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Phosphotriester formation by the haloethylnitrosoureas and repair of these lesions by *E. coli* BS21 extracts

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ABSTRACT

The alkylation of phosphates in DNA by therapeutically active haloethyl-nitrosoureas was studied by reacting N-chloroethyl-N-nitrosourea (CNU) with dTpdT, separating the products by HPLC, and identifying them by co-chromatography with authentic markers. Both hydroxyethyl and chloroethyl phosphotriesters of dTpdT were identified; a similar reaction between CNU and dTR yielded 3-hydroxyethyl and 3-chloroethyl dTR as the major products of ring alkylation. A DNA-like substrate for repair studies was synthesized by reacting ¹⁴C-labelled N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea (¹⁴C-CGNU) with poly dT and annealing the product to poly dA. An extract of *E. coli* strain BS21 selectively transferred a chloroethyl group from one of the chloroethyl phosphotriester isomers in this substrate to the bacterial protein; chemical instability of the hydroxyethyl phosphotriesters precluded definite conclusions about the repair of this product.

INTRODUCTION

The haloethylnitrosoureas are effective agents for the treatment of a variety of malignancies, including tumors of the central nervous system (1). The cytotoxic action of these drugs has been attributed to their ability to alkylate DNA, and many different base modifications have been identified (2,3). One such lesion, 1-(3-deoxycytidyl),2-(1-deoxyguanosinyl)ethane, is believed to arise from an initial haloethylation of the O⁶ position of guanine and is a cause of DNA cross-linking (4,5). Lending support to the hypothesis that this is a cytotoxic lesion is the observation that cells proficient in the repair of O⁶-alkylguanine are resistant to the cytotoxic effects of the haloethylnitrosoureas (6). However, it appears that other DNA modifications also contribute to the total cytotoxic effect of these agents (7).

Alkylation of the phosphate backbone is a major consequence of the reaction of nitrosoureas with DNA (8). McCarthy *et al.* showed that methyl phosphotriesters were repaired by an *E. coli* BS21 extract (9), and Margison *et al.* cloned an *E. coli* gene responsible for this transferase activity (10). Later, methylnitrosourea (MNU), was shown to form phosphotriesters in two

steric configurations, one of which was repaired by the *ada* gene product (11,12). In bacteria, acceptance of methyl groups from methyl phosphotriesters by this protein stimulates synthesis of repair enzymes responsible for the adaptive response (13). If a similar phenomenon occurred in mammalian cells, it could be responsible for the development of resistance to treatment by the therapeutic nitrosoureas.

Phosphotriester formation by the haloethylnitrosoureas was postulated by Lown and McLaughlin to explain data showing chain scission of poly A by these agents (14). Conrad *et al.* demonstrated that hydroxyethylnitrosourea reacts with dTpdT to form hydroxyethyl esters of dTpdT and studied the hydrolysis of this phosphotriester (15). Similar chemical investigations of phosphotriester formation by chloroethylnitrosourea do not appear to have been reported.

These studies were undertaken to establish the chemical nature of the phosphotriesters produced by haloethylnitrosoureas and to study the ability of *E. coli* extracts to repair these adducts. We report that both hydroxyethyl and chloroethyl phosphotriesters are produced by chloroethylnitrosourea, that one isomer of the chloroethylphosphotriester is a repairable lesion, and that the hydroxyethylphosphotriesters degrade too rapidly to be assayed for repair.

