Oxidation of Thymine to 5-Formyluracil in DNA: Mechanisms of Formation, Structural Implications, and Base Excision by Human Cell Free Extracts†

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ABSTRACT: Oxidative agents produce several different types of base modifications in DNA, and only a few of these have been properly characterized with respect to mechanisms of formation and biological implications. We have established a procedure using neutral thermal hydrolysis and reverse phase high-performance liquid chromatography to determine the content of the oxidation product 5-formyluracil (5-foU) in DNA. With this method, it is shown that 5-foU residues are formed with high frequency from thymine by quinone-sensitized UV-A photooxidation. Since 5-foU is also induced by ionizing radiation, it appears to be formed under conditions where thymidine radical cations are generated and react with molecular oxygen. It was previously shown that 5-foU is formed directly from [*methyl*-^{3}H]thymine residues in radioactively labeled DNA by two consecutive transmutations of ^{3}H to ^{3}He. The theoretical basis for the kinetics of such conversion is presented in this paper, and the calculated yields are confirmed experimentally by measuring the content of 5-foU in [*methyl*-^{3}H]thymine-labeled DNA aged for different time periods. Such DNA contains virtually only 5-(hydroxymethyl)uracil and 5-foU, apart from normal bases, and is therefore very useful for the investigation of repair enzyme activities involved in the repair of 5-foU-containing DNA. Using this substrate, a DNA glycosylase activity was identified in human cell extracts for the removal of 5-foU. Molecular modeling of DNA containing 5-foU suggests that the formyl group will be kept in plane with the pyrimidine ring because of electrostatic interactions between formyl O and H^6 and formyl H and O^4, with a potential weakening effect on the base-pairing properties of 5-foU relative to thymine.

Guanine oxidized in the 8′ position (8-oxo-7,8-dihydroguanine; 8-oxoG)[1] is considered a principal lesion responsible for the formation of mutations induced by oxidative stress (Wood et al., 1990; Shibutani et al., 1991; Cheng et al., 1992; Lindahl, 1993), and several repair systems counteracting the damaging effects of 8-oxoG have been identified in *Escherichia coli* and mammalian cells (Tchou et al., 1991; Boiteux et al., 1992). However, active oxygen species will produce a multitude of different nucleic acid base modifications (Friedberg et al., 1995; von Sonntag, 1987; Imlay & Linn, 1988; Teebor et al., 1988), and other oxidized bases, e.g. 5-hydroxypyrimidines, will also be formed in significant amounts and have important biological effects (Aruoma et al., 1989; Wagner et al., 1992; Purmal et al., 1994). Recently, 5-formyluracil (5-foU) was identified as a novel type of oxidized thymine lesion in DNA (Kasai et al., 1990; Bjelland et al., 1994). It is a major product induced by ionizing radiation and is formed in yields similar to those of 8-oxoG (Kasai et al., 1990). A potential mutagenic or replication-inhibiting effect of 5-foU was suggested by the finding that 5-foU in DNA is subject to base excision repair in *E. coli* (Bjelland et al., 1994).

In this paper, we describe a method for quantification of 5-foU in DNA and show that 5-foU is a major product induced by quinone-sensitized UV-A photooxidation. It is further shown that protein extracts from human cells contain a DNA glycosylase activity for the removal of 5-foU from DNA. Computer modeling suggests that replacement of the methyl group of thymine with the formyl group might interfere with base pairing to adenine.

MATERIALS AND METHODS

Materials and Reference Compounds. E. coli [*methyl*-^{3}H]thymine-labeled DNA was obtained from DuPont NEN (NET-561). Radioactivity released from the DNA during

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[1] Abbreviations: 5-foU, 5-formyluracil; 5-hmU, 5-(hydroxymethyl)-uracil; HPLC, high performance liquid chromatography; Mops, 3-(*N*-morpholino)propanesulfonic acid; 8-oxoG, 8-oxo-7,8-dihydroguanine.

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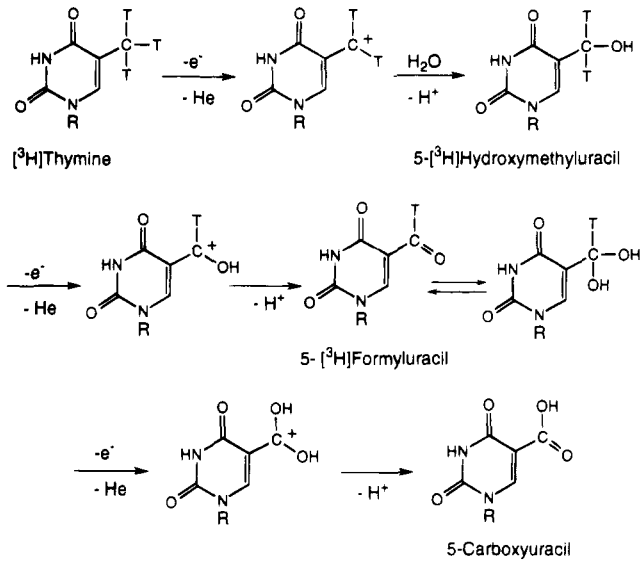

