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Covalent adducts of DNA and the nonprotein chromophore of neocarzinostatin contain a modified deoxyribose

(deoxyribose damage/lability of phosphodiester and *N*-glycosidic linkages/radiomimetic drug/endonuclease S1)

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ABSTRACT When the nonprotein chromophore of neocarzinostatin was allowed to react with either calf thymus DNA or poly(dA-dT)·poly(dA-dT) in the presence of 2-mercaptoethanol and the DNA was precipitated with ethanol, 5% of the fluorescence attributable to the naphthalene rings of the chromophore coprecipitated with the DNA. Most of this fluorescence remained attached to DNA through successive reprecipitations, suggesting formation of covalent adducts between chromophore and DNA. Enzymatically digested poly(dA-dT)·poly(dA-dT)-chromophore adduct contained, in addition to deoxyadenosine and thymidine, several highly fluorescent hydrophobic products, separable by reverse-phase chromatography, all of which contained both adenine and thymine radiolabel, as well as chromophore radiolabel. One such product consistently had twice as much thymine as adenine, suggesting a structure chromophore-d(TpApT), in which the attached chromophore rendered both phosphodiester bonds refractory to endonuclease S1. This adduct fragment was completely hydrolyzed at pH 12, releasing adenine, 3′-dTMP, and 5′-dTMP. At pH 7, the adduct fragment slowly released chromophore and 3′-dTMP with parallel kinetics, leaving a modified d(ApT), which was cleaved by snake venom phosphodiesterase to yield 5′-dTMP and a modified deoxyadenosine. These hydrolysis patterns are unlike those of any previously characterized base or phosphotriester DNA adduct but rather indicate an altered deoxyadenosine sugar. The formation of adducts containing a modified deoxyribose suggests that deoxyribose may be the site of covalent chromophore attachment. Alteration of this same site, possibly the 5′-carbon of the sugar moiety, may account for the extreme lability of the phosphodiester bond.

The DNA-degrading activity of the protein antibiotic neocarzinostatin (NCS) is attributable to the action of a nonprotein chromophore (M_r, 661) that is tightly but reversibly bound to the apoprotein ($K_d \approx 10^{-10}$ M) (1–4). The chemistry of DNA degradation by the chromophore is poorly understood, but the resulting DNA lesions (for review, see ref. 5)—i.e., strand breaks, released bases, fragmented sugars (6), and aldehyde-like functions (6)—are reminiscent of DNA sugar damage induced by x-rays (7). This similarity to x-ray damage, as well as the requirement for a reducing agent and molecular oxygen (8, 9), the high efficiency of the reaction at 0°C (1), the inhibition of the reaction by the free radical scavenger α-tocopherol (8), and the recent demonstration of ESR signals on drug activation (10, 11), suggest that the mechanism of DNA strand scission involves the generation of free radicals.

In this report, we describe an additional DNA lesion whose existence implies a direct chemical reaction between NCS chromophore and DNA: a covalent DNA-chromophore adduct. Furthermore, the chromophore appears to be attached to a modified nucleoside with increased lability of its phosphodiester and *N*-glycosidic linkages.

MATERIALS AND METHODS

Sonicated calf thymus DNA was prepared as described (4). Poly(dA-dT)·poly(dA-dT) from Sigma was used without further purification. [*methyl*-^{3}H]Thymine- and [8-^{3}H]adenine-labeled poly(dA-dT)·poly(dA-dT) were synthesized on an unlabeled primer with *Escherichica coli* DNA polymerase I and appropriately labeled nucleoside triphosphates as described (12). Oligonucleotide markers were from Collaborative Research.

NCS chromophore, 0.22 mM in 80% CH_3OH/20% H_2O/16 mM sodium citrate, pH 4, was extracted from dialyzed lyophilyzed clinical NCS as described (13) and stored at −70°C. NCS [*methyl*-^{3}H]chromophore (18 Ci/mol; 1 Ci = 3.7 × 10^{10} becquerels) was isolated from *Streptomyces carzinostaticus*, var. neocarzinostaticus, grown as described (14) but with addition of [*methyl*-^{3}H]methionine (20 μCi/ml; 1 Ci/mmol) to the production medium. Details of the isolation and characterization of the labeled chromophore will be published elsewhere.

