Oxidation of Arachidonic Acid in Micelles by Superoxide and Hydrogen Peroxide*

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Arachidonic acid was co-oxidized by xanthine oxidase. Both superoxide radical and hydrogen peroxide were required for oxidation, as shown by essentially complete inhibition caused by superoxide dismutase or by catalase. Pure arachidonate, free of lipid hydroperoxides, was susceptible to this co-oxidation, and the presence of lipid hydroperoxides did not accelerate the process. The role of trace metals was indicated by the stimulatory effect of EDTA-Fe and by the inhibitory effect of diethylenetriamine pentaacetate. Initiation of arachidonate co-oxidation was due to a potent oxidant generated by the interaction of H₂O₂ and O₂⁻ in the presence of Fe, rather than to either O₂⁻ or H₂O₂ per se. Hence, mannitol, a scavenger of OH⁻, but not of O₂⁻ or H₂O₂, also inhibited oxidation. Arachidonic acid autoxidation, a much slower process than xanthine oxidase co-oxidation, was barely detectable on the time scale of these observations. Unlike the co-oxidation, autoxidation was autocatalytic and therefore accelerated by hydroperoxide products. Marked quantitative differences in the distribution of isomeric hydroperoxide products of the enzymic co-oxidation, as compared to the autoxidation, were noted and their significance was discussed.

Oxidation of polyunsaturated lipids usually proceeds by a free radical chain mechanism, yielding a mixture of hydroperoxide, carbonyl, and hydrocarbon products. Because this process is the basis of the oxidative polymerization of drying oils and of the rancidification of foods, it has been well studied and has been the subject of several reviews (1-5). Expiration of hydrocarbons, such as ethane and pentane, demonstrates that lipid oxidation can occur in vivo (6-10). Polysaturated lipids are particularly abundant in biological membranes and their oxidation constitutes an obvious threat to the integrity of such membranes. Moreover, this lipid peroxidation in a variety of biological systems generates peroxide products with intriguing and unique properties. Hydroperoxides derived from arachidonic acid, for example, function as modulators of the enzymes involved in prostaglandin biosynthesis (11) and are potent chemotactic agents for neutrophils (12). A lipid hydroperoxide produced from arachidonic acid, 5-hydroperoxyeicosatetraenoic acid, is a proposed intermediate in the biosynthesis of leukotrienes (13-15).

Liver microsomes, which catalyze a NADPH-dependent hydroxylation of a variety of compounds, cause an iron-dependent and NADPH-dependent oxidation of lipids (16)

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which is inhibited by hydroxylatable compounds (17). Superoxide dismutase was seen to inhibit this microsomal lipid oxidation, suggesting the involvement of O₂⁻ (18). Superoxide dismutase inhibition of lipid oxidation has been noted under a variety of circumstances (19-33).

Yet there has been serious disagreement. Kellogg and Fridovich (23) noted that the xanthine oxidase reaction, a known source of O₂⁻ and H₂O₂, caused the oxidation of linoleate. Superoxide dismutase or catalase inhibited this lipid oxidation, indicating a requirement for both O₂⁻ and H₂O₂ (23). Essentially identical results were subsequently noted with phospholipid vesicles and with intact erythrocytes (28) and with vesicles formed from washed erythrocyte stroma (34). These data were interpreted on the basis of an interaction of O₂⁻ with H₂O₂ to generate OH⁻, or a comparably potent oxidant, which then attacked the polysaturated lipid. This interaction had been proposed much earlier (35) and was later modified to include catalysis by iron compounds (36, 37). Thomas et al. (32) reinvestigated this process by studying the oxidation of linoleate by the xanthine oxidase reaction. They reported, in contradiction to the earlier work (23, 28), that linoleate oxidation by O₂⁻ was dependent upon the extent of prior accumulation of lipid hydroperoxide and concluded that the proximal oxidant was generated by interaction of O₂⁻ with lipid hydroperoxide, not with H₂O₂.

It seemed essential that these apparent discrepancies be clarified in order to provide a firm foundation of fact upon which further progress could be based. We now report an investigation of the oxidation of arachidonate by an enzymic source of O₂⁻ plus H₂O₂ under conditions allowing continuous monitoring of the rate of accumulation of conjugated diene products.

