Effect of Heme Compounds on Lipid Oxidation[1]

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ABSTRACT

By polarographic oxygen analyzer, the rate of oxidation of linoleate solutions was catalyzed by low concentrations of heme and heme-proteins and inhibited by higher concentrations. High concentrations of these substances also inhibited lipoxygenase catalysis of linoleate oxidation. Manganese and cobalt salts inhibited heme-catalyzed linoleate oxidation. These combined effects may reflect the oxidative synthesis of antioxidants from heme compounds.

A polarographic oxygen analyzer was used to study the effect of heme compounds on oxidation rates of linoleate emulsion systems. In accord with previous observations of Banks (1), Lewis and Wills (2), and Kendrick and Watts (3), the results depended upon the concentrations of heme compound added. Low levels of hemoglobin, myoglobin or hydroxyhemin were effective catalysts; but at higher levels, they were less effective or ineffective. A representative run is shown in Figure 1. Results were similar with the ferro- and ferri- forms. Addition of a concentrated solution of hemoglobin to a heme-catalyzed rapid oxidizing system stopped the uptake of oxygen (Fig. 2). In a similar fashion, the addition of protoporphyrin to a rapidly oxidizing heme-catalyzed linoleate emulsion brought the oxidation to a halt. Changes in the absorption spectra indicated that the heme compounds were modified during the reactions and suggested that products thus derived might have been the

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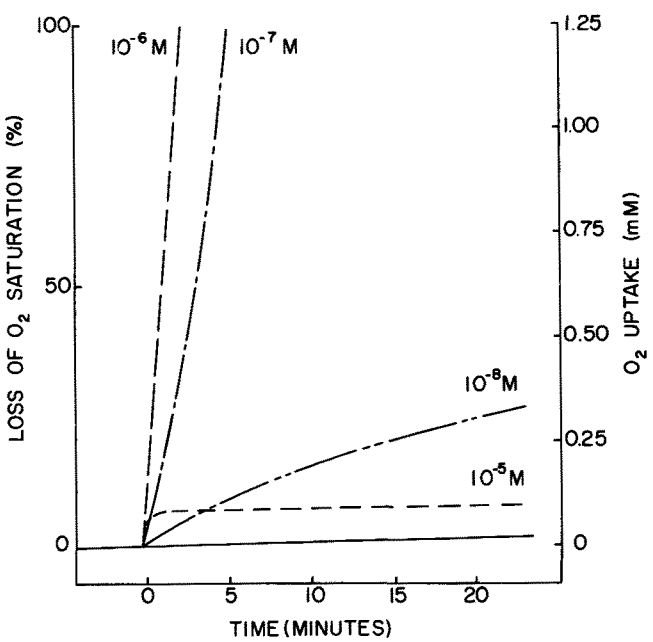

FIG. 1. Effect of different levels of methemoglobin (mHb) on linoleate oxidation. The system contained 0.01 M linoleate (linoleic acid, Hormel) brought to pH 6.5 with 0.5 N KOH, 0.02% Tween 20, pH 6.5 0.1 M phosphate buffer, 24 C. At time zero, mHb was injected to the levels indicated.