MATERIALS AND METHODS

Materials

Deoxythymidine (dTR) was purchased from P-L Biochemicals; calf thymus DNA, from Worthington; and thymidylyl 3'-5' thymidine (dTpdT), poly dT, poly dA, 5'-O-(4,4'-dimethoxytrityl) thymidine 3'-(2-chlorophenyl)phosphate [DMT-dTp(chlorophenyl)], cesium fluoride, dimethylsulfoxide, and 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT), from Sigma. N-(2-chloroethyl)-N-nitrosourea (CNU), and N-[2-chloroethyl-1,2-¹⁴C]-N'-cyclohexyl-N-nitrosourea (¹⁴C-CCNU, specific activity = 24.4 mCi/mmol) were obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. All other chemicals were of reagent grade. Pyridine was refluxed over ninhydrin and redistilled before use while DMSO was refluxed over KOH and redistilled; both solvents were stored over 4A molecular sieves to remove traces of water. The two strains of *E. coli* used in this project were obtained from the Coli Genetic Stock Center. Strain BS21 (CGSC No. 6509), which constitutively expresses the *ada* gene product, was kindly grown and harvested for us by Dr. Dean Rupp of Yale University. Strain PJ1 (CGSC No.

6503), which is *ada*⁻, was kindly grown for us by Dr. Martin Marinus at UMass Medical School.

Synthesis of dTp(HE)dT and dTp(CE)dT

The desired phosphotriesters were obtained by synthesizing a fully protected DMT-dTp(chlorophenyl)dT according to the method of Chattopadhyaya and Reese (16) and displacing the chlorophenyl group by transesterification with the appropriate alcohol in the presence of cesium fluoride as described by Weinfeld *et al.* (12). The synthesis of dTp(CE)dT was straight-forward: DMT-dTp(chlorophenyl)dT (3 mg) was dissolved in 2 ml of methylene chloride; one ml of 1-chloroethanol and 80 mg of cesium fluoride were added; and the reaction was stirred at room temperature for 30 min. The methylene chloride was evaporated under N₂ and the chloroethanol, under reduced pressure. The 5'-protecting DMT group was removed by treatment of the residue with 500 μ l of 80% acetic acid at 37°C for 30 min. The acetic acid was evaporated under reduced pressure and the dTp(CE)dT was purified by HPLC. The yield was approximately 90% of the original DMT-dTp(chlorophenyl)dT.

The corresponding hydroxyethyl phosphotriester is much less stable and it was necessary to reduce the amount of cesium fluoride, the reaction temperature and the time in order to recover dTp(HE)dT from a similar reaction. Three mg of DMT-dTp(chlorophenyl)dT in 2 ml of methylene chloride was mixed with 1 ml of ethylene glycol and 2 mg of cesium fluoride in a shaking ice bath for 5 min. The remaining cesium fluoride was then filtered off, the solvents evaporated, the DMT protecting group removed, and the product purified as above. The yield was approximately 35% of the original DMT-dTp(chlorophenyl)dT.

Synthesis of 3-HEdTR and 3-CEdTR

3-(2-Hydroxyethyl)deoxythymidine (3-HEdTR) was synthesized by reacting dTR (30 mg) dissolved in 1.5 ml DMSO with 150 μ l of 1-bromoethanol in 1.5 ml of 50 mM sodium cacodylate, pH 7.3, which contained 300 mg of K₂CO₃. After incubation for 16 hr at 37°C, the mixture was dried under reduced pressure, redissolved in 3 ml H₂O, and separated on a 2x100 cm Sephadex G-10 column eluted with H₂O. 3-HEdTR, whose structure was established by ultraviolet and mass spectrometry as described below, eluted before the unreacted dTR and was collected, lyophilized, and stored at -20°C until used.

The synthesis and purification of 3-(2-chloroethyl)deoxythymidine (3-CEdTR) was similar, except that 1-bromo-2-chloroethane was substituted for bromoethanol. Unlike 3-HEdTR, 3-CEdTR eluted from the G-10 column after

unreacted dTR. The yields on these syntheses were 65% for the 3-HEdTR and 50% for the 3-CEdTR based on the initial amount of dTR.

