APYRIMIDINIC SITES IN GAMMA-IRRADIATED DNA

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1. Introduction

Saturation of the heterocyclic rings of the nucleic acid bases by the addition of radicals represents a major type of reaction induced by ionizing radiation in DNA in vitro [1,2] and in vivo [3]. Products of the 5,6-dihydroxy-dihydrothymine type and the corresponding hydroperoxy derivatives (symbolized in the following as t′) are formed by γ-irradiation from thymine [4,5]. Saturation of the 5,6-double bond in pyrimidine nucleosides substantially decreases the stability of the ring and the *N*-glycosidic bond. Internal apyrimidinic and apurinic sites would be formed in DNA if spontaneous release of damaged bases occurs. Circumstantial evidence for the presence of such sites in γ-irradiated DNA comes from the observation that endonuclease II of *E. coli* [6] and the apurinic site enzyme from calf thymus [7] both incise γ-irradiated DNA. The radiomimetic effects of drugs such as methyl-methane-sulfonate [8,9] may find an explanation in the formation of apurinic and/or apyrimidinic sites both in DNA exposed to ionizing radiation and DNA treated with radiomimetic drugs. We have now obtained direct chemical evidence which indicates that a fraction of the γ-ray products of the t′-type are released from the DNA backbone under physiological conditions. On the basis of statistical considerations it is likely that the sugar is intact in a nucleotide which has received base damage. The release from γ-irradiated DNA of damaged bases under mild conditions is, therefore, expected to result in internal apyrimidinic or apurinic sites rather than strand breakage.

2. Materials and methods

Growth and labeling of bacteriophage PM-2 with thymine-methyl [3] and the preparation of its double-stranded DNA were carried out by a combination of the procedures of Franklin et al. [10] and Yamamoto, et al [11]. For further purification labeled PM-2 DNA was passed through a column of hydroxylapatite (1.5 X 7.5 cm) using a gradient of 0.1 to 0.6 M sodium phosphate pH 7.4. After dialysis against 5 mM sodium phosphate pH 7.4, the PM-2 DNA was used within a few days. Specific activity ranged from 9 to 25 X 10^6 dpm per A_{260} unit.

The DNA was irradiated under aerobic, non-protective conditions in 0.001 M sodium phosphate pH 7.4 at 0°C with 20 krads of ^{137}Cs γ-rays. The irradiated DNA was incubated in the same buffer at 37°C and samples were with-drawn after different lengths of time up to 30 min. The content in products of the 5,6-dihydroxy-dihydrothymine type (t′) in the 10% TCA precipitable fractions was determined by the alkali-acid degradation assay of Hariharan and Cerutti [12]. Experiments using cetyltrimethylammonium bromide for polymer precipitation in place of TCA showed that the acid treatment had no effect on the values for t′ obtained by the alkali-acid degradation assay. In a series of experiments the acid soluble fraction was further analyzed by chromatography on Dowex 50Wx8 (H^+) and DEAE-Sephadex A25 as described previously [13].

3. Results and discussion

Bacteriophage PM-2 DNA labeled with thymine-methyl [^3H] was irradiated with 20 krads of γ-rays at 0°C and then incubated in dilute phosphate buffer pH 7.4 at 37°C. Acid solubilization of the DNA and the content of the acid precipitable and acid soluble material in products of the 5,6-dihydroxy-dihydro-

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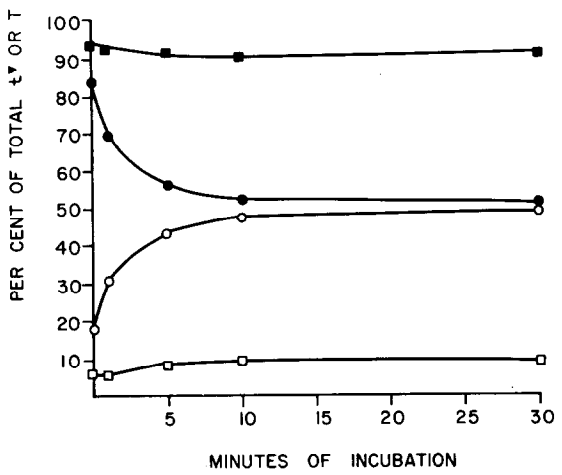

Fig.1. DNA degradation and release of products of the 5,6-dihydroxy-dihydrothymine type (t′) upon incubation of γ-irradiated PM-2 DNA in dilute phosphate buffer pH 7.4 at 37°C. See under Materials and methods for experimental details. DNA degradation: (■–■–■) loss of acid precipitable thymine label (T) and (□–□–□) formation of acid soluble thymine label (T); the data is presented as percent of the radioactivity contained in the total sample. Release of t′: (●–●–●) loss of acid precipitable t′ and (○–○–○) formation of acid soluble t′: the data is presented as percent t′ in a given fraction relative to total t′ in acid precipitable plus acid soluble form.

