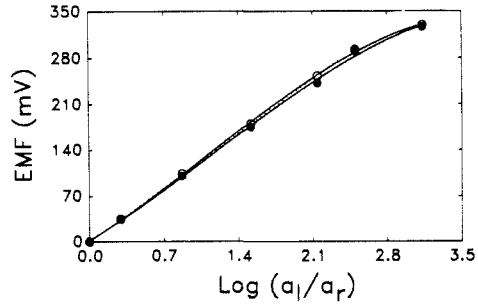

Figure 3. Plot of cell potential (see eq 1) vs log of ratio of the activities of Na^+ on either side of the membrane. Concentration of NaCl on the left side was 2 mM; concentration on the right side was varied between 2 mM and 5 M. ●: Nafion membrane. ○: ultrathin-film composite membrane; film thickness was ca. 1.5 μm.

were also 0.2 M in Bu_4NClO_4, which served as the supporting electrolyte.[7]

The electropolymerization cell contained the Au/Anopore working electrode, an Ag wire quasi-reference, and a Pt foil counter electrode. The polymerization solution was vigorously degassed with purified Ar. Polymerization was initiated by scanning the working electrode potential (200 mV s^{-1}) once from 0 to −2.75 V and back.[8] The voltammetric wave consisted of a single cathodic peak, with no anodic return wave. The poly(DVB/EVB) film which had formed across the membrane surface was rinsed with copious quantities of acetone and air-dried.

Figure 2 shows electron micrographs of cross sections of typical ultrathin-film composite membranes. The poly(DVB/EVB) films are uniformly coated across the Anopore support-membrane surfaces and have uniform film thicknesses.[9] Film thickness was approximated from such micrographs. Films with thicknesses ranging from 3.0 μm to 100 nm have been prepared via this method.[10] The chemical identity of the poly(DVB/EVB) films was established by using Fourier transform infrared spectroscopy.[11]

We have used gas-transport,[1b,c] voltammetric, and potentiometric[12] experiments to prove that the composite membranes are defect-free. Only the potentiometric measurements will be discussed here. Potentiometric data were obtained from sulfonated[13] versions of the poly(DVB/EVB)-based composites. The following cell was employed:

Ag|AgCl|(NaCl, 2 mM)|composite|(NaCl, 2 mM–5 M)|Ag|AgCl

The potential of this cell is given by[12]

$$E_{cell} = \left(2t + \frac{RT}{F}\right) \ln (a_r/a_l) \qquad (1)$$

where t_+ is the transference number for Na^+ in the membrane, and the a terms are the Na^+ activities. If the poly(DVB/EVB-SO_3^-) films are defect-free (and cation permselective), the composite membranes will show cation transference numbers of unity.

Figure 3 shows potentiometric data, plotted according to eq 1; the dotted curve was calculated with the assumption that t_+ = 1.0. The experimental data are for an 1100 equiv wt Nafion[14] and an ultrathin-film composite membrane. Nafion is one of the most cation permselective materials known.[14] This is reflected in the enormous concentration range over which the Nafion data fall on the t_+ = 1.0 line. Remarkably, the ultrathin-film composite membrane data are essentially identical with the Nafion data (Figure 3). These data indicate that the composite membranes are permselective and defect-free.

In closing, it is worth noting that the method described here should be applicable to any of the vast number of materials that can be synthesized electrochemically.[15] Other microporous support membranes could also be employed.

Acknowledgment. We acknowledge invaluable discussions with Drs. David Moll and Richard Fibiger of the Dow Chemical Company. This work was supported by the Air Force Office of Scientific Research, The Office of Naval Research, and the Dow Chemical Company.

(7) DMF was dried over CaH_2 prior to use. Bu_4NClO_4 was recrystallized from ethyl acetate/pentane and dried in vacuo.

(8) Potentials are reported vs Ag quasi-reference. Positive feedback was employed to correct for uncompensated solution resistance. Martin, C. R.; Rubinstein, I.; Bard, A. J. *J. Electroanal. Chem.* **1983**, *151*, 267.