Preparation of Adduct. Reaction mixtures (0.6 ml) were either 600 μM calf thymus DNA or 300 μM poly(dA-dT)·poly(dA-dT)/10 mM 2-mercaptoethanol/50 mM Tris·HCl, pH 8/20 μM NCS chromophore. Chromophore, in 50 μl of 80% methanol, was added last, and the mixture was incubated at 22°C for 15 min. To precipitate the DNA, 0.6 ml of 2 M NaOAc and 3 ml of EtOH were added, and the solution was chilled to −20°C for 10 min. DNA was pelleted by centrifugation at 8000 × g for 15 min, and the supernatant, containing chromophore not covalently bound to DNA, was discarded. The precipitate was washed with 70% EtOH, and the chilling and centrifugation were repeated to pellet any dislodged precipitate. Precipitated DNA was then dissolved in 0.6 ml of the desired buffer.

Enzymatic Digestion. Poly(dA-dT)·poly(dA-dT)-chromophore adduct was dissolved in 0.15 M NaOAc/5 mM EDTA, pH 5, and incubated with crude DNAse II (200 Kunitz units; Sigma; type V) at 37°C for 5 hr. $ZnCl_2$ was then added to 7 mM, along with 1.2 units of endonuclease S1 (Calbiochem) and incubation was continued for 16 hr. These enzyme preparations contained considerable phosphatase activity. Enzymatic hydrolysis of poly(dA-dT)·poly(dA-dT) was very nearly complete; 95% of the adenine label and 98% of the thymine label were recovered as the corresponding deoxynucleosides.

High-Pressure Liquid Chromatography. Enzyme digests and other samples in 0.6–1.1 ml of aqueous buffer were adjusted to pH ≈5 with 2 M HOAc or 2 M NaOAc, filtered through glass wool, and loaded onto an analytical Waters μBondapak C_{18} column preceded by a 2-cm guard column

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Abbreviations: NCS, neocarzinostatin; HPLC, high-pressure liquid chromatography.

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packed with Porasil C_{18}. Samples were eluted at 1 ml/min with a 20-min wash of aqueous buffer (5 mM NH_4OAc/5 mM HOAc, pH 4.75) followed by a linear or convex 2-hr gradient of 0–70% MeOH containing the same acetate buffer. Absorbance (254 nm) and fluorescence (excitation at 340 nm, emission ≥ 418 nm) were constantly monitored. Radioactivity in eluate fractions was assayed after addition of Scintiverse (Fisher). Separation of 3'- and 5'-dTMP was achieved by placing two C_{18} columns in series and eluting at 1 ml/min with aqueous buffer.

Treatment of Purified Adducts. Eluate fractions containing specific adduct peaks were frozen in liquid nitrogen and evaporated to dryness at reduced pressure, and the residues were dissolved in a small volume of 0.1 M NaOAc/HOAc, pH 4.8, and frozen. Aliquots of the purified adducts were added to solutions containing excess buffer at the desired pH, and chemical or enzymatic hydrolysis was carried out at 37°C. Where indicated, samples were adjusted to an apparent pH of 12 with 1 M NaOH. *Crotalus adamanteus* venom phosphodiesterase and *E. coli* alkaline phosphatase were from Sigma. Enzyme treatments were carried out in 55 mM Tris base/5 mM $MgCl_2$, pH 8.5.

After incubation, samples were adjusted to pH 5 and subjected to high-pressure liquid chromatography (HPLC). Samples treated with 1 M HCl were first evaporated to remove HCl and then redissolved.

Some samples were subjected to descending paper chromatography (15) using 95% ETOH/1 M NaOAc (7:3) and *n*-BuOH/aqueous 30% NH_3/H_2O (85:2:12) as solvents.

