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Excited-State Controlled Peroxide Formation of DNA[1]

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Reactions with water and 2 + 2-cycloadditions of individual bases are the primary steps held responsible for the deformation of DNA at short wavelengths. From the observed data however the influence of atmospheric oxygen on the light-induced reaction of DNA is evident. A plausible explanation for these effects is the formation of reactive oxygen species during the UV irradiation of DNA. In the present work the deformation of DNA by different oxygen species like singlet oxygen ($^1O_2$), superoxideanion (O_2^-), hydroxyradical ($OH^\cdot$), and ozone (O_3) is excluded with the help of chemical-trapping experiments. The photoinduced transformation proceeds via excited states of DNA, which react with oxygen to afford peroxide.

INTRODUCTION

During the past 20 years numerous publications have reported studies of the UV-induced deformation of DNA and the individual bases (Fahr, 1969; Wang, 1976; Foote, 1976; Greenstock *et al.*, 1978). These investigations reveal that among other reversible cyclizations reactions and addition of water to the 5,6-double bond of pyrimidine base can be adduced as the cause of the UV-induced mutations of DNA. On the other hand, relatively little is known about the role of atmospheric oxygen during these reactions (Setlow, 1968).

It is quite generally assumed that the light-induced reaction of DNA is controlled from the excited state (reaction path a, Fig. 1). The photoproducts (i-DNA) formed in a 2 + 2-cycloaddition (reaction path b) (Fahr *et al.*, 1966) partly react back to DNA (reaction path c). In principle the excited state can react directly with water (reaction path d) (Fahr, 1969) in aquatic systems or else interact with oxygen in an energy or electron transfer reaction (reaction path e) (Baumann *et al.*, in press), which could contribute to the formation of oxygen species such as $^1O_2$, O_2^-, $^\cdot OH$, $^\cdot OOH$, H_2O_2, and O_3. These species would then be able to attack the DNA and give irreversible reaction products (reaction path f).

RESULTS AND DISCUSSION

The problem requiring resolution was to discover which of the possible oxygen species can be induced by excited DNA. The following questions must be answered in this connection:

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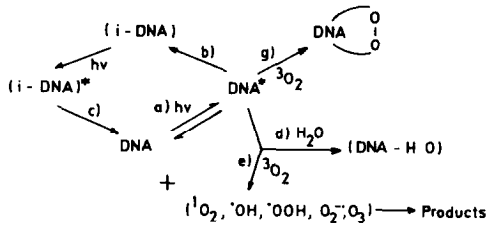

FIG. 1. Photoinduced reaction of DNA at wavelengths above 290 nm.

1. Can DNA be excited under normal conditions?
2. What is the influence of wavelengths, pH, and temperature during the light-induced deformation of DNA?
3. Is the UV deformation of DNA dependent on oxygen partial pressure?
4. What is the influence of inorganic salts on the UV degradation of DNA?

From the results of the irradiations performed in aquatic systems, in which we made allowance for a variety of parameters, it is revealed that the reaction not only varies with wavelength, pH, and temperature but is also dependent on the oxygen partial pressure. By drastically increasing oxygen partial pressure, the relative reaction rate under the experimental conditions can be as much as doubled. A maximum conversion is obtained at pH 12.4, while the deformation can be almost completely blocked in strong acid media (pH 2.0). In contrast, if the experiment is performed with shorter wavelengths ($\lambda > 230$ nm) in the absence of oxygen, then a maximum deformation is recorded in the acid region (pH 3.9). Sodium chloride, potassium chloride, and sodium dihydrogen phosphate salts in concentrations up to 1% at wavelengths above 230 nm produce only a slight change in the conversion rate.

On the basis of absorbance difference diagrams (Fig. 2) (Mauser, 1968), which can be employed to check the uniformity of a photochemical reaction and to draw conclusions about the number and type of independent steps, it may be stated that the photoreaction proceeds nonuniformly.

It is especially significant that the reaction is accelerated with typical triplet sensitizers such as acetone (E_T = 79.0 kcal/mol) when oxygen is excluded (Fig. 3) and can be quenched with *trans*-1,3-pentadiene (E_T = 66.6 kcal/mol).

The rate constant of the triplet rearrangement k_r, which is available from Stern–Vollmer plot, is approximately 0.7×10^7 sec^{-1}. These results show that the triplet-excited states directly or indirectly control DNA formation.

To characterize the possible role of oxygen species (Parlar *et al.*, in press) during the UV-induced reaction of DNA in water at wavelengths greater than 290 nm, which are representative of the troposphere, typical trapping reactions were performed (Table 1).

