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PHOTOCHEMICAL OXIDATION OF CHLORPROMAZINE IN THE DARK INDUCED BY ENZYMICALLY GENERATED TRIPLET CARBONYL COMPOUNDS[1]

Nelson Durán[2], Marcela Haun[3], Adelaide Faljoni[4] and Giuseppe Cilento[5]

Department of Biochemistry, Instituto de Química, Universidade de São Paulo, C.P. 20780, São Paulo, Brazil.

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SUMMARY

Enzymically generated triplet acetone and ethanal transfer energy to chlorpromazine as indicated by (i) suppression of the acetone chemiphosphorescence (ii) concomitant formation of chlorpromazine photoproducts, that is the radical cation and the sulfoxide (iii) inhibition of photoproduct formation by a very efficient competition for triplet carbonyl energy using the sodium salt of 9,10-dibromoanthracene-2-sulfonic acid.

This is the first report of a photooxidation in the dark.

INTRODUCTION

In earlier papers the enzymic generation of electronically excited triplet carbonyl compounds and transfer of their energy to fluorescent acceptors has been reported (1-6). Our next goal has been to induce dark photo processes (7-11) with these excited species.

Chlorpromazine (CPZ)[§] is a most interesting drug as it produces several pharmacological effects (12-14) and it is phototoxic (15-17). It was of interest to examine its behaviour when added to a triplet carbonyl generating system.

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[2]Dr. N. Durán is a FAPESP Visiting Professor (Universidad Católica de Valparaiso, Chile.

[3]M. Haun is a pre-doctoral fellow of CNPq.

[4]Dr. A. Faljoni is a FAPESP post-doctoral fellow.

[5]To whom inquires should be addressed.

[§]Abbreviations used: CPZ, chlorpromazine; CPZO, chlorpromazine sulfoxide; HRP, horseradish peroxidase (type VI); EDTA, ethylenediamintetracetic acid; DBAS, 9,10-dibromoanthracene-2-sulfonate (sodium salt).

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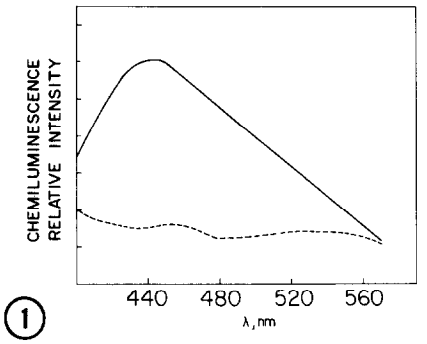


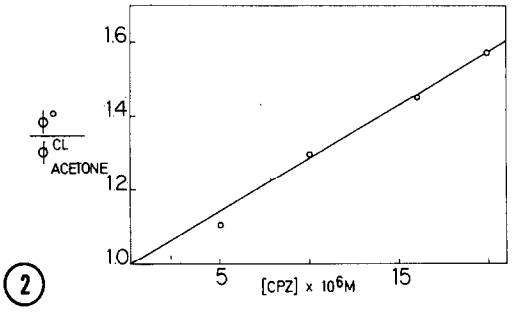


Fig. 1 - Chemiluminescence from the isobutanal/HRP/O_2 system in the absence (———) and presence of CPZ (----).

Fig. 2 - Stern-Vomer plot for the quenching of acetone phosphorescence from the isobutanal/HRP/O_2 by CPZ.

MATERIALS AND METHODS

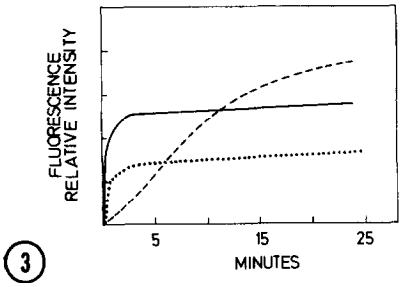
Horseradish peroxidase (HRP)[§] and EDTA were obtained from Sigma, CPZ-HCl, spectrophotometrically pure was kindly furnished by Rhodia Chemical Co. Brazil, Isobutanal, propanal and hydrogen peroxide were from Merck. DBAS was available from earlier work (1,4,18). The standard reaction mixture was: 2.0 μM HRP, 80.0 mM isobutanal or 3.0 mM propanal, 1 mM CPZ, 1 mM EDTA and 0.5M ethanol in 0.1M phosphate buffer pH 6.8. The total volume was 3.0 ml. The peroxidatic (control) reaction mixture was: 8.0 μM HRP, 1 mM CPZ, 1 ml of H_2O_2 (0.1% v/v) in 0.05M acetate buffer pH 4.5 in a total volume of 3.0 ml.