(9) Low-quality films showed "rainbow-like" optical diffraction patterns. High-quality films, which were hundreds of nanometers thick, showed only one color.

(10) Film thickness was varied by varying the monomer concentration. The thinnest films were prepared on a version of Anopore which contains 200-nm-diameter pores which branch at one surface into 20-nm-diameter pores. A complete report is in progress.

(11) FTIR spectra showed peaks at 795 and 833 (meta-disubstituted phenyl ring) and 1512 and 1481 (para-disubstituted phenyl rings) cm^{-1}.

(12) Khedr, M. G. A.; Haleem, S. M. A.; Baraka, A. *J. Electroanal. Chem.* **1985**, *182*, 157.

(13) Espenscheid, M. W.; Martin, C. R. *J. Electroanal. Chem.* **1985**, *188*, 73.

(14) Nafion is a registered trademark of the E. I. du Pont Company. Hsu, W. Y.; Gierke, T. D. In *Perfluorinate Ionomer Membranes*; Eisenberg, A., Yeager, W. L., Eds.; ACS Symposium Series 180; American Chemical Society: Washington, DC, 1982; p 283.

(15) Yamazaki, N. *Adv. Polym. Sci.* **1969**, *6*, 377.

Mechlorethamine Cross-Links Deoxyguanosine Residues at 5′-GNC Sequences in Duplex DNA Fragments

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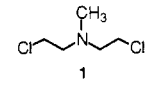
Mechlorethamine (**1**, *N*-methylbis(2-chloroethyl)amine) is the simplest of the nitrogen mustards. The ability of nitrogen mustards to cross-link the two strands of duplex DNA[1] is widely believed to account for their antitumor activity.[2] Isolation from hydrolysates of mechlorethamine-treated DNA of the conjugate[3] **2** first led to the reasonable suggestion that deoxyguanosine residues of adjacent base pairs are preferentially cross-linked,[3,4] a notion which persists.[5] We report here that mechlorethamine cross-links duplex DNA fragments through the distal deoxyguanosine residues at the sequence 5′-GNC (N=G or C) in preference to 5′-GC or 5′-CG sequences.



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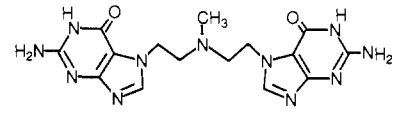

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Self-complementary DNAs I and II (Figure 1) were treated with **1**.[6] Analysis of the product mixtures by denaturing poly-

(1) Geiduschek, E. P. *Proc. Natl. Acad. Sci. U.S.A.* **1961**, *47*, 950.

(2) (a) Pratt, W. B.; Ruddon, R. W. *The Anticancer Drugs*; Oxford University Press: New York, 1979. (b) Gale, E. F.; Cundliffe, E.; Reynolds, P. E.; Richmond, M. H.; Waring, M. J. *The Molecular Basis of Antibiotic Action*; Wiley: New York, 1981.

(3) (a) Brookes, P.; Lawley, P. D. *Biochem. J.* **1961**, *80*, 496. (b) Brookes, P.; Lawley, P. D. *J. Chem. Soc.* **1961**, 3923.

(4) Kohn, K. W.; Spears, C. L.; Doty, P. *J. Mol. Biol.* **1966**, *19*, 266.

(5) (a) Mattes, W. B.; Hartley, J. A.; Kohn, K. W. *Nucleic Acids Res.* **1986**, *14*, 2971. (b) Kohn, K. W.; Hartley, J. A.; Mattes, W. B. *Nucleic Acids Res.* **1987**, *15*, 10531.

