Biochemical Pharmacology, Vol. 28, pp. 2123–2128

0006-2952/79/0715-2123 $02.00/0

MECHANISM OF ACTION OF 2-HALOETHYLNITROSOUREAS ON DEOXYRIBONUCLEIC ACID

NATURE OF THE CHEMICAL REACTIONS WITH DEOXYRIBONUCLEIC ACID

J. WILLIAM LOWN and LARRY W. MCLAUGHLIN
Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

(*Received* 2 *October* 1978; *accepted* 19 *December* 1978)

Abstract—1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) covalently cross-links DNA under physiological conditions. Methyl substitution at either carbon atom of the 2-chloroethyl portion of the molecule prevents cross-linking. Haloalkyl nitrosoureas including 3-chloropropyl, 4-chlorobutyl and 5-chloropentyl, although they readily alkylate DNA, exhibit no ability to cross-link DNA. 3-(2-Chloroethyl)-1-methylcytosine hydrochloride and N^4-(2-chloroethyl)-1-methylcytosine hydrochloride, similar to intermediates suggested in the cross-linking process, alkylate PM2-CCC-DNA readily. These two cytosine derivatives also cyclize readily to give 3,N^4-ethano-1-methylcytosine closely similar to a species isolated from the treatment of poly·C with BCNU. A number of processes including the extent of DNA alkylation, measured with [^{14}C]CCNU labeled in the ethylene portion of the molecule, as well as concomitant DNA single strand scission, and intramolecular alkylation and/or hydrolysis of the chloroethyl cytidine intermediate were investigated as to their effects upon the interstrand cross-linking process.

Many active antineoplastic drugs which are bifunctional alkylating agents have been shown to produce DNA interstrand cross-links, an event generally lethal to the cell compared with alkylation which is repaired more readily. 2-Chloroethyl nitrosoureas, while not obviously bifunctional, induce significant interstrand cross-linking [1, 2]. The suggested mechanism of this cross-linking involves chloroethylation of an appropriate base followed by a second alkylation at the carbon bearing the chlorine atom [1–3].

In addition to cross-linking, DNA degradation in the form of alkali labile sites has been reported for chloroethyl nitrosoureas [4–7]. These lesions appear to result from a combination of phosphate alkylation as well as base alkylation followed by depurination or depyrimidation and production of labile apurinic sites [7].

These observations necessitated a more detailed study of the structural requirements for interstrand cross-linking and of the effects of the combination of DNA single strand scission and interstrand cross-linking as they apply to antileukemic activity.

EXPERIMENTAL

Materials

[^{14}C]CCNU labeled in the ethylene portion of the molecule was a gift from Dr. Gerald Goldenberg, Manitoba Institute of Cell Biology, Winnipeg, Manitoba. Ethidium bromide was purchased from the Sigma Chemical Co., St. Louis, MO 63178, and λ-DNA (mol. wt 31×10^6) was obtained from Miles Biochemicals, Miles Laboratories, Inc., Elkhart, IN 46515.

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. The i.r. spectra were recorded on a Nicolet 7199 F.T. spectrophotometer, and only the principal, sharply defined peaks are reported. The n.m.r. spectra were recorded on Varian A-60 and A-100 analytical spectrometers. The spectra were measured on approximately 10–15% (w/v) solutions in appropriate deuterated solvents with tetramethylsilane as standard. Line positions are reported in parts per million from the reference. Mass spectra were determined on an Associated Electrical Industries MS-9 double-focusing high-resolution mass spectrometer. The ionization energy, in general, was 70 eV. Peak measurements were made by comparison with perfluorotributylamine at a resolving power of 15,000. Microanalyses were carried out by Mrs. D. Mahlow of this department.

N^4-(2-chloroethyl)-1-methylcytosine hydrochloride. This was prepared according to the method of Ueda and Fox [8]. It exhibited a double m.p. at 160° and 272–275° (lit. [8] m.p., 163–164° and 271–273°).

3,N^4-ethano-1-methylcytosine hydrochloride. This was prepared according to the method of Ueda and Fox [8] by heating a small amount of the above chloroethyl derivative on a heating block for a few minutes at 170°: m.p., 272–275° (lit. [8] m.p., 271–273°).

