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Role of Iron and Ascorbic Acid in the Oxidation of Methyl Linoleate Micelles

Keiji YAMAMOTO, Mareyuki TAKAHASHI, and Etsuo NIKI*
Department of Reaction Chemistry, Faculty of Engineering,
The University of Tokyo, Hongo, Tokyo 113

Oxidations of methyl linoleate micelles in aqueous dispersions induced by iron and ascorbic acid have been studied aiming specifically at elucidating the mechanism of initiation reactions. It was suggested that the initial radicals were generated by the decomposition of hydroperoxide by iron and that ascorbic acid functioned as a reducing agent for ferric ion to give more reactive ferrous ion.

The oxidations of lipids, especially of polyunsaturated fatty acids and their esters, have received renewed attention recently in connection with the oxidative deteriorations of foods and a variety of pathological events in biological systems. These non-enzymatic, random oxidations proceed by a free radical chain mechanism and one of the key steps is the initial generation of free radicals. The free radicals may be formed in vivo by various routes such as thermal and photochemical cleavages of labile compounds, irradiation, and intake of exogeneous radicals. Iron has been suggested to play an important role in vivo[1,2] and it has been known that the combination of iron and ascorbic acid can initiate a free radical autoxidation of lipids and in fact this system has been often used as an initiating system in model experiments. However, it is not clearly understood how the initiation reaction takes place in this particular system in spite of its frequent applications. Furthermore, the functions of ascorbic acid are complicated and both prooxidant and antioxidant effects of ascorbic acid have been reported.[3] In this study, we have tried to understand the mechanism of the initiation reaction induced by iron and ascorbic acid.

The oxidation of methyl linoleate micelles in the aqueous dispersions was carried out at 37 °C as reported previously.[4] The rate of oxygen uptake was measured from the decrease of oxygen concentration in the aqueous dispersions with an oxygen electrode. Triton X-100, tetradecyltrimethylammonium bromide (TTAB), and sodium dodecyl sulfate (SDS) were used as non-ionic, cationic, and anionic surfactant, respectively.

The rate of spontaneous oxidation of methyl linoleate micelles in 0.01 M (M = mol dm^{-3}) Triton X-100 aqueous dispersions was quite small, but the addition of ferrous sulfate induced the free radical chain oxidations. As shown in Fig. 1, the rate of oxidation increased with increasing concentration of ferrous sulfate, the kinetic order being 0.53, suggesting that the rate of chain initiation is directly proportional to the concentration of ferrous ion. The addition of t-butyl hydroperoxide increased the rate of oxidation and the kinetic order of the rate of oxidation on the concentration of ferrous ion was 0.50 in the presence of of 1 mM t-butyl hydroperoxide. Ferric chloride also induced the oxidation of

