Vol. 80, No. 3, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 14, 1978 Pages 490-495

PHOTOCHEMICAL-LIKE EFFECTS IN DNA CAUSED BY ENZYMICALLY ENERGIZED TRIPLET CARBONYL COMPOUNDS

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Received December 21,1977

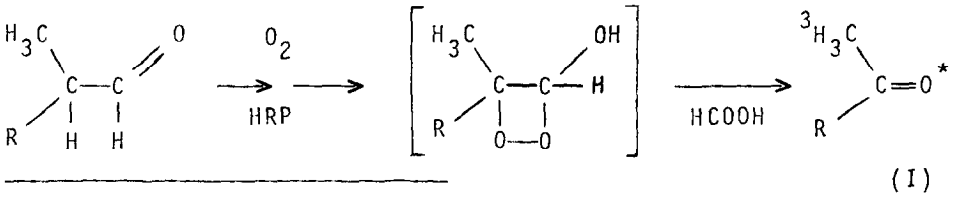
SUMMARY

The same circular dichroism spectrum as that of DNA conformationally altered by UV irradiation is observed when native DNA is added to an enzymic system which produces an eletronically excited triplet carbonyl compound.

INTRODUCTION

The enzymic formation of electronically excited species other than those which occur in bioluminescence is under active investigation in this laboratory (1-15). The "photochemistry without light" of these enzymically energized species may be important for understanding certain normal or pathological processes.

The enzymic formation in high yield of triplet acetone (I; R=CH_3) and of triplet ethanal (I; R=H) has been achieved in this laboratory by the peroxidase catalysed aerobic oxidation of IBAL[2] and of propanal (13,14):



(I)

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[2]Abbreviations: IBAL, isobutanal; HRP, horseradish peroxidase; CD, circular dichroism.

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Furthermore, we have shown that the energy can be transferred to certain foreign acceptors and have thus paved the way for more detailed studies of "photochemistry in the dark" (14).

In this work, we report our finding that DNA, upon addition to the above enzymic systems, becomes altered in the same manner as upon UV irradiation, as judged by the CD spectrum.

MATERIAL AND METHODS

Calf thymus DNA, HRP (Type VI), catalase, and superoxide dismutase were obtained from Sigma Chemical Co., sodium benzoate was from Aldrich Chemical Co. and EDTA was from Harleco Co., IBAL, from Aldrich Chemical Co., was purified by distillation under nitrogen. Sodium 9,10-dibromoanthracene-2-sulfonate was available from earlier work (12).

The complete reaction mixture consisted of: 20 μM HRP, 50 μg/ml DNA, 20 μM EDTA and 80 mM substrate in 0.83 M phosphate pH 7.4 buffer; the total volume was 3.0 ml. This mixture was used for chemiluminescence, CD and emission spectra.

Light emission was determined in a Perkin-Elmer MPF-4 Fluorescence Spectrometer. A Hamamatsu TV Photocounter C-767 was used for following chemiluminescence as a function of time.

CD measurements were made in a Cary 60 Spectropolarimeter equipped with the Model 6001 CD attachment. Circular cells of 1.0 cm optical path were used. The specific ellipticity, (ψ), was expressed in degrees.cm^2.$decimoles^{-1}$.

RESULTS

If DNA is present in the peroxidase/O_2 system, which oxidizes IBAL to triplet acetone and formic acid, it undergoes alteration as shown by the CD spectrum (Fig. 1). The alteration is concomitant with the reaction and no further change is observed following O_2 depletion. No alteration occurs if both peroxidase and IBAL are omitted, or if IBAL is absent. On the other hand, exactly the same modified CD spectrum of DNA was obtained if catalase (580 units), superoxide dismutase (100 units) or benzoate (10 mM) were also present in the system. After a few hours, IBAL alone induced the same kind of alteration, albeit

