SELECTIVE PHOTOCHEMICAL ALKYLATION OF PURINES IN DNA

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Received April 30, 1974

SUMMARY

Ultraviolet irradiation of DNA in the presence of 2-propanol and di-tert-butyl peroxide leads to the selective production of 8-α-hydroxy-iso-propyladenine and -guanine, while no pyrimidine dimers are formed.

Photochemical reactions of purines and purine nucleosides with a variety of organic substrates have been reported in recent years.[1-7] It has been found that in purines of nucleic acids these reactions result in the formation of the appropriate 8-substituted derivatives; for example, 8-α-hydroxy-alkyl purines were produced in photochemical reactions of purines with alcohols.[3,4] We have shown recently that similar reactions take place in the purine moieties of DNA,[8] which was irradiated in the presence of 2-propanol with ultraviolet (UV) light of $\lambda > 260$ nm or of longer wavelengths ($\lambda > 290$ nm), using acetone as a photosensitizer. In both cases thymine dimers were also formed in the DNA. These dimers result either from direct light-absorption by the heterocyclic bases or from energy transfer from the excited ketone photosensitizer.[9-11] On the other hand, we observed that selective alkylation of purines occurred when mixtures of purines and pyrimidines were irradiated with UV light of $\lambda > 290$ nm in the presence of 2-propanol, using organic peroxides as photoinitiators.[12]

We now report the selective production of 8-α-hydroxy-iso-propyl-adenine and -guanine in native DNA, without any formation of pyrimidine dimers, following UV irradiation of the nucleic acid in the presence of 2-propanol and di-tert-butyl peroxide [$(Bu^tO)_2$] as a photoinitiator.

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EXPERIMENTAL

Escherichia coli M55-B46, a purine-requiring strain, and E. coli $15T^-$, a thymine-requiring strain, were used for the preparation of [^{14}C]-purine-, and [^{14}C] or [^{3}H]-thymine labeled DNA, respectively. DNA was isolated by the method of Thomas et al.[13] RNA was eliminated ($\leq$ 1% alkali-labile counts) from the DNA preparation by treatment with pancreatic ribonuclease I together with ribonuclease T_1.[14] The specific activities of the purine and thymine labeled DNA were 1.5×10^4 cpm/µg and 1.5×10^6 cpm/µg, respectively. Zone sedimentation was carried out in linear concentration gradients of 5 to 20% (w/v) sucrose in 0.9M NaCl, 0.1M NaOH, and 0.1mM EDTA. Samples of DNA (0.1 ml, ca. 1µg), gently layered on top of the gradient, were predenaturated by addition of 0.2 ml of 0.5M NaOH (10 min at room temperature), and sedimented in a Spinco SW50 rotor in a Beckman Model L2-65B ultracentrifuge at 4°C (49,000 rpm, 120 min). Following centrifugation, fractions were collected (0.18 ml) from the top of the tube into counting vials, using toluene scintillation reagent.

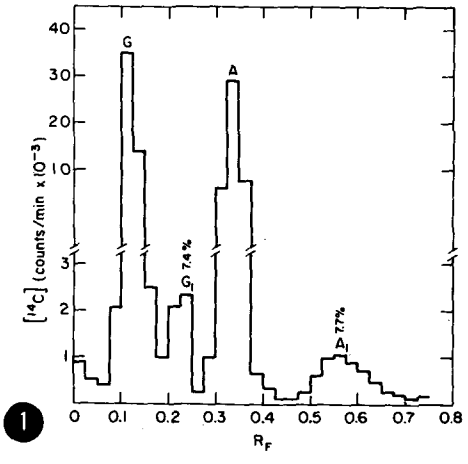
Irradiation was carried out at room temperature in 0.02M phosphate buffer solutions (pH 6.8), using a Wild universal lamp equipped with an Osram 200W high pressure mercury vapor burner and a filter containing a 8×10^{-4}M solution of 5-bromodeoxyuridine ($\lambda > 300$ nm; incident dose rate 5×10^{-6} einstein. cm^{-2}. min^{-1}). Prior to the addition of labeled DNA, the phosphate buffer was flushed for 10 min with oxygen-free nitrogen. Careful agitation was achieved by hand tilting of the samples during irradiation.