HPLC Separations

Individual compounds were purified and mixtures were separated on an Alltech Spherisorb 5 μm C_{18} HPLC column. Samples were eluted at 1 ml/min with 20 ml of 23% methanol, a 5 ml gradient of 23% to 26% methanol, 30 ml of 26% methanol, a 5 ml gradient of 26% to 50% methanol, and 15 ml of 50% methanol, all in 50 mM KH_2PO_4 , pH 6.0. Absorption of the eluant was monitored at 265 nm with a Perkin-Elmer LC-55 spectrophotometer and recorded on a Hewlett Packard 3390A reporting integrator.

Ultraviolet and Mass Spectrometry

Ultraviolet absorption spectra were obtained on a Beckman Model 35 spectrophotometer. The molecular weight of each substituted nucleoside was determined by the electron impact method using a Varian MAT311A mass spectrometer with 70 eV electrons. Molecular weights of substituted dinucleotides were determined by positive ion fast atom bombardment mass spectrometry.

Reactions with Haloethylnitrosoureas

Deoxythymidine and dTpdT were dissolved separately in 100 mM sodium cacodylate, pH 7.3, at a concentration of 10 mg/ml and an equal volume of a 40 mg/ml solution of CNU in 100% ethanol was added to each. The mixtures were incubated at 37°C for 3 hr, filtered and assayed by HPLC.

A substrate for repair assays was generated by a similar reaction. Poly dT, 3 A_{265} optical density units, was dissolved in 5 μl of 100 mM sodium cacodylate buffer, pH 7.3, and 3 μCi of ^{14}C -CCNU dissolved in 3 μl of 100% ethanol was added. After 4 hr of incubation at 37°C, the alkylated polymer was recovered by precipitation with three volumes of 95% ethanol. Unreacted ^{14}C -CCNU and its degradation products were removed by repeated dissolution and ethanol precipitation of the polymer until no radioactivity was detected in the supernatant. The alkylated poly dT was then annealed to an equal number of OD units of poly dA by heating the mixture to 65°C for 15 min followed by slow cooling to room temperature. The final substrate contained 3.7 ODU of poly dT:poly dA in 900 μl of 100 mM sodium cacodylate, pH 7.3, with a specific activity of 50,000 cpm per ODU.

Bacterial Cell Extracts

E. coli (400-500 mg wet weight) were suspended in 3 ml of ice cold 70 mM Hepes/KOH, pH 7.8, containing 1 mM tetrasodium EDTA, 3 mM dithiothreitol, and 5% glycerol. Cells were lysed in an ice bath with a Tekmar sonic disruptor (250 W model) using pulsating sonication at 30% maximum intensity

for 2 X 30 sec. Cell debris was removed by centrifugation at 30,000 g for 15 min at 4°C. The resulting supernatant was stored at -70°C in 100 µl aliquots without further treatment. The BS21 extract contained 18 mg/ml protein, as determined by the Bradford assay (17), and 9 units of O⁶-alkyltransferase per mg protein as determined by the method of Pegg *et al.* (18). The PJ1 extract contained 5.6 mg/ml protein and less than 0.45 units of O⁶-alkyltransferase, the limit of detectability in our assay.

Phosphotriester Repair Assay

The removal of phosphotriesters was assayed essentially as described by McCarthy and Lindahl (11). Alkylated poly dT:poly dA was incubated at 37°C with the indicated amounts of bacterial protein in a total volume of 250 µl of 70 mM Hepes/KOH, pH 7.6. After 10 min, 50 µl of an 8 mg/ml solution of calf thymus DNA was added as carrier and the incubation was stopped by the addition of 300 µl of 0.8 M TCA. The precipitated macromolecules were rinsed with 300 µl of 95% ethanol to remove residual TCA, and resuspended in 300 µl of the original buffer. Bacterial proteins were then digested at 37°C for 1 hr by the addition of 4 µl of a 20 mg/ml solution of Proteinase K. This treatment was repeated twice to completely digest the precipitated proteins. Proteinase K-sensitive counts were recovered by re-precipitating the macromolecules with TCA as before and counting the supernatant. The pellet was again rinsed with 300 µl of 95% ethanol and redissolved in 500 µl of 100 mM sodium cacodylate, pH 7.3.

