Identification of 2-Deoxyribonolactone at the Site of Neocarzinostatin-Induced Cytosine Release in the Sequence d(AGC)†

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ABSTRACT: Neocarzinostatin- (NCS) induced release of cytosine from the deoxycytidylate residues of d(AGC) sequences of duplex oligonucleotides leaves a damaged sugar residue with intact phosphodiester linkages [Kappen, L. S., Chen, C., & Goldberg, I. H. (1988) *Biochemistry 27*, 4331–4340]. In order to isolate and characterize the sugar damage product, drug-treated duplex d(AGCGAGC*G) (the single target C* residue has ^3H in its 5- and 5′-positions) was enzymatically digested to mononucleosides. High-pressure liquid chromatographic analysis of the digest revealed drug-induced products which could be cleanly separated by thin-layer chromatography (TLC) into two components: product a (R_f 0.47) and product l (R_f 0.87). The more polar product a was further purified by adsorption onto DEAE-Sephadex A-25. After elution with HCl and lyophilization, this material behaved like product l on TLC. Readjustment to alkaline pH caused its quantitative conversion back to product a. On electrophoresis product l behaved like a neutral compound, and the negatively charged product a migrated just behind formate. On the basis of the various chemical and biochemical characteristics of the lesion and apparent ^3H abstraction by NCS from the C-1′ position (see reference cited above), it appears that the two interconvertible products a and l are respectively the acid (carboxylate) and lactone forms of 2-deoxyribonic acid. The structure of the sugar damage product was confirmed by gas chromatography/mass spectrometry. The amount of 2-deoxyribonolactone recovered is about 60% of the cytosine released on a molar basis, showing that it is the major, if not the only, product associated with cytosine release. It is proposed that abasic site formation involves abstraction of a hydrogen atom by a free radical form of NCS to generate a carbon-centered radical at C-1′ of deoxyribose. Upon addition of dioxygen to form a peroxyl radical intermediate, the latter degrades to release the base and a sugar oxidized at C-1′ to the lactone.

Upon activation by thiol in the presence of oxygen the antibiotic neocarzinostatin primarily generates direct DNA strand breaks at thymidylate and deoxyadenylate residues [reviewed in Goldberg (1987)]. The strand breaks, bearing 3′-phosphate and 5′-nucleoside 5′-aldehyde termini, result from the specific oxidation of C-5′ of the deoxyribose moiety by a presumed free radical form of the drug. In addition, NCS[1] produces abasic lesions in DNA, and those at deoxycytidylate residues in the preferred sequence d(AGC) have been implicated in mutagenesis in *Escherichia coli* (Povirk & Goldberg, 1985, 1986). These abasic lesions differ from ordinary apurinic lesions produced by simple hydrolytic cleavage of the base from the sugar in that their phosphodiester linkages are much more labile to alkali-induced hydrolysis but resistant to cleavage by certain apurinic/apyrimidinic (AP) endonucleases and cannot be protected from alkali-induced cleavage by prior treatment with sodium borohydride (Kappen et al., 1988). These features suggested that the sugar residue at the NCS-induced abasic site is not deoxyribose or its derivatives with an aldehyde group at C-1′. Instead, it appeared that selective oxidation had occurred at C-1′, since tritium from 1′,2′-labeled deoxycytidylate in d(AGC) was abstracted into the activated NCS (Kappen et al., 1988). However, because of the necessity to invoke a rather sizable tritium isotope selection effect and because the abstracted tritium could not be unambiguously assigned to the 1′-position, only chemical characterization of the sugar product at the abasic site could establish the structure of the lesion and clarify the mechanism leading to its formation. In this paper we show that 2-deoxyribonolactone is the main, if not the only, product generated at the abasic site and invoke a mechanism involving peroxyl radical formation at C-1′ in its formation.

Materials and Methods

Synthesis of oligodeoxynucleotides and preparation of radiolabeled substrates using [5-^3H]dCTP (22.8 Ci/mmol) and [5′,5-^3H]dCTP (55.5 Ci/mmol; 5′-^3H, 62%; 5-^3H, 38%) from New England Nuclear were as previously described (Kappen et al., 1988). Nuclease S_1 from *Aspergillus oryzyae* and *E. coli* alkaline phosphatase were products of Sigma. Phosphodiesterase I from *Crotalus adamanteus* venom was purchased from Worthington.