During the irradiation of DNA in water at wavelengths greater than 290 nm no endoperoxides could be detected in the presence of dimethylanthracene. If dimethylfurane is used as trapping agent for $^1O_2$ the ozonide is formed in very small concentrations along with other undetermined reaction products; however, it is also produced in similar concentrations in the absence of DNA. In contrast to

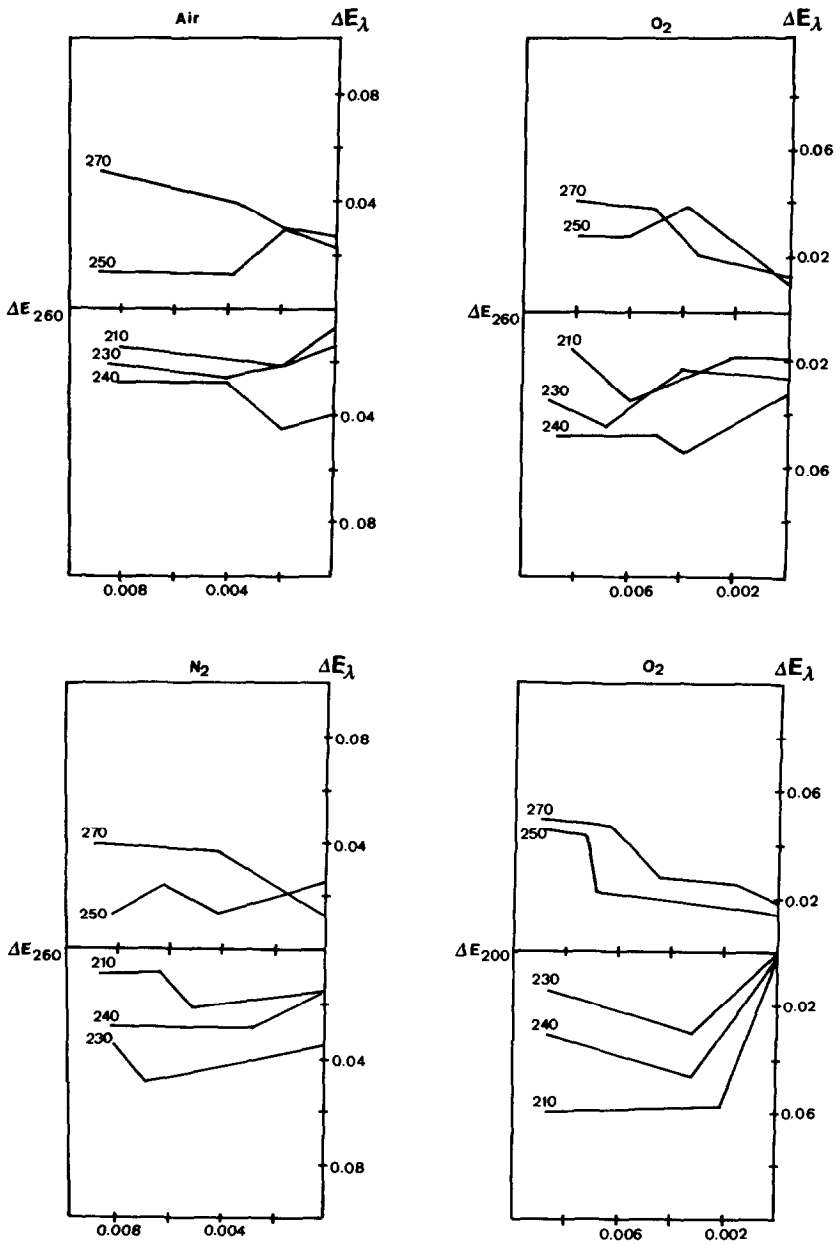

FIG. 2. Absorbance difference diagrams of the photoreaction of DNA.