RESULTS

Formation of Chromophore-DNA Adducts. We had previously shown that both untreated and 2-mercaptoethanol-inactivated (8) NCS chromophore bind reversibly to DNA (4). Both forms of chromophore-DNA complexes were completely dissociated by EtOH precipitation of the DNA (ref. 13 and Fig. 1). However, when DNA was present during treatment of chromophore with 2-mercaptoethanol, 5% of the 420-nm fluorescence attributable to the naphthalene rings of the chromophore coprecipitated with the DNA (Fig. 1). About 20–30% of the fluorescence in the precipitate was lost on reprecipitation of the DNA, but the rest remained attached to DNA through repeated precipitations. Phenol extraction, $CHCl_3$ extraction, and extensive dialysis also failed to dissociate the DNA-chromophore complex. These results strongly suggested formation of covalent DNA-chromophore adducts.

Adduct formation, as judged by coprecipitation of chromophore fluorescence with DNA, occurred with unaltered efficiency in complete darkness but showed an absolute requirement for 2-mercaptoethanol. Furthermore, no adduct was formed when chromophore was incubated with 2-mercaptoethanol before addition of DNA (Fig. 1). Thus, DNA-chromophore adducts are not photoadducts but appear to result from sulfhydryl-initiated oxidation–reduction reactions of the NCS chromophore. All synthetic DNAs tested, including poly(dA-dT)·poly(dA-dT), poly dA·poly dT, and poly(dG-dC)·poly(dG-dC), formed chromophore adducts.

In experiments with [*methyl*-^{3}H]chromophore, only 2% of the radiolabel coprecipitated with DNA. Thus, the fluorescence assay, in which 5% of the fluorescence coprecipitated, appears to overestimate the formation of adduct. The presence of 1 M NaCl or ethidium bromide at 100 μg/ml in the reaction mixture inhibited formation of the [*methyl*-^{3}H]chromophore-DNA adduct 57% and 81%, respectively. Similar inhibition by NaCl (8) and ethidium bromide (16) of NCS-induced DNA strand breakage has been reported. These results suggest that reversible intercalative binding of chromophore to DNA (4, 13) precedes both adduct formation and strand breakage.

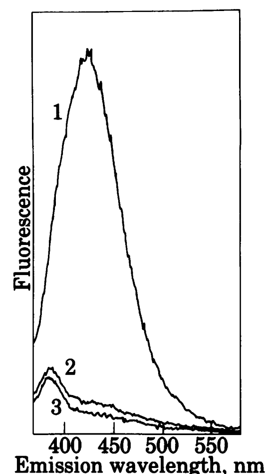

FIG. 1. Irreversible binding of NCS chromophore to DNA. Chromophore was allowed to react with calf thymus DNA in the presence of 2-mercaptoethanol. The DNA was then precipitated with EtOH, pelleted, and dissolved in 20 mM Tris base, pH 8, and its fluorescence spectrum (excitation, 340 nm) was recorded (spectrum 1). A second sample was treated identically, except that chromophore was incubated for 15 min in the presence of 2-mercaptoethanol before addition of DNA (spectrum 2). Both samples had 20 times the fluorescence of spectrum 1 in the initial reaction mixture. Spectrum 3, buffer only.

Enzymatic Digestion and Purification of Adducts. Isolation of low-molecular-weight compounds containing substituents from both DNA and putative adduct-forming agents provides much stronger evidence for adduct formation than simple persistent binding. For isolation of such compounds, enzymatic digestion of adduct-containing DNA (17) has been widely used. Since a large fraction of the NCS chromophore-DNA adduct was unstable at neutral and alkaline pH (data not shown), we chose enzymes with low pH optima. With crude DNAse II/nuclease S1 (both of which contained phosphatase activity), DNA was digested to the level of single nucleosides.

