REACTIVITIES OF DIPHENYLFURAN (A SINGLET OXYGEN TRAP) WITH SINGLET OXYGEN AND HYDROXYL RADICAL IN AQUEOUS SYSTEMS

Kiyoshige Takayama, Toshikazu Noguchi, Minoru Nakano
Department of Biochemistry, School of Medicine, Gunma University
Maebashi, Gunma 371, Japan
Toshihiko Migita
Department of Chemistry, Gunma University, Kiryu, Gunma 376, Japan

Received February 14,1977

Summary: It has been studied whether 2,5-diphenylfuran is a specific singlet oxygen trap in aqueous systems. With certain $^1O_2$ generating systems (Rose Bengal photooxygenation and NaOCl-H_2O_2 systems) and ·OH generating systems (Fenton's reagent and acetaldehyde-xanthine oxidase system), diphenylfuran was chiefly converted in all cases to cis-dibenzoylethylene, but not to trans-dibenzoylethylene. Low but detectable conversion of diphenylfuran to a hydroperoxide, probably a distinct $^1O_2$-derived reaction in aqueous media, was found only in the Rose Bengal photooxygenation system.

The evolution of $^1O_2$ in biological systems such as NADPH-dependent microsomal lipid peroxidation (1,2), myeloperoxidase-H_2O_2-halide possibly involved in the microbicidal activity of polynuclear lecocytes (3) and acetaldehyde-xanthine oxidase have been demonstrated by the characteristic emission spectra for $2\ ^1\Delta_g \longrightarrow 2\ ^3\Sigma_g^-$ transition (1) or with $^1O_2$ traps (especially furan analogues) followed by the analysis of their products. (2-4). However, one of the $^1O_2$ traps, 1,3-diphenylisobenzofuran, appears to be not specific for $^1O_2$ because it is converted to 1,2-dibenzoylbenzene by a radical mediated oxidation as well as by the $^1O_2$ reaction (5,6). Even though the reactivities of several furan

Abbreviations used: $^1O_2$, singlet molecular oxygen; DPF, 2,5-diphenylfuran; cis-DBE, cis-1,2-dibenzoylethylene; trans-DBE, trans-1,2-dibenzoylethylene; NMR, nuclear magnetic resonance; IR, infrared.

ISSN 0006-291X

analogues with $^1O_2$ in organic solvents are well documented, their behavior in aqueous systems, especially in the systems in which other oxidants as well as $^1O_2$ could be generated, have not yet been studied in detail.

Recently, the conversion of DPF to cis-DBE has been reported to be a specific $^1O_2$-derived path way. (2.3). If this is true, DPF could prove to be an excellent indicator of $^1O_2$.

The present work was undertaken to test the reaction of DPF with $^1O_2$ or ·OH in aqueous media, using $^1O_2$ (or ·OH) generating- and acetaldehyde xanthine oxidase systems.

RESULT AND DISCUSSION

An ascorbate-Fe^{2+}-EDTA system (modified Fenton's reagent (7)) was employed as the ·OH generating system, while both Rose Bengal photooxygenation and NaOCl-H_2O_2 systems served as aqueous $^1O_2$ generating systems.

Under our experimental conditions (see Experimental section), DPF was largely converted in all instances to cis-DBE and other minor products, but not to trans-DBE (Table I).

Even though Fenton's reagent produces $^1O_2$ during the interaction of three components (·OH, O_2^- and H_2O_2) via the cycle of Haber-Weiss (8,9) and related reactions (1,10) (eq 1-4), the concentration of $^1O_2$ should be much lower than that in a certain $^1O_2$ generating system (Rose Bengal photooxygenation or NaOCl-H_2O_2 system).

$$Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^- \quad (1)$$

$$Fe^{3+} + O_2^- \xrightarrow{2H^+} Fe^{3+} + H_2O_2 \quad (2)$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + \cdot OH + {}^-OH \quad (3)$$

$$H_2O_2 + O_2^- \longrightarrow \cdot OH + {}^-OH + O_2(^1\Delta_g \text{ or } {}^3\Sigma_g^-) \quad (4)$$

However, Nilsson and Kearns (11) have been unable to demonstrate the presence of $^1O_2$ in the system in which O_2^-, ·OH and H_2O_2

Table I. Reactions of DPF in ·OH and $^1O_2$ Generating Systems.