Drug Reaction. Extraction of NCS chromophore from the native drug and treatment of oligonucleotides were essentially as reported (Kappen et al., 1988). The substrate d(AGCGAGC*G) (the single target C* residue has ^3H label at the 5- and 5′-positions) annealed to an excess (0.4–1.5 A_{260} units) of the 12-mer d(CICT)$_3$ was treated with NCS chromophore at 0 °C for 1 h in a standard reaction that contained 50 mM Hepes/NaOH, pH 8.0, 0.2 mM EDTA, and 5 mM glutathione.

Analysis of Sugar Damage Products. In order to isolate the sugar damage product, the reacted oligonucleotide was recovered by ethanol precipitation after the addition of tRNA and 0.3 M sodium acetate and digested to the level of mo-

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[1] Abbreviations: NCS, neocarzinostatin; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; HPLC, high-pressure liquid chromatography; TLC, thin-layer chromatography; GC/MS, gas chromatography/mass spectrometry.

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nonucleosides by a combination of either (a) nuclease S_1 and alkaline phosphatase or (b) nuclease S_1, phosphodiesterase I, and alkaline phosphatase. Nuclease S_1 digestion was in 10–20 mM sodium acetate, pH 4.5, and 0.2 mM $ZnCl_2$. The pH was then adjusted to 7.7 with Hepes/NaOH, pH 10.1, before the addition of the other enzyme(s). Digestion with enzyme combination b also received 15 mM $MgCl_2$. Enzyme digestions were at 37 °C for 6–12 h. Completion of digestion was ascertained by the absence of nucleotides and the generation of 2′-deoxycytidine on TLC analysis of an aliquot of the reaction.

The enzyme digest was subjected to HPLC using a μBondapak C_{18} column. Elution (1 mL/min) was isocratic with triethylammonium bicarbonate, pH 7.6. The radioactive peaks were located by liquid scintillation counting of aliquots of the fractions. The fractions of interest were lyophilized and dissolved in H_2O.

Thin-layer chromatographic analysis of the HPLC fractions on cellulose plates (with fluorescent indicator) was in the following solvent systems: (a) 1 M sodium acetate–ethanol (3:7) and (b) 2-propanol–NH_4OH–H_2O (7:1:2). Added nonradioactive markers, such as cytosine and cytidine, were located under UV light. Electrophoresis was performed on Whatman 3MM paper in 0.1 M ammonium carbonate, pH 8.8, at 1000 V for 90 min. In both electrophoresis and TLC, radioactivity in 1-cm strips was determined by liquid scintillation counting.

Estimation of Cytosine. Release of radioactive cytosine was quantitated both by HPLC (retention time 5 min) and by TLC system a (R_f 0.57) described above.

Alkali and Acid Treatment. Alkali treatment of the isolated sugar fragment was at room temperature in 0.1 M NaOH for 20–60 min. The enzyme digest was made 0.23 M in NaOH or adjusted to pH 12.0 with NaOH. The samples were then neutralized with HCl. Acid treatment was in 0.1 M HCl at room temperature (maximum 2 h). The samples were either directly lyophilized or neutralized with NaOH.

Purification of the Sugar Damage Product(s) for GC/MS Analysis. In standard reaction A of 20 mL, a mixture of 150 A_{260} units of d(GAGCGAGCG) which has two target C residues and trace amounts of the 8-mer d(AGCGAGC*G) (C*, 5-^{3}H, 1.8×10^5 cpm) annealed to 300 A_{260} units of the 12-mer d(CICT)$_3$ was treated with NCS chromophore (73 μM). Another NCS reaction (B) was set up in 0.5 mL with d(AGCGAGC*G) [C* has ^{3}H (2.6×10^6 cpm) in its 5- and 5′-positions] annealed to 0.75 A_{260} unit of d(CICT)$_3$. The oligomers were recovered by ethanol precipitation and digested to mononucleosides by enzyme combination b. The enzyme digest of reaction A (4 mL) was mixed with the digest of reaction B (0.2 mL) which also contains ^{3}H-labeled sugar damage products to serve as markers in the purification steps to follow.