When enzymatic digests of either calf thymus DNA or *E. coli* [*methyl*-^{3}H]thymine-labeled DNA containing chromophore adducts were subjected to reverse-phase HPLC, the fluorescence chromatogram contained a forest of unresolvable peaks, eluting at 45–55% methanol (much later than unaltered nucleosides) and corresponding to a broad peak of [^{3}H]thymine radioactivity (not shown). In an attempt to reduce the number of possible adduct species, similar experiments were carried out with poly(dA-dT)·poly(dA-dT). In this case, the chromatogram contained several discrete fluorescent peaks. Experiments with [*methyl*-^{3}H]thymine- and [8-^{3}H]adenine-labeled poly(dA-dT)·poly(dA-dT) showed that every fluorescent peak contained both adenine and thymine (Fig. 2). In experiments with [*methyl*-^{3}H]chromophore (Fig. 3), the close coincidence of radioactivity and fluorescence profiles verified that fluorescence was attributable to the NCS chromophore. Most of the peaks (peaks 1 and 4 in particular) contained equal amounts of adenine and thymine radiolabel, suggesting that they consisted of endonuclease S1-resistant dinucleoside phosphates with a covalently attached chromophore. However, peak 5 consistently contained twice as much thymine as adenine, suggesting a structure chromophore-d(TpApT), in which the attached chromophore rendered both phosphodiester bonds refractory to endonuclease S1. Similarly, peak 7 had almost twice as much adenine as thymine, suggesting that it consisted primarily of an analogous chromophore-d(ApTpA) structure.

Hydrolysis of the Chromophore-d(TpApT) Adduct. In a preliminary experiment, [^{3}H]adenine-labeled adducts purified as in Fig. 2 were pooled and incubated for 16 hr at pH 12 at 37°C.

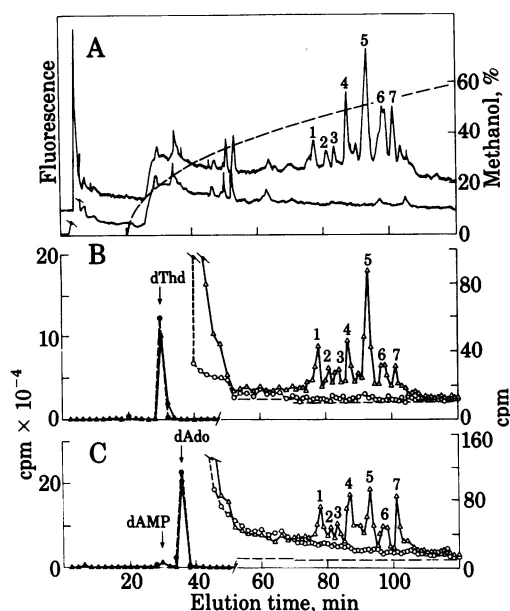

FIG. 2. Reverse-phase HPLC of endonuclease S1 digests of poly(dA-dT)·poly(dA-dT)-chromophore adducts. (*A* and *B*) Fluorescence (upper trace in *A*) and radioactivity (△) profiles of adduct formed with [*methyl*-^{3}H]thymine-labeled poly(dA-dT)·poly(dA-dT). Control (○ and lower trace in *A*), sample was treated identically, except that chromophore was omitted from the reaction mixture. (*C*) An identical experiment with [8-^{3}H]adenine-labeled poly(dA-dT)·poly(dA-dT). The fluorescence chromatograms for the two experiments were superimposible. The specific activity of the adenine label was exactly twice that of the thymine label. (*A*) ---, MeOH in elution solvent. The assay efficiency for 2-ml fractions (0-70 min) was 38% lower than that for 1-ml fractions (71–120 min). (*B* and *C*) ---, Background radioactivity.

