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

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Received September 20, 1993

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 recognized² (two to four nucleotides) presumably occur frequently in all genomes. Increasing this selectivity by targeting lower frequency sites3 requires the development of affinity4 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 crosslinked DNA as a duplex, due to rapid renaturation initiated at

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(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,
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(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. ; 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.

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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.



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 crosslinks. 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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⁽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.

CGCGCGCG

CGACGTCG

CIAATTCG CGAATTCI

CGAATTCG



Figure 3. Interstrand cross-linking of 5'-32P-radiolabeled, EcoRIlinearized 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 crosslink 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.



Acknowledgment. We thank the NIH (GM45804 and AG00417) for financial support and Professor T. Davis, Mr. M. J. Mosher, and Mr. B. H. Chang for the generous gift of linearized plasmid.

and lane integrals were normalized to facilitate comparison.

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.