FIGURE 1: Proposed mechanism of formation of methyl-oxidized derivatives of thymine in [*methyl*-^{3}H]thymine-labeled DNA as a result of the transmutation of ^{3}H to ^{3}He. T, tritium; R, DNA chain.

storage was removed by ethanol precipitation and several washes of the pellet with 70% ethanol. ColE1 [6-^{3}H]-thymine-labeled DNA with a specific activity of 200 000 dpm/μg was prepared by incorporation of [6-^{3}H]thymidine from Amersham Corp. (TRK. 61) as described (Seeberg, 1978). *E. coli* [2-^{14}C]thymine-labeled DNA was obtained from Amersham Corp. (CFB. 170). 5-foU was prepared by oxidation of 5-(hydroxymethyl)uracil (5-hmU) (Brossmer & Ziegler, 1966; Bjelland et al., 1994). Thymine and 5-hmU were obtained from Sigma. Calf thymus uracil DNA glycosylase was a gift from Drs. H. Krokan and P. H. Guddal. Human alkyl base DNA glycosylase (Anpg) was kindly provided by Dr. T. O'Connor.

Quantification of 5-foU in DNA by Neutral Thermal Hydrolysis and HPLC. Complete hydrolytic removal of 5-foU was achieved by incubation of the DNA in 10 mM sodium cacodylate, pH 7, or 10 mM potassium phosphate, pH 7 (100 μL), at 100 °C for 5 h (see Results). For quantification, the DNA was precipitated with ethanol and the supernatants were concentrated by evaporation and subjected to HPLC together with marker compounds as described (Bjelland et al., 1994).

Theoretically Estimated Amounts of Methyl-Oxidized Forms of Thymine in [methyl-^{3}H]Thymine-Labeled DNA as a Function of Time. When the thymine methyl group in [*methyl*-^{3}H]thymine-labeled DNA contains three tritium atoms, these will decay one by one, yielding the following DNA base residues in aqueous solution (Figure 1),

$$\overset{\lceil A \rceil}{RCT_3} \rightarrow \overset{\lceil B \rceil}{RCT_2OH} \rightarrow \overset{\lceil C \rceil}{RCT{=}O} \rightarrow \overset{\lceil D \rceil}{RCOOH}$$

where A is [*methyl*-^{3}H]thymine, B is 5-[*hydroxymethyl*-^{3}H]-hmU, and C is 5-[*formyl*-^{3}H]foU. Decay of the last tritium in C yields an unidentified residue D whose label is lost and therefore cannot be detected radiochemically. However, D is tentatively identified as 5-carboxyuracil (Figure 1).

The mole fractions at time t for all entities from A to D are called $X_A(t)$ to $X_D(t)$, normalized so that $X_A(t) + X_B(t) + X_C(t) + X_D(t) = 1$. The time rate of change for each product $X_A'(t)$ to $X_D'(t)$ is determined by the rate of their formation

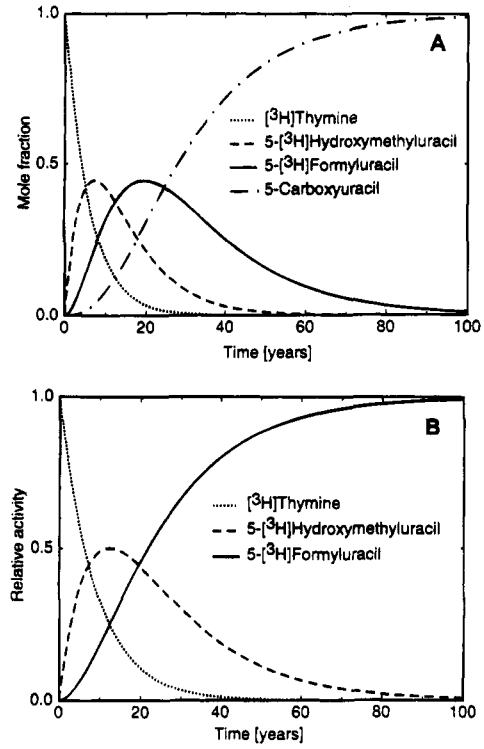

FIGURE 2: Kinetics of formation of methyl-oxidized thymine derivatives in [*methyl*-^{3}H]thymine-labeled DNA. The different functions follow from the proposed model presented in Figure 1. (A) Mole fraction of the different species formed as a function of time. (B) Relative radioactivity contributed by each species formed.

