

GENERATION OF ELECTRONICALLY EXCITED TRIPLET SPECIES IN BIOCHEMICAL SYSTEMS

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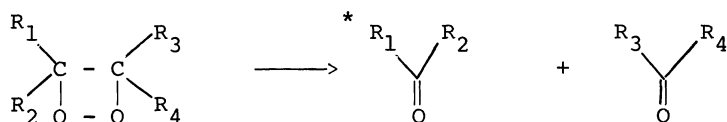
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Abstract - Triplet carbonyl species are generated in high yields by the peroxidase(oxidase)-catalyzed oxidation of appropriate substrates. As inferred from sensitized emission and induced photochemical reactions, these enzyme generated excited species can efficiently transfer their energy to appropriate acceptors. Our results open the possibility of understanding some of the multiple biological roles that peroxidases play and provide a rationale for the apparent occurrence *in vivo* of photochemical-like processes in the absence of light.

INTRODUCTION

The generation of electronically excited singlet states in biological systems is attested to by the widespread phenomenon of bioluminescence. Although sporadically considered earlier, substantial progress in the search for biochemically-generated triplet species was made only as of the late seventies (1, 2). The initial difficulty in detecting triplet states was by no means surprising as these states are easily quenched and not emissive. This reflects the long intrinsic lifetime of triplet species, a property which, at the same time, makes them potentially useful (or detrimental) in biological systems. In this regard White and co-workers (3,4) have pointed out several photochemical-like transformations which occur *in vivo* in the absence of light.

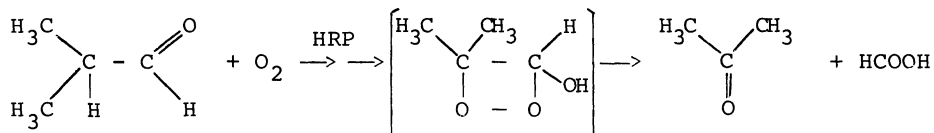
The synthesis of 1,2-dioxetanes (5) and dioxetanones (6), coupled with the observation that their cleavage gives rise to an excited carbonyl product (5-11)



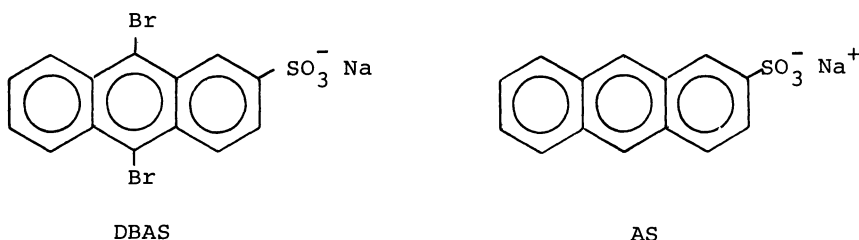
paved the way for the elucidation of the chemistry of several bioluminescent processes and provided us with a clue for the search for triplet species in biochemical systems. Thus, in bioluminescent processes, the cyclic peroxide intermediate bears extensively conjugated, easily ionizable substituents, features which favor formation of the excited carbonyl in the singlet state (12-15). In contrast, the cleavage of simple dioxetanes and dioxetanones generates the excited carbonyl species overwhelmingly in the triplet state (16,11).

ENZYME-GENERATED TRIPLET ACETONE

We therefore searched for enzymic processes which generate products of the type expected from the cleavage of a hypothetical simple dioxetane/dioxetanone intermediate. The literature indeed provided several examples, of which the most appealing appeared to be the horseradish peroxidase ("HRP") catalyzed oxidation of isobutanal to acetone and formic acid (17)



since it would allow us to exploit the available knowledge of the chemistry of dioxetane generated triplet acetone. Since the latter elicits fluorescence from 9,10-dibromoanthracene (8,18,11), but not from anthracene (16), we assayed "aqueous soluble" 2-sulfonate derivatives DBAS and AS in the above enzymatic system.



In fact, sensitized emission could be observed with DBAS, even in the μM concentration range but not with AS, consistent with the presence of long-lived triplet acetone (19).

Furthermore, when chelating agents such as EDTA or pyrophosphate are present, the enzymatic reaction itself becomes emissive with a ratio of emitted photons per molecule of oxygen consumed of at least 1×10^{-7} . As shown in Fig.1 the emission spectrum matches the acetone phosphorescence spectrum (19).

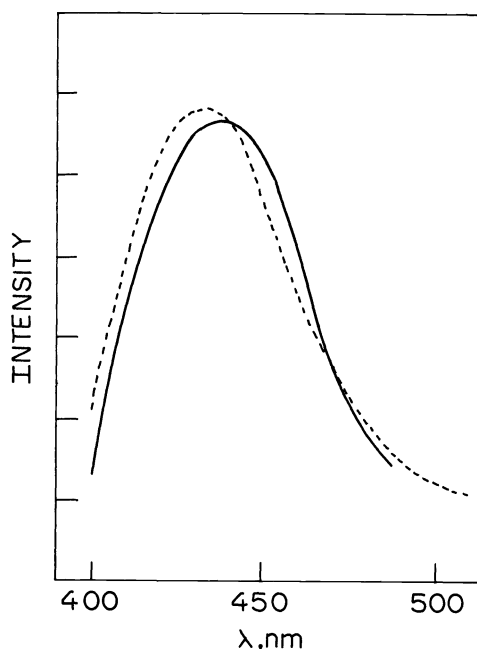


Fig. 1 - Spectrum of the emission from the peroxidase-catalyzed aerobic oxidation of isobutanol (—) at 35°C and from the thermolysis of tetramethyldioxetane at 53°C in deionized water under nitrogen (----).