The remaining DNA was digested to the nucleoside level with a mixture of nucleases as previously described (19). Synthetic optical density markers were added and 400 µl of the digest was separated by HPLC as described above; addition of the markers is important since slight variations in conditions resulted in changes in retention times. Fractions of 0.5 ml were collected, 3.5 ml of scintillation fluid was added, and the samples were counted in a Beckman LS1800 scintillation counter. Profiles of radioactivity vs time were plotted on a Hewlett-Packard 85B computer and analyzed by a program which calculates the total radioactivity above background in each peak.

RESULTS

Since previous studies have shown that haloethylnitrosoureas substitute DNA bases with hydroxyethyl and haloethyl groups (2,3), it was anticipated that they would react with dTpdT to form hydroxyethyl and chloroethyl phosphotriesters. Marker amounts of dTp(HE)dT and dTp(CE)dT were synthesized by

transesterification of 5'-DMT-dTp(chlorophenyl)dT according to Weinfeld *et al.* (12) who used this approach for the synthesis of the methyl phosphotriesters of dTpdT. These synthetic products, like the methyl phosphotriesters, eluted from a C₁₈ column as twin peaks which presumably represent the two optical diastereoisomers of each phosphotriester. These phosphotriesters had UV spectra identical to the original dTpdT as expected; mass spectrometric data (see Table 1) verified the nature of the substituent alkyl group in each compound. Both dTp(HE)dT and dTp(CE)dT eluted with the late peak in the HPLC system used to identify the products of DNA alkylation by the haloethyl-nitrosoureas (19).

Ring alkylation by the nitrosoureas has been reported to occur at the 3 position of dTR (8). Therefore, 3-HEdTR and 3-CEdTR were expected as products of the reaction between dTR and the chloroethylnitrosoureas; marker amounts of these compounds were synthesized by reacting dTR with 1-bromoethanol and 1-bromo-2-chloroethane, respectively. Fig. 1 shows the ultraviolet absorption spectra of these compounds at acidic, neutral, and basic pH. These spectra are identical to each other and to those of 3-ethylthymidine reported by Singer (20), establishing the position of ring alkylation; the nature of the substituent group was verified by mass spectrometry. Table 1 lists the properties of the marker compounds synthesized in this report.

The reaction of GNU with dTpdT was then investigated to determine if dTp(HE)dT and dTp(CE)dT were indeed produced by phosphate alkylation with a haloethylnitrosourea. GNU was used in these studies because of its relatively high solubility in aqueous solutions. Panel C in Fig 2 shows the HPLC profile of the reaction mixture; controls of unreacted dTpdT and of incubated

Table 1 - Products of dTR and dTpdT Alkylation by GNU

Compound	HPLC R.T. (a)	Mass Spec. Form. Wt.	% Yield (b)
dTp(HE)dT ₁	12.4	590	0.20
dTp(HE)dT ₂	14.8	590	0.18
dTp(CE)dT ₁	43.9	608	1.08
dTp(CE)dT ₂	45.3	608	1.16
N ³ -HEdTR	5.2	286	1.92
N ³ -CEdTR	18.5	304	0.24

(a) HPLC retention time expressed in min.

(b) Expressed as percent of original dTpdT or dTR.

CNU are shown in Panels A and B, respectively. Two pairs of peaks, one pair at approximately 12 min, and the other pair at approximately 44 min, constitute the major products of this reaction. Panel D, which shows the co-chromatography of the dTpdT/CNU reaction mixture with the synthetic phosphotriesters, identifies the peaks in the 12 min region as the diastereoisomers of dTp(HE)dT and those in the 44 min region as the isomers of dTp(CE)dT. Several small peaks are apparent in Panel C which are not present in either Panels A or B. These may represent alkylation products of either or both of the thymine rings of the dimer.