**MATERIALS AND METHODS**

Xanthine oxidase was prepared from unpasteurized cream (38) and was assayed by ultraviolet spectrophotometry (39). It was stored at -20°C in 50 mM potassium phosphate, 0.1 mM EDTA at pH 7.8. Superoxide dismutase was isolated from bovine erythrocytes and was assayed in terms of its ability to inhibit the reduction of cytochrome c by xanthine oxidase (40). Bovine liver catalase was purchased from the Worthington Biochemical Corporation and horse heart cytochrome c, type III, from Sigma. Arachidonic acid was from Na-Chex Preparations, Inc. and sodium octyl sulfate was from Schwartz/Mann. Acetaldehyde from Eastman was freshly distilled before use. All other compounds used were obtained from commercial sources at the highest available level of purity.

In order to permit continuous spectrophotometric monitoring of its oxidation, arachidonic acid was dispersed in the form of mixed micelles with sodium octyl sulfate. Sodium octyl sulfate was chosen for this purpose, rather than longer chain alkyl sulfonates such as sodium dodecyl sulfate, because it has less of a tendency to denature proteins. We found that sodium octyl sulfate at 10 mM did not interfere with the xanthine oxidase reaction, but at 100 mM it acted as a strong oxidation inhibitor. Optically clear stock dispersions of arachidonate (6.0 mM) were prepared by adding it to a solution containing 0.11 M

1 Dr. J. A. Reynolds, personal communication.
sodium octyl sulfate, 50 mM sodium pyrophosphate, and 0.1 mM EDTA for 15 min at 9.0, followed by heating at 25°C was followed by 15-fold dilution into the sodium pyrophosphate-buffered reaction mixtures. Potassium salts caused precipitation of the octyl sulfate and were avoided.

Reaction mixtures usually contained 50 mM sodium pyrophosphate, 6.0 mM sodium octyl sulfate, 0.33 mM xanthine oxidase, 0.11 mM EDTA, 20 mM acetaldehyde, and 1.7 × 10⁻⁷ M xanthine oxidase at pH 9.0 and 38°C. Where indicated, H₂O₂ was also present at 0.05 mM. Reactions were initiated, after thermal equilibration, by adding the xanthine oxidase. Accumulation of conjugated diene products was monitored at 233 nm in a Gilford model 2000 recording spectrophotometer, equipped with a thermostatted cell compartment. Temperature equilibration prior to initiation of the reaction was critical, since the micellar configuration was responsive to temperature (41) and caused an apparent temperature-dependent change in absorbance. Preparative scale oxidations were performed in partially filled Erlenmeyer flasks continually agitated in a water bath at 38°C. At intervals, 3.0-ml samples of such large scale reactions were temporarily transferred into cuvettes and their absorbance was recorded at 233 nm.

The correspondence between increased absorbance at 233 nm and the formation of conjugated diene hydroperoxides was verified by HPLC (2 TLC), and repeated scanning of the absorption spectrum with a Hitachi 100-80 recording spectrophotometer. Samples were prepared for HPLC and TLC by acidification with H₂SO₄, followed by extraction with chloroform/methanol. Extracts were then reduced to dryness in vacuo, and redissolved in organic solvent. Silica TLC plates were developed with hexane/isopropyl alcohol:acetic acid (200:10:5), followed by spraying with 4% KI in acetic acid followed 4 min later with 1% aqueous starch (23). Fatty acid hydroperoxides were separated by normal phase HFLC using a Whatman Magnum 9 10-mm silica column (9 mm, inner diameter, × 50 cm). The solvent used was acetic acid/isopropyl alcohol:water (21:4:584), and the flow rate was 3 ml/min, with detection by uv at 295 nm (43).

RESULTS

Co-oxidation of Arachidonate by Xanthine Oxidase—Xanthine oxidase, while catalyzing the aerobic oxidation of acetaldehyde, caused a co-oxidation of arachidonate. This co-oxidation, followed in terms of absorbance increase at 233 nm, was entirely dependent upon the simultaneous presence of xanthine oxidase, acetaldehyde, and arachidonate. Omission of any one of these components eliminated detectable changes in absorbance. We may conclude that spontaneous autooxidation of arachidonate was not significant on the time scale and under the conditions of these observations and that the conversion of acetaldehyde to acetate did not contribute noticeably to the rates followed at 233 nm.