and breakdown, which, for each one, is determined by the rate of tritium decay as follows:

$$X_A'(t) = -3\lambda X_A(t) \quad (1)$$

$$X_B'(t) = 3\lambda X_A(t) - 2\lambda X_B(t) \quad (2)$$

$$X_C'(t) = 2\lambda X_B(t) - \lambda X_C(t) \quad (3)$$

$$X_D'(t) = \lambda X_C(t) \quad (4)$$

where λ is the decay constant of tritium. $\lambda = \ln 2/t_{1/2} = 0.056\,516$ years^{-1} when the half-life of tritium $t_{1/2}$ is 12.262 years. The initial condition is

$$X_A(t = 0) = 1$$

The solution of the set of differential equations 1−4 is

$$X_A(t) = e^{-3\lambda t} \quad (5)$$

$$X_B(t) = 3(e^{-2\lambda t} - e^{-3\lambda t}) \quad (6)$$

$$X_C(t) = 3(e^{-3\lambda t} - 2e^{-2\lambda t} + e^{-\lambda t}) \quad (7)$$

$$X_D(t) = 1 - e^{-3\lambda t} + 3e^{-2\lambda t} - 3e^{-\lambda t} \quad (8)$$

The behavior of eqs 5−8, i.e., mole fraction of the different species as a function of time, is shown in Figure 2A. To compare the mathematical solution with the observed activity of 5-foU (normalized activity), we calculate the corresponding relative activities $a_i(t)$,

$$a_i(t) = n_i X_i(t)/S; \quad i = A, B, \text{ or } C$$

where

$$S = \sum_i n_i X_i(t); \quad i = A, B, \text{ or } C$$

is the normalization and n_i is the number of ^3H per molecule. Since there are originally three tritium atoms in each [*methyl*-^{3}H]thymine residue, it follows that

$$S = 3e^{-\lambda t}$$

and finally that

$$a_C(t) = e^{-2\lambda t} - 2e^{-\lambda t} + 1 \quad (9)$$

for 5-foU,

$$a_A(t) = e^{-2\lambda t} \quad (10)$$

for thymine, and

$$a_B(t) = 2(e^{-\lambda t} - e^{-2\lambda t}) \quad (11)$$

for 5-hmU, giving the relative radioactivity contributed by each species as a function of time. Equations 9−11 are visualized in Figure 2B.

Photooxidation of DNA. DNA samples (*E. coli* DNA or *Eco*RI-linearized ColE1 DNA) were denatured by being heated to 100 °C for 5 min, followed by rapid cooling on ice, and treated similarly as previously described for the photooxidation of thymidine (Decarroz et al., 1986). A 1 mL solution of DNA and menadione (0.5 mM; Sigma, M-5625) in 1 mM potassium phosphate, pH 7.25, was treated with O_2 for 15 min, followed by irradiation under constant oxygen bubbling for 40 min with a 360 nm emitting lamp (Philips E/70/2 HPW 125 W HO) placed 15 cm from the solution (20 J/m^{2}s). The DNA was subsequently precipitated with ethanol, followed by three washes with 1 mL of 70% ethanol.

Computer-Assisted Molecular Modeling. Version 22 of the CHARMM program (Brooks et al., 1983), with a preliminary version of the CHARMM22 all-atom potential function for nucleic acids, was used for all calculations.

Isolation of Human Mononuclear Peripheral Blood Cells and Preparation of Cell Extracts. Mononuclear human blood cells were isolated by Isopaque-Ficoll density gradient centrifugation as described (Bøyum et al., 1991). Donor blood from healthy individuals was used. The mononuclear cell fraction contained 75−85% lymphocytes and 15−25% monocytes, which gives 60−70% monocyte-derived protein in the final extract. The separated cells were washed twice in 0.9% NaCl, concentrated by low-speed centrifugation (as prepared from 40 mL of blood; 5 × 10^7 cells), resuspended by vortex mixing without addition of buffer, and placed on ice. The cells were plasmolyzed by addition of 250 μL of 84% (w/v) sucrose (dissolved in 40 mM Tris-HCl, pH 8/10 mM EGTA). After incubation on ice for 5 min, 1 mL of 50 mM Mops, pH 7.5/100 mM KCl/1 mM EDTA/1 mM dithiothreitol was added, and the cells were lysed by freezing in liquid nitrogen, followed by gentle thawing at ice temperature repeated three times. Cell debris was removed by high-speed centrifugation in a microcentrifuge. Protein concentration was determined by the method of Bradford (1976) with lysozyme as standard. The protein extracts contained 2−6 mg of protein/mL. Heat inactivation was performed by incubation at 100 °C for 40 min.