α-Terpinene and Limonene, which yield in the presence of $^1O_2$ specific conversion products, e.g., Ascardial and Terpinolene, a strong $^1O_2$ quencher does not react under these conditions. Analysis of the products in comparison to those of Rose Bengal shows that DNA has no effect on these reactions. Experiments with imidazole in combination with *N,N*-dimethyl-*p*-nitrosoaniline, which afford transannular peroxides in the presence of $^1O_2$ and should influence the absorption band of *N,N*-

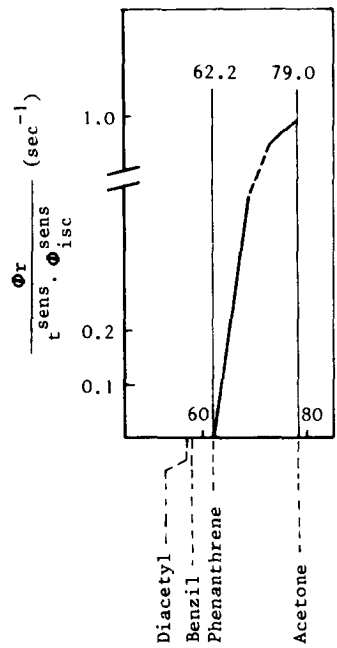

FIG. 3. Energy frequency rate ($\sim\phi_r/t^{sens}\cdot\phi_{isc}^{sens}$) of the photodeformation of DNA in the presence of triplet sensitizers. ϕ_r = quantum yield of product formation; t^{sens} = half-time of the sensitizers; ϕ_{isc}^{sens} = quantum yield of the sensitizers of intersystem.

dimethyl-*p*-nitrosoaniline at 440 nm, could not be carried out because of their photoinstability. When thiourea and allylthiourea were used as trapping agents for $^1O_2$ the anticipated aminosulfonic acids could not be detected. An attempt to quench the reaction with N_3^{3-} ions also produced no effect in this case. The relative DNA breakdown rate is not altered under these conditions. If the reaction is carried out in D_2O, which is known to increase the lifespan of singlet oxygen, then the degradation rates are not altered. These studies show that the participation of singlet oxygen in the photoinduced deformation of DNA can be excluded. The question of whether OH radicals are produced can be answered based on the results of the following trapping experiments: benzene, which is known to react to phenol; ethanol, which is transformed into acetone; and 2-propanol, which gives acetone in the presence of $^{\cdot}OH$, were stable under the reaction conditions. Addition of *N,N*-dimethyl-*p*-nitrosoaniline also gave no assessable results because of its photoinstability. These specific reactions exclude the presence of $^{\cdot}OH$ in the reaction solution. If it is assumed that ozone accounts for the deformation, then a secondary photoinduced reaction should lead to the formation of H_2O_2 or $^{\cdot}OH$; and their formation was excluded. In addition, the mass spectroscopic studies of the reaction solution in a special gas-phase mass analyzer system showed the probability of the presence of ozone to be remote.

The question of whether the peroxide anion ($O_2^{\cdot-}$) is formed during the photolysis of DNA in aqueous systems was answered by the results of the reactions with hydroxylamine. The expected nitrite could not be identified. Peroxide dismutase,

TABLE 1

TRAPPING REACTIONS OF OXYGEN SPECIES POTENTIALLY FORMED DURING IRRADIATION OF DNA AT WAVELENGTHS ABOVE 290 nm

Compound used during DNA irradiation with wavelengths greater than 290 nm (concn in mol/liter)	Oxygen species	Expected compound[a]	Result[b]	Reference
9,10-Dimethylanthracene ($2.5–7.5 \times 10^{-5}$)	$^1O_2$	Endoperoxide	n.d.[c]	Nathan *et al.*
2,5-Dimethylfurane ($2.5–7.5 \times 10^{-5}$)	$^1O_2$	Ozonide	Slight ozonide formation; also detectable in blank	Foote *et al.*, Kearns, Zepp *et al.*
Terpinolene ($1.0–5.0 \times 10^{-4}$)	$^1O_2$	Hydroxylated product	n.d.	Klein *et al.*
α-Terpinene ($1.0–5.0 \times 10^{-4}$)	$^1O_2$	Ascaridole	Different products in fingerprint analysis by comparison with reaction with Rose Bengal	Schenck *et al.*, Foote *et al.*
Limonene ($1.0–5.0 \times 10^{-4}$)	$^1O_2$	Products	Different products in fingerprint analysis by comparison with reaction with Rose Bengal	Schenck *et al.*, Gollnick
Imidazole + *N,N*-dimeyl-*p*-nitrosoaniline ($1.0 \times 10^{-2} + 5.0 \times 10^{-5}$)	$^1O_2$	Transanular peroxide	Bleaching of band at 440 nm in presence of *N,N*-dimethyl-*p*-nitrosoaniline. Cannot be performed because of photoinstability	Kraljic *et al.*
D_2O solvent	$^1O_2$	—	Longer lifespan of $^1O_2$, hence increased DNA breakdown rate	Davidson *et al.*, Nilsson *et al.*
Allylthiourea ($1.0–5.0 \times 10^{-3}$)	$^1O_2$	Iminoaminosulfonic acid	n.d.	Kramer *et al.*, Kraljic *et al.*