(6) DNA was synthesized (Applied Biosystems Model 380A) and was purified by denaturing PAGE (20%, 25:1 acrylamide/bisacrylamide, 40% urea) and radiolabeled.[7,8] DNAs (0.75 OD/100 μL 250 mM aqueous sodium cacodylate (pH 8)) were incubated 1 h at 37 °C with **1** (2.5 mM for I, II; 0.25 mM for III). Cross-linked DNA was isolated by ethanol precipitation, resuspension in 5 M urea, and 20% denaturing PAGE (19:1 acrylamide/bisacrylamide, 50% urea, 0.35 mm thick, 41 × 37 cm). Piperidine cleavage products were evaluated by PAGE, gel drying, and densitometry;[8] bands were assigned by reference to a Maxam–Gilbert G-lane.[10]

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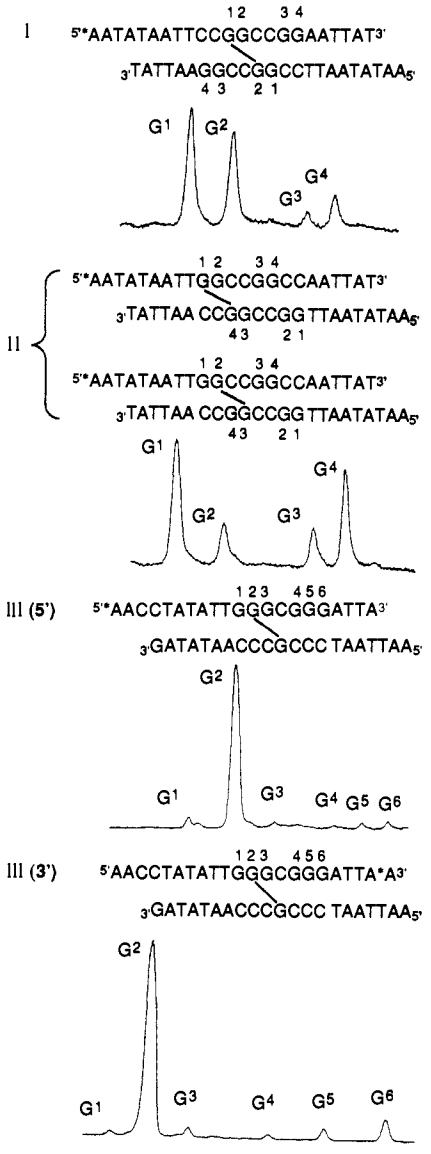

Figure 1. Densitometer scans of the electrophoretic analyses of the G-containing regions of I, II, and III. Autoradiograms of III were overexposed to allow detection of minor cleavage products. Lettering indicates residue cleaved. Radiolabeled phosphate (^{32}P) denoted by asterisk.

acrylamide gel electrophoresis (PAGE) revealed an ca. 1% yield of interstrand cross-linked DNA, identified by electrophoretic mobility comparison to mitomycin C cross-linked samples.[8] A single major cross-linked product was obtained from I; II afforded two major products of similar electrophoretic mobility.[9] The major cross-linked products, isolated by PAGE, were cleaved by heating with aqueous piperidine;[10] denaturing PAGE of the fragment mixtures indicated that virtually all radioactive products were of strand lengths corresponding to cleavage at G residues. Only traces of cross-linked or ca. single strand length DNA remained, suggesting N7 of deoxyguanosine as the near-exclusive site of alkylation. For I, predominant cleavage was at G^1 and G^2 in a 1:1 ratio. For II, cleavage occurred at all four G residues, in a ratio of roughly $3(G^1):1(G^2):1(G^3):3(G^4)$ (Figure 1).

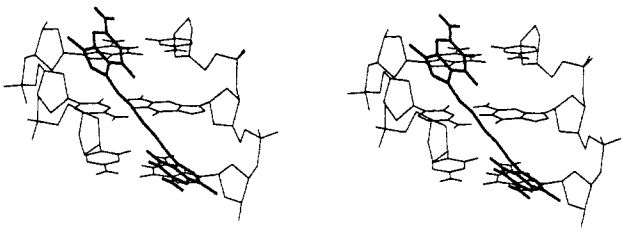

Figure 2. Computer-generated[13,14] stereoview of two guanines linked through N7 by a fully extended pentylene chain (bold) superimposed on distal residues of the DNA duplex sequence 5'-GCC in the B conformation.