This result -at first surprising- does however have some analogies (20) and suggests that triplet acetone is generated within the enzyme and shielded by the latter from deactivating oxygen collisions. The modest 2-3 fold overall increase of the emission between the beginning of the reaction (ca. 2 mM O_2) and the point where oxygen is depleted (Fig. 2), further confirms protection (21).

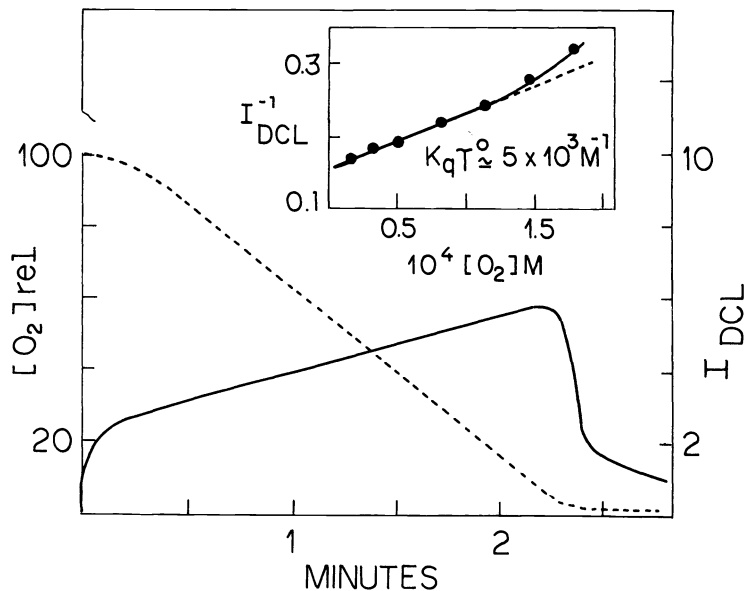


Fig. 2 - Oxygen consumption and emission intensity during peroxidase catalyzed aerobic oxidation of isobutanol. The insert shows a plot of the reciprocal of the emission intensity vs. oxygen concentration.

When HRP is replaced by hemin, triplet acetone is still formed, as indicated by sensitized emission, but, being unprotected, it does not phosphoresce due to quenching by oxygen (22). The *in situ* formation of triplet acetone has been firmly substantiated by quenching experiments with D- and L-tryptophan. The Stern-Volmer plots (Fig. 3) exhibit a strong chiral discrimination (23)

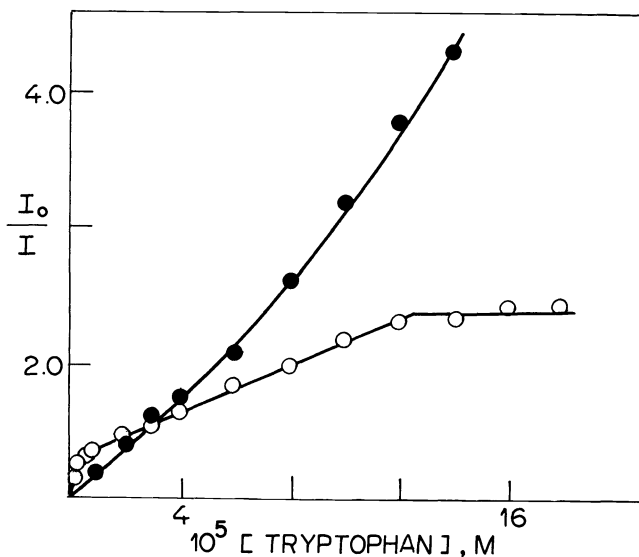


Fig. 3 - Stern-Volmer plot for the quenching of the enzymically-generated acetone phosphorescence by L-tryptophan (-o-) and by D-tryptophan (-●-).

indicating that the quenchers encounter the excited species within the enzyme. The Stern-Volmer behavior of L-tryptophan may also reflect heterogeneity of the triplet acetone population. As expected, D- and L-tryptophan are equally efficient at quenching triplet acetone generated via thermolysis of dioxetanes (monitored by transfer to DBAS).

In the presence of chelating agents, the sensitized DBAS emission can be seen even with the dark adapted eye. From the y-intercept/slope ratio of a double reciprocal plot of the effect of DBAS concentration upon the intensity of emission, $k_{ET}\tau^0$ is found to be $2 \times 10^5 \text{ M}^{-1}$ (21). AS quenches both the acetone phosphorescence ($K_{sv} = 2 \times 10^5 \text{ M}^{-1}$) and the DBAS-sensitized emission ($K_{sv} = 1.7 \times 10^5 \text{ M}^{-1}$). However, even though the donor does transfer to AS, the latter does not fluoresce. These results indicate energy transfer from the donor to the T_2 level of DBAS and AS. In DBAS, the heavy bromo atoms favour the spin-forbidden reverse intersystem crossing to S_1 to the extent that it can compete with internal conversion to the T_1 state and fluorescence is seen. This is analogous to the behavior of triplet acetone, generated from the thermolysis of dioxetanes, towards 9,10-dibromoanthracene (23,25).