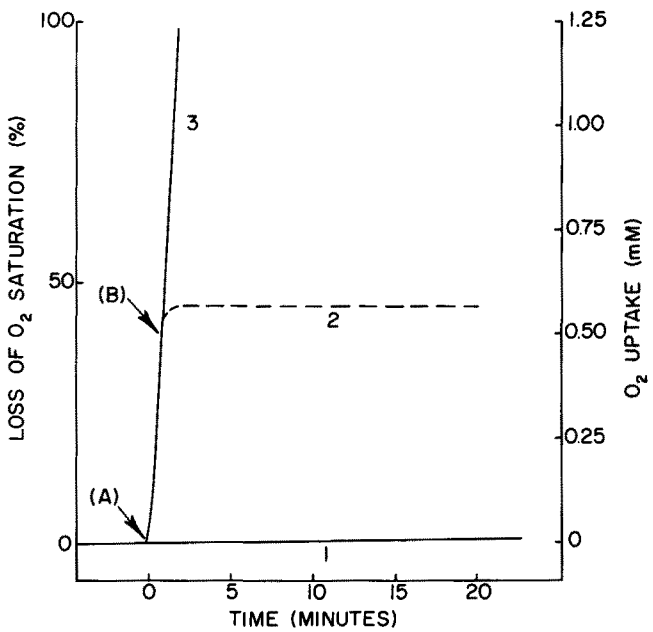

FIG. 2. Effect of adding methemoglobin (mHb) to a rapidly oxidizing linoleate system. At A, mHb was added to 10^{-6} M to 2 and 3. At B, additional methemoglobin was injected to 10^{-5} M to 2. The same cessation of oxygen uptake occurred when protoporphyrin was added to 10^{4} M instead of mHb to 10^{-5} M.

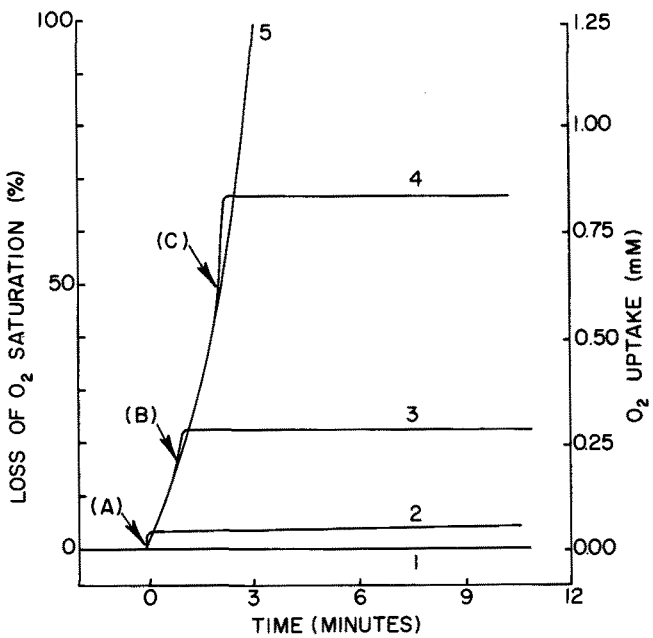

FIG. 3. Effect of addition of methemoglobin (mHb) to a lipoxygenase-catalyzed (Worthington) linoleate system (see Fig. 1). 1, Control; 2, mixture of 10^{-5} M mHb and 0.002% lipoxygenase added at A; 3, 0.0002% lipoxygenase added at time zero, then 10^{-5} M mHb at B; 4, same as 3 but mHb added after more oxidation had occurred, at C; 5, 0.002% lipoxygenase at A.