When CNU was reacted with dTR to determine the sites of ring alkylation, the major product was identified as 3-HEdTR by co-chromatography of the reaction mixture with marker 3HEdTR and 3-CEdTR. The yields of these products in reactions of CNU with dTpdT or dTR are listed in Table 1.

A double-stranded DNA-like substrate containing these phosphotriesters was generated to assay for the ability of *E. coli* extracts to repair these lesions. Poly dT was treated with ^{14}C -CCNU under conditions similar to those used to alkylate dTR and dTpdT with CNU. The alkylated poly dT was annealed to poly dA after extensive washing to remove the decomposition products of unreacted ^{14}C -CCNU.

Fig. 3 shows the HPLC separation of an aliquot of this substrate digested to the nucleoside level. Panel A demonstrates that 40% of the radioactivity co-elutes with 3-HEdTR (6-7 min region) and another 40% co-elutes with dTp(CE)dT (50-54 min region). It is noteworthy that no radioactive material

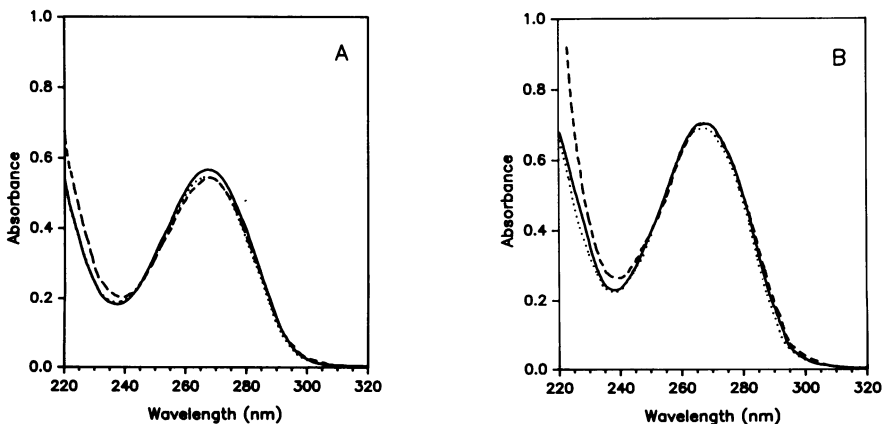


Figure 1. Ultraviolet spectra of 3-(2-hydroxyethyl)deoxythymidine (panel A) and 3-(2-chloroethyl)deoxythymidine (panel B) in acidic (\cdots , pH 1), neutral ($—$, pH 7), and basic ($---$, pH 12) solution.