These results have recently been substantiated with micelle-solubilized 9,10-dibromoanthracene and anthracene (26). In the spectrum of the sensitized dibromoanthracene fluorescence, the well-resolved vibrational bands are noteworthy (Fig. 4); when the enzymatic reaction has almost depleted the available oxygen, a new emission, ascribed to the expected (27) phosphorescence,

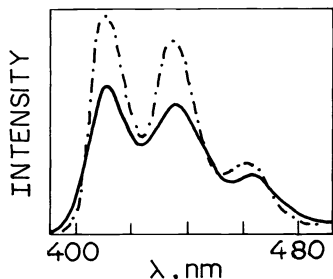
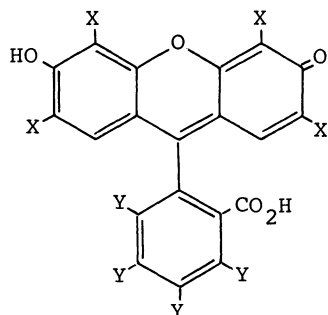


Fig.4 - Spectrum of the emission elicited by $10 \mu\text{M}$ 9,10-dibromoanthracene during the enzymic generation of triplet acetone (—) and of the fluorescence of the spent reaction mixture (-----); $\lambda_{\text{exc}} = 383 \text{ nm}$. Dibromoanthracene solubilized by 3 mM Brij-35.

is seen in the red. Anthracene gave no evidence for this emission, presumably as a result of the intrinsically poor radiative properties of its triplet.

Triplet-triplet energy transfer to an upper triplet of the acceptor as the first step of a sensitized emission has also been suggested to occur with xanthene dyes (28). Thus fluorescein 1a, eosine 1b, and Rose Bengal 1c quench the enzyme-generated acetone phosphorescence, the corresponding K_{sv} values



- 1a X = H, Y = H
 1b X = Br, Y = H
 1c X = I, Y = Cl

(2.3×10^5 , 1.8×10^4 and $4.2 \times 10^4 \text{ M}^{-1}$) and $k_{ET}\tau^0$ values (9.8×10^4 , 3.3×10^4 and $6.7 \times 10^4 \text{ M}^{-1}$, respectively) being quite similar. However, when the emission intensity at infinite acceptor concentration is corrected for ϕ_F of the acceptor (0.92 for fluorescein, 0.19 for eosine and 0.02 for Rose Bengal (29), the relative populations of the S_1 state are found to be 1:15:100,

respectively. These results are consistent with a heavy atom effect, presumably exerted on reverse intersystem crossing (30). Long-range Förster-type radiationless energy transfer would be expected to show the reverse order since the overlap between the acetone phosphorescence spectrum and the acceptor absorption band decreases upon going from fluorescein to eosine to Rose Bengal. Similar results have recently been obtained with triplet acetone generated by tetramethyldioxetane thermolysis (31).

Enzyme-generated triplet acetone elicits phosphorescence emission from biacetyl (21) (Fig.5), thus confirming the occurrence of triplet-triplet energy transfer processes. It also elicits fluorescence from other acceptors such as

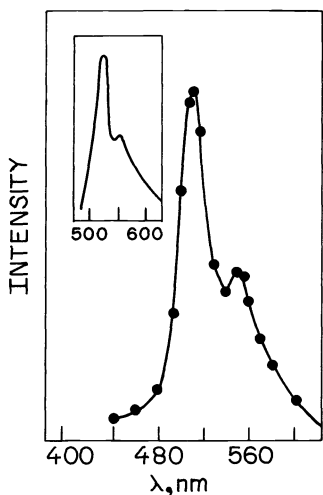


Fig.5 - Spectrum of the emission elicited during the enzymic generation of triplet acetone in the presence of 2 mM biacetyl. The insert shows the biacetyl phosphorescence spectrum.

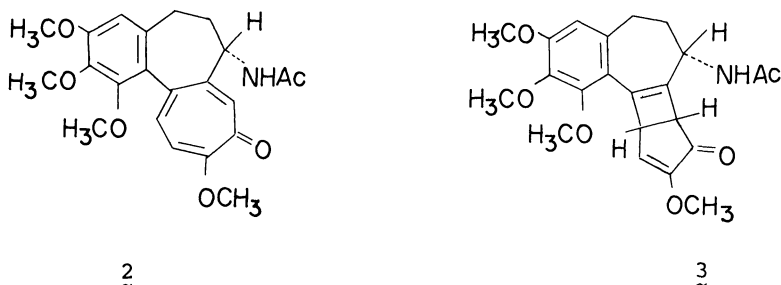
flavins (32), micelle-solubilized chlorophyll (26), and even chloroplasts (33), though the mechanism has not been fully ascertained. Typically, the K_{sv} and/or $k_{ET}\tau^0$ values for enzyme generated triplet acetone are roughly one order of magnitude higher than those for triplet acetone generated via thermolysis of tetramethyldioxetane (31).

An intriguing question is why the apparent Stern-Volmer quenching constants for a number of triplet energy acceptors are larger than that for O_2 , a molecule which might be expected to diffuse readily in proteins (34). In some cases the acceptor may bind to the acting enzyme near the site where triplet acetone is generated. An example may be quenching by 3,5-dihalogenotyrosines, which leads to linear Stern-Volmer plots when triplet acetone is generated by thermolysis of tetramethyldioxetane and to upward curving plots when it is generated enzymically (35).

INDUCED PHOTOCHEMISTRY

Enzyme-generated triplet acetone is always accompanied by some isopropanol (up to 7%); all controls indicate that the latter is formed by reduction of triplet acetone (21). Since triplet acetone is able to elicit emission from several acceptors, it should also be able to sensitize or promote photochemistry, thus providing the biochemical counterpart of "photochemistry without light" accomplished with dioxetanes (36).