N_3^- (1.0×10^{-4}–1.0×10^{-2})	$^1O_2$	—	No slowing of DNA breakdown	Gollnick *et al.*, Foote *et al.*, Hasty *et al.*
Hydroxylamine (1.0×10^{-3}–1.0×10^{-4})	O_2^-	NO_2^-	n.d.	Elstner *et al.*
Peroxide dismutase	O_2^-	O_2^- trapping	Cannot be performed because of photoinstability	McCord *et al.*
Tetrazolium salts	O_2^-	O_2^- trapping	Cannot be performed because of photoinstability	Altmann
Crocin	O_2^-	O_2^- trapping	Cannot be performed because of photoinstability	Montalbini *et al.*
Cytochrome *c*	O_2^-	O_2^- trapping	Cannot be performed because of photoinstability	Bors *et al.*
Riboflavin	O_2^-	—	See text	Spikes *et al.*
Benzene (1.0×10^{-5})	$^{\cdot}OH$	Phenol	n.d.	Mill *et al.*
Ethanol (1.0–8.0×10^{-3})	$^{\cdot}OH$	Acetaldehyde	n.d.	Bors *et al.*
2-Propanol (5.0×10^{-4}–2.0×10^{-2})	$^{\cdot}OH$	Acetone	n.d.	Bors *et al.*
Methional (1.0–2.0×10^{-3})	$^{\cdot}OH$	Ethylene	n.d.	Beauchamp *et al.*
N,*N*-Dimethyl-*p*-nitrosoaniline	$^{\cdot}OH$	OH adduct	Cannot be performed because of photoinstability	Hetada *et al.*
—	H_2O_2	$^{\cdot}OH$	n.d.	
—	O_3	MS–gas analysis	n.d.	
Cumene (excess)	$^{\cdot}OOH$	Acetophenone, hydroxycumene	Identified	
Styrine (5.0×10^{-4})	$^{\cdot}OOH$	Side chain reactions (Benzaldehyde)	Also detectable in blank	

[a] Capillary GC data: SE 30 (30 cm) 60–220°C temperature program (1 ml N_2/min); Methylphenylsilane, 60–220°C temperature program (1 ml N_2/min).
[b] Product identification and determination of fingerprints were carried out in the case of $^1O_2$ with Rose Bengal and $OH^{\cdot}$ radicals by photolysis of H_2O_2.
[c] Not detected.

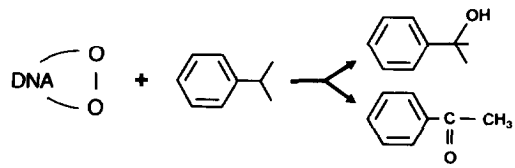

FIG. 4. Oxidation reaction of cumene in the presence of DNA peroxide.

tetrazolium salts, crocine, and cytochrome *c* are unsuitable as trapping agents for O_2^- under irradiation conditions because they themselves absorb and are photolytically unstable. Support for the absence of O_2^- was obtained from studies with riboflavin, which in the presence of ethylenediaminetetraacetate (EDTA) or methionine gives O_2^- by photoinduction. When the experiments were carried out in the presence of riboflavin the relative deformation rate of DNA increased. In follows that DNA is able to react with O_2^-. Assuming that DNA can form O_2^- by light induction in the presence of EDTA or methionine in analogy to riboflavin, then the photodegradation of DNA should be accelerated in the presence of these substances. However, they had no effect on the reaction. Thus, DNA cannot generate superoxide anions (O_2^-) even in the presence of EDTA or methionine. It can do so only if riboflavin is present in the reaction solution. The experiments showed, however, that DNA can take the role of EDTA or methionine during the photolysis of riboflavin and contribute to the generation of superoxide (O_2^-).

If it is assumed that DNA reacts with ground state oxygen ($^3O_2$) to form peroxides from the excited state, then these should react with cumene to give cumyl alcohol and acetophenone, analogously to the humic substances in natural waters (Mill *et al.*, 1980). Experiments with cumene in the presence of DNA led to the identification of the above-mentioned products. These products were indeed unambiguously identified (Fig. 4).