The fluorescence and absorption spectra were measured in a Perkin-Elmer Spectrophotometer MPF-4 and a Zeiss DMR-21 recording spectrophotometer, respectively. Oxygen consumption was measured with a Yellow Spring Model 53 Oxygen Monitor.

Chromatography was carried out on Whatman Nº 1 paper. The solvent was the upper phase of a biphasic system resulting from a 50:50:12; v/v/v mixture of n-butanol, water and glacial acetic acid (14).

RESULTS

When 1 mM CPZ was present in the triplet acetone generating system, that is, isobutanal/HRP/O_2 the rate of O_2 uptake was not affected during the first half of the reaction and only moderately slowed at lower O_2 tension. However, the acetone phosphorescence emission was completely suppressed (Fig. 1). A Stern-Vomer plot of this quenching effect is linear; the slope, that is $k_q\tau$, is $2.9 \times 10^4\ M^{-1}$ (Fig. 2). As a result of this quenching effect CPZ is altered. One of the products is CPZO, identified in the reaction mixture by its fluorescence maximum at 380 nm (λ_{exc}=335nm)



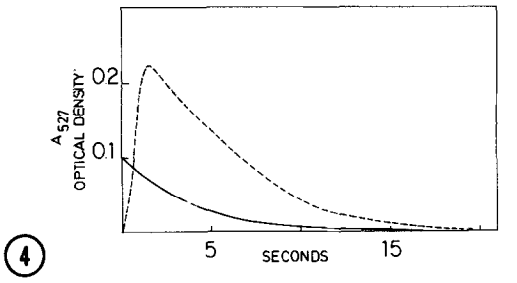


Fig. 3 - CPZO formation following the addition of CPZ to the isobutanal/HRP/O_2 (———) and to the propanal/HRP/O_2 (····) system. The dotted line represent CPZO formation in the HRP/H_2O_2/CPZ system.

Fig. 4 - CPZ radical cation formation followed at 527 nm when CPZ was added to the isobutanal/HRP/O_2 system (----) and to the propanal/HRP/O_2 system (———).

and also by paper chromatography. With the latter technique when the initial CPZ concentration was 1.8×10^{-4}M only CPZO was detected. In one experiment at 40^{o}C the disappearance of CPZ was greater than expected on the basis of the oxygen present.

CPZO was also formed when CPZ was added to the propanal/HRP/O_2 system which is known to generate triplet ethanal (4). The sulfoxide can also be formed peroxidatically (19) but at much slower rate (Fig. 3).

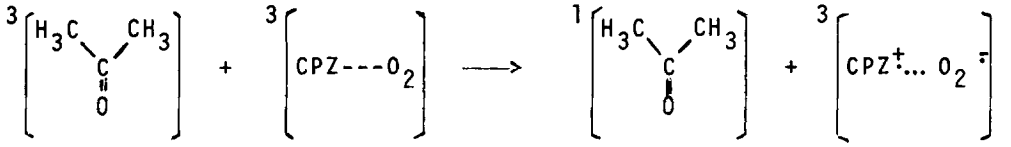
A double reciprocal plot of the effect of CPZ concentration upon the appearance of CPZO fluorescence is linear; the intercept/slope ratio, k_T, is 1.6×10^{4} M^{-1}. DBAS, which is very efficient abstractor of triplet carbonyl energy (1-4), inhibited CPZO formation; the Stern-Vomer plot is linear with a slope of 1.2×10^{5} M^{-1}. Stern Volmer plots (minimun of four points) were linear with correlation coefficients > 0.95.

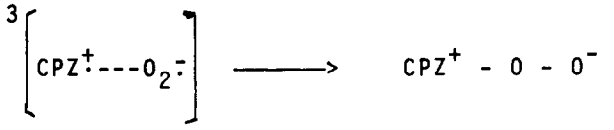
Another product formed as a result of the quenching effect of CPZ was the radical cation, $CPZ^{+\cdot}$ (λ_{max} = 527 nm), which decayed with a half life of 4-5 sec. (Fig. 4). Peroxidatically $CPZ^{+\cdot}$ formation was much slower. No alteration of CPZ was observed when the latter was added to the reacted isobutanal/HRP/O_2 system. Catalase had no effect upon the rate of CPZO and $CPZ^{+\cdot}$ formation.