3-(2-Chloroethyl)-1-methylcytosine hydrochloride. 3-(2-Hydroxyethyl)-1-methylcytosine, prepared according to the method of Mizuno *et al.* [9], was dissolved in ethanol saturated with HCl. After removal of the ethanol a 30-mg sample of the hydrochloride salt was added to 50 μl of thionyl chloride in 600 μl of dry hexamethyl phosphoramide at 0°. The solution was very slowly allowed to warm to room temperature and stirred overnight. One ml ethanol was then added; the mixture was stirred an additional hour and then added to 7 ml ether. The white solid was recrystallized twice from ethanol/ether without heating. The yield was 15 mg (45%), m.p. 215° and 269–272°.

Anal. Calc. for $C_7H_{10}N_3OCl \cdot HCl \cdot \frac{1}{4}H_2O$ [mol. wt

187.0512 (free base)]: C, 36.77; H, 5.08; N, 18.38. Found (187.0519, mass spectrum): C, 36.63; H, 4.97; N, 17.99. P.m.r. D_2O (HOD 4.7δ) δ 3.5 (s, 3H, CH_3); 3.9 (t, 2H, CH_2); 4.5 (t, 2H, CH_2); 6.2 (d, 1H, CH); 7.8 (d, 1H, CH). I.r. ν_{max} (EtOH) 3320 (N—H); 1630 (C=O); 1560 (C=N) cm^{-1}.

This compound could also be converted to 3,N^4-ethano-1-methylcytosine hydrochloride by heating a small amount on a heating block for a few minutes at 220°, m.p. 269–272° (lit. [8] m.p. 271–273°).

1-(3-Chloropropyl)-1-nitrosourea, 1-(4-chlorobutyl)-1-nitrosourea, 1-(5-chloropentyl)-1-nitrosourea and 1,3-bis-[1-(chloromethyl)ethyl]nitrosourea were prepared as described previously [2, 10]. 1,3-Bis(2-chloropropyl)nitrosourea was prepared according to the method of Johnston *et al.* [11].

Methods

Fluorescence assay for determining CLC sequences in DNA produced by nitrosoureas. All measurements were performed on a G. K. Turner & Associates model 430 spectrofluorometer equipped with a cooling fan to minimize fluctuations in the xenon lamp source. Wavelength calibration was performed as described in the manual for the instrument. One-cm-square cuvettes were used. The excitation wavelength was 525 nm and the emission wavelength was 600 nm. The 100 × scale of medium sensitivity was generally used, and water was circulated between the cell compartment and a thermally regulated bath at 22°. The fluorometric method of detecting CLC sequences in λ-DNA has been described [12, 13]. Cross-linking of DNA creates a nucleation site which allows renaturation of λ-DNA after heat denaturation (96°/3 min) and rapid cooling and thus provides intercalation sites for ethidium. That this assay procedure detects the formation of CLC-DNA as a result of a chemical cross-linking event has been confirmed by experiments with the enzyme S_1-endonuclease [12, 14]. This enzyme specifically cleaves single-stranded DNA and is essentially inactive on duplex DNA and, therefore, distinguishes DNA which is renaturable by virtue of a chemical cross-link and DNA which separates into single strands on heating. A 20-μl aliquot was taken at intervals from the reaction mixture (50 mM potassium phosphate, pH 7.2; 1.0 A_{260} units of λ-DNA; 5 mM nitrosourea; total volume, 200 μl) at 37° and added to the standard assay mixture (which was 20 mM potassium phosphate, pH 11.8, 0.4 mM EDTA, and 0.5 μg/ml of ethidium). The fluorescence after the heating and cooling cycle compared with control times 100 gives the percentage of CLC-DNA in a sample. For a standard set of conditions (i.e. type and concentration of DNA, pH, ionic strength, and the temperature), the accuracy of the CLC assay is determined by the precision of the fluorescence readings. Overall accuracy of the CLC assay is estimated at ± 2 per cent.

Fluorescence determination of alkylation of PM2-CCC-DNA by nitrosoureas. A 20-μl aliquot was taken at intervals from the reaction mixture [50 mM potassium phosphate, pH 7.2, 1.2 A_{260} units of PM2-CCC-DNA (90% CCC), 5 mM nitrosourea in a total volume of 200 μl at 37°] and added to the standard assay mixture (which was 20 mM potassium phosphate, pH 11.8, 0.4 mM EDTA, and 0.5 μg/ml of ethidium). The fluorescence after heating at 96°/3 min followed by rapid cooling was compared with the initial value.