Several photochemical-like processes have been accomplished, among them chlorpromazine photooxidation (37), single-strand breaks in DNA (38), phytochrome phototransformations (39) and tryptophan photooxidation (23). Especially noteworthy, both for its importance and the solid evidence accumulated, is the conversion of colchicine (2) into α, β and γ -lumicolchicines (40), mainly the β -isomer 3:



The transformation is accompanied by quenching of the acetone phosphorescence ($K_{sv} = 4.6 \times 10^3 \text{ M}^{-1}$). The formation of β -lumicolchicine and of some γ -isomer has been fully confirmed using ^3H -labelled colchicine (Fig. 6).

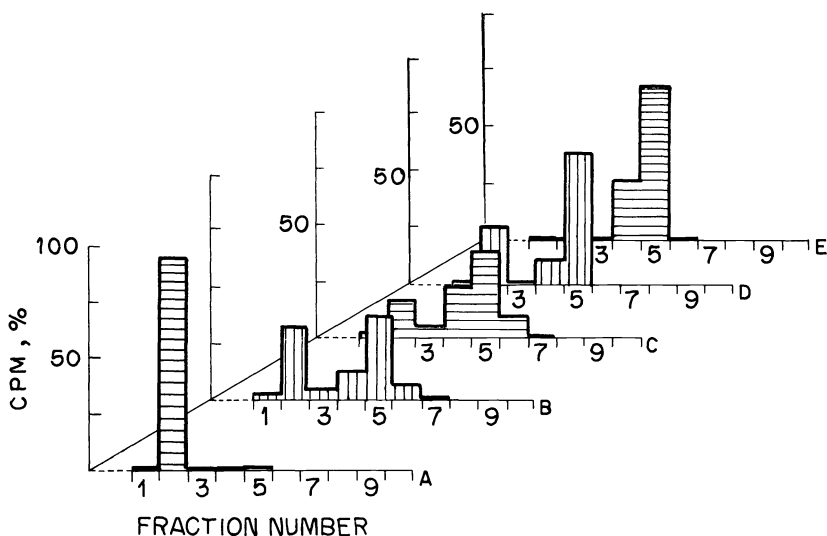
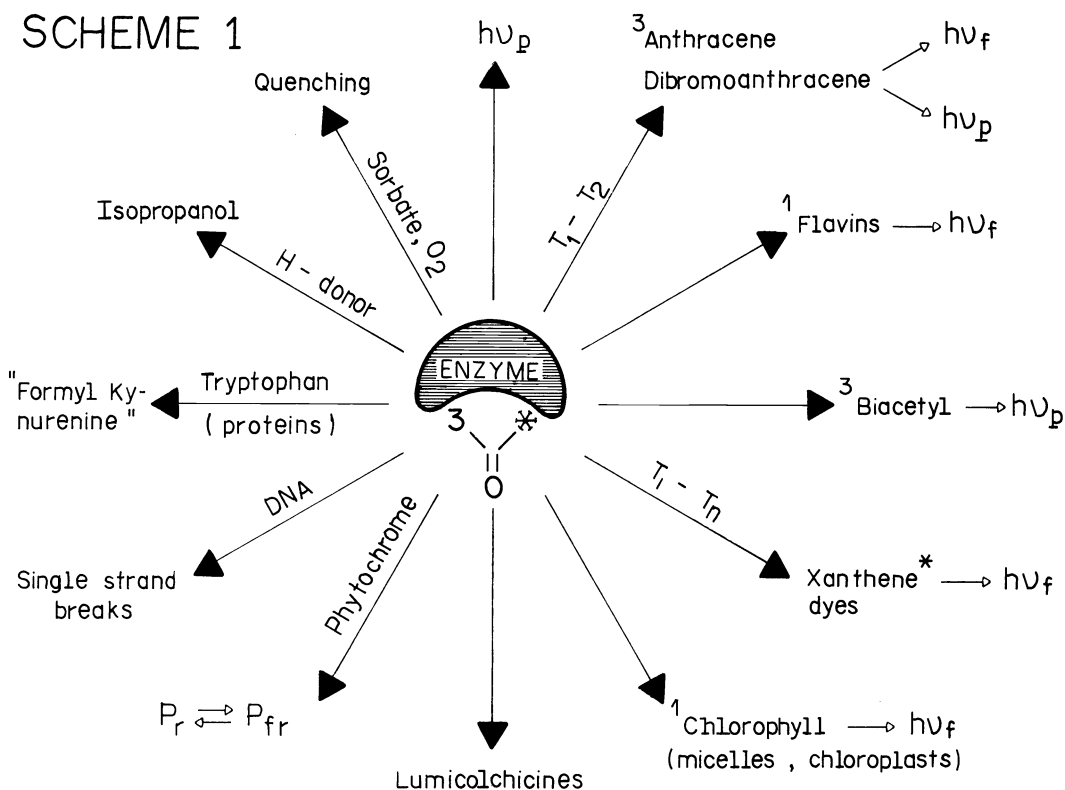


Fig. 6 - Formation of lumicolchicine from the exposure of [ring C, methoxy- ^3H]-labelled colchicine to the enzymic system which generates triplet acetone and to UV light. Thin layer chromatogram of colchicine exposed to: (A) isobutanal; (B) isobutanal and peroxidase for 15 min.; (C) isobutanal and peroxidase for 30 min; (D) UV light for 60 min; (E) UV light for 255 min. Fraction: 2, colchicine; 3, α -colchicine; 4, γ -colchicine; 5, β -colchicine; 6, unknown.