The adducts were almost completely hydrolyzed, releasing mainly material that chromatographed as adenine and dAMP. However, when [^{3}H]adenine-labeled peak 5, tentatively identified as chromophore-d(TpApT), was hydrolyzed, ^{3}H label was found almost exclusively as free adenine (Fig. 4*D*). The identity

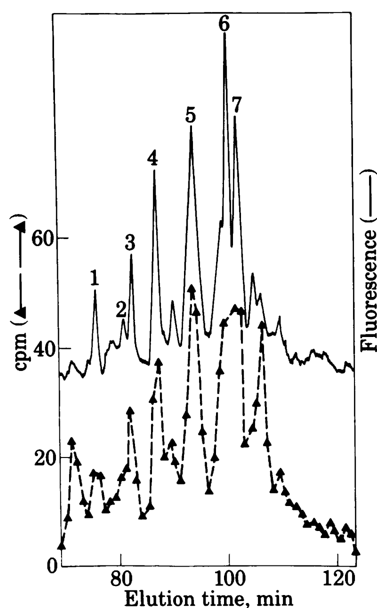

FIG. 3. Reverse-phase HPLC of endonuclease S1 digests of adducts formed between poly(dA-dT)·poly(dA-dT) and [*methyl*-^{3}H]chromophore. Adduct preparation, digestion, and chromatography were as in Fig. 1. Background (11 cpm) has been subtracted from the radioactivity profile.

of adenine was confirmed by paper chromatography with both the EtOH/NaOAc and the BuOH/NH_3 solvent systems; both systems completely resolved adenine from N^6-methyladenine, and the second system has been shown to resolve a variety of alkylated adenines (18).

[^{3}H]Thymine-labeled chromophore-d(TpApT) adduct was completely hydrolyzed at pH 12 to yield, almost exclusively, a 1:1 mixture of 3′-dTMP and 5′-dTMP (Fig. 4*A*). Little, if any, (<5%) hydrolysis of authentic d(TpApT) was seen under these conditions; furthermore, release of dTMP from chromophore-d(TpApT) at pH 12 was nearly complete after 1 hr at 22°C (not shown). dTMP cochromatographed with thymine on HPLC but was well separated by EtOH/NaOAc paper chromatography. All the thymine label from hydrolyzed chromophore-d(TpApT) was converted by bacterial alkaline phosphatase to a compound that chromatographed in both systems as thymidine (not shown). The same products—i.e., dTMP and adenine—were obtained when adenine- or thymine-labeled chromophore-d(TpApT) was hydrolyzed for 16 hr at 37°C in 1 M HCl.

Most of the chromophore-d(TpApT) adduct was also hydrolyzed by a 5-hr incubation at pH 8.5 (Fig. 4) or a 20-hr incubation at pH 7 (Fig. 5), releasing half of the thymine label as 3′-dTMP and half as a compound that also contained adenine label and eluted just ahead of authentic d(ApT) (Fig. 4 *B* and *E*). This compound appeared to be a slightly modified form of d(ApT), since it could be cleaved by snake venom phosphodiesterase to yield 5′-dTMP (Fig. 4*C*) and a compound, presumably a modified deoxyadenosine, that contained the majority of the adenine label and eluted just ahead of authentic deoxyadenosine (Fig. 4*F*). Both the modified d(ApT) and modified deoxyadenosine peaks were rather broad, suggesting some heterogeneity in structure. In addition, there appeared to be a hydrolysis-resistant fraction of adduct, since no further loss of thymine-labeled adduct was seen after 10 hr at pH 8.5. When [^{3}H]chromophore-labeled chromophore-d(TpApT) was hydrolyzed at pH 8.5, label appeared primarily in a compound that eluted even later than intact adduct; no chromophore label was found in the position of the modified d(ApT).

The time course of hydrolysis of [^{3}H]thymine-labeled chromophore-d(TpApT) at pH 7 (Fig. 5) showed that 3′-dTMP and modified d(ApT) were released from the intact adduct with identical kinetics. Since the modified d(ApT) contains no chromophore, it appears that release of chromophore, 3′-dTMP, and modified d(ApT) from the intact adduct occurs simultaneously.

