THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 266, No. 14, Issue of May 15, pp. 9039-9042, 1991
Printed in U.S.A.

Singlet Oxygen Production from the Reactions of Ozone with Biological Molecules*

(Received for publication, November 30, 1990)

Jeffrey R. Kanofsky‡§¶ and Paul Sima‖

From the ‡Medical Service and the ‖Research Service, Edward Hines, Jr. Department of Veterans Affairs Hospital, Hines, Illinois 60141 and the §Departments of Medicine and Molecular and Cellular Biochemistry, Loyola University Stritch School of Medicine, Maywood, Illinois 60153

The reaction of ozone with a number of biological molecules was found to produce singlet oxygen in high yield. At pH 7.0, the reaction of ozone with an equimolar amount of biological molecule produced the following singlet oxygen yields (mole of singlet oxygen/mole of ozone): cysteine, 0.49 ± 0.02; methionine, 1.13 ± 0.11; reduced glutathione, 0.33 ± 0.02; albumin, 1.00 ± 0.05; uric acid, 0.64 ± 0.09; ascorbic acid, 0.96 ± 0.007; NADPH, 1.07 ± 0.07; NADH, 0.95 ± 0.01. Thus, singlet oxygen may be an important intermediate in the biochemical damage caused by ozone.

Ozone, an important toxin in the polluted urban atmosphere, is detrimental to both plants and animals. In spite of a large research effort over the past few decades, the mechanisms by which ozone causes biochemical damage have not yet been fully elucidated (1, 2). Both lipids and amino acids have been proposed as important biochemical targets for ozone, in part because the rate constants for the reactions of ozone with polyunsaturated fatty acids and with certain amino acids are extremely large (1–5). NADH and NADPH have also been proposed to be critical biochemical targets for ozone, both because the ozone-reaction rate constants are extremely large and because the ozone-oxidation products are not substrates for the normal NADH or NADPH oxidation-reduction cycle (6). Protection against ozone-induced biological damage is believed to be mediated by certain biological antioxidants. These include ascorbic acid, uric acid, and α-tocopherol (4, 5, 7, 8).

Recent work has emphasized the contribution of secondary reactions to the aggregate biochemical damage caused by ozone. Hydrogen peroxide, hydroperoxides, hydroxyl radical, and superoxide anion have been proposed as important intermediates in these secondary reactions (9–13). Little work has been reported, however, on the potential role of singlet oxygen as an intermediate of importance in ozone toxicity. This is surprising, since the reaction of ozone with many types of organic molecules produces singlet oxygen in high yield (14–17). Examples of organic compounds reacting with ozone to produce singlet oxygen include tertiary amines, sulfides, sulfoxides, phosphites, hindered olefins, certain ethers, and certain aldehydes (14–17). A common feature in these reactions is that one atom from the ozone is incorporated into the oxidized product, while the remaining two oxygen atoms from the ozone are released as a molecule of singlet oxygen.

Since we could find no reports in the literature of singlet oxygen yields for the reactions of ozone with biomolecules, we decided to measure the singlet oxygen yields for a number of potential target biomolecules. Linoleic acid was selected for study as a model unsaturated fatty acid. The five most reactive amino acids (cysteine, tryptophan, methionine, tyrosine, and histidine) were selected for study, because most of the ozone damage to proteins is believed to take place at the side chains of these amino acids (3, 18). The reactions of ozone with NADH, with NADPH, and with certain biological antioxidants (α-tocopherol, uric acid, and ascorbic acid), believed to be important in the detoxification of ozone, were also studied.

EXPERIMENTAL PROCEDURES

Chemiluminescence Spectrometer—Singlet oxygen was identified from its characteristic 1270-nm chemiluminescence (19, 20). The chemiluminescence spectrometer used to measure the singlet oxygen light emission has been described previously (19). Since many of the reactions studied in this paper were very fast, the electronic detection system of the spectrometer was modified to more accurately respond to the rapid changes in light emission. The chopping wheel and lock-in amplifier, employed in prior studies, were not used. The output of the infrared detector (EO817L, North Coast Optical Systems and Sensors, Santa Rosa, CA) was coupled to a Keithley metrabyte DAS-8 high speed analog to digital converter (Taunton, MA). The output of the infrared detector was sampled at 50 Hz, and the resulting data points were stored on an IBM AT computer (Boca Raton, FL). Another modification of the apparatus for these studies was the addition of a 25-mm diameter, 25-mm focal length lens in front of the infrared detector. This improved the light collection efficiency from the samples and also decreased the relative amount of off-axis light reaching the infrared detector.

