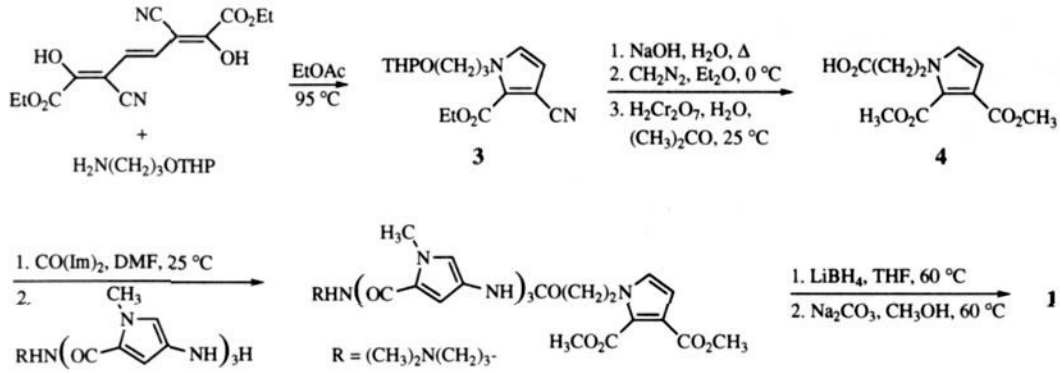

Figure 2. Synthesis of pyrrole–oligopeptide conjugate **1**.[11]

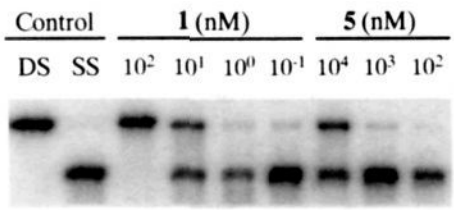

Figure 3. Interstrand cross-linking of 5′-^{32}P-radiolabeled, *Eco*RI-linearized pBluescript II KS (−) plasmid by pyrrole–oligopeptide conjugate **1** and **5**. Agents were incubated at the concentrations of **1** or **5** shown with 22 ng of plasmid in a total volume of 100 μL at 25 °C for 12 h in 50 mM HOAc/NaOAc (pH 5.0) and analyzed on a 0.8% agarose gel.[13]

indicates that N2 is the site of attachment on DNA. This lesion is likely **2**.

The pyrrole–oligopeptide conjugate **1** is thus shown to cross-link DNA efficiently at sites bearing the distamycin binding sequence adjacent to a pyrrole cross-linking sequence. The interstrand cross-linking reactions of **1** are, in many important respects, different from those of the most closely related analogs, the psoralen–oligonucleotide conjugates.[6] The latter recognize the *major* groove, presumably cross-linking *thymidine* residues at the sequence 5′-d(*TA*) by a *photochemical* reaction on the *interior* of the helix (intercalation). In contrast, the conjugate **1** recognizes the *minor* groove, cross-linking *deoxyguanosine* residues at 5′-d(*CG*) by a *substitution* reaction on the *exterior* of the helix. The method described herein, supplemented with the eventual development of agents which recognize other sequences in the minor groove, has the potential of affording a general approach to the sequence-controlled delivery of interstrand cross-linking agents to DNA.

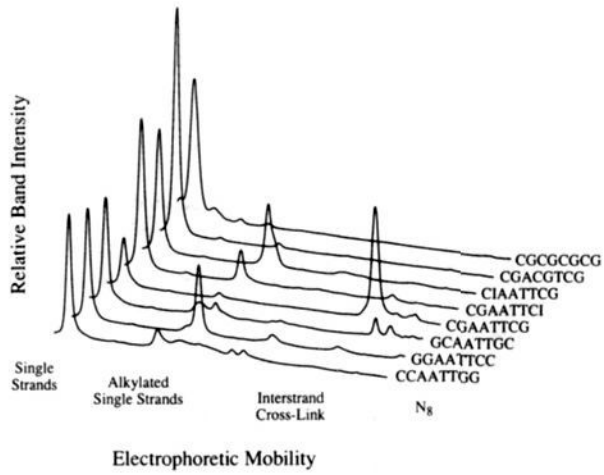

Figure 4. Reaction of conjugate **1** with 5′-^{32}P-radiolabeled, synthetic DNA 5′-d(GATN$_8$ATC)$_2$, N$_8$ as shown. Reaction conditions: DNA (0.2 OD, 8 μM in duplex), **1** (40 μM) in 50 mM HOAc/NaOAc (pH 5.0), 100 mM NaCl, 5 mM $MgCl_2$, 25 °C, for 13 h; 25% DPAGE was followed by phosphorimager analysis. Single-strand bands were aligned and lane integrals were normalized to facilitate comparison.

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Note Added in Proof: While this work was in review, Kutyavin et al. (*J. Am. Chem. Soc.* **1993**, *115*, 9303) reported efficient, interstrand cross-linking DNA sequences controlled by chlorambucil-modified ODNs which function by a triplex mechanism.