DISCUSSION

The hydrolysis products of the isolated adduct (summarized in Fig. 6), particularly the release of 3′-dTMP and modified d(ApT), show that the proposed adduct structure chromophore-d(TpApT) is correct. In addition, the release of adenine and of both 3′- and 5′-dTMP by rather mild alkaline hydrolysis, and the spontaneous release of 3′-dTMP at pH 7, indicate that the *N*-glycosidic linkage and the phosphodiester linkages of the deoxyadenosine in the chromophore-d(TpApT) adduct have been rendered unusually labile. Previous studies of various DNA adducts (19) suggest that modifications of bases or phosphates would not be expected to result in such lability or pattern of product formation. Modifications of adenine may facilitate depurination and depurinated sites in the DNA backbone are alkali labile, but base modification would not be expected to make the 5′-deoxyribose-phosphate linkage susceptible to pH 7 hydrolysis, while the *N*-glycosidic bond remains intact. Similarly, modification of a phosphate (e.g., formation of an alkyl phosphotriester) might labilize a single phosphodiester bond but should not affect the stability of the adjacent phosphodiester and *N*-glycosidic bonds. Since our experimental conditions re-

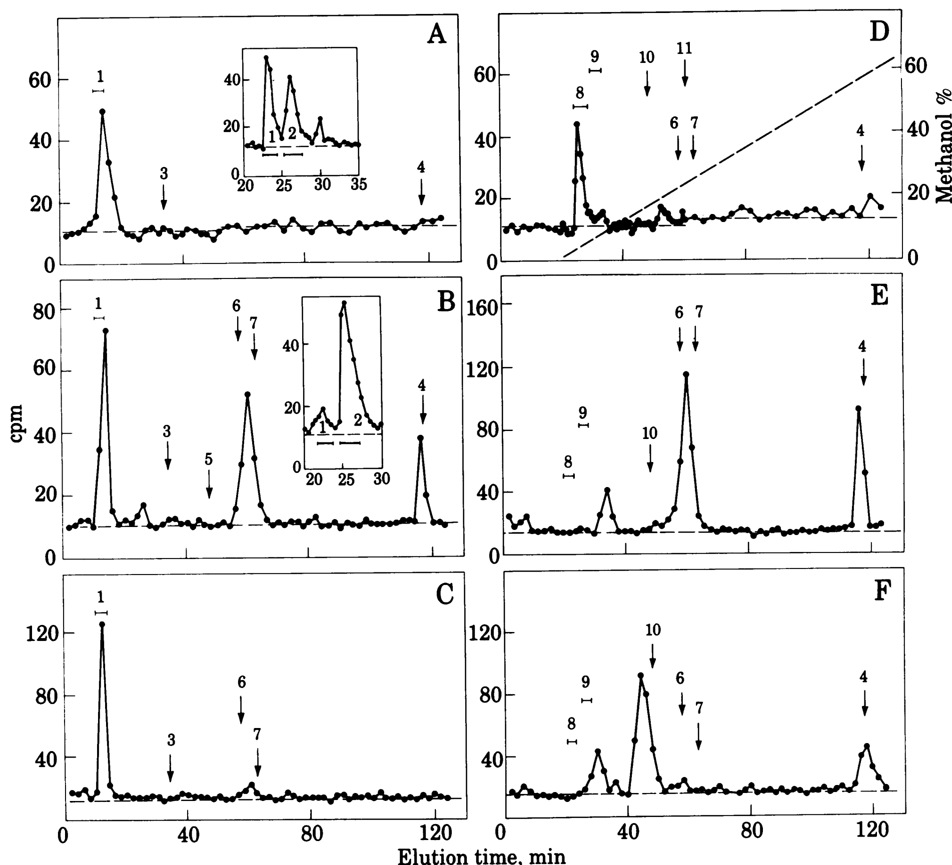

FIG. 4. Hydrolysis of chromophore-d(TpApT) adduct (peak 5 in Fig. 2) and analysis of products by reverse-phase HPLC. (*A*) Thymine-labeled adduct incubated for 16 hr at pH 12. (*Inset*) An identical sample chromatographed on two columns in series to separate 5'-dTMP and 3'-dTMP. (*B*) Thymine-labeled adduct incubated for 5 hr in 55 mM Tris base/5 mM $MgCl_2$, pH 8.5. (*Inset*) Material eluting as dTMP was recovered and rechromatographed. (*C*) Material eluting near d(ApT) (55–63 min) in *B* was recovered and treated for 5 hr with venom phosphodiesterase at 0.03 units/ml. (*D*) Adenine-labeled adduct hydrolyzed for 16 hr at pH 12. (*E*) Adenine-labeled adduct incubated for 5 hr at pH 8.5. (*F*) Same as *E*, except venom phosphodiesterase at 0.03 units/ml was added. – – –, Background radioactivity. (*D*)———, MeOH gradient (0–70% in 2 hr). Markers: 1, 5'-dTMP; 2, 3'-dTMP; 3, thymidine; 4, adduct; 5, d(pApT); 6, d(TpA); 7, d(ApT); 8, adenine; 9, dAMP; 10, deoxyadenosine; 11, d(TpApT).