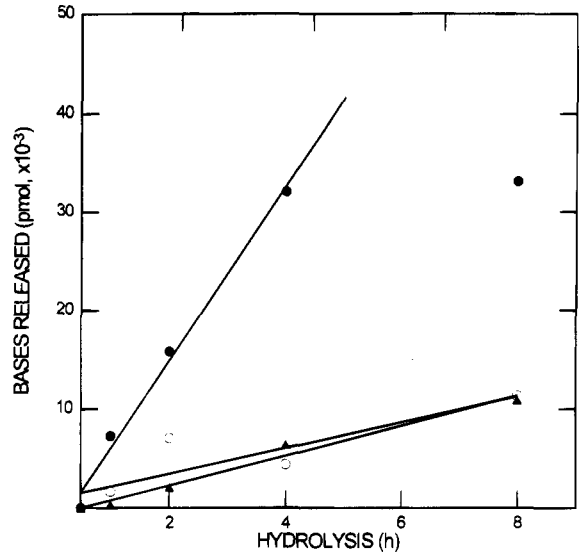

FIGURE 3: Release of 5-foU, 5-hmU, and thymine from [*methyl*-^{3}H]thymine-labeled DNA by heating at 100 °C, pH 7, as a function of time. Amount of material released by each species represents the average of two HPLC analyses. ●, 5-foU; ○, 5-hmU; ▲, thymine.

Enzymatic Assays for 5-foU DNA Glycosylase Activity. Substrate DNA was incubated with protein extract/enzyme in 100 μL of 70 mM Mops, pH 7.5/1 mM EDTA/1 mM dithiothreitol/5% (v/v) glycerol (reaction buffer) at 37 °C, followed by precipitation with ethanol as in the alkyl base DNA glycosylase assay (Bjelland & Seeberg, 1987). HPLC analysis of thymine-derived oxidation products was performed as indicated according to methods previously described (Bjelland et al., 1994). Enzymatic release of ethanol-soluble radioactivity from [*methyl*-^{3}H]thymine-labeled DNA was measured essentially as described for the alkyl base DNA glycosylase assay (Bjelland & Seeberg, 1987).

RESULTS

Kinetics of 5-foU Formation in [methyl-^{3}H]Thymine-Labeled DNA. 5-foU has previously been identified as a major product accumulating in [*methyl*-^{3}H]thymine-labeled DNA due to two consecutive transmutations of ^{3}H to ^{3}He with 5-hmU as an intermediate after the first transmutation (Teebor et al., 1984; Bjelland et al., 1994; Figure 1). The 5-foU residue is still radioactively labeled due to the third remaining ^{3}H in the methyl group.

Due to the electron-withdrawing properties of the formyl group, the N-glycosylic bond of 5-formyldeoxyuridine is labilized compared to those of thymidine and 5-(hydroxymethyl)deoxyuridine (Hansch et al., 1991). Accordingly, heating of aged [*methyl*-^{3}H]thymine-labeled DNA for an increasing period of time at 100 °C, pH 7, yielded progressive release of 5-foU (Figure 3). After 4 h, 5-foU was quantitatively removed as opposed to thymine or 5-hmU, which was released much more slowly with time. Hydrolysis for 5 h at 100 °C and pH 7 followed by ethanol precipitation and reverse phase HPLC was therefore adopted as standard conditions for quantification of 5-foU residues in DNA (Figure 4). Previously, neutral thermal hydrolysis has been employed for quantification of alkylated residues such as 3-methyladenine and 7-methylguanine in DNA (Frei et al., 1978; Beranek et al., 1980).