Under these conditions unreacted PM2-CCC-DNA returns to register after heat denaturation because of topological constraints. Alkylated PM2-CCC-DNA shows a decrease in fluorescence because of thermally induced depurination followed by alkaline strand scission of the apurinic site in the assay medium [15]. The ratio of the decrease in fluorescence (after the heating and cooling cycle) to that of the control is a measure of the extent of alkylation. In a control experiment it was shown that none of the components interfered with the ethidium fluorescence. Provided no single strand cleavage of PM2-CCC-DNA is observed (detected by the characteristic rise in fluorescence before the heating/cooling cycle), this technique can be used to measure levels of alkylation not readily observed using λ-DNA.

Binding of [^{14}C]*CCNU to λ-DNA.* Duplicate 100-μl mixtures containing the desired concentration of [^{14}C]CCNU were incubated at 37° and pH 7.2 with 1.0 A_{260} λ-DNA (mol. wt 31 × 10^6). After a 6-hr incubation, a 10-μl aliquot was transferred to the assay solution and the extent of interstrand cross-linking was measured. A 1-μl aliquot was transferred to 10 ml of a toluene based liquid scintillation mixture (Scinti-Verse, Fisher Scientific Co., Edmonton, Alberta) and counted on a Beckman LS 100c (Serial No. 1000930) scintillation counter using a [^{14}C]toluene standard (New England Nuclear, Dorval, Quebec, 4 × 10^5 dis./min/ml) to determine initial CCNU concentration. The remaining reaction mixture was dialyzed at 4° against three 1000-ml vol. of 20 mM potassium phosphate (pH 7.0) containing 2 mM EDTA. The DNA nucleotide equivalent concentration of the dialysate was determined by u.v. absorption at 260 nm assuming an extinction coefficient of 7000. A 100-μl aliquot of the dialysate was then counted as described above to determine the concentration of DNA bound radioactivity. A 100-μl aliquot of the dialysis solution was used to determine background counts.

The ratio of initial CCNU concentration to DNA bound radioactivity (corrected for a 1.0 A_{260} DNA concentration) is used to determine the hydrolyzed drug/bound drug ratio. The ratio of DNA nucleotide equivalent concentration after dialysis to DNA bound radioactivity is used to determine the binding ratio.

Conversion of N^4*-(2-chloroethyl)-1-methylcytosine to 3,*N^4*-ethano-1-methylcytosine.* The p.m.r. (D_2O, pH 7.2) of the chloroethyl derivative shows a sharp resonance for the methylene protons at δ 3.6 (s, 4H). Under the same conditions, the cyclized product exhibits a close A_2B_2 pattern centered at δ 4.25 (m, 4H).

A 1-ml D_2O reaction mixture containing 65 mmoles of the chloromethyl compound in deuterated 200 mM potassium phosphate (pH 7.2) was incubated at 37° in the p.m.r. probe of a Varian A-100 analytical spectrometer. The rate of intramolecular cyclization was obtained by monitoring the changes in the areas of the signals listed above.

Conversion of 3-(2-chloroethyl)-1-methylcytosine to 3,N^4*-ethano-1-methylcytosine.* The p.m.r. (D_2O, pH 7.2) of the chloroethyl derivative shows a resonance for one of the ring protons at δ 7.8 (d, 1H). Under the same conditions, the cyclized product exhibits a similar doublet for one of the ring protons shifted slightly

upfield. While the inner peaks of the two doublets overlap, the outer resonances are cleanly separated.

A 1-ml D_2O reaction mixture containing 65 mM of the chloroethyl compound in deuterated 200 mM potassium phosphate (pH 7.2) was incubated at 37° in the p.m.r. probe of a Varian A-100 analytical spectrometer. The rate of intramolecular cyclization was obtained by monitoring the changes in the areas of the signals listed above.

