PRODUCTION OF PYRIMIDINE DIMERS IN DNA IN THE DARK

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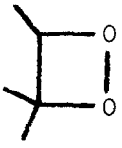
SUMMARY

Trimethyl-1,2-dioxetane was decomposed (70°C) in the presence of E.coli DNA. Pyrimidine dimers were detected in the hydrolysate of the reaction mixture. Assuming the existence in cells of reactions which produce electronically excited molecules of sufficient energy these results demonstrate the possibility that pyrimidine photodimers are produced in cells which are not exposed to u.v.-light.

INTRODUCTION

The generation of electronically excited molecules through chemical reaction of ground state reactants is a familiar phenomenon in biological systems since it is the basis of bioluminescence (1). Recently consideration has been given to the possibility that chemically-produced excited state molecules may be intermediates in biochemical pathways having other functions such as biosynthesis (2,3). It must also be considered that excited species produced inadvertently could act detrimentally to biological systems.

In light of the fact that pyrimidine dimers can be produced in DNA by means of triplet energy transfer from appropriate sensitizers (4,5), the existence of chemically-produced excited molecules in cells would introduce the possibility that pyrimidine dimers can be produced in the DNA in the absence of light. We report here that the excited state products of the dark (thermal) decomposition of trimethyl-1,2-dioxetane (TDO) can induce the formation of pyrimidine dimers in DNA in vitro.



TDO

RESULTS

3-Bromo-2-methyl-2-butyl hydroperoxide (6) dissolved in tetraglyme and kept at 0°C was dehydrobrominated using potassium t-butoxide to give trimethyl-1,2-dioxetane (7) which was distilled (vacuum pump) out of the reaction vessel as it was formed and trapped as a yellow solid in a receiver flask cooled to ~100°K (Some t-butanol is collected along with the TDO). To determine whether or not the thermal decomposition of TDO yields electronically excited products in water solution, the yellow product (an oily liquid at room temperature) was dissolved in a small volume of a water acetone mixture 5:1) to which was added a small amount of 2-anthracene sulfonate in one test and rhodamine-B in another. When these solutions were heated on a hot plate, fluorescences characteristic of the added dyes were easily observed in dim light.

To the yellow oil (~0.8 ml) obtained from 10 g of the bromohydroperoxide was added 6 A (260 nm) units of E.coli 15 T^- DNA labelled with thymine-2-^{14}C (specific activity 23,400 cps/A unit) dissolved in 1 ml of 0.01 M phosphate buffer, pH 7.0. The mixture (cooled to 0°C) was gently bubbled with nitrogen for 10 minutes and then heated at 70°C (water bath) for 20 minutes under an atmosphere of nitrogen. The light amber reaction mixture was evaporated under a stream of nitrogen to a sticky brown solid which was hydrolysed in 1/2 ml of 98 per cent formic acid at 175°C sealed tube for 30 minutes. The hydrolysate was evaporated to dryness, taken up in a small amount of buffer, spotted on Whatman No. 1 paper and chromatographed (descending) using a n-butanol-acetic acid-water (80/12/30 v/v) mixture. For a control, about 4 A units of untreated DNA was hydrolysed and chromatographed in the same way. The dried chromatograms were analyzed for distribution of radioactivity using a Baird Atomic RSC-363 scanner. The results are shown in Figure 1.

The minor bands in the chromatogram of the DNA treated with the dioxetane (Fig. 1a) occur at R_f values corresponding to those reported for the cis-syn-thymine dimer ($\hat{T}T$) (0.24-0.31) (8) and the uracil-thymine dimer ($\hat{U}T$)

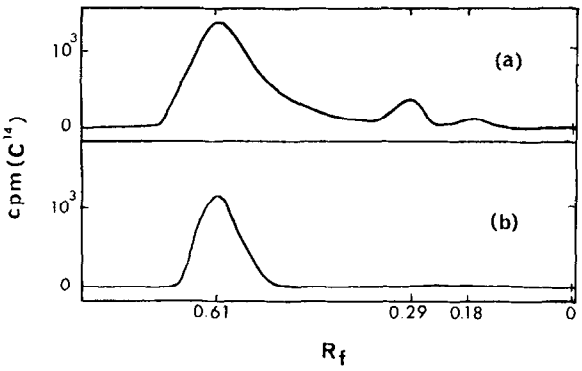
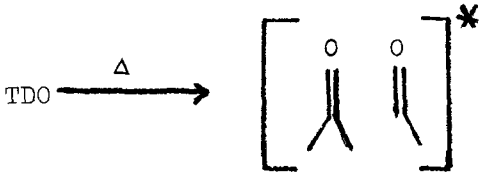


Fig. 1. Radiochromatogram of hydrolysate of DNA (thymine-C^{14}) (a) treated with TDO, (b) untreated.