After the pH was adjusted to 7.0 with HCl, the digest was freed of proteins by centrifugation through a Centricon-10 microconcentrator (Amicon) and then lyophilized and redissolved in H_2O for chromatography on DEAE-Sephadex A-25. The columns (9 × 1.5 cm diameter) were equilibrated with 0.01 M Tris-HCl, pH 7.0, followed by H_2O or 0.01 M Tris-HCl, pH 8.0. The unadsorbed substances were washed through with H_2O, and those adsorbed were eluted with 0.1 M HCl. Fractions of 2 mL were collected at 40 mL/h. An aliquot of the fraction was used to determine the radioactivity. The radioactive peak fractions were lyophilized, and the residue was dissolved in H_2O. Additional purification was achieved by repeating the anion-exchange chromatography on the product(s) from the first column after acidification or alkalinization (see Results). The acidified samples were lyophilized, and the residue was dissolved in H_2O. If the solution was still acidic, the pH was adjusted to 7.0 with NaOH. The alkalinized samples were neutralized with HCl prior to its application onto the column. At the final step of purification from the seventh column (8 × 1 cm diameter), the material eluted with HCl was directly lyophilized and redissolved in H_2O.

The purified product was treated with 0.1 M NaOH for 1.75 h at room temperature prior to the preparation of its TMS derivatives. Derivatization was in 1:1 acetonitrile–*N*,*O*-bis-(trimethylsilyl)trifluoroacetamide (1% trimethylchlorosilane) at 80 °C for 45 min. GC/MS analysis was performed on a Hewlett-Packard 5890 gas chromatograph–5970A mass spectrometer. The TMS derivatives were introduced through a 30 m long DB5 column (J and W Scientific) under the following conditions: 10 °C/min, 80–200 °C; 30 °C/min, 200–300 °C; 300 °C for 7 min.

Preparation of 2-Deoxyribonolactone. 2-Deoxyribose was oxidized with bromine (Pravdic & Fletcher, 1971). The product was alkalinized and purified by chromatography on DEAE-Sephadex A-25 as described above. 2-Deoxyribonic acid was detected by spraying the chromatogram (TLC) with bromcresol-purple (Ganshirt et al., 1965).

Results

Previous work has shown that cytosine release from the d(AGC) sequences of oligonucleotides by NCS leaves the damaged sugar residue with intact phosphodiester linkages in a full-length molecule (Kappen et al., 1988). In order to isolate and characterize the damaged sugar moiety, d(AGCGAGC*G) (the single target C* residue carries ^{3}H label in its 5- and 5′-positions) annealed to d(CICT)$_3$ was used as a substrate for the drug. Choice of d(CICT)$_3$ was made on the basis of our earlier finding that placement of an I residue (instead of G) opposite the target C residue enhanced cytosine release by NCS severalfold (Kappen et al., 1988). HPLC analysis of the enzyme digest of drug-treated oligonucleotide gave in addition to the main peak of deoxycytidine (retention time 9 min) a broad peak of radioactivity encompassing retention times of 4–6 min (data not shown). In a control reaction containing no drug there was no radioactivity in the 4–6-min fractions. The individual 4–6-min HPLC fractions of the drug reaction were subjected to TLC analysis (Figure 1). Figure 1A shows a major radioactive peak in the 4-min fraction migrating as a material (product a) with an R_f of 0.47. The 6-min product (Figure 1B) is distinctly different from the 4-min product in its chromatographic mobility (product l, R_f 0.87). Generally, the radioactivity in the 6-min fraction exceeded that in the 4-min fraction, and occasionally the latter was barely detectable. The 5-min fraction had a chromatographic pattern similar to that shown in Figure 1B but with a greater amount of radioactivity in the cytosine region (not shown). It is clear from the HPLC elution profile and the migration on TLC that the 4-min product is more polar than the 6-min product. The formation of the 4-min and the 6-min products was dependent on both nuclease and alkaline phosphatase treatments. Furthermore, when the ^{3}H label was only in the base (cytosine) of the oligomer, the 4-min and 6-min radioactive products were absent. Direct TLC analysis of the enzyme digest of NCS-treated oligomer also gave two radioactive spots identical with those produced by the 4-min and 6-min HPLC fraction products.