RESULTS AND DISCUSSION

2-Chloroethylnitrosoureas result in significant DNA interstrand cross-linking (BCNU cross-links 42 per cent of λ-DNA in 6 hr) under physiological conditions of pH 7.2 and 37°. As reported earlier [2], the extent of cross-linking is strongly dependent on the nature of the halogen in the nitrosourea. Fluoroethyl, bromoethyl and iodoethyl nitrosoureas exhibit little ability to form interstrand cross-links, while hydroxyethyl and methoxyethyl derivatives do not cross-link DNA. Chloroethyl derivatives appear to be more active than the corresponding bromoethyl and iodoethyl compounds in the leukemia L1210 test system [11]. Recently Johnston *et al.* [16] investigated a series of 2-chloroethyl and 2-fluoroethyl analogues of MeCCNU. They reported that the 2-fluoroethyl compounds were, in four of five examples, "clearly inferior to the corresponding 2-chloroethyl compounds". A similar conclusion was reached [17] using Lewis Lung Carcinoma, a solid tumor system.

Since the DNA interstrand cross-linking ability of 2-haloethylnitrosoureas, reported in a previous publication [2], appears to be generally in accord with reported L1210 *in vivo* activities [11], a more detailed study of the effects of structure on the cross-linking phenomenon was undertaken.

DNA interstrand cross-linking of chloroalkyl nitrosoureas

Four nitrosoureas were synthesized, all of which contained a chlorine atom on a primary carbon atom. They included 1-(2-chloroethyl)-1-nitrosourea (CNU), 1-(3-chloropropyl)-1-nitrosourea (CPNU), 1-(4-chlorobutyl)-1-nitrosourea (4-CBNU) and 1-(5-chloropentyl)-1-nitrosourea (5-CPNU) [10]. While all of these derivatives alkylate DNA significantly (Table 1), only the 2-chloroethyl derivative was able to produce covalent interstrand cross-links (CNU cross-links 36 per cent of λ-DNA in 6 hr). We have observed* that the extent of DNA interstrand cross-links induced by bifunctional alkylating agents such as epoxides and aziridines increases with increasing distance between the alkylating sites. If cross-linking by chloroethyl nitrosoureas involves chloroethylation of one base followed by substitution of chloride by a second nucleophilic site, one might expect chloropropyl, chlorobutyl or chloropentyl nitrosoureas to induce some interstrand cross-links.

Steric effects were then investigated by preparing three appropriately substituted nitrosoureas: 1,3-bis(2-chloroethyl)-nitrosourea (BCNU); 1,3-bis(2-chloropropyl)nitrosourea (BCNU-β-Me) and 1,3-bis[1-(chloromethyl)ethyl]nitrosourea (BCNU-α-Me) [10]. Alkylation of PM2-CCC-DNA is observed for all three compounds (Table 1); however, only the unsubstituted chloroethyl derivative BCNU forms interstrand cross-links (42 per cent cross-linked λ-DNA in 6 hr). The loss of cross-linking ability by chain lengthening or chain branching parallels the structure activity studies of Johnston *et al.* [11] who observed that similar structure modification results in low activity or loss of activity in the leukemia L1210 test system.

Table 1. DNA alkylation and rate of aqueous decomposition of selected nitrosoureas

Compound	$T_{1/2}$* (min)	% Alkylated PM2-CCC-DNA after 180 min of reaction
CNU	8	65†
CPNU	6	81
4-CBNU	5	82
5-CPNU	5	73
BCNU	79	70†
BCNU-α-Me	22	42
BCNU-β-Me	74	52

* See Ref. 10.

† Observed after 60 min of reaction after which time extensive DNA cross-linking obscures measurement of alkylation [2].

Factors affecting DNA interstrand cross-linking by 2-chloroethylnitrosoureas

The extent of interstrand cross-linked DNA produced by chloroethylnitrosoureas is considerably lower than that observed for many other bifunctional alkylating agents under comparable conditions. (Compare CCNU with mitomycin C, Table 2.) Four processes could explain these observed low levels of cross-linking: (i) the extent of initial alkylation of the DNA is low, (ii) labilization of the chlorine atom to allow chloroethylated bases to complete the cross-link occurs only at sites in the DNA which are infrequently alkylated, (iii) intramolecular alkylation or hydrolysis competes favorably with interstrand cross-linking for the intermediate chloroethylated base, and (iv) DNA degradation occurs concomitantly with alkylation and cross-linking and causes cross-linking, as measured spectrofluorometrically, to appear lower.