Based on oxygen consumption and concomitant disappearance of colchicine, $\phi_{\text{chemiexcitation}} \times \phi_{\text{transfer}} \times \phi_{\text{photochemistry}}$ is 0.065, a remarkable value even without optimization; clearly, the three steps must be efficient. Indeed, upon direct irradiation the quantum yield is only 0.003 (41). Since excitation to the singlet state does not meet Förster criteria, enzyme-generated triplet acetone must excite colchicine to the triplet state. The importance of this photochemical process accomplished in the dark stems from the fact that lumicolchicine is formed from colchicine in *Colchicum autumnale* L. even in parts not exposed to light, such as the corm of plants grown in the dark. The transformation is strongly endergonic and there is no evidence that this or related transformations can be accomplished either thermally or enzymically.

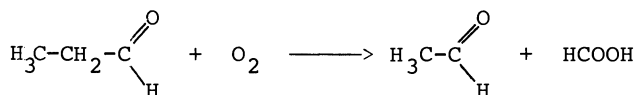
In conclusion, the main processes observed with enzyme-generated triplet acetone are summarized in Scheme 1.

SCHEME 1



PEROXIDASE-CATALYZED OXIDATION OF LINEAR ALDEHYDES

The HRP catalyzed oxidation of propanal leads to acetaldehyde and formic acid (42).



Although this system is not emissive, the formation of an excited species -most certainly triplet acetaldehyde- is attested to by sensitized emission. While DBAS and flavins emit very weakly, strong fluorescence is elicited from xanthene dyes (42), as well as from micelle-solubilized chlorophyll (26) (Fig. 7), chloroplasts (43) or thylacoid membrane fractions enriched in either photosystem I or II (44). Based on oxygen consumption, $\phi_{\text{chemiexc.}} \times \phi_{\text{transfer}}$ is 6×10^{-4} for the S_1 state populating of chlorophyll-a in micellar CTAB.

Sensitized emission is also elicited by higher homologues of propanal (43), though the lower solubility of pentanal and hexanal leads to a reduced rate of reaction -and therefore of excited state formation. Chloroplasts excited in this way do not promote the Hill reaction, a result which points to selective excitation of certain chlorophylls. Based on oxygen consumption and assuming $\phi_{\text{F}} \sim 0.01$ for the fluorescence of chlorophyll in chloroplasts, the efficiency of populating the S_1 state of chlorophyll, *i.e.*, the product $\phi_{\text{chemiexc.}} \times \phi_{\text{transfer}}$, reaches the value 2×10^{-4} in the case of pentanal as substrate.

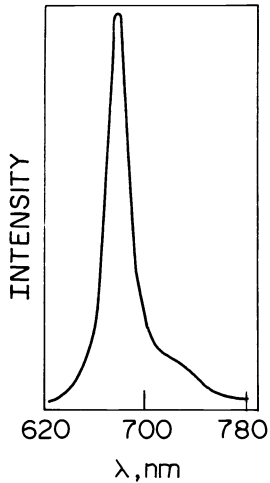


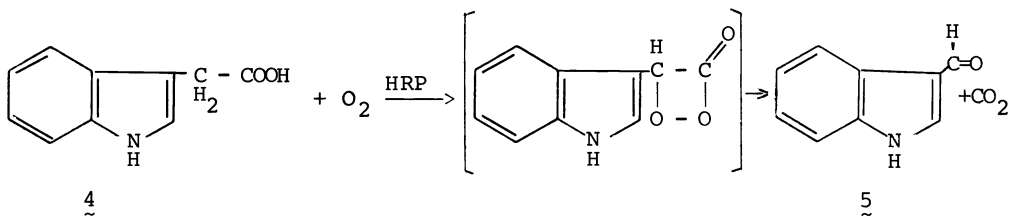
Fig.7 - Spectrum of the emission elicited by 3.5 μ M chlorophyll during the peroxidase catalyzed aerobic oxidation of propanal. The spectrum matches perfectly that from optically excited chlorophyll. CTAB, 0.8 mM, was present to solubilize chlorophyll.

When the C_2 - C_6 aldehydes are compared at the same (low) concentration which allows solubilization a plot of the total sensitized emission (with eosine, lb, as acceptor) against chain length of the substrate shows a well-defined maximum at C_4 , the sequence being $C_4 > C_3 >> C_5 > C_6 > C_2$ (45). The relevance of this result for the fact that the multiple biological activities of linear aliphatic acids have a maximum at C_4 remains to be ascertained.

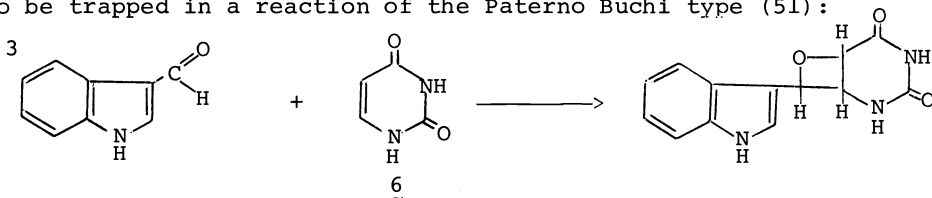
The non-emissivity of enzyme-generated excited aliphatic aldehydes is probably due to "self-quenching" by the excess (homologous) substrate (46). This feature makes the triplet aldehyde generating system a very useful alternative to the triplet acetone generating system for the study of sensitized emissions processes that are only weakly emissive. A recent example is the use of the former system to elicit emission from microsomes (47).

