ABSTRACT

The effects of the presence in DNA of covalently bound guanine adducts of the carcinogen 4-nitroquinoline-1-oxide on the pyrimidine dimer-DNA glycosylase, purified from bacteriophage T4-infected Escherichia coli, were investigated. E. coli DNA, labeled in thymine, photoreversed by silver nitrate, and irradiated by 254 nm monochromatic light, was the substrate. 4-Nitroquinoline-1-oxide was reduced to 4-hydroxyaminoquinoline-1-oxide and then reacted with irradiated DNA in the presence of seryl-AMP, yielding covalently bound adducts in DNA. These were assayed by high performance liquid chromatography. Enzyme activity was assayed by measuring release of labeled free thymine directly photoreversed DNA after the reaction. Glycosylase activity was reduced against carcinogen-modified DNA, with the Vm, 38% of that against the control DNA; the Km was unaffected. Therefore, as with other modified purines, 4-nitroquinoline-1-oxide guanine modifications can reduce enzymic incision at thymine dimers. Left un repaired, pyrimidine dimers are both mutagenic and carcinogenic. This is consistent with the possibility that interference with enzymic initiation of DNA excision repair of UV damage may be an indirect mechanism of mutagenesis by stable carcinogen-DNA adducts.

INTRODUCTION

Damaged sites are removed from DNA by the excision repair pathway. In many instances this is initiated by DNA glycosylases that incise glycosylic bonds linking modified bases to their respective sugars. A great variety of such enzymes, each with its own particular substrate, has been purified and characterized (1). Because an organism may be exposed to many genotoxins simultaneously, differing types of base modifications may all be present at a given moment. If one type of base damage interacts with a DNA glycosylase that initiates repair of another, persistence of that glycosylate substrate might result, with eventual subsequent mutagenesis.

PD DNA glycosylase2 initiates excision repair of UV-induced pyrimidine dimers by cleaving the glycosylic bond linking the 5'-pyrimidine to its deoxyribose (2, 3). This enzyme has shown no activity against any covalently bound carcinogen-DNA adducts (4, 5). Previous work demonstrated reduction of incision of thymine photodimers by PD glycosylase when guanine adducts of the carcinogen AAAF were present in DNA (5). It is of importance to determine if this was unique to DNA-AAAF adducts or if it is a general mechanism resulting from covalent binding of carcinogens to bases. This would indicate that other stable carcinogen-purine DNA moieties can reduce incision of pyrimidine dimers, possibly resulting in persistence of photodamage. Therefore the procarcinogen 4NQO was activated to the ultimate carcinogen amino-acetyl-4HAQ and reacted with DNA to yield stable adducts with guanine. The presence of these moieties resulted in reduced incision of thymine dimers by PD glycosylase. Although the results cannot be extrapolated to other systems, these experiments indicate the possibility of interference with glycosylase-mediated excision repair of pyrimidine photodamage as a general pathway of indirect mutagenesis by carcinogen alterations of DNA purines.

MATERIALS AND METHODS

DNA Preparation and Modification. Escherichia coli was grown, and its DNA was labeled with [3H]thymidine to a specific activity of 10,000-15,000 dpm/µg and purified as described previously (6). [14C]Guanine-labeled E. coli DNA was synthesized by nick translation (7). 2.0 µCi of [8-14C]dGTP (New England Nuclear NET-626) were added to a 4.0 ml reaction mixture; the final DNA specific activity was 7000 dpm/µg. The DNA was photosensitized in the presence of AgNO3 and irradiated as described previously (5). 4NQO was reduced to 4HAQ according to the method of Ikenaga et al. (8). The irradiated DNA was treated with 4HAQ according to the method of Yoshida et al. (9) in the presence of seryl-AMP, which was synthesized according to the method of Berg (10). The DNA was extracted as described (9) and dialyzed into 100 mM NaCl, 10 mM Tris-HCl, pH 7.8, 10 mM EDTA.

Analysis of DNA Damage. The degree of guanine modification of irradiated E. coli DNA was assayed by enzymic digestion of [14C]-guanine-labeled molecules to deoxynucleosides, followed by analysis of the labeled products by high performance liquid chromatography (11). The quantity of reacted DNA was measured according to the method of Burton (12). Thymine dimers were assayed by the method of Carrier and Setlow (13).

Enzymology. PD glycosylase was purified from bacteriophage T4-infected E. coli (purchased as a frozen paste from the New England Enzyme Center, Boston, MA) by the procedure of Friedberg et al. (14). Measurement of alkali-mediated enzymic release of radioactive material from UV-irradiated poly(dA):poly(dT) was used as the assay during the purification procedure (15). Enzyme fraction 4 was used for all subsequent studies. The reaction conditions and the assay used in these enzymological studies were those of Radany and Friedberg (16). The material released following photoreversal by monochromatic 254 nm light was identified as thymine by paper chromatography (13). Other procedures were performed as described previously (5). Then specific activity of the enzyme was 1.8 units/µl with a unit defined as that quantity of enzyme releasing 1 pmol of free thymine per minute under standard reaction conditions.