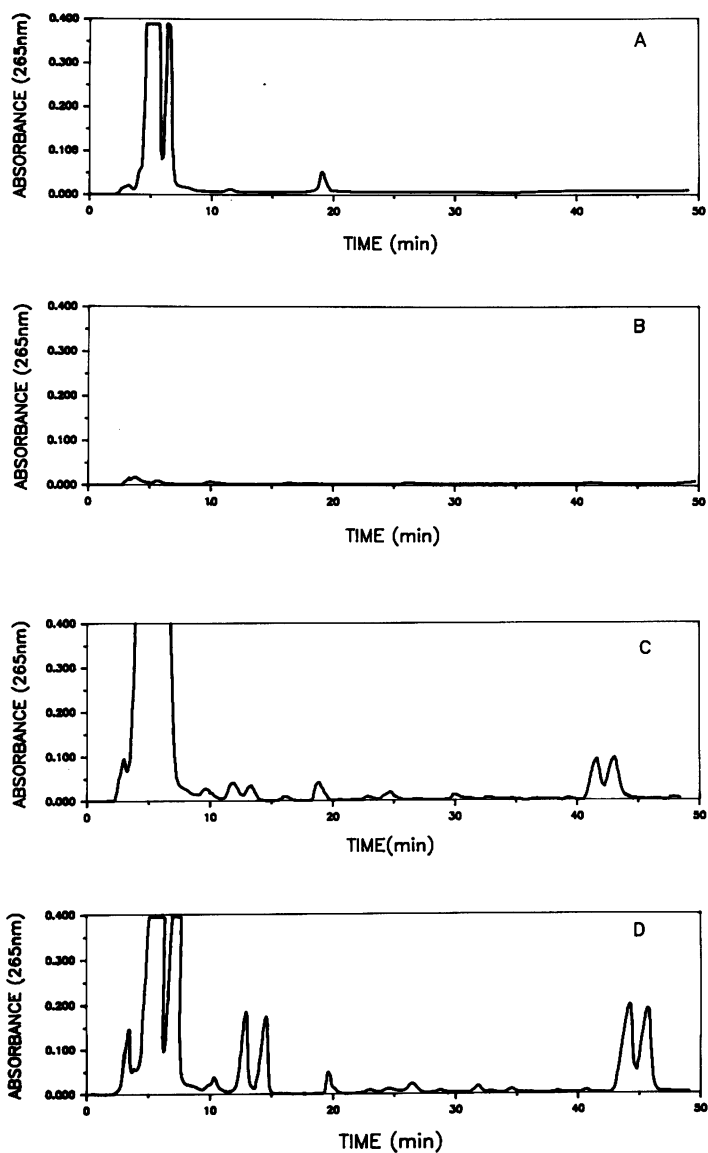


Figure 2. HPLC elution profiles of: unreacted dTpdT (panel A); CNU after incubation alone (panel B); reaction mixture of dTpdT and CNU (panel C); and co-chromatography of the reaction mixture of dTpdT and CNU with synthetic markers (panel D). The dTp(HE)dT isomers appear at 12-15 min and the dTp(CE)dT isomers, at 41-46 min. See text for reaction conditions and elution system.

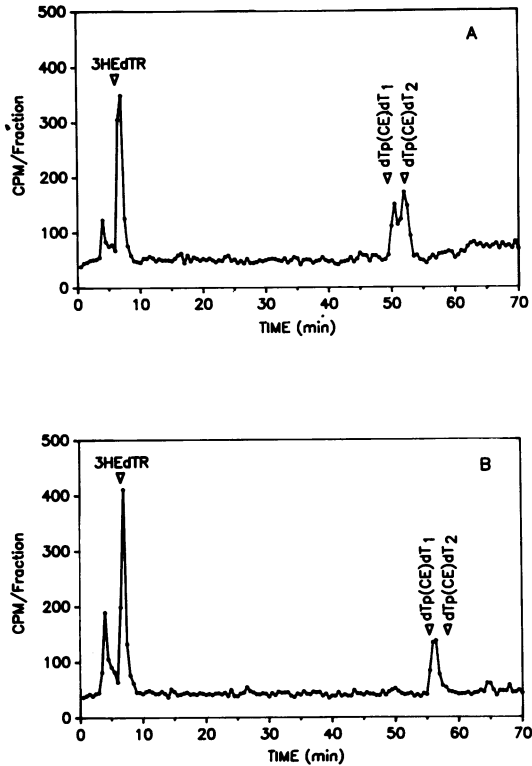


Figure 3. Typical HPLC elution profile of digested ^{14}C -CCNU-poly dT:poly dA substrate. The substrate in panel A was incubated alone and that in panel B, with 200 μg protein of *E. coli* BS21 extract. Retention times of added optical density markers are indicated. See text for sample preparation and elution system.

eluted at the position of the dTp(HE)dT isomers (12-14 min). This probably reflects the extreme lability of this compound to even mildly basic conditions (see Discussion). When the double-stranded substrate was treated with 200 μg of *E. coli* strain BS21 extract prior to DNA digestion, the resulting chromatogram, depicted in Fig. 3 Panel B, demonstrates complete removal of the more retained isomer of dTp(CE)dT. An equal amount of the PJ1 extract had no effect on the amounts of either isomer of dTp(CE)dT.