sult in only one adduct per thousand nucleotides, adducts on adjacent phosphates are extremely unlikely. Furthermore, the expected products of phosphotriester hydrolysis (i.e., alkyl-nucleoside phosphodiesters and unaltered nucleosides) are not found after hydrolysis of chromophore-d(TpApT).

On the other hand, sites of deoxyribose damage in DNA are known to hydrolyze in a manner similar to that found for the chromophore-d(TpApT) adduct. The closest precedent for such hydrolysis is seen in γ-irradiated DNA, where free radical-induced sugar damage leads to release of free base and an oxidized sugar, leaving both 3' and 5' phosphate termini on the DNA (20). This process does not require alkali. However, other forms of oxidized deoxyribose in DNA have been shown to release either 3'- or 5'-phosphate groups as well as free base via alkali-catalyzed β-elimination reactions (21). Evidence for oxidation

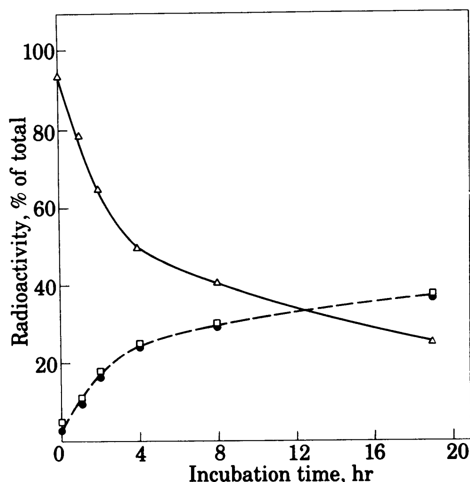

FIG. 5. Kinetics of chromophore-d(TpApT) hydrolysis. Thymine-labeled adduct was incubated in 70 mM sodium phosphate, pH 7, at 37°C for various times and subjected to HPLC. △, Fraction of radioactivity eluting as intact adduct; (□), 3'-dTMP; ●, modified d(ApT). At pH 8 (not shown), 50% hydrolysis was seen at 30 min.

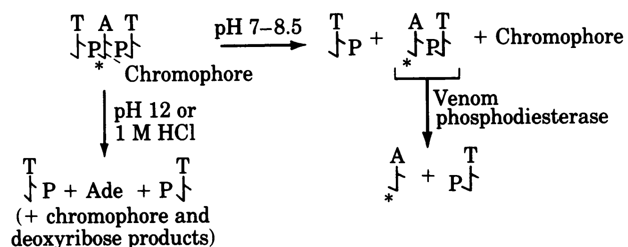

FIG. 6. Adduct hydrolysis patterns. Incubation of intact adduct at pH 12 or in 1 M HCl releases adenine as well as both 3'- and 5'-thymidine monophosphates. Incubation at pH 7 or pH 8.5 releases, simultaneously, chromophore, 3'-dTMP, and a modified (*) d(ApT) that is a substrate for venom phosphodiesterase. The adduct has been drawn with the chromophore attached to the deoxyadenosine sugar, although the evidence for such a linkage is not conclusive.