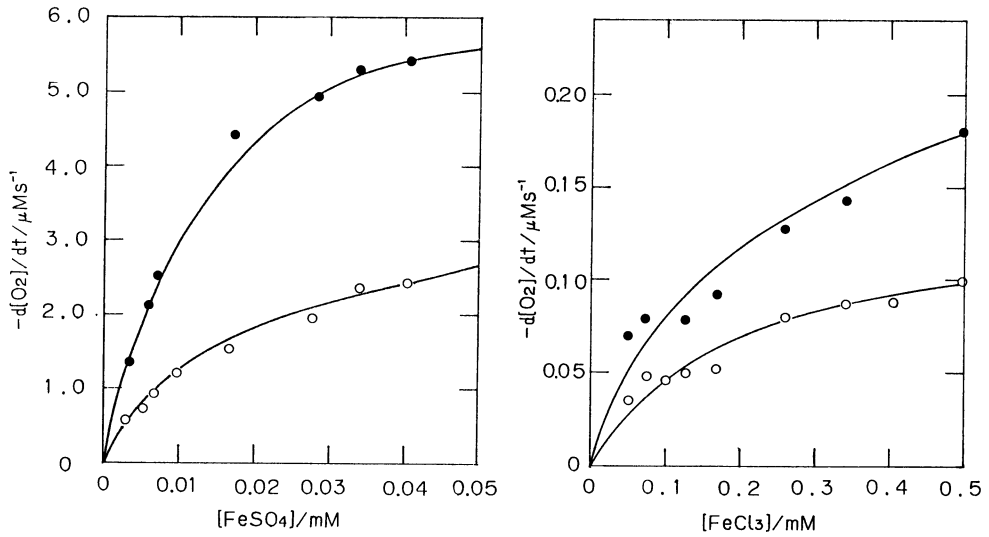

Fig. 1. Rate of oxidation of 144 mM methyl linoleate in 10 mM Triton X-100 aqueous dispersions induced by iron at 37 °C. About 5 x 10^{-5} M methyl linoleate hydroperoxide was present initially. ○: without added hydroperoxide. ●: with 1 mM added t-butyl hydroperoxide.

methyl linoleate micelles, but the rate of oxidation was much smaller than that induced by ferrous sulfate by a factor of about 100. Substantially the same results were obtained with ferrous and ferric ammonium sulfate. In the presence of both ferrous and ferric ions, the rate of oxidation increased monotonously with increasing ratio of ferrous ion.

Figure 2 shows the effect of electronic charge of micelle surface on the ferrous ion-induced oxidation of methyl linoleate micelles. When the surface charge was either neutral or negative, the addition of ferrous sulfate induced the oxidation without any appreciable induction period. On the other hand, when the micelle had a positive charge on its surface, ferrous ion did not induce the oxidation. However, even in this case, the addition of t-butyl hydroperoxide to the aqueous phase induced a fast oxidation without induction period.

These results suggest that ferrous ion decomposes lipid hydroperoxide (LOOH) contained initially in methyl linoleate (methyl linoleate contained about 1 mM, i. e., 0.03 mol% hydroperoxides initially and 5 x 10^{-5} M hydroperoxide in the aqueous dispersions) to give an alkoxyl radical, which must initiate free radical

$$LOOH + Fe^{2+} \longrightarrow LO\cdot + {}^{-}OH + Fe^{3+} \qquad (1)$$

chain oxidation. In fact, when methyl linoleate was treated before the addition of ferrous ion with triphenylphosphine to reduce the hydroperoxide, ferrous ion did not induce the oxidation. The results shown in Fig. 2 may be interpreted as follows; that is, when the micelle surface has a positive charge, ferrous ion may not be able to access to and interact with the methyl linoleate hydroperoxide located within the lipid region of the micelles, but ferrous ion may react with t-butyl hydroperoxide in the aqueous region to give a neutral t-butoxyl radical which can attack methyl linoleate independent of the electronic charge of the micelle surface. The lower rate of oxidation induced by ferric ion than that by

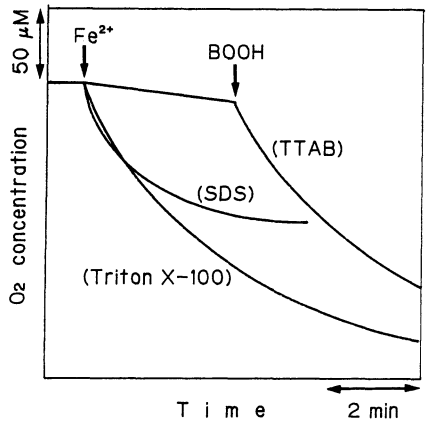

Fig. 2. Effect of surface charge of micelles of 144 mM methyl linoleate in aqueous dispersions on its oxidation induced by 10 uM ferrous sulfate at 37 °C. Surfactant = 10 mM, t-butyl hydroperoxide = 1 mM.

ferrous ion is apparently due to the lower rate of decomposition of hydroperoxide by ferric ion than by ferrous ion. The rate constants for the decomposition of t-butyl hydroperoxide by ferrous and ferric ions were obtained as 2.4×10^{2} $M^{-1}s^{-1}$ and 2.4×10^{-3} $M^{-1}s^{-1}$ respectively at 37 °C, suggesting that the rate of chain initiation by ferrous ion is larger than that by ferric ion by a factor of about 10^{5} under similar conditions, in consistent with the results observed here.