RESULTS

The reaction of PD glycosylase with 4HAQ-modified DNA is shown in Fig. 1. The DNA adducts were first photosensitized and irradiated; after removal of the AgNO3 by dialysis, the DNA was incubated with the enzyme. Reversing the sequence, i.e., reaction of the DNA with 4HAQ followed by photosensitized irradiation, resulted in variable increases in the DNA dimer content, rendering meaningful comparisons of the reacted molecules and the unreacted controls impossible. Release of free thymine upon photoreversal was compared between control and 4HAQ-reacted substrates, both of which had thymine dimer contents of 15%. Fig. 1 shows a reduction of PD glycosylase activity by about 50% against substrate molecules containing...
The alteration of PD glycosylase by 4HAQ-DNA adducts is further demonstrated in Fig. 2. Thymine dimer content of both control and 4HAQ-reacted molecules was 15%; 4HAQ-guanine content of the modified molecules was 3%. The $V_{\text{max}}$ was reduced from $0.56 \pm 0.04 \text{ pmol min}^{-1} (\mu \text{g protein})^{-1}$ of dimers incised for the control substrate to $0.21 \pm 0.01 \text{ pmol min}^{-1} (\mu \text{g protein})^{-1}$ for the 4HAQ-reacted DNA. The $K_m$ was unaffected, being $0.12 \pm 0.03 \mu \text{M}$ for thymine dimers in DNA for the control and $0.10 \pm 0.01 \mu \text{M}$ for the modified substrate. Control experiments with $[^{14}C]$guanine-labeled DNA reacted with 4HAQ; •, control DNA. Each point represents the average of four determinations.  

DISCUSSION

Differing types of DNA modifications may be repaired via varying pathways. A standard method of determining if simultaneous repair of two different forms of DNA damage proceeds by a common mechanism or by independent systems is by concomitant exposure of cells to a pair of genotoxins. Should the repair pathways overlap, exposure to both agents would yield less repair synthesis than the two types of damage would elicit were they inflicted separately. If the pathways were totally independent, the sum of the DNA repair syntheses should be additive of that elicited by both agents. In the case of repair of 4NQO and UV damages, contradictory results have been obtained. Exposure of human cells to both forms of damage resulted in additive repair synthesis, indicating separate rate-limiting steps for the different forms of damage (17, 18). However, another group found no additivity in repair synthesis elicited by both damaging agents (19). The multiplicity of variables in these systems makes interpretation difficult. Therefore, we used a well characterized enzyme that initiates excision repair of pyrimidine dimers and investigated the effects of the presence of 4NQO-guanine adducts in the substrate.

The enzyme studied was the denV gene product of bacteriophage T4. This enzyme cleaves the glycosyl bond between the 5'-pyrimidine of the photodimer and the corresponding deoxyribose, leaving this base linked to the DNA by the cyclobutane ring (3). The assay used was the release of free thymine from enzyme-reacted DNA following photoreversal, since it alone is specific for PD glycosylase activity and does not rely on subsequent endonucleolytic DNA incision for its endpoint (16, 20, 21). The cyclobutane photodimer is monomerized by 254 nm irradiation, resulting in release of the 5'-thymine from the DNA (16). The stoichiometric relationship between recovered radioactivity in thymine and photoreversal of thymine dimers ensures the specificity of the assay for PD glycosylase activity. This avoids uncertainties obtained from other measurements of this enzyme (22).

The observed reduction of PD glycosylase activity against 4HAQ-reacted DNA is similar to those obtained previously with substrates containing AAAF-guanine adducts (5, 23). As in these experiments, the $V_{\text{max}}$ of the enzyme was reduced against the modified substrate, while the $K_m$ was unaffected (5, 23). This is consistent with the known major products in DNA resulting from modification by these carcinogens. The major site of AAAF reaction with DNA is the C-8 position of guanine (24). The major adduct of 4NQO binding to DNA is likewise at the C-8 of guanine (25, 26). Additions at the C-8 position cause local denaturation and conformational distortions of DNA (27). Studies with uracil-DNA glycosylase have shown that purine modifications resulting in such distortions or denaturations result in inhibition of the enzyme similar to that found with PD glycosylase (28–30). By contrast, such moieties as 7-methylguanine and 5-azacytosine, which cause few DNA deformities, do not affect uracil-DNA glycosylase activity (29, 31). It appears that these findings apply to PD glycosylase, i.e., that distortions and base loss sites in the substrate DNA result in inhibition of enzymic incision at modified pyrimidines. A possible mechanism is binding of the endonuclease activity for apurinic/apyrimidinic sites, present on the PD glycosylase molecule (15, 32, 33), at sites resulting from loss of carcinogen-modified purines (5). The instability of DNA-4HAQ adducts raises the possibility of substantial numbers of base losses from DNA containing such moieties (11). Attempts to bind 4HAQ to uracil-containing DNA did not result in reproducible yields.
of modified guanine moieties, so the effects of such sites on uracil-DNA glycosylase could not be tested. Therefore comparisons of the extent of inhibition between the different glycosylases could not be made.

The presence of covalent guanine modifications by either of the two carcinogens, 4NQO or AAAF, reduces enzymic incision of DNA pyrimidine dimers. Neither carcinogen-guanine adduct is substrate for this glycosylase. This establishes that an extraneous type of DNA damage can reduce excision repair of pyrimidine dimers that are removed by a different pathway. It therefore appears that this phenomenon may be a general manifestation of the DNA alterations resulting from covalent binding of certain types of carcinogens. Pyrimidine dimers in human cellular DNA are not incised by this type of glycosylase (34). Therefore these results cannot be directly extrapolated beyond Micrococcus luteus and T4-infected E. coli. The finding of this type of interference with initiation of excision-repair with different glycosylases and a variety of DNA alterations establishes the potential for such effects in a plethora of DNA modifications and repair enzymes. It therefore appears that chemical carcinogens can indirectly exert mutagenic effects through inhibition of excision-repair initiation of structurally unrelated types of modified DNA bases.

REFERENCES

Inhibition of Enzymic Incision of Thymine Dimers by Covalently Bound Guanine Adducts of 4-Nitroquinoline-1-oxide in DNA

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