of DNA sugars by NCS (i.e., release of [^{3}H]formic acid from NCS-treated [5'-^{3}H]thymidine-labeled DNA) has been reported (6). Preliminary experiments indicate that the altered deoxyadenosine obtained after venom diesterase treatment of modified d(ApT) is probably deoxyadenosine 5'-aldehyde, since its properties are similar to those of the altered nucleoside found at the 5' end of breaks in drug-treated DNA and identified as thymidine 5'-aldehyde (22). Thus, it appears likely that the chromophore-d(TpApT) adduct contains an oxidized deoxyadenosine sugar, and it is reasonable to consider that the deoxyribose of deoxyadenosine may be the site of covalent chromophore attachment, perhaps as a 5'-phosphoryl acetal. Several properties of the adduct tend to support this possibility. First, the surprising resistance of chromophore-d(TpApT) to endonuclease S1 digestion is most easily explained by an adduct on the deoxyadenosine sugar, as this is the one site where an adduct might impart sufficient steric hindrance to protect both phosphodiester bonds. Even very large DNA base adducts, such as adenine-(2-acetamidophenanthrene) do not inhibit endonuclease S1 action but are recovered from digests as single nucleosides (23). Second, the simultaneous release of chromophore and 3'-dTMP from the adduct suggests that both are attached to the same DNA substituent—i.e., the deoxyadenosine sugar and possibly its 5'-carbon. Third, all DNA substituents except the deoxyadenosine sugar are always recovered in apparently unaltered form, once they are removed from the intact adduct. Since we have not been able to isolate a chromophore-deoxyribose adduct from which all other DNA substituents have been removed, we cannot exclude the possibility that the chromophore damages deoxyribose but concommitantly forms an adduct with DNA bases or phosphates. However, as noted above, such adducts would not by themselves account for the lability of all three linkages to the deoxyadenosine sugar.

Formation of covalent adducts conclusively shows direct chemical reaction between NCS chromophore and DNA. The sequence specificity of NCS-induced DNA cleavage (5) and the reversible intercalative binding of chromophore to DNA ($K_d \approx 5$ μM) (13) also provide evidence for such direct interaction. Furthermore, despite evidence for involvement of radicals, metal chelators and scavengers of the diffusible radical forms of reduced oxygen fail to inhibit NCS-induced DNA degradation (8). All these results, combined with recent evidence for a free radical form of the chromophore itself (10, 11), suggest that such a chromophore radical, rather than diffusible forms of superoxide or hydroxyl radicals, may be the ultimate agent of DNA damage. Indeed, it is difficult to explain formation of adducts with a molecule as unreactive as deoxyribose other than by stereospecific radical attack. Nevertheless, we still cannot exclude a locally generated highly sequestered reduced form of molecular oxygen as the species that initially reacts with deoxyribose. In either case, a subsequent addition reaction between a deoxyribose radical and a double bond of the highly unsaturated centerpiece of the chromophore (2, 3) seems a plausible mechanism of adduct formation.

The chromophore-d(TpApT) adduct, as well as several other chromophore adduct species (results not shown), contains phosphodiester bonds that are stable at neutral pH but labile to alkali. Thus, some of the alkali-labile bonds in NCS-treated DNA may be attributable to sites of adduct formation. Various DNA-chromophore adducts show a wide range of stabilities, and the hydrolysis of chromophore-d(TpApT) at pH 7 suggests that either this form of adduct or another even more labile adduct species may be a normal intermediate in the strand-breakage reaction. More detailed studies of the kinetics of adduct formation and of their stability in intact DNA (i.e., before enzymatic digestion) will be required to determine whether the majority of strand breaks are formed via adduct intermediates.

The biological significance of DNA-chromophore adducts is uncertain. It seems likely that adducts may be responsible for NCS-induced mutagenesis (24, 25). Quantitatively, the adduct is a minor lesion; only 1 in 50 chromophore molecules in a reaction mixture forms a DNA adduct under optimal conditions, whereas the yield of strand breaks can be as high as one per 5 chromophore molecules (13). However, DNA-chromophore adducts may be much more difficult to repair than simple strand breaks, particularly if they are as resistant to repair endo- and exonucleases as they are to endonuclease S1.

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