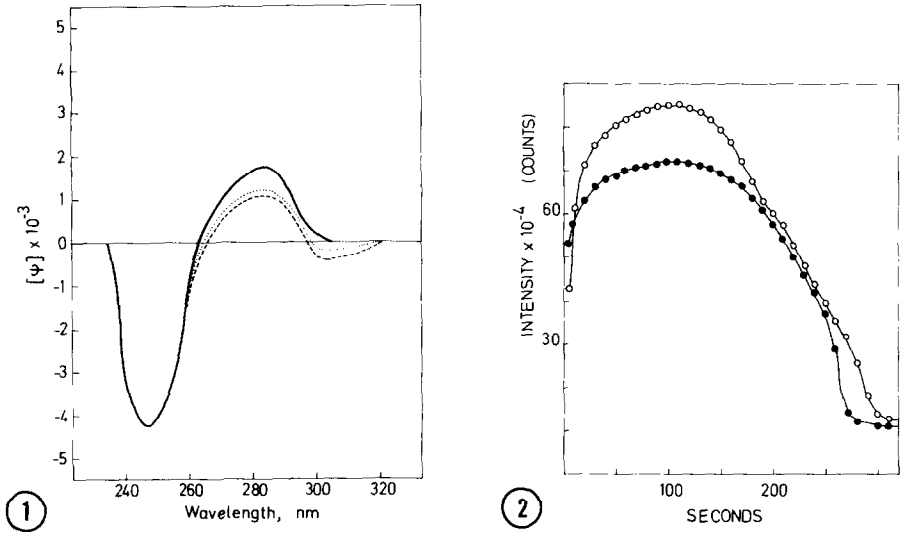

Fig. 1. CD spectrum of DNA: ——— alone; - - - when present present in the reacting IBAL-HRP-O_2 system; ..... when also 9,10-dibromoanthracene-2-sulfonate (33 μM) was also added to the reacting system.

Fig. 2. Photoemission (counts in 10 sec.) as a function of time by the IBAL-HRP-O_2 system in the absence (-o-o-) and presence (-o-o-) of DNA (50 μg/ml).

with much lower efficiency, presumably due to its non-enzymatic oxidation. When 9,10-dibromoanthracene-2-sulfonate (3.3 μM), an efficient acceptor of energy from triplet acetone (13,14) was added to the complete system, DNA alteration was reduced (Fig.1). These results, in addition to being highly reproducible, were also observed with the peroxidase system that generates excited triplet ethanal from propanal.

DNA did not affect the rate of O_2 uptake in the IBAL-HRP system. It did however diminish considerably the acetone phosphorescence emission intensity (Fig. 2).

DNA alteration could also be detected fluorimetrically (16) after elimination of acetone and unreacted IBAL (Fig. 3). Control experiments showed that the peroxidase was not fluorimetrically altered.

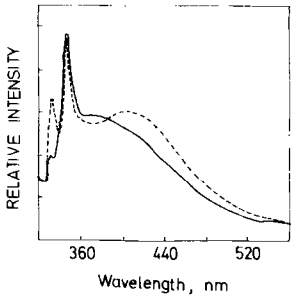

Fig. 3. Fluorescence spectrum of DNA: ——— alone; - - - when present in the reacting IBAL-HRP-O_2 system. Acetone and unreacted IBAL were eliminated by flushing with argon.

DISCUSSION

It is probable that the alteration in DNA originates from the enzymically generated triplet carbonyl compound. This may be inferred from the following evidence: (i) the same CD alteration can be induced by UV irradiation of DNA (17), a photoprocess for which triplet ketones can act as sensitizers (18), (ii) 9,10-dibromoanthracene-2-sulfonate reduces the alteration, (iii) the acetone phosphorescence intensity is considerably reduced in the presence of DNA, (iv) DNA alteration was concomitant with the generation of triplet acetone.

With respect to the last of these points, it may be argued that an intermediate of the reaction may have induced the change. However, all readily conceivable species can be dismissed. Thus, neither the enzymic reaction nor the "new" CD spectrum of DNA were influenced by the presence of either catalase or superoxide dismutase, indicating that the H_2O_2 or the free superoxide are not the active agents. Attack by the HO· radical is also rendered unlikely by the presence of an efficient scavenger of this radical, 0.5 M ethanol, in our reaction mixture.

Moreover, sodium benzoate, another well-know efficient HO· scavenger, had no effect on either the reaction or the "new" CD spectrum of DNA, DNA alteration by $^1O_2$, which would in itself be an interesting result, is also excluded because there is no indication whatsoever of the generation of singlet oxygen in our systems.