There was an autocatalytic aspect to this co-oxidation of arachidonate. Thus, as shown by Line 1 in Fig. 1, the absorbance at 233 nm increased exponentially during the first 15 min of the reaction. The rate of this phenomenon was likely that this reflected the progressive accumulation of H₂O₂. H₂O₂, when added at the outset, did decrease the exponential behavior, while increasing the initial rate. Indeed, as shown by Line 2, Fig. 1, 0.033 mM H₂O₂ gave a completely linear co-oxidation of arachidonate. H₂O₂, by itself, did not cause any oxidation of arachidonate and omission of xanthine oxidase or acetaldehyde completely eliminated increases in absorbance at 233 nm, even though 0.05 mM H₂O₂ and the other components of the reaction mixture were present. The effect of varying the concentration of acetaldehyde on the co-oxidation of arachidonate was explored. Typical saturation behavior was seen with a half-maximal rate achieved at approximately 4 mM acetaldehyde. The effect of temperature was also explored. The rate of co-oxidation of arachidonate varied as would be expected on the basis of the Arrhenius equation with an energy of activation of 10.7 kcal/mol. The Arrhenius plot was linear over the temperature range 25-45°C with a correlation coefficient of

2 The abbreviations used are: HPLC, high pressure liquid chromatography; DTPA, diethylenetriaminepentaacetic acid.

FIG. 1 (left). The effect of H₂O₂ on the enzymic co-oxidation of arachidonate. Reaction mixtures contained 0.33 mM arachidonate, 6.0 mM sodium octyl sulfate, 0.11 mM EDTA, 50 mM sodium pyrophosphate, 20 mM acetaldehyde, 8.3 nM xanthine oxidase, and the following amounts of H₂O₂: Line 1, none; Line 2, 5.6 µM; Line 3, 11 µM; Line 4, 3.5 µM; Line 5, 67 µM; and Line 6, 130 µM. Incubation was at 38°C and pH 9.0.

FIG. 2 (right). The effect of varying the concentration of xanthine oxidase. Reaction mixtures were as described in the legend to Fig. 1, but without added H₂O₂ and with the following concentrations of xanthine oxidase: Line 1, 17 nM; Line 2, 8.2 nM; Line 3, 4.2 nM; and Line 4, 2.1 nM. In the absence of xanthine oxidase there was no detectable increase in absorbance at 233 nm over the period of observation.

0.99. Increasing the concentration of xanthine oxidase increased the rate of co-oxidation of arachidonate. This is shown by the data in Fig. 2. It was often convenient, in subsequent experiments, to eliminate the exponential behavior evident in Fig. 2 by adding 0.05 mM H₂O₂.

Catalase Inhibition of Arachidonate Co-oxidation—It seemed likely that the exponential increase in the rate of co-oxidation of arachidonate reflected accumulation of H₂O₂. This was supported by the effect of exogenous H₂O₂ as shown in Fig. 1. If H₂O₂ was essential for the co-oxidation of arachidonate, then catalase should inhibit this reaction. The results in Fig. 3 demonstrate that this was the case. Catalase inhibited 50% at 0.16 µg/ml and 95% at 4.8 µg/ml. When catalase was added to a reaction containing 0.05 mM exogenous H₂O₂, the rate of arachidonate co-oxidation fell, over the course of a few minutes, as the level of H₂O₂ was decreased to a lower steady state by the balance between the production of H₂O₂ by xanthine oxidase and its removal by catalase. Catalase inhibited to an identical extent as the experiment with exogenous H₂O₂ (see above), when the only source of H₂O₂ was the xanthine oxidase reaction itself and when it was applied after a linear rate had been achieved.

Heat denaturation of catalase markedly diminished, but did not entirely eliminate, its ability to inhibit arachidonate co-oxidation. This is probably a reflection of the known peroxidatic activity of ferrithemins (44).

Superoxide Dismutase Inhibition of Arachidonate Co-oxidation—Superoxide dismutase powerfully inhibited the co-oxidation of arachidonate and did so whether added at the outset or after the reaction was well underway. In the latter circumstance, addition of superoxide dismutase caused a cessation of arachidonate co-oxidation within the few seconds required for its admixture with the other reaction components. As shown in Fig. 4, 5.5 ng/ml caused 50% inhibition and 1.0 µg/ml caused complete inhibition. Since the molecular weight of superoxide dismutase is 32,000 (35) we estimate that 1.7 × 10⁻⁹ M was sufficient to cause 50% inhibition. Superoxide dismutase, inactivated by heating at 100°C, was entirely without effect when tested at 0.48 µg/ml.