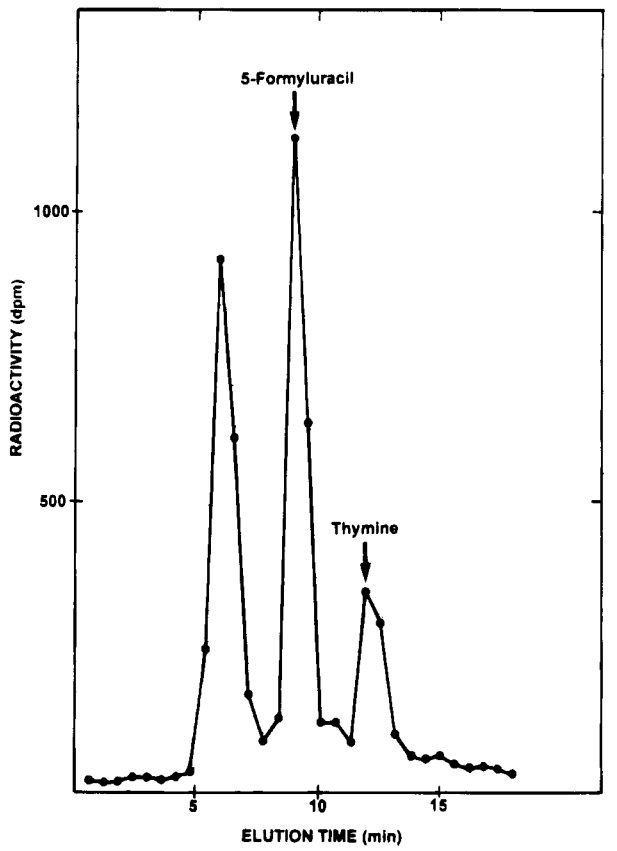

FIGURE 4: Reverse phase HPLC of thymine-derived oxidation products released from quinone/UV-A-photooxidized DNA by neutral thermal hydrolysis. Denatured [6-^{3}H]thymine-labeled ColE1 DNA (1 700 000 dpm) was irradiated with UV-A light (360 nm) in the presence of 2-methyl-1,4-naphthoquinone (menadione) and O_2. Then an aliquot material (95 000 dpm) was subjected to 100 °C for 5 h in 100 μL of 10 mM potassium phosphate, pH 7, followed by DNA precipitation with ethanol. The supernatant was concentrated by lyophilization, dissolved in water with marker compounds added in a total volume of 20 μL, and subjected to HPLC on a Spheri-5 RP-18 column eluted with 97–90% 0.1 M triethylammonium acetate, pH 5.4, in methanol (v/v) (0.5 mL/min; 0.6 min per fraction). The large peak eluting ahead of 5-foU represents some radioactive DNA that escaped precipitation.

On the basis of the proposed mechanism of 5-foU formation (Figure 1), equations were derived to describe the kinetics of formation of 5-foU, and 5-hmU, and the decline in the amount of radiolabeled thymines in the [*methyl*-^{3}H]-thymine-labeled DNA (see Materials and Methods). To test the yields derived from the theoretical calculation, we measured the 5-foU content in 1.95-, 2.47-, and 4.22-year-old batches of such DNA. The values obtained after thermal hydrolysis and HPLC were 0.8, 1.6, and 4.7%, respectively, in terms of radioactivity released as 5-foU which compare to the 1.1, 1.7, and 4.5% values predicted by the calculation (eq 9), thus supporting the transmutation mechanism proposed (Figure 1). Equations 7 and 9 may thus be used to calculate the approximate mole fraction and radioactivity, respectively, of 5-foU in the DNA. The model is based on replacement of each hydrogen by tritium within the methyl group. This assumes a specific activity of 90 Ci/mmol for the thymine residues. The *E. coli* [^{3}H]DNA used in our experiments had a specific activity of 85 Ci/mmol, implying that nearly all thymines are triply tagged.

Induction of 5-foU in DNA by UV-A Photooxidation. Recently, 5-foU was shown to be formed in the DNA by the Fenton-type reaction as well as being induced as a major product following exposure to ionizing radiation (Kasai et al., 1990). Since near ultraviolet photolysis at 350 nm of an aerated aqueous solution of thymidine containing 2-methyl-1,4-naphthoquinone (menadione) had been reported to give rise to 5-formyldeoxyuridine as the most abundant oxidation product (Decarroz et al., 1986), we investigated whether such conditions also might produce 5-foU in DNA. Samples of plasmid DNA, either native or denatured, were subjected to treatment with menadione and UV-A. The DNA was labeled by tritium in the 6′ position of thymine to exclude any formation of 5-foU by the tritium transmutation mechanism (Figure 1). Since the AlkA DNA glycosylase of *E. coli* effectively excises 5-foU from DNA (Bjelland et al., 1994), this enzyme was used as a probe together with HPLC to detect 5-foU formation. Formation of 5-foU was detected in the DNA which was denatured prior to exposure, whereas no detectable 5-foU was induced in the quinone/UV-A-exposed native DNA or in denatured DNA treated with quinone/O_2 in the dark. When the quinone/UV-A-exposed denatured DNA was subjected to neutral thermal hydrolysis for quantitative release of 5-foU, it was observed that 14.1% of the radioactivity recovered after HPLC eluted as 5-foU (Figure 4). It can be calculated that approximately 3% of the thymines is converted to 5-foU by the quinone/UV-A oxidative treatment. A similar amount of 5-foU was also induced in denatured [2-^{14}C]thymine-labeled DNA, as demonstrated by neutral thermal hydrolysis and HPLC (data not shown).