PEROXIDASE-CATALYZED AEROBIC OXIDATION OF INDOLEACETIC ACID

Indole-3-acetic acid ("IAA"; 4) is a plant hormone whose peroxidase-catalyzed oxidation is biochemically important (48,49). Indole-3-aldehyde ("IAL"; 5) is a major product, especially at acidic pHs. Since IAL and CO_2 are the expected products from a hypothetical α -peroxylactone intermediate, we searched for



excited IAL. In a model system -the autoxidation of the phenylthiolester of IAA in dimethylsulfoxide/potassium t-butoxide- chemiluminescence identical to IAL fluorescence was observed and IAL could be isolated from the spent reaction mixture (50). In the enzymatic system, addition of uridine (6) permitted 3 IAL to be trapped in a reaction of the Paternò Buchi type (51):



Since intersystem crossing is very efficient in $^1\text{IAL}^*$ (52), irradiation of a mixture of IAL and uridine at 280 nm leads to the same product (51). These results have been verified by comparative experiments with the systems $[2\text{-}^{14}\text{C}]\text{-IAA/HRP/O}_2\text{/uridine}$ and $[1\text{-}^{14}\text{C}]\text{-IAL/uridine/irradiation}$ at 280 nm and the system $\text{IAA/HRP/O}_2\text{/[}^3\text{H}\text{]-uridine}$ and $\text{IAL/[}^3\text{H}\text{]-uridine/irradiation}$ at 280 nm.

Uridine cannot be replaced by other bases. Enzymically or optically-generated ^3IAL can, however, also be trapped by t-RNA. Based on $2\text{-}^{14}\text{C}$ labelled substrate, the enzymatic reaction leads to 20% incorporation. This provides a minimum for the chemiexcitation yield to ^3IAL . Less radioisotope was incorporated at pH 5.6 than at pH 3.8 and even less at pH 6.8, in agreement with decreased enzymic formation of IAL at higher pH values. By cleaving t-RNA, the same Paternò-Büchi product as reported above was isolated (53). Incidentally, these results support the earlier inference, based on the fact that O_2 consumption is faster than IAL formation, that ^3IAL is generated free in the bulk solution (54).

Enzyme-generated ^3IAL does not excite DBAS or flavins; however, eosine fluorescence is readily elicited (54). Very strong chlorophyll fluorescence is observed provided the chlorophyll is micelle-solubilized (26); in addition to the 680 nm peak, the emission spectrum (Fig. 8) exhibits another strong peak

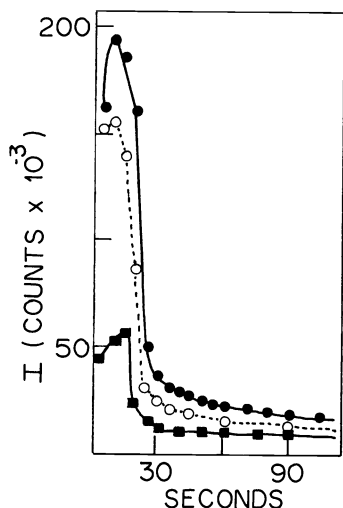


Fig. 8 - Spectrum of the emission elicited by $3.5 \mu\text{M}$ chlorophyll during the peroxidase-catalyzed aerobic oxidation of indole-3-acetic acid. Chlorophyll solubilized by 0.8 mM CTAB.

at 625 nm whose origin has not yet been elucidated. Eosine and chlorophyll appear to be excited by energy transfer, a CIEEL (10) process with the precursor intermediate being unlikely because none of these acceptors speed up IAL formation (pH 3.8).

As shown in Fig. 9, the IAA/HRP/O_2 system also elicits red emission from chloroplasts (33); the number of chlorophylls excited per primary chemiexcitation of the donor is 2×10^{-5} at pH 5.6.

Sensitized emission is also observed with thylakoid membrane fractions enriched in either photosystem-I or photosystem II: the emission sensitized with the photosystem I-enriched fractions is sufficiently intense that the emission spectrum can be registered on conventional equipment. The spectrum is identical to that observed upon direct excitation (44).

Among the systems assayed, the IAA/HRP/O_2 system is the only one which elicits emission from t-RNA^{Phe} (53). The emission spectrum suggests that the emitter is the thiouridine group and indeed, no emission is elicited from yeast t-RNA, which is known to lack thiouridine. Magnesium ion, which affects the t-RNA^{Phe} structure, reduced label incorporation, but increased the sensitized emission.

By analogy to the IAA/HRP/O_2 system, one may infer that the α -peroxidase system of higher plants, which oxidizes fatty acids to the next lower aldehyde and CO_2 (55), generates the aldehyde in an excited state. Evidence for an α -hydroperoxide has indeed been presented (56).

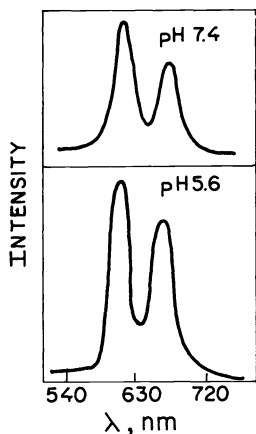
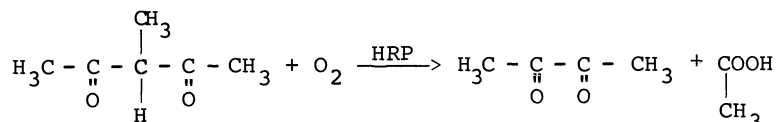


Fig.9- Temporal behavior of the emission elicited from chloroplasts during the peroxidase-catalyzed aerobic oxidation of indole-3-acetic acid at pH 5.6: (-●-●-) in the absence and (-o-o-) in the presence of the 630 nm cut-off filter. No significant red emission is observed if any one of the components is omitted. Lower curve: emission from the enzymatic system without filter.

