Figure 2. Plot of molar ratio of III/II as a function of equilibration time for vesicles prepared from III plus 40 mol % of cholesterol (O) and a 1/1 mixture of I/II plus 40 mol % of cholesterol (•); the equilibration temperature was maintained at 60 ± 1 °C. In all cases, equal molar ratios of symmetrical dimers were produced (±5%). The percentage of cholesterol is based on phospholipid monomer content.

DNA Interstrand Cross-Linking by Reductively Activated FR900482 and FR66979

Jinsuk Woo, Snorri Th. Sigurdsson, and Paul B. Hopkins*

Department of Chemistry
University of Washington
Seattle, Washington 98195

Received November 9, 1992

Several hypotheses1-3 have been offered to account for the in vivo DNA alkylating activity of the antitumor antibiotic substances FR9004821 (1) and FR669792 (2). We report herein that the DNA interstrand cross-linking reactions of 1 and 2 share in common with mitomycin C3 greatly enhanced efficiency in a reducing medium and selectivity for dG-to-dG cross-linking at 5'-d(CG) with the participation of both dG exocyclic amino groups. We also provide preliminary evidence favoring mitosene-like structures 7 and 8 for these cross-links, analogous to lesion 9 previously derived from mitomycin C.6 These observations support the hypothesis of reductive activation of 1 and 2 to form mitosene-like, reactive intermediates (e.g., 4, 5)1 which are responsible for interstrand cross-linking.


1199

0002-7863/93/1515-1199$04.00/0 © 1993 American Chemical Society
The DNA duplexes [5'-d(TATAAN₆TTATA)₂] (Figure 1) admixed with FR900482 or FR66979 were treated with sodium dithionite, the reagent of choice for in vitro mitomycin C activation. The largest nucleotide sequence unique to the most efficiently interstrand cross-linked DNAs (N₆ = TACGTA, TTCGAA, TCGGAG) was the dinucleotide 5'-d(CG), the preferred site of cross-linking for reductively activated mitomycin C. Cross-linking efficiency with FR66979 greatly exceeded that with FR900482. In the absence of dithionite, interstrand cross-linking was negligible (Figure 1). The absence of deoxyguanosine in enzymatic digests of electrophoretically homogeneous samples of N₆ = TACGTA cross-linked with either substance indicated that deoxyguanosine residues on opposite strands at 5'-d(CG) were bridged in the cross-links, as with mitomycin C. Substitution of one deoxyinosine residue, which lacks the N₂ amino group of dG, for one of the two dG residues at the duplex sequence 5'-d(CG) in N₆ = TACGTA greatly reduced cross-linking with both substances, revealing that both N₂ amino groups of dG residues at 5'-d(CG) participate in cross-linking as for mitomycin C and other pyrrole-derived bifunctional electrophiles. A further similarity of both agents to mitomycin C was the relative efficiency of cross-linking as a function of flanking sequence, 5'-d(ACGT) > 5'-d(TCGA) = 5'-d(CCGA) (Figure 1). The longest wavelength UV absorption of 7 was bathochromically shifted by some 40 nm (λₘₐₓ = 370 nm, n = +) relative to 1 as expected for conversion of a hydroxybenzene-carboxaldehyde to a hydroxyindolecarboxaldehyde (1 → 7). No absorbance of comparable wavelength was present in 8. NaBH₄ treatment of 7 returned 8 (RP-HPLC analysis). Preparation of larger samples of 7 and 8 to permit more rigorous structure assignment is underway.

The DNA interstrand cross-linking activities of reductively activated FR900482, FR66979, and mitomycin C thus share features most simply accounted for by the intermediacy of the analogs of FR900482 or FR66979 were separately analyzed by RP-HPLC (snake venom phosphodiesterase, calf intestinal alkaline phosphatase, DNase I). Each returned, in addition to dA, dC, and dT, a predominant, single, more strongly retained substance, tentatively proposed herein to be 7 and 8, respectively. Electrospray ionization MS of these substances afforded molecular ions (proton and sodium ion adducts) as required for 7 and 8, the analogs of 9 derived from mitomycin C. The longest wavelength UV absorption of 7 was bathochromically shifted by some 40 nm (λₘₐₓ = 370 nm, n = +) relative to 1 as expected for conversion of a hydroxybenzene-carboxaldehyde to a hydroxyindolecarboxaldehyde (1 → 7). No absorbance of comparable wavelength was present in 8. NaBH₄ treatment of 7 returned 8 (RP-HPLC analysis). Preparation of larger samples of 7 and 8 to permit more rigorous structure assignment is underway.

The DNA interstrand cross-linking activities of reductively activated FR900482, FR66979, and mitomycin C thus share features most simply accounted for by the intermediacy of the analogs of 4 and 5, respectively, the analogs of 6. The existence of significant alternative alkylation pathways involving nucleophile-activated or other intermediates of unspecified structure remains for now a topic of speculation.

Acknowledgment. This work was supported by the NIH (GM45804, GM32681, and AG00417). P.B.H. is a Cope Scholar.

Supplementary Material Available: DPAGE of dI substitution experiment, RP-HPLC of digest, and mass and UV spectra of the putative 7 and 8 (4 pages). Ordering information is given on any current masthead page.

References

(7) FR900482 was a gift of Fujisawa Pharmaceutical Co. Ltd. FR66979 was prepared from FR900482 by NaBH₄ reduction. Representative experimental details are described elsewhere: Kirchner, J. J.; Sigurdsson, S. Th.; Hopkins, P. B. J. Am. Chem. Soc. 1992, 114, 4621.
(8) Partially 5'-phosphodiester synthetic DNA (0.5 OD₅₂₈, 2 nmol duplex) and 64 µg (200 nmol) of FR900482 or 13 µg (40 nmol) of FR66979 in 25 µL of aqueous pH 7.6 Tris buffer (200 mM) were combined, sparged at 25 °C with argon, and then treated sequentially with five aliquots of 5 µL of 40 mM (FR900482) or 5 µL of 8 mM (FR66979) Na₂S₂O₄ (200 mM and 40 nmol per aliquot, respectively) at 5-min intervals. The solution stood at 25 °C for 16 h. The nucleic acid products were precipitated with ethanol and analyzed by DPAGE.