thymine type (t′) were determined as described under Materials and methods. As shown in fig. 1, the amount of acid soluble radioactivity increased within 30 min from 6 to 9% while the t′ content of the acid soluble material increased by 32% from 17% to 49% (of the total t′, i.e., acid soluble t′ plus acid precipitable t′). Within the same time period the t′-content of the acid precipitable DNA decreased from 83% to 51%. It follows that approximately one third of the t′ originally present in the DNA is selectively released from the backbone under very mild conditions generating internal apyrimidinic sites. Because of the design of our experiments and the specificity of the alkali-acid degradation assay it cannot not be excluded that a urea fragment remains attached to the sugar portion of part of the residues following t′ release and that 'unclean' apyrimidinic sites are formed.

The release of t′ from the polymer remains incomplete. The thymine products of the t′-type which are released and those which remain attached to the polymer must differ in their exact chemical structure. This is not surprising since numerous position- and stereo-isomers of 5,6-dihydroxy-dihydrothymine and the corresponding hydroperoxy derivatives have been identified following irradiation of thymine and thymidine [14,15]. It is conceivable that differences in the local structure of the DNA may influence the stability of the ring damaged thymine products.

The acid soluble material released during irradiation at 0°C and during 30 min of postirradiation incubation at 37°C was analyzed by chromatography on Dowex 50Wx8 (H^+). The elution profiles are shown in fig. 2. Three and four radioactivity peaks are discernable in the elution profiles of the material solubilized during irradiation and postirradiation incubation, respectively. The peak fractions were further analyzed by chromatography on DEAE-Sephadex A25 (see 'Materials and methods') and their t′ content was determined by the alkali-acid degradation assay. Peak I was shown to

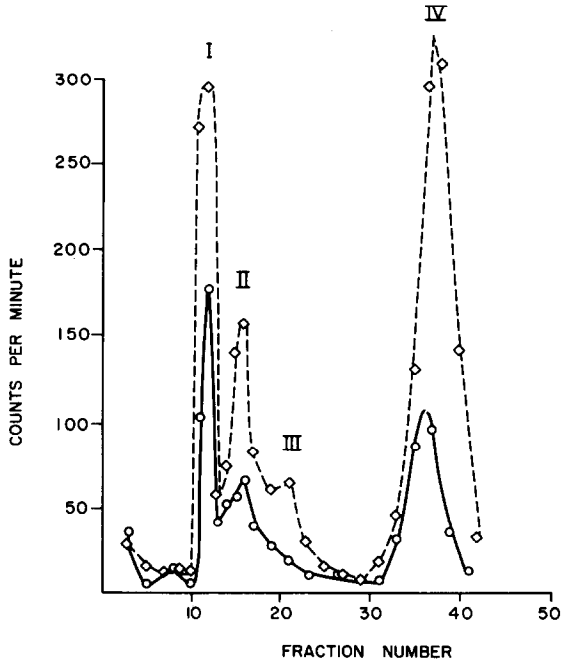

Fig.2. Chromatography on Dowex 50Wx8 (H^+) of the acid soluble material formed during γ-irradiation of PM-2 DNA and within 30 min of postirradiation incubation. See under Materials and methods for experimental details. (○–○–○) soluble material formed during irradiation (◇–◇–◇) soluble material formed within 30 min postirradiation incubation at 37°C.

consist of oligonucleotides and did not contain t'. Peaks II and III contained uncharged material and predominantly consisted of t'. Peak IV was identified as thymine. Radiation products of the t'-type produced by γ-irradiation of thymine-methyl [^{14}C] eluted mostly at the position of Peak II of the Dowex 50Wx8 (H^+) chromatogram. The release during postirradiation incubation of acid soluble oligonucleotides and intact thymine in addition to the material formed during radiation is most likely due to fragmentation of damaged sugar residues. The presence of labile sugar damage in γ-irradiated DNA and nucleotide model systems has been described previously. [16,17].

It follows from our results that products are formed in DNA by γ-rays involving the base residues which are labile even under physiological conditions. Apyrimidinic sites formed by the spontaneous release of the damaged thymine and probably also t'-products remaining in the DNA are expected to be labile in alkali and form a class of lesions which may be detectable as 'alkali labile bonds' in γ-irradiated DNA. The presence of 'alkali-labile bonds' in irradiated DNA has been observed by many investigators [18,19] but the labile lesions have not been chemically identified.

Acknowledgement

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