Quantitative measurements of singlet oxygen yield were made by comparing the time-integrated 1270-nm emission from the system under study with the time-integrated emission from the hydrogen peroxide plus hypohalous acid reaction at pH 7.0 (21, 22).

Ozone—Ozone was produced by passing oxygen through a model 03V5-0 OREC ozone generator (Ozone Research & Equipment Corp., Phoenix, AZ). Ozone was trapped in acid solution, because these solutions were stable for several hours. The outflow of the ozone generator was bubbled through a 1 mM perchloric acid solution for at least 20 min. An aliquot of the ozone solution was then transferred to a quartz cuvette. Additional 1 mM perchloric acid solution was added to increase the volume to 2 ml. The concentration of ozone was then determined from the absorbance at 260 nm using an absorption coefficient of 3300 cm^{-1} M^{-1} (23).

Reagents—Ascorbic acid, cysteine, deuterium oxide (99.8%), glutathione (reduced), histidine, human albumin, linoleic acid, methionine, NADH, NADPH, sodium lauryl sulfate, α-tocopherol, tyrosine, tryptophan, and uric acid were obtained from Sigma. Oxygen (ultra high purity, 99.8%) was obtained from Matheson Gas Products, East Rutherford, NJ. Hydrogen peroxide (30% stabilized reagent grade, J.

* This work was supported by Grant GM32974 from the National Institutes of Health and the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom reprint requests and correspondence should be addressed: Box 278, Hines, IL 60141.

T. Baker Inc., Superoxol) was assayed using the method of Cotton and Dunford (24). Hypochlorous acid was distilled from a 5.25% commercial solution (Clorox) and assayed as previously described (21). Other chemicals were reagent grade. Water was glass-distilled. Phosphate buffer made with deuterium oxide solvent was adjusted to an apparent pH of 6.6 using a glass electrode to give the desired p^{2}H of 7.0 (25).

The reaction of ozone with α-tocopherol required the use of a detergent, sodium lauryl sulfate, to disperse the α-tocopherol. In preliminary experiments, it was found that ozone reacted with impurities in the sodium lauryl sulfate to produce singlet oxygen. This singlet oxygen production could be greatly reduced by treating the sodium lauryl sulfate with ozone before doing the experiment. Ozone was bubbled through pH 7.0, 100 mM sodium phosphate buffer for 30 min. Sodium lauryl sulfate 10% (w/v) was then added and the solution kept until the 260-nm ozone absorption decayed to zero. This ozone-treated sodium lauryl sulfate solution produced very little singlet oxygen when additional ozone was added.

Experimental Conditions—Experiments were done at 25 °C. A cuvette containing 2 ml of ozone in perchloric acid solution was placed in the chemiluminescence spectrometer. The reaction was then initiated by the rapid injection of 2 ml of sodium phosphate buffer, pH 7.0, containing the reactant. The light emission was then integrated for 4 s. The perchloric acid lowered the buffer pH by less than 0.02 units. All experiments were done at least three times. Results are reported as the mean ± standard error.

RESULTS

The reactions of ozone with many of the biological molecules studied produced 1270-nm chemiluminescence characteristic of singlet oxygen. The kinetics of the light emission from the reactions of ozone with NADPH and with cysteine are shown in Fig. 1. The duration of the emission was determined by the reactant mixing time, roughly 0.5 s. Spectral analyses of the near infrared emission from the ozone-NADPH and the ozone-cysteine reactions show a peak near 1270 nm (Table I) consistent with singlet oxygen. Spectral analyses of the near infrared emission from the other ozone-biomolecule reactions studied were very similar to those shown in Table I (data not shown). Further support for the assignment of the near infrared emission to singlet oxygen comes from the very large increase in light emission caused by substituting deuterium oxide for water (Table II).