Figures 3 and 4 show pictorially the effect of ascrobic acid on the rate of iron-induced oxidations of methyl linoleate micelles in the aqueous dispersions. Ascorbic acid had only a minor effect on the initial rate of oxidation induced by ferrous sulfate and it retarded the oxidation as its concentration increased. The rate of oxidation of methyl linoleate micelles induced by ferrous ion decreased gradually with time as the ferrous ion was oxidized to ferric ion, but the addition of ascorbic acid resumed the fast oxidation. On the other hand, ascorbic acid increased the rate of oxidation induced by ferric ion. This must be ascribed to the reduction of ferric ion by ascorbic acid to give a more reactive ferrous ion. It has been found experimentally that ferric ion was reduced rapidly by ascorbic acid to give ferrous ion. Cysteine and glutathione which reduced ferric ion also accelerated the oxidation in the presence of ferric ion.

Ascorbic acid can scavenge oxygen radicals by itself and acts as a chain-breaking antioxidant in the free radical chain oxidation of lipids in the absence of iron.[5] The above results suggest that ascorbic acid is a double edged sword and may function as both antioxidant and prooxidant, depending on the conditions, by scavenging oxygen radicals and by reducing ferric ion respectively. In the oxidation of methyl linoleate micelles in the presence of ferrous ion, ascorbic acid must function primarily as a radical scavenger. On the other hand, in the presence of ferric ion, ascorbic acid functions as a reducing agent and accelerates the oxidation unless the concentration of ascorbic acid is much higher than that of ferric ion.

The formation of hydrogen peroxide was observed when ascorbic acid was dissolved in water. Hydrogen peroxide is decomposed by iron by Fenton mechanism

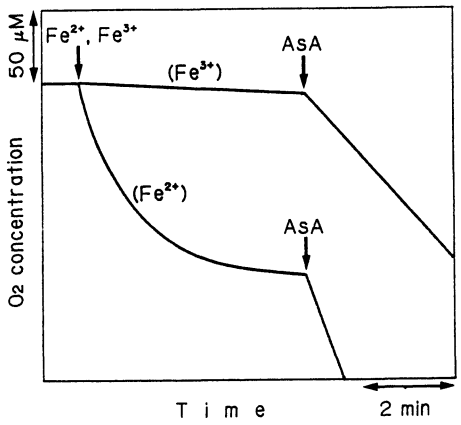

Fig. 3. Effect of addition of 100 μM ascorbic acid on the oxidation of 144 mM methyl linoleate in 10 mM Triton X-100 aqueous dispersions in the presence of 10 μM iron at 37 °C

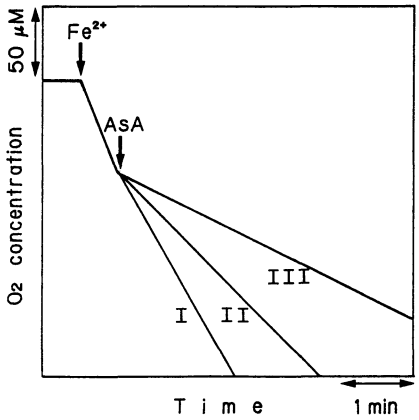

Fig. 4. Effect of addition of ascorbic acid (I: 10 μM, II: 100 μM, III: 1 mM) on the oxidation of 144 mM methyl linoleate in 10 mM Triton X-100 aqueous dispersions in the presence of 10 μM ferrous sulfate at 37 °C

and may contribute to the chain initiation, but the amount of hydrogen peroxide formed under the present reaction conditions was found to be very small compared with the hydroperoxide contained initially.

In conclusion, iron initiates the free radical chain oxidation of lipids primarily by decomposing hydroperoxide and generating alkoxyl radicals. Ferrous ion decomposes hydroperoxide much faster than ferric ion. Ascorbic acid contributes in chain initiation by reducing ferric ion to ferrous ion, but it can also act as a chain-breaking antioxidant. The relative importance of the dual functions of ascorbic acid must depend on its concentration, presence or absence of ferrous and ferric ions, and their structure and concentrations. In the biological systems, the local concentrations at specific site are apparently important. The direct initiation by iron-oxygen complex or ferrous-ferric complex [1] may contribute at the very early stage, but once even a minute amount of hydrogen peroxide and/or hydroperoxides are accumulated, their redox decomposition by iron must be more important.

References

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