The protein dependence and specificity of the repair of the more retained isomer of dTp(CE)dT is shown in Fig. 4 and Table 2. Each sample contained the same amount of substrate incubated with 0 to 168 μg of bacterial protein

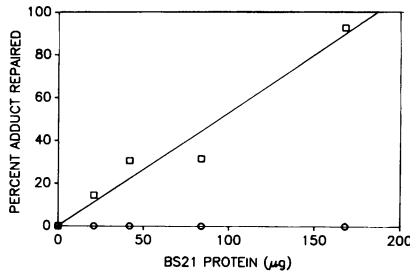


Figure 4. Percentages of each dTp(CE)dT isomer removed from ¹⁴C-CCNU-poly dT: poly dA by *E.coli* BS21 extract. The more retained isomer is indicated by □, and the less retained isomer, by ○. Data were calculated by subtracting the radioactivity associated with each derivative peak from that of the corresponding peak in a control sample incubated without extract.

and then digested as described in Materials and Methods. Fig. 4 shows the percent of the original radioactivity of each isomer of dTp(CE)dT which is lost after treatment with the indicated amounts of BS21 protein; the more retained isomer disappears in a protein-dependent fashion, but the less retained isomer is not affected at all.

Table 2 demonstrates that the radioactivity removed from the more retained isomer of dTp(CE)dT appears in a Proteinase K-sensitive fraction following treatment with the bacterial protein. A small amount of radioactivity is released spontaneously during the incubation, but as the amount of BS21 extract is increased, more becomes sensitive to Proteinase K treatment. At the same time, the amount of radioactivity associated with dTp(CE)dT₂ decreases; total recovery of radioactivity for all of these samples is the same (92-100%). The results demonstrate that, as the more retained isomer of dTp(CE)dT is repaired, the radioactivity is transferred to a Proteinase

Table 2 - Selective Loss of Radioactivity in the dTp(CE)dT Peak with a Corresponding Increase in Proteinase K Sensitive Fraction

BS21 Extract (µg protein)	Spontaneously Released cpm	Proteinase K Released cpm	Cpm Co-eluting With:		
			3-HEdTR	dTp(CE)dT ₁	dTp(CE)dT ₂
0	30	86	677	229	337
21	125	122	671	227	288
42	134	168	669	239	234
84	128	182	658	235	231
168	191	265	684	257	25

K-sensitive fraction; neither the less retained isomer of dTp(CE)dT nor the 3-HEdTR is affected by the bacterial extract.

DISCUSSION

The results described above demonstrate that CNU reacts with dTpdT to form both hydroxyethyl- and chloroethylphosphotriesters, as expected. Conrad *et al.* reported that the half-life of dTp(HE)dT at pH 7 and 37°C is only about 60 min and becomes significantly less as the pH is increased (15). Thus, the amount of dTp(HE)dT measured in the reaction between dTpdT and CNU is probably an underestimate of the total amount originally formed. Since this reaction was carried out at pH 7.3 for 3 hours, the observed ratio of dTp(HE)dT/dTp(CE)dT of 0.18/1 may reflect an actual ratio as high as 1.4/1.

Only dTp(CE)dT was found in enzymatic digests of poly dT reacted with ¹⁴C-CCNU. The most likely explanation for this is that chain scission occurred at the sites of hydroxyethylphosphotriester formation. It was observed during reactions of ¹⁴C-CCNU with poly dT that between one-third and one-half of the polymer became ethanol soluble after alkylation. This could only be due to extensive single-strand breakage such as that produced by the degradation of dTp(HE)dT. The introduction of similar single-strand breaks in cellular DNA would have profound effects on cell survival and mutagenesis, particularly if such breaks were clustered. Therefore, cellular resistance to the action of haloethylnitrosoureas is probably at least partly dependent on the ability of cells to repair hydroxyethylphosphotriesters or the single-strand breaks they produce.