In general, it was not possible to study the effects of singlet oxygen quenchers upon the 1270-nm emission from the ozone plus biomolecule reactions, because most singlet oxygen quenchers react rapidly with ozone. Thus, reductions in chemiluminescence caused by the addition of a singlet oxygen quencher to the system under study are in many cases due to

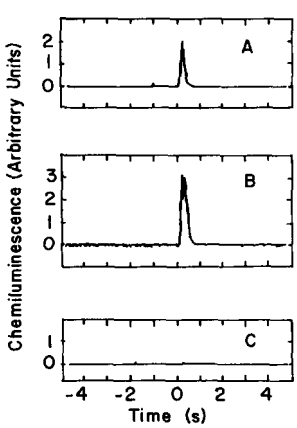

FIG. 1. **Chemiluminescence at 1270 nm from the reaction of ozone with cysteine and with NADPH.** Conditions were 200 μM ozone, pH 7.0, 50 mM sodium phosphate, 0.5 mM sodium perchlorate. *A*, cysteine, 200 μM, average of nine experiments; *B*, NADPH, 200 μM, average of five experiments; *C*, control, no organic material added, average of four experiments.

TABLE I

Spectral analysis of the near infrared emission from the reactions of ozone with NADPH and with cysteine and from the reaction of hydrogen peroxide with hypochlorous acid

Filter[a]	System		
	Hydrogen peroxide + hypochlorous acid[b,c]	Ozone + NADPH[c,d]	Ozone + cysteine[c,e]
nm			
1070	0.00 ± 0.01	−0.02 ± 0.02	−0.01 ± 0.02
1170	−0.003 ± 0.005	0.00 ± 0.01	−0.01 ± 0.01
1268	1.00 ± 0.004	1.00 ± 0.07	1.00 ± 0.05
1370	0.10 ± 0.003	0.05 ± 0.003	0.08 ± 0.01
1475	0.01 ± 0.004	0.02 ± 0.01	0.02 ± 0.01

[a] The interference filters for wavelength selection had bandwidths of 50 nm.

[b] 2 mM hydrogen peroxide, 2 mM hypochlorous acid, pH 7.0, 100 mM sodium phosphate, 50 mM sodium chloride.

[c] The light emission through the 1268-nm filter was always assigned a value of 1.00. These values have been corrected for differences in transmission among the various interference filters and for the wavelength dependence of their infrared detector sensitivity.

[d] 200 μM ozone, 200 μM NADPH, pH 7.0, 50 mM sodium phosphate, 0.5 mM sodium perchlorate.

[e] 200 μM ozone, 200 μM cysteine, pH 7.0, 50 mM sodium phosphate, 0.5 mM sodium perchlorate.

TABLE II

Effect of deuterium oxide solvent and of histidine on the 1270-nm emission from the reaction of ozone with biological molecules

Compound	H_2O solvent/ ^2H_2O solvent[a]	Histidine/ ^2H_2O solvent[b]
Cysteine	0.038 ± 0.004[c]	0.08 ± 0.01
Tryptophan	0.036 ± 0.008[c]	
Methionine	0.030 ± 0.003[c]	
Tyrosine	0.042 ± 0.007[c]	
Histidine	0.040 ± 0.008[c]	
Glutathione (reduced)	0.034 ± 0.004[c]	0.17 ± 0.005
Albumin	0.044 ± 0.004[c]	
α-Tocopherol	0.06 ± 0.003[d]	
Linoleic acid	0.029 ± 0.009[c]	
Uric acid	0.047 ± 0.005[c]	
Ascorbic acid	0.039 ± 0.004[c]	
NADPH	0.042 ± 0.006[c]	0.22 ± 0.01
NADH	0.052 ± 0.004[c]	0.21 ± 0.02

[a] Ratio of 1270-nm emission from water solvent to 1270-nm emission from deuterium oxide solvent.

[b] Ratio of 1270-nm emission from sample containing 2 mM histidine (deuterium oxide solvent) to 1270-nm emission from sample without added histidine (deuterium oxide solvent).

[c] Conditions were 25 μM ozone, 25 μM compound, pH or p^{2}H 7.0, 50 mM sodium phosphate, 0.5 mM sodium perchlorate.

[d] Conditions were 200 μM ozone, 200 μM α-tocopherol, pH or p^{2}H 7.0, 50 mM sodium phosphate, 0.5 mM sodium perchlorate, 1% (w/v) sodium lauryl sulfate.

ozone consumption in addition to any singlet oxygen-quenching effect. For cysteine, glutathione, NADPH, and NADH, however, the rates of the ozone-biomolecule reactions are so fast ($k \sim 10^9$ M^{-1} s^{-1}) that it was possible to add sufficient histidine to quench a large fraction of the singlet oxygen and still not consume a significant quantity of ozone via the ozone-histidine reaction. The results of these histidine quenching experiments are shown in Table II and are consistent with singlet oxygen as the source of the chemiluminescence.