Since data on ^{3}H abstraction by NCS from deoxyribose of the substrate and several other chemical and biochemical

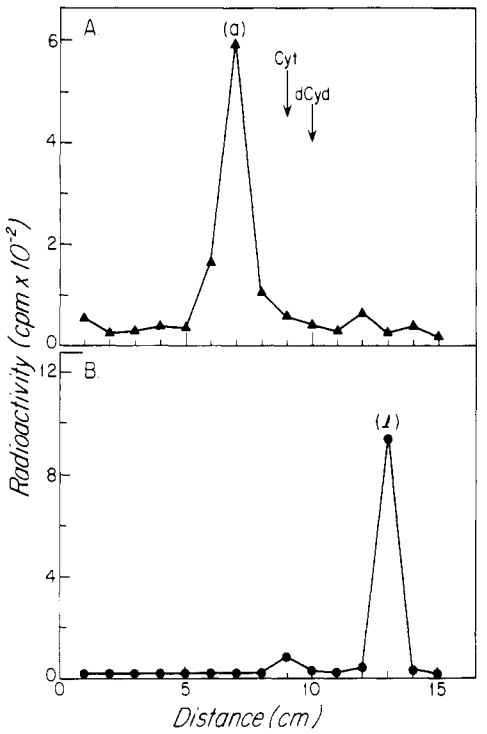


FIGURE 1: Thin-layer chromatographic analysis of NCS-induced sugar damage products. d(AGCGAGC*G) (C* has ^3H at 5- and 5′-positions), after treatment with 30 μM NCS, was digested enzymatically, and the digest was analyzed by HPLC. Fractions of retention times of 4 min (A) and 6 min (B) were analyzed by TLC in solvent system a as given under Materials and Methods. Arrows indicate the positions of marker cytosine (Cyt) and 2′-deoxycytidine (dCyd).

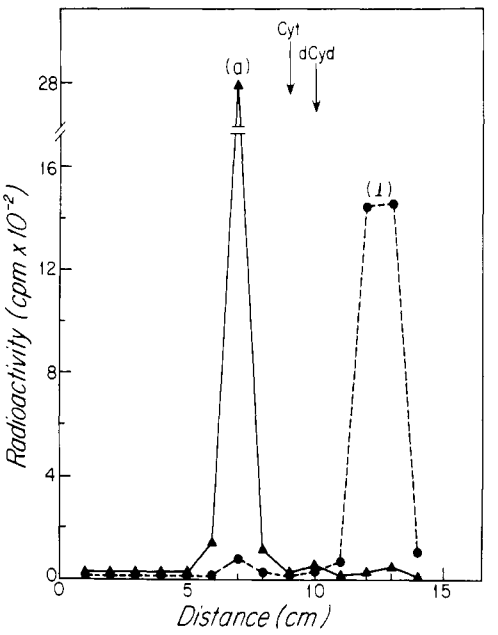


FIGURE 2: Effect of alkaline pH treatment on purified sugar damage product. Substrate, same as in Figure 1, was treated with 44 μM NCS. Enzyme digest of the drug-treated oligomer was made alkaline then neutralized, and subjected to HPLC analysis. The material with retention time of 4 min was further purified on a DEAE-Sephadex A-25 column. The HCl-eluted material was lyophilized and redissolved in H_2O (pH 7.0). This product, with (▲) and without (●) subsequent alkali treatment, was analyzed on TLC in solvent system a.

characteristics of the lesion had suggested the possibility of C-1′ attack (Kappen et al., 1988) and consequent generation of a C-1′ carbonyl moiety, we suspected that the two products with R_f 0.47 (product a) and 0.87 (product l) were respectively the carboxylate and the lactone forms of the oxidized sugar. In support of this formulation, alkali treatment of the enzyme digest caused the disappearance of radioactivity from the faster moving material (R_f 0.87) with concomitant increase in radioactivity in the region of the product with R_f 0.47.

In subsequent experiments the enzyme digest was made alkaline (and then neutralized) prior to HPLC analysis to convert the presumed lactone to the carboxylate. The product with retention time 4 min was then further purified by anion-exchange chromatography. The 4-min material (after alkali treatment and readjustment of pH to 8.0) adsorbed onto a DEAE-Sephadex A-25 column. On elution from the column with HCl and lyophilization, it behaved like product l on TLC (Figure 2) and no longer bound to DEAE-Sephadex. Alkali treatment quantitatively converted this material to a substance with the same mobility as product a of Figure 1. Similarly, the alkali-treated material in Figure 2, on another round of reacidification and lyophilization, reverted to one with R_f 0.87 (data not shown). This interconversion of the two products with alteration of pH suggests that the material with R_f 0.47 is 2-deoxyribonic acid and that with R_f 0.87 is its lactone.