CONCLUSIONS

The results of these experiments confirm that the UV deformation of DNA at wavelengths above 290 nm is controlled solely by the excited states via biradical intermediate stages which are able to react with ground state oxygen ($^3O_2$) to form peroxides (Fig 1, reaction path g). Finally, it is suggested that in addition to intramolecular dimerizations and additions of water the photoinduced oxidation with ground state oxygen should be given emphatic consideration during damaging of DNA by UV light. The DNA peroxides formed can react irreversibly to polar end products in widely different ways. Although their biological significance is unknown, the photoinduced oxidations of DNA should be considered both in the blocking of reduplication and during the change of the genetic information.

REFERENCES

ALTMANN, P. (1976). In *Progress in Histochemistry and Cytochemistry,* Vol. 913. Gustav Fischer Verlag, Stuttgart.

BAUMANN, R., HERRMANN, M., AND KORTE, F. (1985). *Z. Naturforsch.*, in press.

BEAUCHAMP, C. O., AND FRIDOVICH, I. (1970). *J. Biol. Chem.* **245,** 4641.

BORS, W., SARAN, M., LENGFELDER, E., SPÖTTL, R., AND MICHEL, C. (1974). *Curr. Top. Radiat. Rest. Quart.* **9,** 247.

McCord, J. M., and Fridovich, I. (1969). *J. Biol. Chem.* **244**(22), 6049.
Davidson, R. S., and Pratt, J. R. (1982). *Tetrahedron Lett.* **36,** 3729.
Elstner, E. F., and Heupel, A. (1976). *Anal. Biochem.* **70,** 621.
Fahr, E. (1969). *Angew. Chem. Int. Ed. Engl.* **8,** 578.
Fahr, E., Kleber, R., and Böblinger, E. (1966). *Z. Naturforsch. B* **21,** 219.
Foote, C. S. (1976). In *Free Radicals in Biology* (W. A. Pryor, ed.), p. 85. Academic Press, New York.
Foote, C. S., Wüsthoff, M. T., and Schenck, G. O. (1967). *Tetrahedron* **23,** 2583.
Foote, C. S., and Wexler, S. (1965). *Tetrahedron Lett.* **46,** 4111.
Foote, C. S., Fujimoto, T. T., and Chang, Y. C. (1972). *Tetrahedron Lett.* **45.**
Gollnick, K. (1982). *La Chimica e l'Industria* **64,** 156.
Gollnick, K., Haisch, D., and Schade, G. (1972). *J. Amer. Chem. Soc.* **94,** 1747.
Greenstock, C. L., and Wiebe, R. H. (1978). *Photochem. Photobiol.* **28,** 863.
Hasty, N., Merkel, P. B., Radlick, P., and Kearns, D. R. (1972). *Tetrahedron Lett.* **1,** 49.
Hatada, M., Kraljic, I., El Samahy, A., and Trumbore, C. N. (1974). *J. Phys. Chem.* **78,** 888.
Kearns, D. R. (1971). *Chem. Rev.* **71,** 395.
Klein, E., and Rojahn, W. (1965). *Tetrahedron* **21,** 2173.
Kraljic, I., and El. Mohsni, S. (1978). *Photochem. Photobiol.* **28,** 577.
Kraljic, I., and Kramer, H. E. A. (1978). *Photochem. Photobiol.* **27,** 9.
Kramer, H. E. A., and Maute, A. (1968). *Ber. Bunsenges. Phys. Chem.* **72,** 1092.
Mauser, H. (1968). *Z. Naturforsch. B* **23,** 1025.
Mill, T., and Hendry, D. G. (1980). Science **207,** 886.
Montalbini, P., Koch, F., Burba, M., and Elstner, E. F. (1978). *Physiol. Plant Pathol.* **12,** 211.
Nathan, R. A., and Nathan, A. H. (1974). *J. Chem. Soc. Chem. Commun.,* 674.
Nilsson, R., Merkel, P. B., and Kearns, D. R. (1972). *Photochem. Photobiol.* **16,** 117.
Parlar, H., Baumann, R., and Korte, F. (1985). *Naturwissenschaften,* in press.
Schenck, G. O., and Gollnick, K. (1964). *Ann. Chem.,* 674.
Schenck, G. O., and Ziegler, W. (1944). *Naturwissenschaften* **32,** 157.
Setlow, R. B. (1968). *Photochem. Photobiol.* **7,** 643.
Spikes, J. D., and Schwartz, H. M. (1978). *Photochem. Photobiol.* **28,** 921.
Wang, S. Y. (1976). *Photochemistry and Photobiology of Nucleic Acids,* Academic Press, New York.
Zepp, R. G., Wolfe, N. L., Baughman, G. L., and Hollis, R. C. (1977). *Nature (London)* **267,** 421.