Binding of [^{14}C]*CCNU to* λ*-DNA.* To determine the extent of binding to DNA, [^{14}C]CCNU labeled in the ethylene portion of the molecule was allowed to react with λ-DNA under physiological conditions (see Experimental). Previous work involved a similar study with mitomycin C [12]. The results from that study as well as those from the present one are listed in Table 2. As can be observed from Table 2, even at twice the concentration, the extent of alkylation by CCNU is much lower than that observed for mitomycin C. Additionally, the amount of nitrosourea which hydrolyzes is far greater than in the case of mitomycin C. Both of these observations suggest that CCNU is much less selective in its reaction with nucleophiles, even reacting to a large extent with water. As the concentration of CCNU increases, the extent of binding increases as is expected; however, even at binding ratios which approach those of mitomycin C, the amount of cross-linked DNA is below 50 per cent.

Intermolecular reactions of 2-chloroethylcytidine derivatives. Previous work by Ludlum *et al.* [3] has

* J. W. Lown and L. W. McLaughlin, unpublished results.

Table 2. DNA interstrand cross-linking by CCNU and mitomycin C

	CCNU			Mitomycin C*
Drug concentration (M)	$2.19 \pm 1.2 \times 10^{-4}$	$1.03 \pm 0.2 \times 10^{-3}$	$6.53 \pm 0.1 \times 10^{-3}$	1.2×10^{-4}
% Cross-linked DNA	5 ± 1	39 ± 1	47 ± 1	84 ± 1
Nucleotide equivalents				
Bound drug	2131 ± 74	484 ± 12†	73 ± 7	50
Hydrolyzed drug				
Bound drug	3271 ± 148	3498 ± 21	3331 ± 335	42

* Data from Ref. 12.
† Ratio of interstrand cross-lined:total alkylation = 1:430.

indicated that the cytidine residues of nucleic acids are alkylated most extensively by chloroethyl nitrosoureas. The two most likely positions for chloroethylation appeared to be the 3 position and the N^4 position of cytidine. The 3 position is known to be alkylated by a variety of alkylating agents [18]. Although the 3 position of cytidine in the DNA helix would appear to be inaccessible to alkylation because of hydrogen bonding, nevertheless treatment of DNA *in vitro* or *in vivo* with *N*-methyl-*N*-nitrosourea [19], *N*-ethyl-*N*-nitrosourea [18] or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [20] has resulted in the isolation of small amounts of *N*-3 alkylated cytidine residues. Chloroethylation by nitrosoureas might well occur at the 3 position prior to cross-linking, as suggested by Ludlum *et al.* [3]. Chloroethylation of the N^4 position provides a nitrogen half-mustard which appeared to be the most likely intermediate chloroethylcytidine residue which would retain alkylating activity. While the N^4 position of cytidine is not generally observed to be alkylated significantly after treatment of DNA with a variety of alkylating agents [18], recent research by Singer [21] has indicated that treatment of cytidine with *N*-ethyl-*N*-nitrosourea results in significant amounts of N^4-ethylated cytidine. The observed low levels of cross-linking by chloroethylnitrosoureas at relatively high levels of alkylation suggested that such a less reactive site might be involved in the cross-linking phenomenon.

Two model compounds were prepared to test alkylating activity: 3-(2-chloroethyl)-1-methylcytosine hydrochloride and N^4-(2-chloroethyl)-1-methylcytosine hydrochloride (see Fig. 2.) As indicated in Fig. 1, both compounds show significant, although relatively low levels of alkylation of PM2-CCC-DNA. While labilization of the chlorine atom to nucleophilic displacement can be explained for the N^4-(2-chloroethyl) derivative, the basis for the alkylating activity observed for the 3-(2-chloroethyl) analogue is unclear. In light of the observation that 3-chloropropyl, 4-chlorobutyl and 5-chloropentyl nitrosoureas show no cross-linking activity, it appears that labilization of the chlorine atom to nucleophilic displacement is a prerequisite for cross-linking to occur.