The electrophoretic mobilities of the purified product(s) are also compatible with the conclusion drawn above. On electrophoresis the product with 6-min retention time on HPLC behaved like a neutral compound, whereas the 4-min product migrated to a distance of 21 cm under conditions where marker formate moved 24 cm (data not shown). Furthermore, the purified material eluted by HCl from DEAE-Sephadex (after drying) comigrated with the internal marker thymidine. Alkali treatment quantitatively converted this material to a negatively charged substance with electrophoretic mobility identical with that of the 4-min product (data not shown).

Conclusive evidence for the identity of the sugar damage product(s) comes from GC/MS analysis. The ability to interconvert the lactone and the acid forms was made use of in their purification by DEAE-Sephadex chromatography. While the carboxylate form of the sugar damage product and inorganic phosphate (generated from the degradation of nucleotides) adhered to the column, nonanionic substances, including the lactone, were washed through with H_2O. The sugar acid and inorganic phosphate could be separated from each other chromatographically after conversion of the former to the neutral lactone by acidification and lyophilization. By alternating cycles of DEAE chromatography of the lactone or carboxylate forms of the sugar damage product, sufficient amounts of purified material were obtained for GC/MS analysis. The mass spectrum of the TMS derivatives of the NCS-produced sugar damage product (Figure 3A) gave a fragmentation pattern identical with that obtained for authentic 2-deoxyribonic acid (Figure 3B), and both patterns are in agreement with the mass spectrum reported for 2-deoxy-*erythro*-pentonic acid (Petersson, 1970). Evidence for the identity of the radioactive drug product a with authentic 2-deoxyribonic acid comes from their similar chromatographic behavior on alteration of pH and their identical mobilities on TLC in solvents a and b.

It is important to establish that 2-deoxyribonolactone is the major sugar product associated with cytosine release. The multistep procedures used above in the analysis of deoxyribonic acid and its lactone are not suitable for their quantitation. An alternate procedure involved treatment of the enzyme digest directly with alkali and selective adsorption of the carboxylate onto a small DEAE-Sephadex column and its elution with HCl. The acid eluate was evaporated to dryness to remove any volatile radioactive products. The only radioactive substance recovered in the dried material was 2-deoxyribonolactone as ascertained by TLC analysis. In a typical reaction