Ozone spontaneously decomposes in aqueous solution via a chain radical reaction involving hydroxyl radicals and superoxide anions (26–28). Under the conditions used in our studies (200 μM ozone, pH 7.0, phosphate buffer), ozone had a half-life of 4.8 ± 0.2 min. Only 1% of the ozone decomposed during the 4-s reaction period. As shown in Fig. 1 and Table III, the

TABLE III

Singlet oxygen yields and rate constants for the reactions of ozone with potential target molecules

Compounds	Rate constant at pH 7.0	Singlet oxygen yield
	$M^{-1}\ s^{-1}$	*mol/mol of reactant*
None, phosphate buffer control		0.000 ± 0.004[a]
Cysteine	1.6×10^9 [b]	0.49 ± 0.02[a]
Tryptophan	7×10^6 [b]	0.13 ± 0.01[a]
Methionine	4×10^6 [b]	1.13 ± 0.11[a]
Tyrosine	2.8×10^6 [b]	0.12 ± 0.02[a]
Histidine	1.9×10^5 [b]	0.09 ± 0.02[a]
Glutathione (reduced)	7×10^8 [b]	0.33 ± 0.02[a]
Albumin		1.00 ± 0.05[c]
α-Tocopherol	7.5×10^5 [d]	0.14 ± 0.01[a,e]
Linoleic acid	1×10^6 [d]	0.047 ± 0.002[a]
Uric acid	1.4×10^6 [d]	0.64 ± 0.09[a]
Ascorbic acid	6×10^7 [d]	0.96 ± 0.07[a]
NADPH		1.07 ± 0.07[a]
NADH	$\sim 10^9$ [f]	0.95 ± 0.01[a]

[a] Conditions were pH 7.0, 50 mM sodium phosphate, 0.5 mM sodium perchlorate, 200 μM ozone, 200 μM biological molecules.

[b] Values from Pryor *et al.* (3).

[c] Conditions were pH 7.0, 50 mM sodium phosphate, 0.5 mM sodium perchlorate, 25 μM albumin, 25 μM ozone.

[d] Values from Giamalva *et al.* (4).

[e] The phosphate buffer contained 1% (w/v) sodium lauryl sulfate to disperse the α-tocopherol. This value has been corrected for the small amount of singlet oxygen generated from the reaction of ozone with impurities in the sodium lauryl sulfate (0.01 ± 0.001 mol/mol of ozone).

[f] From competitive oxidation studies, the rate constant is of the same order of magnitude as that for cysteine (see Mudd *et al.* (6)).

spontaneous decomposition of ozone did not generate significant quantities of singlet oxygen during the 4-s observation period used.

DISCUSSION

The biological molecules producing high yields of singlet oxygen in this study contain functional groups which are known to react with ozone to generate singlet oxygen. Ozone attacks the sulfur atom of methionine, cysteine, and glutathione (18).

$$O_3 + RSR' \rightarrow RSOR' + O_2\ (^1\Delta_g) \quad (1)$$

Methionine sulfoxide was the only methionine-oxidation product detected by Mudd *et al.* (18). This correlates with our observation that 1.13 ± 0.10 molecules of singlet oxygen were generated for each molecule of methionine oxidized. Oxidized glutathione and cystine are major oxidation products of reduced glutathione and cysteine, respectively (18, 29). These oxidation products could result from the reaction of an intermediate sulfoxide with a second molecule of substrate (30, 31).

$$RSOH + RSH \rightarrow RSSR + H_2O \quad (2)$$

Human albumin contains 584 amino acids with 1 cysteine (32). Assuming all amino acids are equally accessible to ozone, the fraction of ozone molecules reacting with cysteine will be given by:

$$\text{Fraction} = \frac{K_{\text{cysteine}}}{\sum_{i=1}^{584} K_i} \quad (3)$$

where K_{cysteine} is the rate constant for the ozone-cysteine reaction and K_i is the rate constant for the reaction of ozone with amino acid number i in human albumin (3, 32). Using the rate constants measured by Pryor *et al.* (3), 95% of the ozone molecules should react with cysteine. This is consistent with the high yield of singlet oxygen measured in this study and with past work showing some cysteine-containing enzymes, oxidized with ozone, can be reactivated by treatment with low molecular weight thiols (31).