Intramolecular reactions of 2-chloroethylcytidine derivatives. The possibility that intramolecular alkylation or cyclization competes favorably with cross-linking was investigated by comparing the rates at which 3-(2-chloroethyl)-1-methylcytosine and N^4-(2-chloroethyl)-1-methylcytosine alkylate intramolecularly to form 3,N^4-ethanocytosine (Fig. 2). The cyclizations were observed to follow first-order kinetics (Fig. 3). The N^4-substituted compound in aqueous buffer (pH 7.2) at 37° has a half-life of 16 min and complete conversion to the cyclic compound occurred within 150 min. The 3-substituted derivative under the same conditions has a half-life of 53 min. The faster rate of the N^4 derivative may result from nitrogen mustard labilization of the chlorine atom to nucleophilic dis-

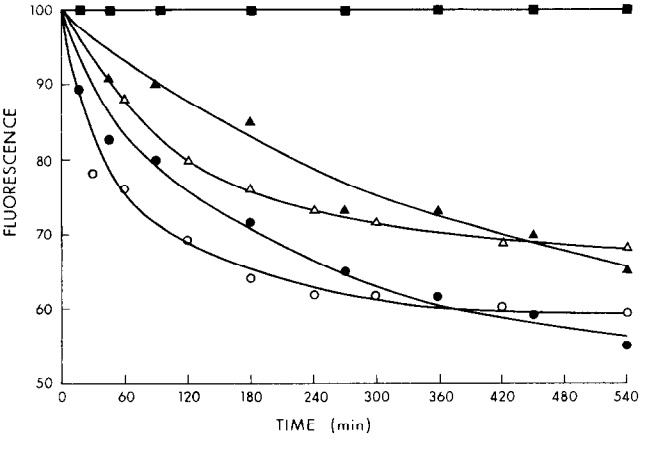

Fig. 1. Reaction of drug with 1.0 A_{260} PM2-CCC-DNA, pH 7.2, 37°. Key: N^4-(2-chloroethyl)-1-methylcytosine hydrochloride (▲) 5 mM; (●) 10 mM; and 3-(2-chloroethyl)-1-methylcytosine hydrochloride (△) 5 mM; (○) 10 mM.

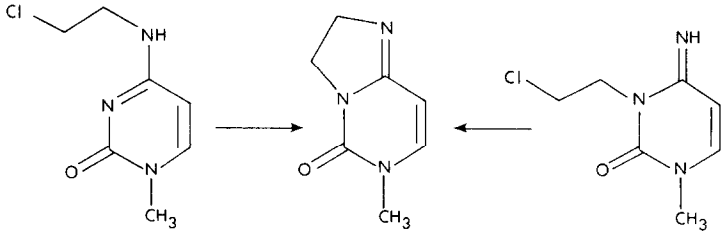

Fig. 2. Conversion of N^4-(2-chloroethyl)-1-methylcytosine or 3-(2-chloroethyl)-1-methylcytosine to 3,N^4-ethano-1-methylcytosine.

placement. The results from both compounds suggest that, although hydrogen bonding to the 3 or N^4 positions in the DNA helix may decrease the rate of such cyclizations after initial chloroethylation, intramolecular alkylation may compete favorably with interstrand cross-linking. No 3 or N^4 hydroxyethylated cytosine was observed to be formed during either reaction. Although a hydroxyethylated cytidine monophosphate derivative has been isolated after treatment of poly C with BCNU [3], the necessary hydrolysis of the chlorine atom appears to occur prior to alkylation. This observation is in accord with a recent report by Ludlum and Tong [22] concerning 3-(2-fluoroethyl)cytidine. A possible explanation for the formation of hydroxyethylated bases involves nucleophilic attack at the carbon bearing the nitrogen of the oxadiazoline suggested [10, 23] as an intermediate in the decomposition of 2-chloroethylnitrosoureas.

Effects of DNA single strand scission on interstrand cross-linking

The effects of single strand scission occurring concomitantly with cross-linking were then investigated. Previous studies have shown that chloroethyl nitrosoureas result in significant alkaline induced single strand scission [7]. It has also been observed that hydroxyethyl nitrosoureas result in extensive single strand scission most probably through phosphate alkylation [7]. To determine the relative effects of single strand scission on DNA cross-linking, a compound mixture was prepared. Equivalent amounts of 3-(cyclohexyl)-1-(2-hydroxyethyl)-1-nitrosourea (CHNU) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) were allowed to react with λ-DNA at pH 7.2 and 37°. The mixture was then assayed for ability to produce interstrand cross-links. As can be observed in Fig. 4, addition of the effective single strand scission agent CHNU results in an apparent decrease in the extent of cross-linked DNA as compared to that observed for BCNU alone. Cross-linking as measured spectrofluorometrically involves heat denaturation under alkaline conditions followed by renaturation of DNA which has been chemically cross-linked. Decreasing the molecular weight of the DNA by single strand scission results in smaller fragments of DNA renaturing after heating and an apparent decrease in cross-linked DNA.