These results are inconsistent with predominant cross-linking of 5'-CG sequences (absence of G^3 fragment from I) or predominantly 5'-GC sequences[4,5] (failure to account for G^1 fragment of II). The data are consistent with predominant cross-linking of distal deoxyguanosine residues at the duplex sequence 5'-GNC (N=G or C).[11] Thus, the single product resulting from linkage in I of G^1 and G^2 on opposite strands affords equal fragmentation at G^1 and G^2. The two distinct 5'-GNC-containing sequences in II provide two interstrand cross-linked products, linked G^1-to-G^4 and G^2-to-G^3 in an ca. 3:1 ratio, accounting for the observed fragment ratios.

This conclusion was confirmed in a third duplex, III (Figure 1). Sequential cross-linking, isolation of the major cross-linked product,[9] and cleavage of III in three radiolabeled forms (5'-labeled top strand, 5'-labeled bottom strand, 3'-labeled top strand) pinpointed the isolated, central G on one strand and G^2 on the other as the predominant linked residues (Figure 1). 5'-GNC was thus cross-linked in preference to 5'-GC, 5'-CG, and 5'-CGG.

Mechlorethamine cross-links distal deoxyguanosine residues at 5'-GNC in preference to 5'-GC, despite the latter possessing the minimal N7-to-N7 atom spacing in B-DNA.[12] Space-filling and computer models[13] reveal that considerable distortion would accompany an N7-to-N7 cross-link by a single mustard (e.g., **2**) at 5'-GNC. Figure 2 shows the best superposition on deoxyguanosine residues in B-DNA possessing the distal 5'-GNC relationship of two guanine residues linked through N7 by a fully extended pentylene tether.[14] One attractive hypothesis is that cross-linking sequence preference reflects sequence preferences in the initial monoalkylation of duplex DNA.[5] Preferential monoalkylation of the 5'-most deoxyguanosine residue in 5'-GG over 5'-GC followed by cross-link formation at the closest deoxyguanosine N7 atom on the opposing strand would then result in cross-linking at 5'-GGC in preference to 5'-GC. Alternatively, conversion of monoadducts to cross-links at 5'-GNC faster than at 5'-GC would equally account for these observations.[15]

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(8) Weidner, M. F.; Millard, J. T.; Hopkins, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 9270.

(9) Other, less abundant, cross-linked products are formed in these reactions as evidenced by a "smear" of radioactivity surrounding the major product band. Because the electrophoretic mobility of interstrand cross-linked DNA fragments is significantly impacted by the location of the cross-link within the fragment (Weidner, M.; Millard, J. T.; Hopkins, P. B. Manuscript in preparation), and we have isolated only the major product, it should not be concluded that 5'-GNC sequences represent the sole sites of cross-linking.

(10) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499.

(11) Interestingly, in the only structurally defined N7-to-N7 interstrand cross-linked duplex previously reported, the cross-link is at a 5'-GNC sequence (5'-GAC): Ojwang, J.; Grueneberg, D.; Loechler, E. L. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 556.

(12) Arnott, S.; Campbell-Smith, P.; Chandresekharan, P. In *CRC Handbook of Biochemistry*; CRC Boca Ratan, FL, 1976; Vol. 2, pp 411–422.

(13) Still, W. C. et al. *Macromodel Version 2.5*; Department of Chemistry, Columbia University, New York 10027.

(14) Generated by using the "flexible superimposition" option[13] varying only terminal torsional angles (bonds linking guanine N7 to terminal tether atoms).

(15) This same monoalkylation sequence preference is exhibited by other mustards (L-phenylalanine mustard, chlorambucil, phosphoramide mustard, etc.)[5], suggesting that preferred cross-linking at 5'-GGC by mustards may be general. The model in Figure 2 leads to the predictions that mustard cross-linking may be preferred at bent or flexible duplex DNA sequences and that the cross-linked DNAs themselves may possess a significantly reorganized, perhaps bent geometry.