(0.19-0.22) (9). That the material responsible for the band at R_f = 0.29 consisted primarily of $\hat{T}\hat{T}$ was shown by its efficient conversion to monomer upon irradiation in solution with 254 nm light. The major band at R_f = 0.61 (8) is due mainly to thymine. It is the only band present in the control (Fig. 1b). The presence of still other products in the hydrolysate of the treated DNA is suggested by the tailing of the major band (Fig. 1a). The bands assigned to $\hat{T}\hat{T}$ and $\hat{U}\hat{T}$ account for about 6.5% and 0.8%, respectively, of the total radioactivity. Thus the $\hat{T}\hat{T}$ and $\hat{U}\hat{T}$, which arises from the deamination of $\hat{C}\hat{T}$, are formed in the ratio of about 4 to 1 since only thymine is labelled.

DISCUSSION

Kopecky and coworkers have shown that the thermal decomposition of TDO in organic solvents gives acetaldehyde and acetone (6) and that some fraction of one or both of these products is produced in an electronically excited state (Eq. (1)) (7).



(1)

Indeed White et al. have used TDO as a source of excited state molecules which can transfer electronic energy to various acceptor molecules which subsequent-

ly undergo "photochemical reactions without light" (3,10). We chose TDO as a source of excited state molecules in our experiment on the basis of the energy required to excite the DNA bases. The lowest lying triplet states of both acetaldehyde (11) and acetone (12) possess sufficient electronic energy to excite the lowest triplet levels of the DNA bases (12). Indeed, triplet excitation transfer from acetone to the nucleotides has been demonstrated (12). Furthermore, acetone has been used to sensitize the formation of pyrimidine photodimers in DNA (13) and tRNA (14). The mechanism of sensitization undoubtedly involves the transfer of triplet excitation from the sensitizer to the pyrimidines in the polynucleotide (4).

The experiments with the fluorescent dyes show that heating TDO in water solution yields electronically excited products with finite yield. Thus it is not surprising, considering the facts mentioned above, that heating TDO in the presence of DNA leads to the formation of pyrimidine photodimers in the DNA. By comparison with the photosensitized process using acetone or acetophenone the efficiency of production of "free" electronically excited product molecules upon heating TDO in water is estimated to be at least 0.01. The results of White and coworkers indicate efficiencies on the order of 0.01-0.1 for organic solvents (3,10).

The ratio of $\hat{T}\hat{T}$ to $\hat{C}T$ produced using TDO is 4:1. The ratio reported for the acetone sensitized case (313 nm) is 7:1 (13). The apparent difference remains unexplained at this time but may simply reflect the fact that the experiment with TDO was carried out at 70°C, near the melting point of DNA, while the photosensitization experiment was done near room temperature.

Pyrimidine dimers act as both lethal and premutational lesions in phage and bacteria (15,16,17). These organisms possess enzymic systems which can repair the pyrimidine dimer damage in the dark (18). Pyrimidine dimers are formed in mammalian cells upon irradiation with ultraviolet light (19) and it has recently been shown that mammalian cells also possess excision systems which repair dimers (20). This repair serves to protect the skin from the

effects of damage due to sunlight since the absence of a functional repair system has been shown to be the molecular basis for the human skin disease, xeroderma pigmentosum (20-23), a genetic disease associated with high sensitivity to sunlight and greatly increased susceptibility to skin cancer (24). There appear to be excision repair systems in phage and bacteria which are capable of repairing lesions other than pyrimidine dimers (17,25,26). Thus excision repair may be functional not only in skin but also in tissues which are not susceptible to ultraviolet light-induced damage but in which chemical modification occurs in the DNA. For example, this may be the basis for the de Sanctis-Cacchione syndrome (27), a clinical form of xeroderma pigmentosum in which neurological disorders occur in addition to the skin symptoms.

The present results show that pyrimidine dimers may be produced in cells in the absence of ultraviolet light and that this could require the presence of DNA repair systems in cells which are not exposed to light. No reaction capable of yielding electronically excited products of sufficient energy to induce DNA photochemistry is known to occur in a biological system. Thus, at this time it would certainly be unwise to formulate, for example, the molecular basis of the de Sanctis-Cacchione syndrome in terms of pyrimidine dimer formation in the brain. However, it would be equally unwise to discount pyrimidine dimers as possible DNA lesions in cells not exposed to ultraviolet light.

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