Reaction System	Reaction Time (min)	cis-DBE[a] (mg)	comp.B[b] (mg)	comp.C[c] (mg)	Hydro-peroxide[d] (mg)	DPF[e] Recoverd (mg)
Fenton's System	120	5.8 (28%)	0.5	0.7	none	12.6 (63%)
Rose Bengal/hν	150	10.3 (49%)	3.3	2.5	1.7	negligible
OCl^--H_2O_2	10	3.7 (18%)				13.0 (65%)

a. Yield (mol%) was based on DPF initially used. NMR spectrum in $CDCl_3$ had peaks at δ7.25 (doublet, 2H), 7.52 (multiplet, 5H) and 7.95 (multiplet, 5H). The principal IR peaks (KBr) were at 3000, 1660, 1595, 1440, 1390, 1010, 1000, 890, 760, and 708 cm^{-1}. R_f=0.34
b. Yellow crystalline material with the same migration as trans-DBE (R_f=0.45). A distict compound from trans-DBE on IR spectral information.
c. Oily material. R_f=0.55.
d. R_f=0.14.
e. Recovery (%) was based on DPF initially used. R_f=0.72.

could be generated. Thus the conversion of DPF to cis-DBE in Fenton's reagent or a similar agent would appear to be in the main promoted ·OH. However, the results obtained by King et al (2) who used a similar system (but in the presence of added H_2O_2 under N_2 and at lower pH) in that the latter does not point to the evidence for the oxidation of DPF. This clearly indicates that both ·OH and O_2 are required for the oxidation of DPF.

In the Rose Bengal photooxygenation system, DPF was largely converted to cis-DBE and in lesser extent to other products including a hydroperoxide. However, attempt to detect a hydroperoxide with another $^1O_2$ generating system (NaOCl-H_2O_2) was unsuccessful, probably because of difficulties in separation and visualization. Since an exposure of 2,5-dimethylfuran to a Rose Bengal photooxygenation system yields 2-hydroxy-5-hydroperoxy-2,5-dimethyldihydrofuran (a hydroperoxide analogue of dimethyl-

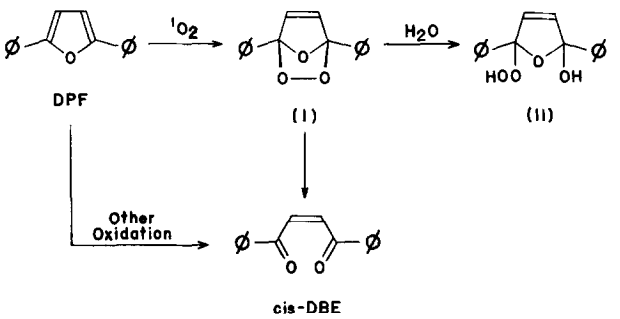

Fig. 1. Oxidation products of DPF.

furan) and diacetylethylene as the chief products (unpublished data), a hydroperoxide derived from DPF would appear to be 2-hydroxy-5-hydroperoxy-2,5-diphenyldihydrofuran (compound II) which could be formed from DPF through an endoperoxide (compound I) (Fig. 1). Even if the compound I is produced from DPF by $^1O_2$ way, it should be easily converted to cis-DBE (which is also a chief product of DPF in the modified Fenton's system) rather than the compound II.

When DPF was exposed to the acetaldehyde-xanthine oxidase system, it was oxidized to cis-DBE, but not to the compound II. Since this system is known to produce ·OH (12), the results obtained do not serve as evidence for the generation of $^1O_2$ in the system.

Many biological oxidation systems may produce ·OH (from H_2O_2 in the presence of metals or by the interaction of O_2^- with H_2O_2) and other free radicals. Therefore, it must now be asked whether the conversion of DPF to cis-DBE in myeloperoxidase-H_2O_2-halide system (3) or in NADPH-dependent microsomal lipid peroxidation system (2) might be caused by $^1O_2$.

Compound II (a hydroperoxide) appears to be a distinct $^1O_2$-

derived product of DPF. However, it may be difficult to detect this compound in enzyme systems in which $^1O_2$ is generated in low yield and DPF is present in limited concentrations. Therefore, DPF must be used with great care in the detection of $^1O_2$ generated in aqueous system.

EXPERIMENTAL SECTION

DPF and trans-DBE were obtained from W.H.Curtin & Co. and Aldrich Chemical Co., respectively. cis-DBE was prepared from trans-DBE (13) and also kindly supplied by Dr. P.B.McCay. Photox, polymer bound Rose Bengal, was a gift from Dr. A.P.Schaap.

1. Rose Bengal photooxygenation system A mixture of DPF (20 mg, 0.09 mmole) in acetone (25 ml), Photox (360 mg), and 25 ml of 0.5 M potassium phosphate (pH 7.8) was transferred to a water-cooled immersion Pyrex cylinder (14) and irradiated with a 500 W tungsten lamp at a distance of 10 cm with vigorous stirring. Oxygen was bubbled into the solution during irradiation. After 2.5 hours the Photox was removed by filtration and the filtrate was extracted with two 50 ml portions of ether. Combined ether extracts were dried over anhydrous Na_2SO_4 and concentrated to a small volume under reduced pressure at room temperature. An aliquot of the concentrated extract was applied on a thin layer plate (Silica Gel G) and chromatographed in n-hexane-dioxane (3:1 v/v). The chromatogram revealed two thick spots corresponding to cis-DBE (compound A) and trans-DBE (compound B) and two other thin spots corresponding to DPF and a compound (compound C) after spraying with 0.5% 2,4-dinitrophenylhydrazine in 2 N HCl. In addition hydroperoxide was visualized by 4% KI in glacial acetic acid. To estimate the property of the product, the combined ether extract was applied on preparative thin layer plates (Silica Gel G) and irrigated in n-hexane-dioxane. The zones corresponding to compound A, compound B, compound C and hydroperoxide were excised from the chromatoplates and the associated compound was then eluted with acetone. The identification of compounds A and B was then completed by IR or/and NMR spectral informations using cis-DBE and trans-DBE, respectively, as reference compounds. Compound C and a hydroperoxide were not further investigated because their quantities were small.