Since superoxide dismutase scavenges O₂⁻, but not H₂O₂, while catalase scavenges H₂O₂ but not O₂⁻, and since either superoxide dismutase or catalase could cause virtually complete inhibition of arachidonate co-oxidation by xanthine oxidase, it follows that both O₂⁻ and H₂O₂ are required for this co-oxidation.
Inhibition of Arachidonate Co-oxidation by Mannitol—Oxidations exhibiting a dual dependence upon both $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ have repeatedly been observed to be inhibited by scavengers of $\text{OH}^-$. (35, 45–50). We tested the effects of mannitol, which reacts very rapidly with $\text{OH}^-$ (51), but which scavenges neither $\text{O}_2^-$ nor $\text{H}_2\text{O}_2$. Mannitol at 20 mM caused 32% inhibition and at 40 mM caused 50% inhibition of arachidonate co-oxidation. We conclude that $\text{O}_2^-$ and $\text{H}_2\text{O}_2$, generated by the xanthine oxidase reaction, interacted in a way which produced a potent oxidant which could be scavenged by mannitol and which was responsible for initiating the oxidation of arachidonate.

The Role of Metals—The simplest explanation for the production of a potent oxidant from $\text{O}_2^-$ plus $\text{H}_2\text{O}_2$ would be the reduction of $\text{H}_2\text{O}_2$ to $\text{OH}^- + \text{OH}^-$ by $\text{O}_2^-$. This reaction, frequently referred to as the Haber-Weiss reaction, has been explored and its rate found to be very low (52). Others have, however, found evidence for catalysis of the Haber-Weiss reaction by iron complexes (53, 54). We explored the possibility of such catalysis by testing the effects of added EDTA-Fe(II), which at the pH of the reaction mixtures would oxidize to EDTA-Fe(III). Fig. 5 demonstrates that micromolar levels of EDTA-Fe(II) markedly stimulated the co-oxidation of arachidonate by the xanthine oxidase reaction. Arachidonate co-oxidation, in the presence of exogenous 25 mM EDTA-Fe(II), remained fully susceptible to inhibition by superoxide dismutase or by catalase and fully dependent on the xanthine oxidase reaction.

Ferrous salts plus $\text{H}_2\text{O}_2$ do constitute a source of $\text{OH}^-$ by the Fenton reaction (55), and high levels of EDTA-Fe(II) (50 µM) did cause arachidonate oxidation in reaction mixtures containing 0.05 mM $\text{H}_2\text{O}_2$ and lacking xanthine oxidase. However, even with 50 mM EDTA-Fe(II), the addition of xanthine oxidase increased arachidonate oxidation 8.3-fold. Commercially available EDTA and sodium pyrophosphate contain impurities of iron, and we suspect that the co-oxidation of arachidonate seen in the absence of exogenous EDTA-Fe(II) was dependent upon this endogenous Fe. Indeed, adding EDTA, per se, increased arachidonate co-oxidation to an extent which suggested a 0.5% impurity of Fe in the EDTA.

If the co-oxidation of arachidonate by xanthine oxidase was entirely dependent upon trace impurities of iron, as well as upon $\text{O}_2^-$ and $\text{H}_2\text{O}_2$, then a chelating agent which inactivated the iron impurities should eliminate this co-oxidation. Previous studies have suggested that DTPA, unlike EDTA, does not inactivate iron salts (56). When 0.1 mM EDTA was replaced by 0.1 mM DTPA, the rate of arachidonate co-oxidation was inhibited 46%, and when 0.2 mM EDTA was replaced by 0.2 mM DTPA this inhibition increased to 75%. This effect of DTPA, as well as the effects of superoxide dismutase, catalase, and mannitol, suggest that $\text{OH}^-$, or a species of comparable reactivity, generated by an iron-catalyzed interaction of $\text{O}_2^-$ and $\text{H}_2\text{O}_2$, was the root cause of arachidonate co-oxidation.