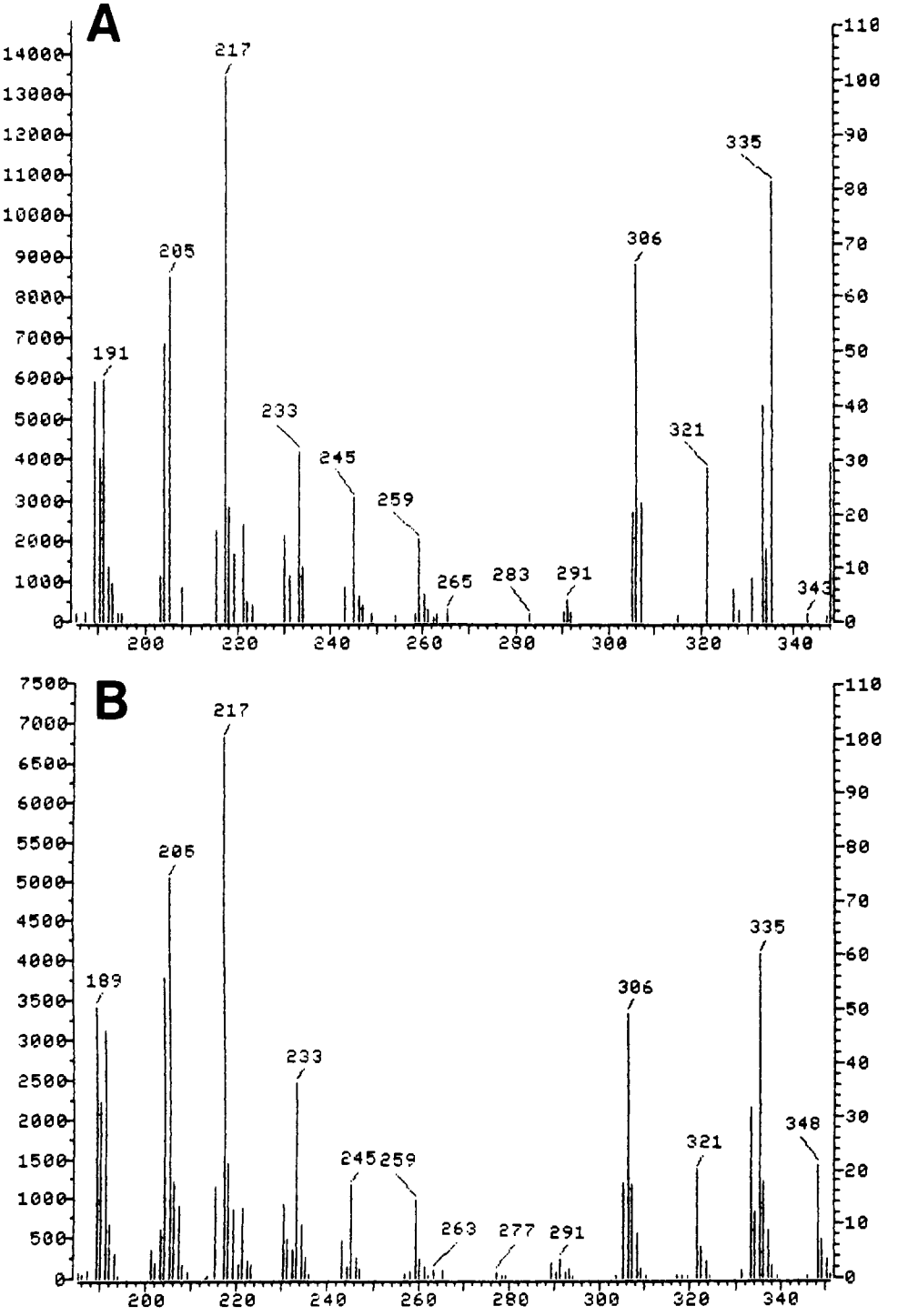


FIGURE 3: Partial mass spectra of the TMS derivatives of (A) sugar damage product purified from NCS reaction and (B) 2-deoxyribononic acid. The retention time of the material on gas chromatography was 12.67 min.

containing the 8-mer d(AGCGAGC*G) (the only target C* residue has ^3H at its 5- and 5′-positions) annealed to $d(CICT)_3$ and 59 μM NCS, 25% of the total cytosine was released; 2-deoxyribonolactone recovered from the same reaction amounted to 57% of the released cytosine on a molar basis.

Discussion

The biologically active component of the protein antitumor antibiotic neocarzinostatin is a labile nonprotein chromophore (NCS chromophore) composed of three discrete structural components (Hensens et al., 1983; Edo et al., 1985): (1) a substituted naphthoic acid moiety, (2) an amino sugar (*N*-methyl-α-D-fucosamine), and (3) a novel, highly strained bicyclo[7.3.0]dodecadienediyne epoxide containing two acetylenic bonds. Accumulated evidence supports a model for NCS chromophore binding to DNA involving intercalation of the naphthoate moiety and electrostatic interaction of the positively charged amino sugar moiety with the negatively charged DNA backbone, so as to place the bicyclic core with its reactive diyne–ene system in the minor groove of DNA (Napier & Goldberg, 1983). Recent studies using a series of hexameric oligodeoxynucleotides suggest that the drug prefers 5′-d(GNT)-3′ sequences, intercalating between G and N and cleaving at T (Lee & Goldberg, 1989). It has been proposed that activation of NCS chromophore by thiol (or sodium borohydride) involves thiol adduction at C-12 of the chromophore bicylic core, opening of the epoxide, and rearrangement of the diyne–ene system to form a presumed C-2, C-6 diradical