The chloroethylphosphotriesters, as exemplified by dTp(CE)dT, are much more stable compounds. However, Yamauchi *et al.* (21) have recently demonstrated the ability of phosphate esters to alkylate DNA bases. It seems possible that chloroethylphosphotriesters could, therefore, transalkylate other sites in DNA. The significance of such transalkylation would depend on the modified nucleoside produced. Other secondary reactions of the chloroethyl phosphotriesters could lead to DNA-DNA or DNA-protein cross-linking reactions.

The data presented above indicate that the *E. coli* phosphotriester alkyltransferase produced by the *ada* gene can remove the chloroethyl group from one isomer of dTp(CE)dT in a double-stranded substrate and transfer it to bacterial protein. Due to the instability of dTp(HE)dT discussed above, no information could be obtained concerning repair of hydroxyethylphosphotriesters, but it seems probable that hydroxyethyl groups could also be removed from one isomer. In bacteria, removal of alkyl groups from phospho-

triesters induces the adaptive response. If a similar general response occurred in mammalian cells, it could be an important mechanism for the development of resistance to the haloethylnitrosoureas.

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REFERENCES

1. Montgomery, J.A. (1981) In: Prestayko, A.W., Crooke, S.T., Baker, L.H., Carter, S.K., and Schein, P.S. (eds.), Nitrosoureas: Current Status and New Developments, Academic Press, NY, pp. 3-8.
2. Ludlum, D.B., and Tong, W.P. (1981) *ibid.* 85-94.
3. Ludlum, D.B., and Tong, W.P. (1985) In: Muggia, F.M. (ed.), Cancer Chemotherapy, Vol. II, Martinus Nijhoff, pp. 141-154.
4. Tong, W.P., Kirk, M.C., and Ludlum, D.B. (1982) *Cancer Res.* 42, 3102-3105.
5. Tong W.P., Kirk, M.C., and Ludlum, D.B. (1983) *Biochem. Pharmacol.* 32, 2011-2015.
6. Erickson, L.C., Laurent, G., Sharkey, N.A., and Kohn, K.W. (1980) *Nature (Lond.)* 288, 727-729.
7. Kacinski, B.M., Rupp, W.D., and Ludlum, D.B. (1985) *Cancer Res.* 45, 6471-6474.
8. Singer, B. (1979) *J. Natl. Canc. Inst.* 62, 1329-1339.
9. McCarthy, J.G., Edington, B.V., and Schendel, P.F. (1983) *Proc. Natl. Acad. Sci USA* 80, 7380-7384.
10. Margison, G. P., Cooper, D. P., and Brennand, J. (1985) *Nuc. Acid Res.* 13, 1939-1952.
11. McCarthy, T.V., and Lindahl, T. (1985) *Nucl. Acid Res.* 13, 7067-7077.
12. Weinfeld, M., Drake, A.F., Saunders, J.K., and Paterson, M.C. (1985) *Nucl. Acid Res.* 13, 2039-2053.
13. Teo, I., Sedgwick, B., Kilpatrick, M.W., McCarthy, T.V., and Lindahl, T. (1986) *Cell* 45, 315-324.
14. Lown, J. W., and McLaughlin, L. W. (1979) *Biochem. Pharmacol.* 28, 1631-1638.
15. Conrad, J., Muller, N., and Eisenbrand, G. (1986) *Chem-Biol Interact.* 60, 57-65.
16. Chattopadhyaya, J.B., and Reese, C.B. (1980) *Nucl. Acids Res.* 8, 2039-2053.
17. Bradford, M. (1976) *Anal. Biochem.* 72, 248-254.
18. Pegg, A. E., Perry, W., and Bennett, R. A. (1983) *Biochem. J.* 197, 195-201.
19. Ludlum, D.B. (1987) *Pharmacol. Ther.* 34, 145-153.
20. Singer, B., and Grunberger, D. (1983) In: *Molecular Biology of Mutagens and Carcinogens*, Plenum Press, NY, p328.
21. Yamauchi, K., Hayashi, M., and Kinoshita, M. (1975) *J. Org. Chem.* 40, 385-386.