Ozone reacts with some sterically hindered olefins to give singlet oxygen (16).

$$RR'C{=}CR''R''' + O_3 \rightarrow RR'C(\text{O})CR''R''' + O_2\ (^1\Delta_g) \quad (4)$$

NADH, NADPH, uric acid, and ascorbic acid all have non-aromatic carbon-carbon double bonds and react with ozone to give singlet oxygen in high yield. For NADH (and by analogy NADPH), Mudd *et al.* (6) found ozone to attack one of the carbon-carbon double bonds on nicotinic acid producing a diol. Hydrolysis of the epoxide produced by Reaction 4 should yield the diol detected by Mudd *et al.* For uric acid, the ozone oxidation products have not been fully characterized but include a large amount of urea and a small amount of allantoin (7). Fragmentation of the unstable epoxide produced in Reaction 4 could produce these products.

Singlet oxygen emission was easily detectable in this study because the concentration of biological molecules used was very low. Under these conditions most singlet oxygen molecules will be inactivated through collision with solvent molecules and the lifetime of singlet oxygen will be relatively long, about 4 μs in water solvent (33). In contrast, in intact biological systems (*e.g.* cells, cell membranes, and human plasma), the concentration of biological molecules is very large. Under these conditions, most of the singlet oxygen generated would be expected to interact (chemically or physically) with the various biological molecules present. Recent estimates of the singlet oxygen lifetime in cells, human red cell membranes, and human plasma are <0.5, 0.1, and 1 μs, respectively (34–37). Thus, in intact biological systems, the intensity of any singlet oxygen emission would be much smaller than that observed in the current study.

An important consequence of the very short singlet oxygen lifetime in living cells is that exogenously added singlet oxygen quenchers will be ineffective in limiting biochemical damage, unless these quenchers are present at the extremely high concentrations necessary to significantly lower the singlet oxygen lifetime within the cell. Thus, some earlier studies showing that singlet oxygen quenchers fail to limit biochemical damage (10, 38) in cell cultures do not rule out singlet oxygen as an important biochemical mediator.

Uric acid and ascorbic acid have been proposed to act as protective compounds which limit ozone toxicity (4, 7, 8). The protective action of these compounds is not likely the result of a direct reaction with ozone, since the reaction of ozone with ascorbic acid and with uric acid generates singlet oxygen, a well established oxidative toxin, in high yield.

There has been considerable controversy about the role of singlet oxygen as a biochemical intermediate (39). This is due, in part, to the fact that the mechanisms proposed for the biochemical generation of singlet oxygen often involve the reaction between two highly reactive precursors, for example hydrogen peroxide and hypochlorous acid (19–22, 39). As a consequence, the singlet oxygen generation will have a quadratic dependence on the concentration of these precursors. It is not clear that sufficiently high concentrations of precursors are generated in biological systems to produce significant amounts of singlet oxygen. In contrast, the reaction of ozone with various biological molecules should produce high yields of singlet oxygen even at very low ozone concentrations.

The results of the current study are not sufficient to establish a significant role for singlet oxygen as a mediator of

biological damage from ozone. Not all biological molecules react with ozone to yield singlet oxygen. The amount of singlet oxygen actually generated in intact biological systems will depend upon many factors. This study does suggest, however, that additional work is needed to evaluate the role of singlet oxygen in ozone toxicity. Experiments with intact biological systems, while technically more difficult than the current study, should provide some of the needed information.

Acknowledgments—We thank Brian Dunlap and John Schaefer for help with the construction of apparatus and Anita Osis for help with the preparation of the manuscript.