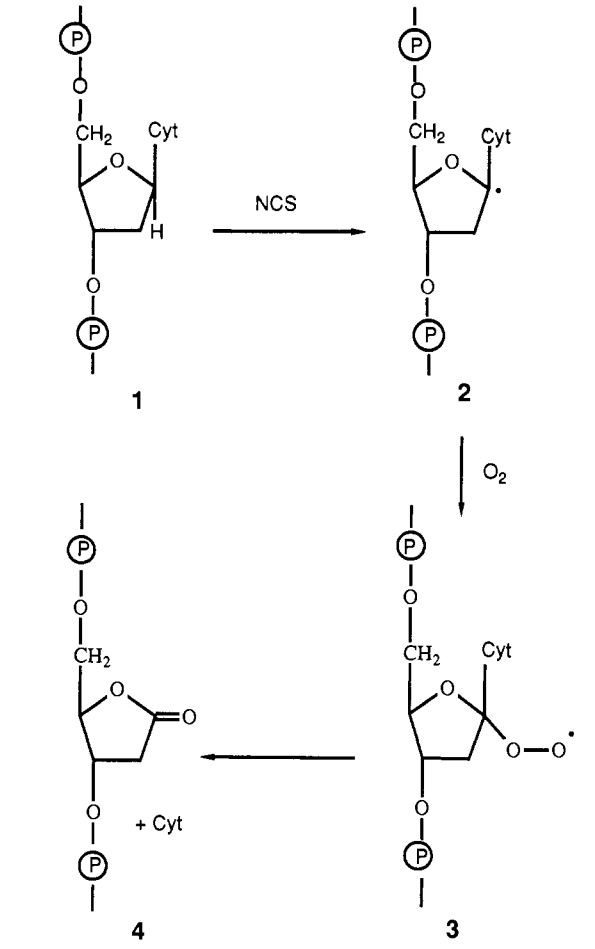
species (Myers, 1987; Hensens et al., 1983). Support for involvement of a diradical form of NCS chromophore in the DNA damage comes from recent ^1H NMR and mass spectroscopic studies showing that DNA is the likely source of the two hydrogen atoms abstracted by the drug and incorporated into its C-2 and C-6 positions (Chin et al., 1988).

Earlier work has shown that in the process of DNA strand breakage activated NCS chromophore attacks C-5′ of thymidylate (and to a lesser extent, deoxyadenylate) residues by abstracting a hydrogen atom into the drug to form a carbon-centered radical at C-5′ of the deoxyribose (Charnas & Goldberg, 1984; Kappen & Goldberg, 1985). Following dioxygen addition to form a peroxyl radical species, an aldehyde is generated at C-5′ leading to a strand break with 3′-phosphate and 5′-nucleoside 5′-aldehyde termini. In the absence of oxygen, instead of strand breakage, a covalent adduct forms between the drug and the deoxyribose, presumably at C-5′. It is clear that the source of one of the hydrogens incorporated into the activated drug comes from C-5′ at the break site.

NCS chromophore also induces base release with the formation of alkali-labile, abasic sites in the DNA (Bose et al., 1980; Kappen & Goldberg, 1983; Boye et al., 1984). These lesions can account for up to 25% of the total strand breaks generated (Povirk & Houlgrave, 1988) and are most frequent when glutathione (not dithiothreitol or 2-mercaptoethanol) or sodium borohydride is the activating agent (Povirk & Goldberg, 1985; Kappen et al., 1988; Povirk & Houlgrave, 1988). We have shown that abasic lesions occur with an especially high frequency at deoxycytidylate residues in d(AGC) sequences and have atypical responses to enzymatic or chemical hydrolysis when compared with abasic sites generated by acid-induced depurination (Povirk & Goldberg, 1985; Kappen et al., 1988). It was also found that the alkali-labile sites at deoxycytidylate residues in d(AGC) can be entirely accounted for by the release of cytosine base with the formation of an abasic lesion and that these lesions exist in otherwise intact strands, i.e., full-length molecules possessing intact phosphodiester linkages on either side of the lesion (Kappen et al., 1988). The latter finding permitted a strategy in the isolation of the sugar damage product involving nuclease digestion of the damaged strand. The identification of the DNA damage product as 2-deoxyribonolactone, rather than a derivative resulting from β-elimination of the phosphate moieties (including the carbon-bound oxygen) at C-3′ or C-5′, as found with the 1,10-phenanthroline–cuprous complex (Goyne & Sigman, 1987), confirms that the lesion existed in an unbroken strand of DNA. The inability of sodium borohydride to prevent alkali-induced cleavage at the abasic site is expected for the lactone product. It also appears that the initial product of the oxidation at C-1′, the lactone, remains as such in the DNA as judged by the fact that even after an isolation procedure involving nuclease digestion at pH 8 the main form on TLC analysis is the lactone, not the acid. The resistance of the abasic lesion to cleavage at its 3′-side by AP endonuclease III from *E. coli* (Povirk & Goldberg, 1985; Povirk & Houlgrave, 1988) is understandable, since the enzyme functions by a β-elimination mechanism (Bailly & Verly, 1987), presumably requiring Schiff base formation with an aldehyde group at C-1′.