Does Lipid Hydroperoxide Play a Role in Arachidonate Co-oxidation?—The arachidonic acid used in the studies described above was devoid of hydroperoxide by the criterion of TLC. Furthermore, quantitative HPLC demonstrated that its content of hydroperoxide was less than 0.005%. Nevertheless, since Thomas et al. (52) reported an essential role of hydroperoxides in the co-oxidation of arachidonate by xanthine oxidase, we explored the effect of arachidonate hydroperoxides. Neat arachidonic acid was air-oxidized under the influence of ultraviolet irradiation. The extent of accumulation of hydroperoxide was estimated from its absorbance at 233 nm, applying a molar extinction coefficient of 25,000 (57). This arachidonic acid was then dispersed with sodium octyl sulfate, and its susceptibility to co-oxidation by xanthine oxidase was measured. The results are shown in Table I. It is clear that hydroperoxide did not increase susceptibility to co-oxidation, but rather decreased it substantially. This lack of a stimulatory effect of hydroperoxides was expected. Thus, when $\text{H}_2\text{O}_2$ was present at the outset, the co-oxidation of arachidonate by xanthine oxidase was a linear function of time, until slowed by inactivation of xanthine oxidase after ~45 min. Had hydroperoxides been a required reactant, we should have observed a percentage of the total arachidonate. Other components were 20 mM acetaldehyde, 0.1 mM EDTA, 6.0 mM sodium octyl sulfate, 50 mM sodium pyrophosphate, and 17 mM xanthine oxidase. Incubation was at 38°C and pH 9.0.

| Initial peroxide | Linear rate (Å/min/g nm) |
|------------------|-------------------------|
| 0                | 0.018                   |
| 1.4              | 0.016                   |
| 3.7              | 0.014                   |
| 5.8              | 0.0094                  |
an exponential increase of rate with increased time of reaction, due to the effect of accumulated peroxide products.

While the co-oxidation of arachidonate by xanthine oxidase was not increased by the presence of hydroperoxides the autoxidation of arachidonate, in the absence of xanthine oxidase, did show such an augmentation. This autoxidation was very much slower than the enzymic co-oxidation, but it could be observed over long periods of incubation. Thus, in the usual reaction mixture, such as the one described in the legend of Fig. 2 but lacking xanthine oxidase, we noted an autoxidation of 2.9 nm/min, averaged over the first 1.2 h, and of 7.2 nm/min, averaged over the next hour. In contrast, arachidonate co-oxidation in the complete reaction mixture containing xanthine oxidase was 110 nm/min averaged over the first 1.2 h, and had decreased to 87 nm/min over the next hour.

Superoxide dismutase at 0.5 μg/ml was sufficient to cause 50% inhibition of this autocatalytic autoxidation, implying a role for $O_2^−$.

Product Analysis—TLC and HPLC were used to examine and identify the conjugated diene hydroperoxide products of arachidonate co-oxidation by xanthine oxidase. In complete reaction mixtures, such as those described in the legend to Fig. 4, but lacking superoxide dismutase, 3.9% conversion of arachidonate to hydroperoxide was achieved in 100 min of incubation. TLC confirmed the presence of several peroxide products. HPLC with effluent monitoring at 235 nm showed the six hydroperoxide isomers usually produced by the autoxidation of arachidonate (43).

Conjugated diene hydroperoxides substituted at positions 15, 12, 11, 9, 8, and 5 of the 20-carbon chain were observed. In addition to these major products having trans,cis-diene stereochemistry, minor products tentatively assigned the trans,trans-diene configuration were detected. As in bulk phase autoxidation of arachidonate, the 15-hydroperoxide and 5-substituted hydroperoxide (trans,cis stereochemistry) were the major products formed in xanthine oxidase co-oxidation. There were, however, striking quantitative differences in the isomeric hydroperoxides observed in autoxidation and enzymic co-oxidation. The ratio of the 5:15 hydroperoxides found from autoxidation was generally less than 0.6, while the same ratio observed in the xanthine oxidase-catalyzed reaction was greater than 1.1 (four experiments). In addition, the relative amounts of the 12-, 11-, 9-, and 8-hydroperoxides formed in the enzymic oxidation were significantly less than was found in autoxidation (see Fig. 6). In fact, the 12-, 11-, 9-, and 8-hydroperoxides amounted to less than 5% of the hydroperoxide mixture in the xanthine oxidase oxidation while in bulk phase arachidonic acid oxidation, these hydroperoxides amounted to 30% of the hydroperoxides formed.