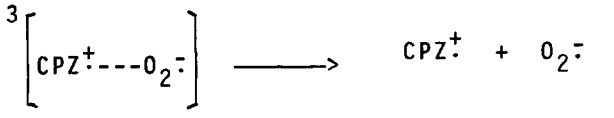
DISCUSSION

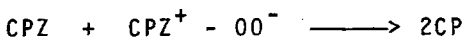
The formation of CPZO and $CPZ^{+\cdot}$, which are known photoproducts of CPZ (20), concomitantly with the suppression of acetone phosphorescence, strongly indicates that energy is being transfered from triplet acetone to CPZ. Further evidence that $CPZ^{+\cdot}$ and CPZO have a photochemical origin are the values of $2.9 \times 10^4\ M^{-1}$ and $1.6 \times 10^4\ M^{-1}$ for $k\tau$ as determined from data in Fig. 2. These values are roughly similar to that obtained for the transfer from triplet acetone to the fluorescent state of DBAS ($7.5 \times 10^4\ M^{-1}$) (4). Formation of $CPZ^{+\cdot}$ by reaction of CPZ with an intermediate to triplet carbonyl generation would have slowed oxygen consumption. Formation of $CPZ^{+\cdot}$ by direct interaction between CPZ and shielded triplet carbonyl is ruled out because the $k\tau$ values are above $10^4\ M^{-1}$ whereas from collisional quenching with a diene (sorbic acid), $k\tau$ is $10^3\ M^{-1}$ (to be published).The photochemical nature of CPZ oxidation is strongly confirmed by the competiting effect of DBAS.

A species must be present which accepts energy from the electronically excited compound with the same efficiency as DBAS and by a long range interaction. CPZ itself is unlikely to be the accepting species as its ε value in the region of acetone phosphorescence is negligibly small. It is possible that the acceptor is a charge transfer complex or contact intermediate (21) of CPZ with oxygen. The charge transfer absorbance (under the conditions of the reaction) in the region of the acetone phosphorescence maximum is of the same order of magnitude of that of 2×10^{-6}M DBAS, a concentration which allows efficient energy transfer (4). The following scheme is tentatively proposed:









The same $[CPZ---O_2]$ and $[CPZ^{+\cdot}---O_2^{-\cdot}]$ intermediates have been proposed for the photochemical oxidation of CPZ (20); similar intermediates have recently been postulated in the photochemical induced oxidation of steroidal isoxazolidines by molecular oxygen (22). Furthermore, photooxidation by specific irradiation into the charge transfer band of a donor-oxygen complex is known (23,24). It is also known that $CPZ^{+\cdot}$ can be converted to CPZO (20). The energy transfer step although spin forbidden is efficient in view of the long lifetime of the donor. In addition it is possible that the interaction of the triplet charge transfer state with singlet states (21) partially removes the spin restriction. It is feasible that an upper triplet can be reached at the expense of another triplet species by a long range process (25,26):

$$T + T_1 \longrightarrow S + T_2$$

The formation of $[CPZ---O_2]$ complexes is expected theoretically since CPZ is a donor (27,28) and oxygen an acceptor (21,30). This complexation has indeed been observed polarographically (31) and has now been confirmed spectrophotometrically by us using the method of Tsubomura and Mulliken (21).
The proposed energy transfer process could occur because the triplet donor is protected from O_2 deactivation, as oxygen is both a very efficient triplet quencher and it is also a participant of the reaction. The formation of two molecules of CPZO from the peroxide intermediate is in analogy with the behaviour of methionine in photooxygenation (32).

The present work has shown that photooxidation of CPZ may occur in a "dark" biochemical process and in a very efficient manner as judged from the high yield of CPZO on the basis of O_2 consumption. However, the disappearance of CPZ by other routes cannot be excluded. This is the first reported of a "photobiochemistry without light" effect. CPZO is a major metabolite of CPZ (13) but at present it would be premature to ascribe its formation to a dark photochemical process.

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