NCS chromophore is able to oxidize specifically C-1′ of deoxyribose at target sites in certain sequences, such as the deoxycytidylate residue in d(AGC). The finding of 2-deoxyribonolactone as the DNA sugar damage product, as well as the earlier observation that tritium from probably C-1′ is abstracted into NCS chromophore, clearly supports a mechanism (Scheme I) involving generation of a carbon-centered radical (**2**) at C-1′ by a free radical form of the drug. Further, the requirement for oxygen (or its substitute, misonidazole) (Kappen et al., 1988) in the formation of the abasic sites suggests that, as in the case of the lesion at C-5′, dioxygen adds to the carbon-centered radical to form a peroxyl radical (**3**), which undergoes base release and carbonyl group formation at C-1′ (**4**).

Scheme I



The question arises as to whether 2-deoxyribonolactone is the only sugar product at the abasic site. Since cytosine release is a measure of abasic site formation, lactone formation and cytosine release should be equivalent. Yet, the former accounted for only about 60% of the latter. Since the quantitation of lactone formation involved a multistep procedure, including an enzymatic digestion, it is likely that loss of some lactone product occurred during the isolation. It is also possible that some small fraction of the cytosine released is not associated with abasic site formation but rather with attack at C-5′ with subsequent cleavage between C-4′ and C-5′ to form 3′-formyl phosphate ended DNA, sugar degradation products, and base (Chin et al., 1987). Finally, the peroxyl radical at C-1′ may, in addition to forming the lactone, cleave the sugar ring between C-1′ and C-2′ releasing the base and forming a fragment with an aldehyde group at C-2′ and intact phosphodiester linkages (von Sonntag, 1987).

Although direct strand breakage at the thymidylate residue [in the sequence d(GCT)] complementary to the deoxyadenylate residue in d(AGC) exceeds abasic site formation at the deoxycytidylate residue in d(AGC) (Kappen et al., 1988), all of the latter are accompanied by breaks at this same thymidylate (Povirk et al., 1989). It thus appears that in

forming abasic lesions the activated NCS chromophore functions as a bistranded reactive agent, generating closely (two nucleotides apart) opposed abasic and direct strand break lesions, almost directly across the minor groove of the DNA from each other. A diradical form of NCS chromophore lying in the minor groove might be expected to act as a bifunctional reagent, attacking C-1′ of deoxycytidylate in one strand and C-5′ of thymidylate two nucleotides to the 3′-side on the complementary strand. Accordingly, it seems likely that these two sites can be the sources of the two hydrogens incorporated into the bicyclic core of NCS chromophore in the DNA damage reaction. However, since not all breaks at thymidylate are accompanied by the abasic lesion, the second hydrogen may also possibly come from another, yet unidentified, site. It is also conceivable, however, that hydrogen atom abstraction by NCS chromophore from C-1′ of deoxycytidylate is as frequent as from C-5′ of thymidylate but that the carbon-centered radical formed at the former is more readily repaired than the latter by hydrogen atom donation by radical scavenging thiol.

In accord with earlier mentioned work, it is possible to propose a model for the interaction between NCS chromophore and d(AGC)·(GCT) in which the naphthoate moiety intercalates between the DNA base pairs so as to place the bicyclic core containing the diyne–ene structure in the minor groove, so that the activated diradical form can simultaneously attack the appropriate deoxyribose carbons of target nucleotides in each of the complementary strands. The structurally related antibiotic calicheamicin has also been shown to generate closely opposed lesions on the complementary DNA strands (Zein et al., 1988). The lesion on one strand appears to possess a nucleoside 5′-aldehdye at the 5′-end of a break; the opposing lesion remains to be characterized.

Acknowledgments

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Registry No. NCS, 9014-02-2; d(AGCGAGCG), 117919-04-7; 2-deoxyribonolactone, 34371-14-7; cytosine, 71-30-7.

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