centration of ADP which inhibited the cysteine-Fe-catalyzed oxidation was very stimulating to systems containing NADH and Fe³⁺.

The lack of added effect when NADH was added to the ascorbate-Fe system might indicate that under the conditions used, the system was operating near maximal activity in the presence of the ascorbate-Fe and that additional reducing equivalents supplied via the NADH-enzyme system did not add much more activity. Unlike the situation with cysteine, ADP did not show significant inhibition with the ascorbate-Fe system. This might be due to the fact that ascorbate formed a strong complex with Fe³⁺, which might prevent it from interacting with the ADP.

The close correlation between the production of lipid hydroperoxides and later breakdown products (Fig. 1) was not observed when cysteine was added to the system. In this case, a very rapid buildup of the lipid hydroperoxides which reached a peak approximately where the control sample was coming out of its lag phase was observed. Under the conditions of our assays, the lipid hydroperoxides dropped to a very low level within 40 min in the assay medium to which the cysteine had been added. Obviously, cysteine has a major role to play in the breakdown of lipid hydroperoxides. Since it also supports the production of TBA-reactive substances, it is likely that it participates in the breakdown by a mechanism involving free radicals. Sulfur-containing amino acids are susceptible to attack by free radicals (Hultin, 1986).

The reactivity of the system in the presence of various additives may give insight as to the nature of the reaction. EDTA and sodium tripolyphosphate most likely function because they can form complexes with iron ions. One would presume that they form a stronger complex with Fe²⁺ than does ADP. The Fe in these complexes must, however, be unavailable for the oxidation reaction. Svingen et al. (1979) have shown that EDTA-chelated iron participates in the decomposition of lipid hydroperoxides but not in the initiation reaction.

It is important to keep in mind that the order of addition of components may have an important effect on what is observed. For example, many investigators have found that histidine can inhibit lipid oxidation in aqueous systems. We found that this depended on the order of addition. If the Fe³⁺ and the ADP were put into the reaction medium first, histidine had no effect. However, if the iron was added in the presence of histidine but before ADP, the histidine was extremely inhibitory. Presumably, it formed a complex which did not give up the iron to the ADP very readily even though it was incapable of extracting the iron from an ADP-Fe complex already formed.

The effectiveness of propyl gallate indicated that the reaction proceeded via free radical intermediates. That sulfhydryl groups were involved was indicated by the inhibitory action of the p-hydroxymercurobenzoate. Reduced glutathione slightly stimulated the reaction and may behave in a manner similar to that of cysteine, although it was not as effective. The use of active oxygen intermediate scavengers indicated that these compounds could stimulate the reaction. Superoxide dismutase was a scavenger of superoxide anion which was the first reduction product of molecular oxygen; catalase was a scavenger for the two electron-reduced product, H₂O₂. Dimethylsulfoxide and thiourea will remove the hydroxyl free radical, and diphenyluran is capable of reacting with and removing singlet oxygen.

Based on the observations above, a proposed reaction pathway is suggested in Fig. 9. Some form of reduced iron reacts with molecular oxygen to form an intermediate (here indicated as the Fe²⁺O₂²⁻, dioxygen ferrous ion complex, in equilibrium with Fe²⁺O₂, perferrosyl iron). This releases superoxide and Fe³⁺ which forms a complex with ADP whose function is to keep the Fe³⁺ soluble and/or modify its oxidation-reduction potential. On reduction, the Fe²⁺ is released from the ADP complex. The function of the enzyme (ENZ) is to reduce the Fe³⁺ to Fe²⁺ using electrons from NADH. This reduction of iron is suggested to be the primary function of the membrane enzyme and occurs in two other places in the scheme, indicated by ENZ.

Superoxide is dismutated to O₂ and H₂O₂ which can then further react with O₂⁻ to produce the hydroxy free radical (·OH) via a Fenton-type reaction. Superoxide dismutase inhibited the reaction by removing superoxide to prevent its reaction with the H₂O₂ formed while the inhibitory effect of catalase was due to its ability to remove H₂O₂. The hydroxy free radical may then abstract hydrogen from lipid to initiate lipid oxidation which then proceeds through the usual process to form a hydroperoxide. Hydroxy free radical scavengers destroyed the ultimate initiator, ·OH. The hydroperoxide can be decomposed by ferrous iron or ADP-complexed Fe³⁻ to produce a variety of free radicals and singlet oxygen. The effectiveness of the singlet oxygen scavenger, diphenyluran, indicated that initiation of the reaction could also be accomplished by singlet oxygen. Cysteine in the presence of Fe⁺⁺ can also decompose lipid hydroperoxides to produce free radicals. These products may then be involved with further propagation reactions by interacting with other molecules of lipid (LH) to maintain the chain reaction.

The main suggestion of this reaction scheme is that the role of the enzyme is simply to maintain iron in the reduced state. Although we do not have direct proof for this, we have been able to demonstrate a non-enzymic lipid oxidation which occurs very rapidly in the presence of Fe⁺⁺ and the extent of which (as measured by TBA-reactive substances) is proportional to the amount of Fe⁺⁺ added to the reaction mixture (Shewfelt and Hultin, 1983). Presumably, in this case, the reaction stops because the enzyme is not available to reduce the Fe⁺⁺ formed during the reaction to Fe⁺⁺.

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REFERENCES


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