Base Excision of 5-foU by Human Cell Free Extracts. Monocytes generate oxygen radicals through the respiratory burst and are thus probably more heavily exposed to active oxygen species than most cells (Halliwell & Gutteridge, 1989). Accordingly, a blood cell fraction enriched for monocytes was chosen as the cell source to assay for the presence of 5-foU-releasing activity in human cells. Incubation of aged [*methyl*-^{3}H]thymine-labeled DNA with cell free extracts from such cells resulted in efficient release of 5-foU, where no release was observed with buffer alone or with heat-inactivated extract (Figure 5A,B). Analysis of enzyme dependence of this reaction showed that 7% of the 5-foU in the substrate (1.8 fmol) was excised by 1 μg of protein extract after 2 h of incubation, which gives a maximum release of 5-foU of 0.014 fmol min^{-1}(μg of protein)$^{-1}$ (Figure 5C). Release of 5-foU was also effected by the cell extract on DNA damaged by UV-A photooxidation, although 100 times more extract was needed for similar amounts of 5-foU excision, which can be ascribed to lower enzymatic efficiency on denatured DNA (data not shown).

Since 5-foU DNA glycosylase activity is a function of the AlkA enzyme in *E. coli* (Bjelland et al., 1994), it was of interest to investigate whether the 5-foU DNA glycosylase function might be an activity of the alkyl base DNA glycosylase enzyme in human cells (Anpg; Chakravarti et al., 1991; Samson et al., 1991; O'Connor, 1993). However, incubations of aged [*methyl*-^{3}H]thymine-labeled DNA with purified Anpg did not result in any detectable release of ethanol-soluble radioactivity or 5-foU as analyzed by HPLC (data not shown). Uracil DNA glycosylase from calf thymus also failed to release 5-foU from DNA (data not shown).

Base-Pairing Characteristics of 5-foU. To evaluate possible structural implications of having 5-foU residues in DNA, energy minimization and molecular dynamics simulations of a modified B-DNA were done (Brooks et al., 1983). The results obtained indicate that the 5-formyl group tends