DISCUSSION

The co-oxidation of arachidonate, to conjugated dieneic hydroperoxides, by xanthine oxidase was dependent upon both $O_2^−$ and $H_2O_2$. Thus, catalase, a selective $H_2O_2$ scavenger, caused virtually complete inhibition of oxidation, as did superoxide dismutase, a selective $O_2^−$ scavenger. This co-oxidation exhibited an initial exponential phase due to the accumulation of $H_2O_2$. Addition of $H_2O_2$ gave an enzymic co-oxidation which was linear within the period of observation. The species actually initiating arachidonate oxidation appeared to be $OH^-$, or something of comparable reactivity, since mannitol, which scavenges $OH^-$, but not $O_2^−$ or $H_2O_2$, inhibited the enzymic co-oxidation. These results are most readily accommodated by proposing an interaction of $O_2^−$ and $H_2O_2$ which generated $OH^-$ or its equivalent. Endogenous iron salts and complexes were important for this interaction as shown by the accelerating effect of EDTA-Fe and by the inhibition by DTPA. Several reaction schemes can be proposed for this iron-catalyzed production of a potent oxidant from $O_2^−$ plus $H_2O_2$.

In Schemes I and II, hydroxyl radical is the oxidant, while in Scheme III, either hydroxyl or (FeO)$^{3+}$ could play this role. Spin-trapping studies have suggested hydroxyl radical as the oxidant in comparable systems (58, 59) yet (FeO)$^{3+}$ has also been shown to be capable of hydrogen atom abstraction from inactivated organic compounds (60, 61).
Pryor and coworkers reported that the co-oxidation of linoleate by xanthine oxidase was inhibited by superoxide dismutase and was dependent upon the presence of hydroperoxides (32). They accommodated their results by proposing the reaction $\text{O}_2^- + \text{ROOH} \rightarrow \text{O}_2 + \text{OH}^- + \text{O}-\text{O}_2$. We, in contrast, have noted no dependence of the enzymic co-oxidation upon the presence of hydroperoxides. Indeed peroxides, when introduced by prior autoxidation (Table I), slightly diminished the rate of enzymic co-oxidation. Autoxidation of arachidonate was, however, autocatalytic and thus was probably accelerated by lipid hydroperoxides.

An explanation of the apparent discrepancy between our results and those of the Pryor group may now be proposed. While the latter reported a single LOOH-dependent pathway for oxidation of arachidonic acid involving $\text{O}_2^-$ in the xanthine oxidase system, two distinct oxidative processes were at work in our system: a major co-oxidation and a minor autoxidation (seen only in the absence of the xanthine oxidase reaction). Unlike the co-oxidative pathway, the pathway for autoxidation gave an autocatalytic rate, implying a dependence on peroxides. $\text{O}_2^-$ was also implicated in this autoxidation, since superoxide dismutase at 0.5 pg/d inhibited 50%.

Hence, the oxidation reported by Pryor's group appears equivalent to the minor autooxidative pathway of arachidonate observed in our system, rather than to its enzymic co-oxidation. Their reaction systems contained 1.72 mM ethanol to solubilize the fatty acid, as well as 0.1 mM DTPA as a chelator. Ethanol is an excellent scavenger of OH$^-$, and has been seen to inhibit the co-oxidation of methionine to ethylene by the xanthine oxidase system (35). DTPA at 0.1 mM would partially convert endogenous Fe into a chelate inactive with respect to catalyzing the interaction of $\text{O}_2^-$ with H$_2$O$_2$ (50% inhibition of co-oxidation in our system). These components of the reaction mixtures used by Pryor’s group must account for the differences between the results reported by them, and those obtained by us.

The enhancement of 5-hydroperoxy-icosatetraenoic acid in the enzymic co-oxidation described here over the amounts of this isomer found in the bulk phase oxidation of arachidonic acid is of chemical and biological interest.