In summary, the effects of the electrophiles from nitrosoureas on DNA are summarized in Fig. 5. Alkylation of the internucleotide linkages results in type I single strand scission as reported previously [7]. Alkylation of the bases results in depurination followed by a slower type II single strand scission involving hydrolysis of labile apurinic sites [7] or further intramolecular or intermolecular alkylation by the chloroethylated base. Intermolecular alkylation produces either intrastrand or interstrand DNA cross-links.

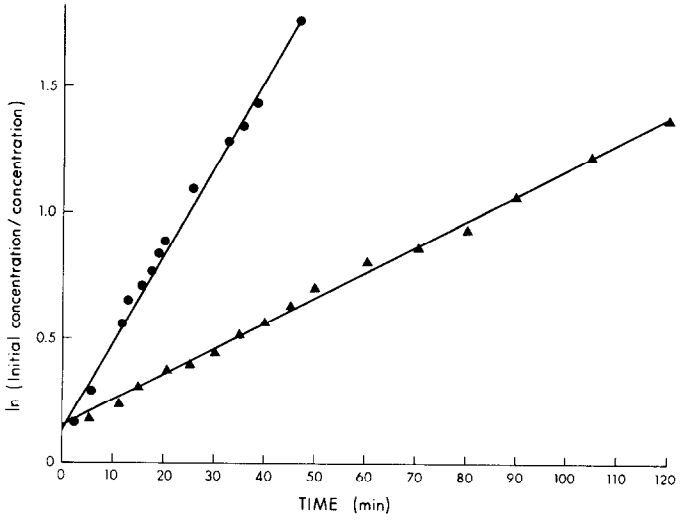

Fig. 3. First-order kinetics for the formation of 3,N^4-ethano-1-methylcytosine from (●) N^4-(2-chloroethyl)-1-methylcytosine or (▲) 3-(2-chloroethyl)-1-methylcytosine.

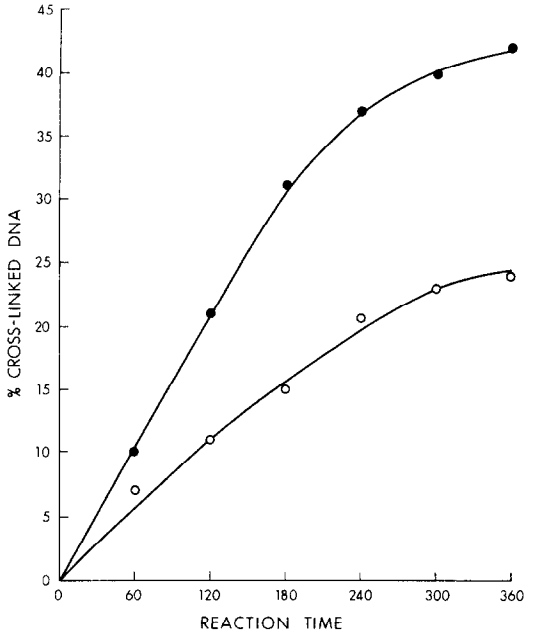

Fig. 4. Reaction of 1.0 A_{260} λ-DNA pH 7.2 and 37° with (●) 5 mM BCNU; and (○) 5 mM BCNU plus 5 mM CHNU.