After irradiation all DNA samples were dialyzed for 24 hrs against 0.1M phosphate buffer (pH 7.0). The purine bases were liberated by mild acid treatment (2% HCl at 100°C for 10 min), and the purine content was analyzed as previously described.[8] Analysis for thymine dimers has been carried out by the method of Setlow and Carrier.[8,15]

RESULTS AND DISCUSSION

Irradiation of [^{14}C]-purine labeled E.coli DNA with UV of $\lambda > 300$ nm

in the presence of 2-propanol and $(Bu^tO)_2$ as photoinitiator led to the conversion of ca. 8% of both of the adenine and guanine moieties into the corresponding 8-α-hydroxy-iso-propyl derivatives A_1 and G_1, respectively (see Figure 1). Longer irradiation periods led to an exponential increase in the formation of the purine photoproducts, as presented in Figure 2. No



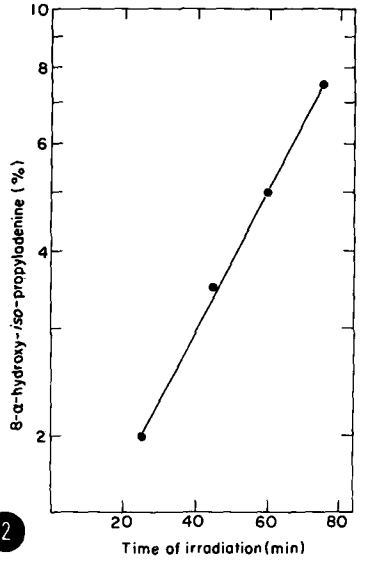


Figure 1. Chromatographic distribution of purines and purine photoproducts after mild acid hydrolysis of irradiated [^{14}C]-purine labeled DNA. Chromatograms were developed with n-butanol-water-ammonia (86:13:1 v/v/v). DNA (12μg/ml, 1.5×10^4 counts.min^{-1}.μg^{-1}) was irradiated at λ>300 nm for 75 min in the presence of 1M 2-propanol and 0.5M $(Bu^tO)_2$. A,G,A_1 and G_1 denote the positions of adenine, guanine, and co-chroamtographed authentic samples of 8-α-hydroxy-iso-propyladenine, and -guanine, respectively.

Figure 2. Conversion of adenine to 8-α-hydroxy-iso-propyladenine as a function of time of radiation.

pyrimidine dimers could be detected in [^{14}C]-thymine labeled native DNA irradiated under similar conditions.* Therefore, the reported photochemical

*The thymine-2-propanol adduct[8] was detected in irradiated single stranded DNA (heat denatured).

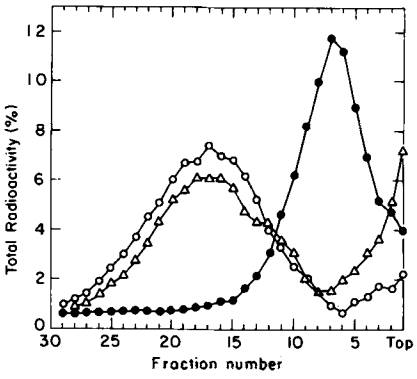


Figure 3. Sedimentation through alkaline sucrose gradients of [^{3}H]-thymine labeled E.coli DNA with 0.5M 2-propanol and 0.5M $(Bu^tO)_2$; (o) non irradiated; (Δ) non irradiated, repeatedly shaken prior to sedimentation; (●) irradiation with λ>300 nm for 45 min. Sedimentation was carried out for 120 min at 49,000 rpm at 4°C.

reactions are selective for purine moieties in native DNA.

Under the conditions of the reaction, the incident light is exclusively absorbed by the photoinitiator. The excited $(Bu^tO)_2$ is fragmented into oxy-radicals,[4,5] which abstract a hydrogen atom from 2-propanol, leading to alcohol free radicals $\dot{C}(CH_3)_2OH$, which are further scavenged by the purine moieties of DNA, to yield the observed photoproducts.[8] The exponential dependence of the photoproduct formation with time suggests that some alteration in the secondary structure of DNA occurs during irradiation. In order to test whether this alteration involves chain breakage, we performed alkaline sucrose gradient sedimentation of the DNA. We observed about 40% decrease in the sedimentation coefficient of a [^{3}H]-thymine labeled DNA following irradiation in the presence of $(Bu^tO)_2$ (see Figure 3), which is a higher effect than that observed when no peroxide is present in the solution. It, therefore, appears that the presence of the peroxide enhanced the production of strand breaks during the irradiation of the DNA. This occurrence of strand breaks is assumed to ease the access of the alcohol free radicals to the purine moieties, thus increasing the formation of purine photoproducts. It is noteworthy that stronger agitation of the DNA

solution led to an increased formation of the photoproducts, possibly due to enhanced formation of strand breaks (see Figure 3).

The development of selective photochemical reactions for the various nucleic acid constituents might lead to the formation of a single-type chemical lesion in irradiated DNA. This is a prerequisite for the study of the correlation between a given radiation-induced lesion and its biological consequences in living systems. This investigation may also contribute to a better understanding of the interaction of nucleic acids with their environment in living organisms under radiation.

ACKNOWLEDGEMENT

We thank Prof. R. Ben-Ishai and Mrs. R. Sharon for supply of [^{3}H]-thymine labeled DNA and for valuable discussions.

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