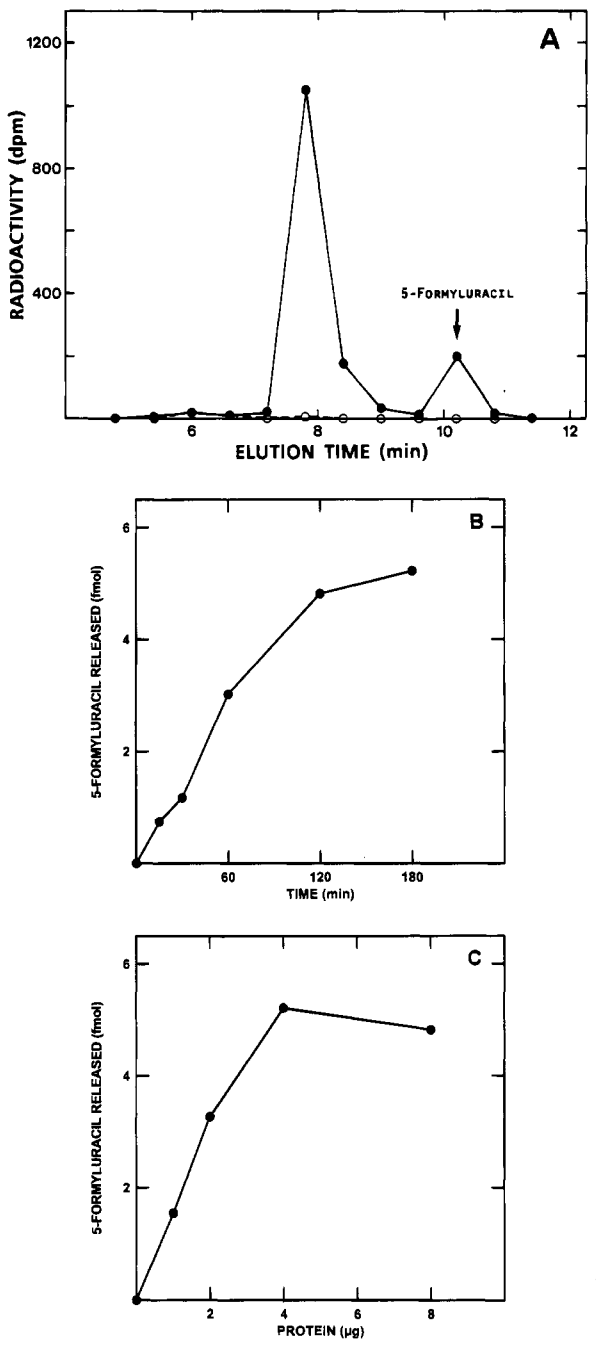

FIGURE 5: Release of 5-foU from DNA by extracts from human mononuclear leucocytes. (A) Cell free extract (80 μg of protein) was incubated with 9-year-old [*methyl*-^{3}H]thymine-labeled ColE1 DNA for 3 h at 37 °C (100 μL of reaction buffer, 15 000 dpm, 0.4 μg). The DNA was precipitated with ethanol, and the supernatant was subjected to reverse phase HPLC as described in the legend to Figure 4; incubation was with active (●) and with heat-inactivated (○) extract. The large peak eluting ahead of 5-foU represents 5-hmU as well as some thymine glycols and 5-hydroxy-5-methylhydantoin (Bjelland et al., 1994). (B) Kinetics of 5-foU excision from aged [*methyl*-^{3}H]thymine-labeled *E. coli* DNA (100 000 dpm, 30 ng), in which 1.64% of the radioactivity was in the form of 5-foU, as determined by HPLC analysis (ph 7.5, 0.5 min per fraction) using 8 μg of protein. (C) Protein dependence of 5-foU excision after 2 h incubation using the same conditions as in part B. The total amount of 5-foU in 30 ng of DNA was 25 fmol. Background values obtained from incubations without enzyme were subtracted, where the spontaneous 5-foU release was estimated to be 0.32 fmol/h.

to stay in the plane of the aromatic ring with the formyl oxygen adjacent to the hydrogen in the 6′ position of the pyrimidine ring and the formyl hydrogen adjacent to the oxygen in the 4′ position of the pyrimidine ring (Figure 6).

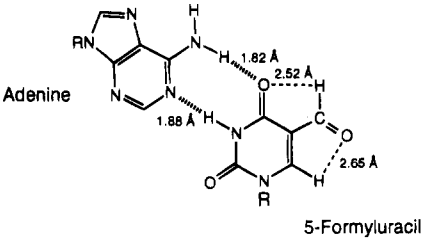

FIGURE 6: Electrostatic interactions of atoms in the formyl group with adjacent atoms in a 5-foU residue in DNA. Energy minimization of a defined double-stranded DNA sequence (the Dickerson oligomer; Drew et al., 1981) *in vacuo* having one of its thymines replaced by 5-foU was conducted by computer-assisted molecular modeling (Brooks et al., 1983).

The replacement of the thymine methyl group with the more polar formyl group may lead to an interference of base pairing to adenine, thus suggesting the possibility of mutagenicity caused by 5-foU residues in DNA.

DISCUSSION

It can be predicted that 5-foU will be formed in DNA by •OH-mediated abstraction of a hydrogen from the methyl group of thymine followed by the addition of oxygen (Teebor et al., 1988). Evidence for such a reaction occurring with the free base and the nucleoside was presented several years ago, when 5-foU and 5-formyldeoxyuridine were produced by UV-B and γ-irradiation of solutions of thymine and thymidine, respectively (Alcántara & Wang, 1965; Cadet & Téoule, 1975a,b). Recently, Kasai et al. (1990) identified 5-foU as one of three major UV-absorbing products formed by γ-irradiation of DNA, the two others being 8-oxoG and 5-hmU. The Fenton reaction also produced 5-foU in DNA (Kasai et al., 1990). Here we show that 5-foU is induced in DNA by quinone-sensitized photooxidation (Figure 4). UV-A light excites menadione and other quinones to a high-energy state (triplet quinone), which is able to abstract electrons (Figure 7; Wagner et al., 1990). Abstraction of an electron from thymidine by triplet quinone yields the thymidine radical cation, which in the presence of oxygen subsequently yields 5-formyldeoxyuridine as a major product (Decarroz et al., 1986). 5-foU was induced in single-stranded but not in double-stranded DNA by this mechanism, probably because of steric hindrance which excludes menadione from making close contacts with the base residues when the DNA is in the double-stranded form. In addition to electron transfer to triplet quinone, the intermediate thymidine radical cation will also be generated by •OH formed by Fenton reaction as well as by ionizing and UV-B and -C radiations (Wagner et al., 1990). Menadione-sensitized photooxidation can thus be regarded as a good model for studying the reactions of the thymidine radical cation in DNA introduced by various oxidative treatments.

Menadione has been under evaluation as a possible cancer chemotherapeutic agent because it is able to generate oxygen damage *in vivo* by redox cycling and stimulation of hydroxyl radical formation (Nutter et al., 1992). It might be expected that this would produce, among other lesions, a certain amount of 5-foU. The results presented here show that menadione-sensitized photooxidation targets 5-foU lesions specifically to single-stranded regions in DNA. Menadione-sensitized photooxidation could thus be particularly damaging for rapidly dividing cells containing a significant proportion of single-stranded regions and therefore has a potential use in cancer therapy of UV-A accessible tumors.