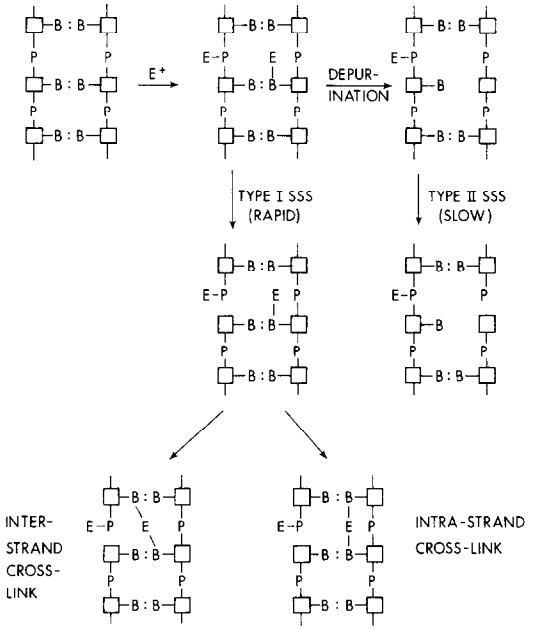

Fig. 5. Effects of electrophiles from nitrosoureas on DNA.

Acknowledgements—This investigation was supported by grants to J.W.L: Grant 1Ro1 CA21488-01, awarded by the National Cancer Institute, DHEW, and Grant A-2305 from the National Research Council of Canada and by the University of Alberta.

REFERENCES

1. K. W. Kohn, *Cancer Res.* **37**, 1450 (1977).
2. J. W. Lown, L. W. McLaughlin and Y. M. Chang, *Bioorg. Chem.* **7**, 97 (1978).
3. D. B. Ludlum, B. S. Kramer, J. Wang and C. Fenselau, *Biochemistry* **14**, 5480 (1975).
4. L. C. Erickson, M. O. Bradley and K. W. Kohn, *Cancer Res.* **37**, 3744 (1977).
5. P. H. Gutin, J. Hilton, V. J. Fein, A. E. Allan, A. Rottman and M. D. Walker, *Cancer Res.* **37**, 3761 (1977).
6. J. Hilton, D. L. Bowie, P. H. Gutin, D. M. Zito and M. D. Walker, *Cancer Res.* **37**, 2262 (1977).
7. J. W. Lown and L. W. McLaughlin, *Biochem. Pharmac.*, in press.
8. T. Ueda and J. J. Fox, *J. Am. chem. Soc.* **85**, 4024 (1963).
9. H. Mizuno, H. Okuyama, H. Hayatsu and T. Ukita, *Chem. pharm. Bull., Tokyo* **12**, 1240 (1964).
10. J. W. Lown, L. W. McLaughlin and J. A. Plambeck, *Biochem. Pharmac.*, **28**, 2115 (1979).
11. T. P. Johnston, G. S. McCaleb, P. S. Opliger and J. A. Montgomery, *J. med. Chem.* **9**, 892 (1966).
12. J. W. Lown, A. Begleiter, D. Johnson and A. R. Morgan, *Can. J. Biochem.* **54**, 110 (1976).
13. A. R. Morgan and V. Paetkau, *Can. J. Biochem.* **50**, 210 (1972).
14. M. H. Akhtar, A. Begleiter, D. Johnson, J. W. Lown, L. W. McLaughlin and S. K. Sim, *Can. J. Chem.* **54**, 2891 (1975).
15. J. W. Lown, K. C. Majumdar, A. I. Meyers and A. Hecht, *Bioorg. Chem.* **6**, 453 (1977).
16. T. P. Johnston, G. S. McCaleb, S. D. Clayton, J. L. Frye, C. A. Krauth and J. A. Montgomery, *J. med. Chem.* **20**, 279 (1977).
17. J. A. Montgomery, G. S. McCaleb, T. P. Johnston, J. G. Mayo and W. R. Laster, Jr., *J. med. Chem.* **20**, 291 (1977).
18. B. Singer, in *Progress in Nucleic Acid Research and Molecular Biology* (Ed. W. E. Cohn), Vol. 15, p. 219. Academic Press, New York (1975).
19. P. D. Lawley and S. A. Shah, *Chem. Biol. Interact.* 7, 115 (1973).
20. P. D. Lawley and C. J. Thatcher, *Biochem. J.* **116**, 693 (1970).
21. B. Singer, *Fedn Eur. Biochem. Soc. Lett.* **63**, 85 (1976).
22. D. B. Ludlum and W. P. Tong, *Biochem. Pharmac.* **27**, 77 (1978).
23. J. A. Montgomery, in *Sixth International Symposium on Medicinal Chemistry* (Abstr.), University of Sussex, Brighton, September 4–7, 1978.