OTHER CHEMIENERGIZING SYSTEMS

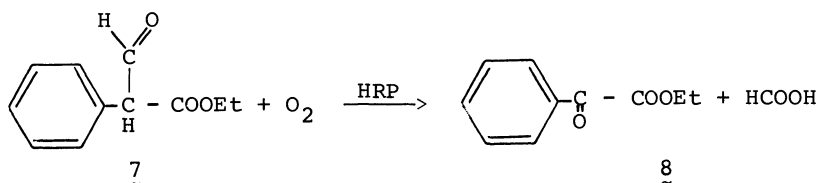
The peroxidase-catalyzed aerobic oxidation of 3-methylacetoacetone leads to triplet biacetyl, identified by its phosphorescence spectrum (57).



As indicated by the fluorescence elicited from micelle-solubilized chlorophyll, chemiexcitation occurs during the oxidation of phenylacetaldehyde to benzaldehyde and formic acid



and of the ethyl ester of α -formylphenylacetic acid (7) to the ethylester of benzoylformic acid (8) and formic acid (58):



The latter system is of special interest because it occurs in the roots of *Datura innoxia* (59).

Sensitized emission of CTAB-solubilized chlorophyll also points to excited state formation in the peroxidase-catalyzed aerobic oxidation of the tuberculostatic and carcinogenic drug isonicotinic acid hydrazide to pyridine-4-aldehyde (60); this reaction does not proceed through a dioxetane.

Excited species are also formed during microsomal lipid peroxidation. Although the emission spectrum indicates singlet oxygen, the occurrence of other species -most likely triplet carbonyls- has been demonstrated by adding chlorophyll to the microsome preparation. The lipid peroxidation induced with *t*-butylhydroperoxide is then accompanied by a red emission similar to that observed in the fluorescence spectrum of microsome-bound chlorophyll (61).

CONCLUDING REMARKS

Both trapping experiments, e.g., uridine in the IAA/HRP/O₂ system and induced photochemistry, e.g. conversion of colchicine into lumicolchicine sensitized by acetone, are consistent with chemiexcitation yields of at least 0.2. This substantial generation of triplet species by enzymatic systems and the resultant photochemistry promoted by them open the possibility of understanding some of the multiple roles that peroxidases play. One can conceive of at least three functional roles for triplet species in vivo. The first is reaction with a biologically important structure, an in vitro example being provided by adduct formation with t-RNA in the IAA/HRP/O₂ system. Secondly, energy transfer may drive photochemical processes, a likely example being the conversion of colchicine into lumicolchicines. The third -not entirely independent of the second and more speculative- is energy transfer to a photochromic molecule to generate a biologically active isomer. Inherent in each of these roles is the possibility of detrimental effects, and indeed endogenously generated excited species can lead to spontaneous mutagenesis in excision-repair deficient strains of bacteria (62).

Finally, we note that the enzymatic generation of excited species with a multiplicity different from that of the ground state is no longer limited to triplet species; the generation $^1\Delta_g \rightarrow ^1O_2$ has recently been unequivocally confirmed (63).

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REFERENCES

1. G. Cilento, Acc.Chem.Res. **13**, 225-230 (1980); Photochem.Photobiol.Rev., **5**, 199-228 (1980). These reviews contain references pertaining to a possible role of excited states in biological processes as considered by other authors.
2. G. Cilento, Chemical and Biological Generation of Excited States, (Eds. W. Adam and G. Cilento) 277-307, Academic Press, New York (1982).
3. E.H. White and C.C. Wei, Biochem.Biophys.Res.Comm. **39**, 1219-1223 (1970).
4. E.H. White, J.D. Miano, C.J. Watkins and E.J. Breaux, Ang.Chem.Int.Ed. Engl. **13**, 229-243 (1974).
5. K.R. Kopecky and C. Mumford, Can.J.Chem. **47**, 709-711 (1969).
6. W. Adam and J.-C. Liu, J.Am.Chem.Soc. **94**, 2894-2895 (1972).
7. F. McCapra, Acc.Chem.Res. **9**, 201-208 (1976).
8. T. Wilson, Chemical Kinetics (Ed. D.R. Hersbach), Ser II, 265-322, MTP Int.Rev.Sci., London.
9. J.W. Hastings and T. Wilson, Photochem.Photobiol. **23**, 461-473 (1976).
10. G.B. Schuster and K.A. Horn, Chemical and Biological Generation of Excited States (Eds. W. Adam and G. Cilento) 229-247, Academic Press, New York (1982).
11. W. Adam, Chemical and Biological Generation of Excited States, (Eds. W. Adam and G. Cilento) 115-112, Academic Press, New York (1982).
12. F. McCapra, I. Beheshti, A. Burford, R.A. Hann and K.A. Zaklika, J.Chem.Soc.Chem.Comm. **24**, 944-946 (1977).
13. O. Shimomura, T. Goto and F.H. Johnson, Proc.Natl.Acad.Sci.USA **74**, 2799-2802 (1977).
14. O. Shimomura, Chemical and Biological Generation of Excited States (Eds. W. Adam and G. Cilento) 249-276, Academic Press, New York (1982).
15. A.P. Schaap and S.D. Gagnon, J.Am.Chem.Soc. **104**, 3504-3506 (1982).
16. N.J. Turro, H.C. Steinmetzer and A. Yekta, J.Am.Chem.Soc. **95**, 6468-6470 (1973).
17. R.H. Kenten, Biochem.J. **55**, 350-360 (1953).