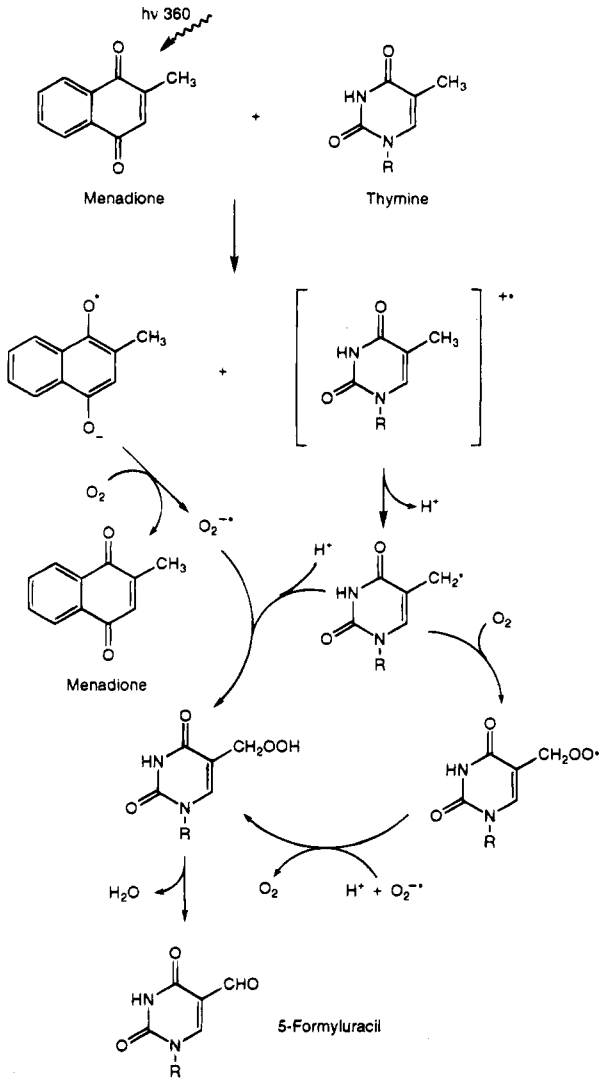

FIGURE 7: Mechanism of formation of 5-foU residues in DNA by quinone-sensitized photooxidation. R, DNA chain.

5-foU residues in DNA were recently identified as a substrate for the AlkA enzyme (3-methyladenine DNA glycosylase II) of *E. coli* (Bjelland et al., 1994). By contrast, no detectable 5-foU DNA glycosylase activity was found to be associated with the Tag enzyme (3-methyladenine DNA glycosylase I), which only removes 3-methyladenine efficiently from DNA (Riazuddin & Lindahl, 1978; Bjelland et al., 1993). Neither could *E. coli* glycosylases previously shown to recognize oxidation damages in DNA, endonuclease III and formamidopyrimidine DNA glycosylase (Doetsch & Cunningham, 1990; Boiteux, 1993), excise 5-foU (Bjelland et al., 1994). Excision of 5-foU by AlkA was a rather surprising observation since the main substrate for AlkA is thought to be 3-alkylated purines and the enzyme was initially assumed to be involved only in the repair of alkylation damage (Lindahl et al., 1988). Nevertheless, the enzyme is shown to have a broad substrate specificity for several types of alkylated bases including O^2-methylated pyrimidines (Thomas et al., 1982; McCarthy et al., 1984; Carter et al., 1988) and was recently shown to work on N^2,3-ethano- and N^2,3-ethenoguanine (Matijasevic et al., 1992) as well as having a low affinity for hypoxanthine residues in DNA (Saparbaev & Laval, 1994). Here we show that a 5-foU DNA glycosylase activity also is present in extracts from human cells. Two different DNA substrates were used, i.e., aged [*methyl*-^{3}H]thymine-labeled DNA and quinone/UV-A-exposed denatured DNA, and significant excision of 5-foU was demonstrated with both types of DNA (Figure 5A–C and data not shown). No 5-foU-releasing activity was found to be associated with purified 3-methyladenine DNA glycosylase from human cells (Anpg), indicating that the 5-foU DNA glycosylase activity represents a new enzyme different from previously characterized glycosylases. However, a recent report presents evidence for excision of 8-oxoG by Anpg (Bessho et al., 1993).

At present, limited data on the biological consequences of 5-foU residues in DNA exist, but the existence of efficient repair enzyme activities in both bacterial and human cells suggests by itself that 5-foU may have mutagenic and/or cytotoxic effects, perhaps analogous to those of 8-oxoG. The modeling suggests that the formyl group might interfere with the O^4 hydrogen bonding to adenine because of electrostatic interactions with the H atom of the formyl group (Figure 6). However, it should be emphasized that these calculations are based on modeling which may not give a precise reflection of the *in vivo* situation. Nevertheless, Kasai et al. (1990) found that 5-foU incorporated during replication in *Salmonella typhimurium* seems to produce AT to GC transitions in DNA which suggest a mutagenic behavior of 5-foU.

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