2. Hypochlorite-hydrogen peroxide system Sodium hypochlorite solution (10%, 10 ml, 13 mmoles) was added dropwise with vigorous stirring to a mixture of DPF (20 mg, 0.09 mmole), acetone (20 ml) and H_2O_2 (30%, 1 ml, 8.8 mmoles). The reaction was conducted at 4° in the dark. Analysis of the products was then processed as in experiment 1. Thin layer chromatography of the reaction mixture gave cis-DBE, unoxidized DPF and three minor compounds (UC). Preparative thin layer chromatography yielded cis-DBE in quite pure state. Authentic cis-DBE, added to the reaction system in place of DPF, was partially converted to three unidentified compounds with the same migrations as UC. This points to decomposition of cis-DBE by excesses of H_2O_2 via the Baeyer-Villiger reaction.

3. Ascorbate-Fe^{2} -EDTA system The system consisted of $FeSO_4$ (45 mg, 0.16 mmole), EDTA (182 mg, 0.49 mmole), ascorbic acid (313 mg, 1.8 mmoles), DPF (20 mg, 0.09 mmole) in acetone (150 ml) and 80 ml of 0.15 M potassium phosphate buffer (pH 7.8) in a 1 l Erlenmeyer flask. Oxygen was bubbled into the solution during incubation at 37° in the dark. At the end of 2 hours the reaction mixture was extracted with two 150 ml portions of ether. The combined ether extracts were washed with 100 ml of water, dried over Na_2SO_4, and concentrated to a small volume. Thin layer chromatographic analysis of the concentrated extract established that three products identical with those obtained with the Rose Bengal system, except for a hydroperoxide, were present. Characterization of the products were carried out as described in experiment 1.

4. Acetaldehyde-xanthine oxidase system DPF (6.6 mg, 0.03 mmole) in acetone(3 ml) was added to a mixture which had consisted of 10 mM acetaldehyde, 0.1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.8) in a total volume of 27 ml. The reaction was started by the addition of 0.84 unit of milk xanthine oxidase (EC 1,2,3,2; specific activity, 1.71 IU/mg of protein (15)) and conducted at 37° in the dark with gentle agitation. At one hour the mixture was extracted twice with an equal volume of ether (or chloroform). The extracts were combined, dried over Na_2SO_4, and evaporated to dryness. An aliquot (50 ul) of the residue in ether (0.2 ml) was applied to a thin layer plate and run as described in the experiment 1. The thin layer chromatographic analysis established that no hydroperoxide was present and that cis-DBE only had been formed. However, a control system performed under identical conditions, but with omission of one of two components (i.e. the enzyme or acetaldehyde), did not in each case yield detectable cis-DBE.

REFERENCES

1. Nakano, M., Noguchi, T., Sugioka, K., Fukuyama, H., Sato, M., Shimizu, Y., Tsuji, Y., and Inaba, H., (1975) J. Biol. Chem., 250, 2404-2406
2. King, M., Lai, E.K., and McCay, P.B., (1975) J. Biol. Chem., 250, 6496-6502
3. Rosen, H., and Klebanoff, S.J., (1976) Fed. Proc., 35, 1391
4. Kellogg, E.W., and Fridovich, I., (1976) J. Biol. Chem., 250, 8812-8817
5. Chan, H.W.S., (1971) J. Am. Chem. Soc., 93, 4632-4633
6. Baldwin, J.E., Swallow, J.C., and Chan, H.W.S., (1971) Chem. Commun., 1407
7. Brodie, B.B., Axelrod, J., Shore, P.A., Udenfriend, S., (1954) J. Biol. Chem., 208, 741-750
8. Haber, F., and Weiss, J., (1934) Proc. R. Soc. Lond., A 147 332-351

9. Weiss, J., (1953) Experientia, 9, 61-62
10. Koppenol, W.H., (1976) Nature, 262, 420-421
11. Nilsson, R., and Kearns, D.R., (1974) J. Phys. Chem., 78, 1681-1683
12. Beauchamp, C., and Fridovich, I., (1970) J. Biol. Chem., 245, 4641-4646
13. Conant, J.B., and Lutz, R.E., (1923) J. Am. Chem. Soc., 45, 1303-1307
14. Walrant, P., and Santus, R., (1974) Photochem. Photobiol., 411-417
15. Massey, V., Brumby, P.E., Komai, H., and Palmer, C., (1969) J. Biol. Chem., 244, 1682-1691