The finding that 5- and 15-hydroperoxy-icosatetraenoic acid are major products in superoxide/hydrogen peroxide-mediated arachidonic acid oxidation relates to anaphylaxis and the inflammatory response. A superoxide-dependent plasma chemotactic factor has been suggested to be a lipid component bound to serum albumin (62) and in vitro incubation of purified arachidonic acid with a superoxide-generating system results in the formation of products that are strongly chemotactic (63). Our experiments provide a direct chemical link between these superoxide-mediated oxidations and arachidonic acid hydroperoxides, compounds known to be strongly chemotactic (12).

We also note that 5-hydroperoxy-icosatetraenoic acid is the proposed intermediate in the biosynthesis of the slow reacting substance of anaphylaxis (14) and, as such, it plays a crucial role in the allergic response. 5-hydroperoxy-icosatetraenoic acid is the major hydroperoxide product formed in superoxide/hydrogen peroxide-mediated arachidonic acid oxidation and incorporation of 5-hydroperoxy-icosatetraenoic acid into cells, into the slow reacting substance of anaphylaxis biosynthetic pathway may have profound biological effects.

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REFERENCES

1. Barber, A. A., and Bernheim, F. (1967) Adv. Gerontol. Res. 2, 355-403.
2. Tappel, A. L. (1972) Ann. N. Y. Acad. Sci. 203, 12-28.
3. Wolman, M. (1975) Isr. J. Med. Sci. 11, Suppl. 1, 47-53.
4. Tappel, A. L. (1975) in Pathobiology of Cell Membranes (Trump, B. F., and Aris, A. V., eds) Vol 1, pp. 145-172, Academic Press, New York.
5. Pryor, W. A. (1978) Photochem. Photobiol. 28, 781-786.
6. Riley, C. A., Cohen, G., and Liebeman, M. (1974) Science 183, 206-210.
7. Dillard, C. J., Dumelin, E. E., and Tappel, A. L. (1977) Lipids 12, 109-114.
8. Konz, J. R., and Elstner, E. F. (1978) Biochim. Biophys. Acta T28, 213-221.
9. Lindblom, T. D., and Anders, M. W. (1978) Biochem. Pharmacol. 27, 563-567.
10. Donovan, D. H., and Menzel, D. B. (1978) EXPERIEN (Basel) 34, 775-776.
11. Moncada, S., Gygiewski, R. J., Bunting, S., and Vane, J. R. (1976) Prostaglandins 12, 715-722.
12. Goetzl, E. J., Woods, J. M., and Gorman, R. R. (1977) J. Clin. Invest. 59, 179-183.
13. Murphy, R. C., Hammarström, S., and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4275-4279.
14. Parker, C. W. (1979) J. Allergy Clin. Immunol. 63, 1-14.
15. Bergant, P., and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3213-3217.
16. Hoehn, P., Norden, K., and Ernster, L. (1964) Biochem. Biophys. Res. Commun. 14, 323-328.
17. Orrénius, S., Dahlner, G., and Ernster, L. (1964) Biochem. Biophys. Res. Commun. 14, 329-334.
18. Pederson, T. C., and Aust, S. D. (1972) Biochem. Biophys. Res. Commun. 48, 789-795.
19. Pederson, T. C., and Aust, S. D. (1973) Biochem. Biophys. Res. Commun. 52, 1071-1078.
20. Goda, K., Chu, T.-W., Kimura, T., and Schap, A. P. (1973) Biochem. Biophys. Res. Commun. 52, 1300-1306.
21. Zimmerman, R., Flosa, L., Wesci, U., and Hartman, H. J. (1973) FEBS Lett. 29, 117-120.
22. Petkau, A., and Chelack, W. S. (1974) Fed. Proc. 33, 1505.
23. Kellogg, E. W., III, and Fridovich, I. (1975) J. Biol. Chem. 250, 8815-8817.
24. Petkau, A., and Chelack, W. S. (1976) Biochem. Biophys. Acta 433, 445-456.
25. Tyler, D. D. (1975) FEBS Lett. 51, 180-183.
26. Pederson, T. C., and Aust, S. D. (1975) Biochem. Biophys. Acta 385, 232-241.
27. Takahama, U., and Mishikimi, M. (1976) Plant Cell Physiol. 17, 111-118.
28. Kellogg, E. W., III, and Fridovich, I. (1977) J. Biol. Chem. 252, 6721-6728.
29. Gutteridge, J. M. C. (1977) Biochem. Biophys. Res. Commun. 77, 379-386.
30. Goldstein, I. M., and Weissman, G. (1977) Biochem. Biophys. Res. Commun. 75, 604-609.
31. Svingen, B. A., O'Neal, F. O., and Aust, S. D. (1978) Photochem. Photobiol. 28, 803-809.
32. Thomas, M. J., Mehl, K. S., and Pryor, W. A. (1978) Biochem. Biophys. Res. Commun. 83, 927-932.
33. Kameda, K., Ono, T., and Inai, Y. (1979) Biochem. Biophys. Acta 572, 77-82.
34. Lynch, R. E., and Fridovich, I. (1978) J. Biol. Chem. 253, 4697-4698.
35. Beauchamp, C., and Fridovich, I. (1970) J. Biol. Chem. 245, 4641-4646.
36. McCord, J. M., and Day, E. D., Jr. (1978) FEBS Lett. 86, 139-142.
37. Gutteridge, J. M. C., Richmond, R., and Halliwell, B. (1979) Biochem. J. 184, 469-472.
38. Wood, W. R., Brady, F. O., Wiley, R. D., and Rajagopalan, K. V. (1975) Arch. Biochem. Biophys. 169, 695-701.
39. Kalckar, H. M. (1947) J. Biol. Chem. 167, 429.
40. McCord, J. M., and Fridovich, I. (1969) J. Biol. Chem. 244, 6049-6055.
41. Tanford, C. (1980) The Hydrophobic Effect, 2nd Ed, John Wiley and Sons, New York.
Oxidation of Arachidonate by $O_2^-$ plus $H_2O_2$