18. N.J. Turro, P. Lechtken, G. Schuster, J. Orell, H.C. Steinmetzer and W. Adam, J.Am.Chem.Soc. 96, 1627-1629 (1974).
19. O.M.M. Faria Oliveira, M. Haun, N. Durán, P.J. O'Brien, C.R. O'Brien, E. J.H. Bechara and G. Cilento, J.Biol.Chem. 253, 4707-4712 (1978).
20. T.L. Gioannini and P. Campbell, Biochem.Biophys.Res.Commun. 96, 106-113 (1980).
21. E.J.H. Bechara, O.M.M. Faria Oliveira, N. Durán, R. Casadei de Baptista and G. Cilento, Photochem.Photobiol. 30, 101-110 (1979).
22. O. Augusto and E.J.H. Bechara, Biochim.Biophys.Acta 631, 203-209 (1980).
23. E. Rivas-Suárez and G. Cilento, Biochemistry 20, 7329-7333 (1981).
24. R. Schmidt, H. Kelm and H.-D. Brauer, Ber.Bunsenges.Phys.Chem. 81, 402-407 (1977).
25. T. Wilson and A.M. Halpern, J.Am.Chem.Soc. 102, 7272-7279 (1980).
26. I.L. Brunetti, G. Cilento and L. Nassi, Photochem.Photobiol. 38, 511-519 (1983).
27. N.J. Turro, M. Grätzel and A.M. Braun, Ang.Chem.Int.Ed. 19, 675-696 (1980).
28. N. Durán and G. Cilento, Photochem.Photobiol. 32, 113-116 (1980).
29. M.J. Wade and J.D. Spikes, Photochem.Photobiol. 14, 221-224 (1971).
30. J. Saltiel, D.E. Townsend and A. Sykes, J.Am.Chem.Soc. 105, 2530-2538 (1983).
31. L.H. Catalani and E.J.H. Bechara, Photochem.Photobiol., in press.
32. M. Haun, N. Durán and G. Cilento, Biochem.Biophys.Res.Commun. 81, 779-784 (1978).
33. L. Nassi and G. Cilento, Photochem.Photobiol. 37, 233-237 (1983).
34. D.B. Calhoun, J.M. Vanderkooi, G.V. Woodrow III and S.W. Englander, Biochemistry 22, 1526-1532 (1983).
35. E. Rivas-Suárez, L.H. Catalani, E.J.H. Bechara and G. Cilento, Photochem.Photobiol. 37, 93-97 (1983).
36. E.H. White, J. Wiecko and D.F. Roswell, J.Am.Chem.Soc. 91, 5194-5196 (1969).
37. N. Durán, M. Haun, A. Faljoni and G. Cilento, Biochem.Biophys.Res.Commun. 81, 785-790 (1978).
38. R. Meneghini, M.E. Hoffmann, N. Durán, A. Faljoni and G. Cilento, Biochim.Biophys.Acta 518, 177-180 (1978).
39. O. Augusto, G. Cilento, J. Jung and P.-S. Song, Biochem.Biophys.Res.Commun. 83, 963-969 (1978).
40. I.L. Brunetti, E.J.H. Bechara, G. Cilento and E.H. White, Photochem.Photobiol. 36, 245-249 (1982).
41. H. Roight and R.M. Leblanc, Can.J.Chem. 51, 2821-2827 (1973).
42. M. Haun, N. Durán, O. Augusto and G. Cilento, Arch.Biochem.Biophys. 200, 245-252 (1980).
43. A. Campa, L. Nassi and G. Cilento, Photochem.Photobiol., in press.
44. L. Nassi and G. Cilento, unpublished.
45. A. Campa and G. Cilento, submitted for publication.
46. W.J. Leigh and J.C. Scaiano, J.Am.Chem.Soc. 105, 5652-5657 (1983).
47. A. Campa and G. Cilento, unpublished.
48. J. Ricard and D. Job, Eur.J.Biochem. 44, 359-374 (1974).
49. R. Nakajima and I. Yamazaki, J.Biol.Chem. 254, 872-878 (1979).
50. N. Durán, K. Zinner, R. Casadei de Baptista, C.C.C. Vidigal and G. Cilento, Photochem.Photobiol. 24, 383-388 (1976).
51. M.P. De Mello, S.M. De Toledo, M. Haun, G. Cilento and N. Durán, Biochemistry 19, 5270-5275 (1980).
52. P.-S. Song and W.E. Kurtin, J.Am.Chem.Soc. 91, 4892-4906 (1969).
53. M.P. De Mello, S.M. De Toledo, H. Aoyama, H.K. Sarkar, G. Cilento and N. Durán, Photochem.Photobiol. 36, 21-24 (1982).
54. C.C.C. Vidigal, A. Faljoni-Alário, N. Durán, K. Zinner, Y. Shimizu and G. Cilento, Photochem.Photobiol. 30, 195-198 (1979).
55. R.O. Martin and P.K. Stumpf, J.Biol.Chem. 234, 2548-2554 (1959).
56. W.E. Shine and P.K. Stumpf, Arch.Biochem.Biophys. 162, 147-157 (1974).
57. C.H.L. Soares and E.J.H. Bechara, Photochem.Photobiol. 36, 117-119 (1982).
58. W. Adam, L.A. Arias Encarnación, G. Cilento, M. Haun and L. Nassi, Photochem.Photobiol., in press.
59. V.S. Kalyanaraman, S. Mahdevan and S.A. Kumar, Biochem.J. 149, 565-576 (1975).
60. L. Nassi and G. Cilento, Arch.Biochem.Biophys., 229, 220-225 (1984).
61. E. Cadenas, H. Sies, A. Campa and G. Cilento, submitted for publication.
62. N.J. Sargentini and K.C. Smith, Carcinogenesis 2, 863-872 (1981).
63. A.U. Khan, J.Am.Chem.Soc. 105, 7195-7197 (1983).