REFERENCES

1. Pryor, W. A., Dooley, M. M., and Church, D. F. (1983) in *Advances in Modern Environmental Toxicology (International Symposium on Biomedical Effects of Ozone Related Photochemical Oxidants, 1982)* (Lee, S. D., Mustafa, M. G., and Melman, M. A., eds) pp. 7–18, Senate Press, Princeton Junction, NJ
2. Mehlman, M. A., and Borek, C. (1987) *Environ. Res.* **42,** 36–53
3. Pryor, W. A., Giamalva, D. H., and Church, D. F. (1984) *J. Am. Chem. Soc.* **106,** 7094–7100
4. Giamalva, D., Church, D. F., and Pryor, W. A. (1985) *Biochem. Biophys. Res. Commun.* **133,** 773–779
5. Giamalva, D. H., Church, D. F., and Pryor, W. A. (1986) *J. Am. Chem. Soc.* **108,** 6646–6651
6. Mudd, J. B., Leh, F., and McManus, T. T. (1974) *Arch. Biochem. Biophys.* **161,** 408–419
7. Meadows, J., and Smith, R. C. (1986) *Arch. Biochem. Biophys.* **246,** 838–845
8. Meadows, J., and Smith, R. C. (1987) *Environ. Res.* **43,** 410–416
9. Whiteside, C., and Hassan, H. M. (1988) *Free Radical Biol. & Med.* **5,** 305–312
10. Morgan, D. L., and Wenzel, D. G. (1985) *Toxicology* **36,** 243–251
11. Madden, M. C., Eling, T. E., and Friedman, M. (1987) *Prostaglandins* **34,** 445–463
12. Hewitt, C. N., Kok, G. L., and Fall, R. (1990) *Nature* **344,** 56–58
13. Meiners, B. A., Peters, R. E., and Mudd, J. B. (1977) *Environ. Res.* **14,** 99–112
14. Eisenberg, W. C., Taylor, K., and Murray, R. W. (1985) *J. Am. Chem. Soc.* **107,** 8299–8300
15. Murray, R. W., and Kaplan, M. L. (1968) *J. Am. Chem. Soc.* **90,** 537–538
16. Murray, R. W., Lin, J., W.-P. and Kaplan, M. L. (1970) *Ann. N. Y. Acad. Sci.* **171,** 121–129
17. Stary, F. E., Emge, D. E., and Murray, R. W. (1976) *J. Am. Chem. Soc.* **98,** 1880–1884
18. Mudd, J. B., Leavitt, R., Ongun, A., and McManus, T. T. (1969) *Atmos. Environ.* **3,** 669–681
19. Kanofsky, J. R. (1983) *J. Biol. Chem.* **258,** 5991–5993
20. Khan, A. U., Gebauer, P., and Hager, L. P. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 5195–5197
21. Kanofsky, J. R. (1984) *J. Photochem.* **25,** 105–113
22. Kanofsky, J. R. (1984) *J. Biol. Chem.* **259,** 5596–5600
23. Hart, E. J., Sehested, K., and Holcman, J. (1983) *Anal. Chem.* **55,** 46–49
24. Cotton, M. L., and Dunford, H. B. (1970) *Can. J. Chem.* **51,** 582–587
25. Salomaa, P., Schaleger, L. L., and Long, F. A. (1964) *J. Am. Chem. Soc.* **86,** 1–7
26. Weiss, J. (1935) *Trans. Faraday Soc.* **31,** 668–681
27. Hoigne, J., and Bader, H. (1976) *Water Res.* **10,** 377–386
28. Forni, L., Bahnemann, D., and Hart, E. J. (1982) *J. Phys. Chem.* **86,** 255–259
29. Freeman, B. A., and Mudd, J. B. (1981) *Arch. Biochem. Biophys.* **208,** 212–220
30. Barton, J. P., Packer, J. E., and Sims, R. J. (1973) *J. Chem. Soc. Perkin Trans.* **II,** 1547–1549
31. Armstrong, D. A., and Buchanan, J. D. (1978) *Photochem. Photobiol.* **28,** 743–755
32. Peters, T., Jr. (1975) in *The Plasma Proteins* (Putnam, F. W., ed), Vol. 1, pp. 133–181, Academic Press, New York
33. Rodgers, M. A. J., and Snowden, P. T. (1982) *J. Am. Chem. Soc.* **104,** 5541–5543
34. Patterson, M. S., Madsen, S. J., and Wilson, B. C. (1990) *J. Photochem. Photobiol. B: Biol.* **5,** 69–84
35. Firey, P. A., Jones, T. W., Jori, G., and Rodgers, M. A. J. (1988) *Photochem. Photobiol.* **48,** 357–360
36. Kanofsky, J. R. (1990) *Photochem. Photobiol.* **51,** 299–303
37. Kanofsky, J. R. (1991) *Photochem. Photobiol.* **53,** 93–99
38. Warren, D. L., Hyde, D. M., and Last, J. A. (1988) *Toxicology* **53,** 113–133
39. Kanofsky, J. R. (1989) *Chem. Biol. Interact.* **70,** 1–28