42. Porter, N. A., Logan, J., and Kontoyiannidou, V. (1979) J. Org. Chem. 44, 3177–3181
43. Porter, N. A., Wolf, R. A., Yarbro, E. M., and Weenen, H. (1979) Biochem. Biophys. Res. Commun. 89, 1058–1064
44. Jones, P., Robson, T., and Brown, S. B. (1973) Biochem. J. 135, 353–359
45. Gregory, E. M., and Fridovich, I. (1974) J. Bacteriol. 117, 166–169
46. Lown, J. W., Begleiter, A., Johnson, D., and Morgan, A. R. (1976) Can. J. Biochem. 54, 110–119
47. Hodgson, I. K., and Fridovich, I. (1976) Arch. Biochem. Biophys. 172, 292–295
48. McCord, J. M. (1974) Science 185, 529–531
49. McCord, J. M., and Day, E. D., Jr. (1978) FEBS Lett. 86, 139–142
50. Buettner, G. R., Oberley, L. W., and Chan-Leuthauser, S. W. H. (1978) Photochem. Photobiol. 28, 693–695
51. Dorfman, L. M., and Adams, G. E. (1973) Reactivity of the Hydroxyl Radical in Aqueous Solutions, National Bureau of Standards, Washington, D. C.
52. Ferradini, C., and Seide, C. (1969) Int. J. Radiat. Phys. Chem. 1, 219–228
53. Ferradini, C., Foos, J., Hovec, C., and Puchelault, J. (1978) Photochem. Photobiol. 28, 697–700
54. Fong, K.-L., McCay, P. B., Poyer, J. L., Misra, H. P., and Keele, B. B., Jr. (1976) Chem.-Biol. Interact. 13, 77–89
55. Walling, C. (1975) Accs. Chem. Res. 8, 125
56. Halliwell, B. (1978) FEBS Lett. 92, 321–326
57. Smith, Karl J., and Porter, Ned A. (1980) J. Am. Chem. Soc., in press
58. Buettner, G. R., Oberly, L. W., and Chan-Lewthauser, S. W. H. (1978) Photochem. Photobiol. 28, 693
59. Finkelstein, E., Rosen, G. M., Rauckman, E. J., and Paxton, J. (1979) Biochem. Pharmacol. 16, 675
60. Groves, J. T., and Van Der Puy, M. (1974) J. Am. Chem. Soc. 96, 5274–5275
61. Groves, J. T., and McClusky, G. A. (1976) J. Am. Chem. Soc. 98, 859–861
62. Petrone, W. F., English, D. K., Wong, K., and McCord, J. M. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 1159–1163
63. Perez, H. D., and Goldstein, I. M. (1979) Fed. Proc. 38, 1170