The observed DNA alteration must be a "dark photochemical" (1,2,19) effect. This is supported by the occurrence of single strand breaks (unpublished), a damage which can be induced photochemically (20,24). Since the triplet carbonyl compound is formed at the active site of the enzyme in our systems, which would impede its reaction with external species (13,14), any detailed mechanistic scheme for the energy transfer to DNA would at present be premature. Whatever the ultimate explanation may be, the fact that a photochemical-like effect can be induced in DNA in the dark by our enzymic reaction is a significant result. Earlier Lamola (25) had induced damage in DNA with chemically generated triplet acetone.

Acknowledgement. The authors wish to express their gratitude to the "Fundaçao de Amparo à Pesquisa do Estado de São Paulo" (BIOQ/FAPESP programme) and the "Financiadora de Estudos e Projetos" (FINEP) Rio de Janeiro for financial support of this work. A.Faljoni is a FAPESP post-doctoral fellow, N.Durán is a FAPESP Visiting Professor and Marcela Haun is a pre-doctoral fellow of the Brazilian "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

The authors are also deeply indebeted to Dr. Frank Quina for a critical reading of the manuscript.

REFERENCES

1. Cilento, G. (1965) Photochem.Photobiol. 4, 1243-1247.
2. Cilento, G. (1973) Quart.Rev.Biophys. 6, 485-501.
3. Cilento, G., Nakano, M., Fukuyama, H., Suwa, K. and Kamiya, I. (1974) Biochem.Biophys.Res.Communs. 58, 296-300.
4. Zinner, K., Casadei de Baptista, R. and Cilento, G. (1974) Biochem.Biophys.Res.Communs. 61, 889-898.
5. Vidigal, C.C.C. and Cilento, G. (1976) Biochem.Biophys.Res. Communs. 62, 184-190.

6. Cilento, G. (1975) J.Theor.Biol. 55, 471-479.
7. Vidigal, C.C.C., Zinner, K., Durán, N., Bechara, E.J.H. and Cilento, G. (1975) Biochem.Biophys.Res.Communs. 65, 138-145.
8. Zinner, K., Durán, N., Vidigal, C.C.C., Shimizu, Y. and Cilento, G. (1976) Arch.Biochem.Biophys. 173, 58-65.
9. Durán, N., Zinner, K., Casadei de Baptista, R., Vidigal, C. C.C. and Cilento, G. (1976) Photochem.Photobiol. 24,383-388.
10. Takayama, K., Nakano, M., Zinner, K., Vidigal, C.C.C., Durán, N., Shimizu, Y. and Cilento, G. (1976) Arch.Biochem.Biophys. 176, 663-670.
11. Zinner, K., Vidigal, C.C.C., Durán, N. and Cilento, G. (1977) Arch.Biochem.Biophys. 180, 452-459.
12. Durán, N., Zinner, K., Vidigal, C.C.C. and Cilento, G. (1977) Biochem.Biophys.Res.Communs. 74, 1146-1153.
13. Durán, N., Faria Oliveira, O.M.M., Haun, M. and Cilento, G. (1977) J.Chem.Soc.Chem.Commun. 442-443.
14. Cilento, G., Durán, N., Zinner, K., Vidigal, C.C.C., Faria Oliveira, O.M.M., Haun, M., Faljoni, A., Augusto, O., Casadei de Baptista, R. and Bechara, E.J.H. (1977) Photochem.Photobiol., in press.
15. Augusto, O. and Cilento, G. Biochem.Biophys.Res.Communs., in press.
16. Patrick, M.H. and Gray, D.M. (1976) Photochem.Photobiol. 24, 507-513.
17. Lang, H. and Luck, G. (1973) Photochem.Photobiol. 17, 387-393.
18. Lang, H. (1975) Nucleic Acid Res. 2, 179-183.
19. White, E.H., Miano, J.D., Watkins, C.J. and Breaux, E.J. (1974) Angew.Chem. (Int.) 13, 229-243.
20. Stafford, R.S., Allison, D.P. and Rahn, R.O. (1975) Nucleic Acid Res. 2, 143-148.
21. Gutter, B., Speck, W.T. and Rosenkranz, H.S. (1977) Biochim. Biophys.Acta 475, 307-314.
22. Yoakum, G.H. (1975) J.Bacteriol. 122, 199-205.
23. Rahn, R.O., Landry, L.C. and Carrier, W.L. (1974) Photochem. Photobiol. 19, 75-78.
24. Favre, A. and Vigny, P. (1975) Photochem.Photobiol. 22, 288-291.
25. Lamola, A.A. (1971) Biochem.Biophys.Res.Communs. 43, 893-898.