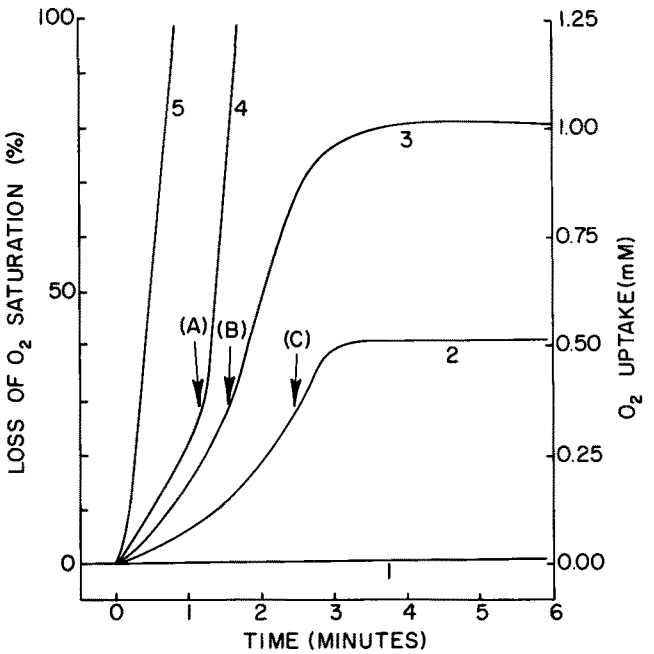

FIG. 4. Inhibition of lipoxygenase by protoporphyrin and methemoglobin (mHb) at pH 8.8. The system differed from that in Figure 1 in the use of 0.1 M borate buffer, pH 8.8. 1, Control; 2, protoporphyrin, 4 x 10^{-4} M, was preincubated with linoleate, 0.0002% lipoxygenase was added at time zero, then 10^{-6} M mHb was added at C; 3, protoporphyrin, 2 x 1^{-4} M was preincubated with linoleate, 0.0002% lipoxygenase was added at time zero, then 10^{-6} M mHb was added at B; 4, 0.0002% lipoxygenase was added at time zero, then 10^{-6} M mHb was added at A; 5, 10^{-6} M mHb was added at time zero.

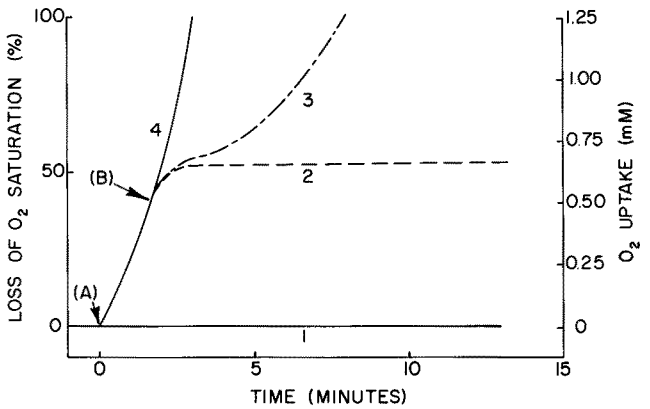

FIG. 5. Manganese inhibition of linoleate oxidation catalyzed by methemoglobin (mHb) at pH 7.5. System contained 0.01 linoleate, 0.1 M Tris buffer, pH 7.5. 1, No addition; 2, 3, 4, 10^{-6} mHb added at time zero; 2, 10^{-4} M Mn^{++} added at B; 3, 10^{-5} M Mn^{++} added at time B.

immediate cause of the cessation by acting as free radical sinks. Kendrick and Watts (3) observed that heme breakdown products had antioxidant activity.

Concentrated heme solutions also strongly inhibited the rate of oxygen uptake by linoleate when this was catalyzed with soybean lipoxygenase (Fig. 3). The mechanism may be similar. An additive effect of protoporphyrin and hemoglobin in inhibiting lipoxygenase is illustrated in Figure 4. At the levels used, the hemoglobin-lipoxygenase effect was additive, but when protoporphyrin was present, addition of hemoglobin to the same level stopped the oxidation.

Both manganese and cobalt salts effectively inhibited heme-catalyzed linoleate oxidation; manganese was somewhat more effective (Fig. 5). These effects appeared to require the presence of some free fatty acid.

The meaningfulness of these combined observations is possibly obscured by insufficient control of what may be a key factor, namely, the exact amount of hydroperoxide present at time zero of each run (Van der Veen, Koide and Olcott, in preparation). The positive catalytic effect of low levels of heme compounds on linoleate emulsions may well depend primarily on their ability to decompose peroxides with the formation of chain-initiating free radicals. In the presence of an excess of porphyrin compounds, derivatives may become free radical scavengers able to interfere with the oxidation if present in sufficient concentration. Nakamura and Nishida (7) recently suggested that the inhibition of linoleate oxidation by high concentrations of hemoglobin may be the result of the formation of a hemoglobin-linoleate combination (1:880 M/M) which effectively reduces the level of free linoleate.

The term "catalyst-inhibitor conversion" has been applied by Uri and his coworkers (4-6) to situations in which heavy metal catalysts at low concentrations become inhibitors at high concentrations. These workers have described, as one example (5), the effect of bis(N-butylsalicylaldimino)-cobalt on the autoxidation of 2,6,10,14-tetramethylpentadecane. Their observations are parallel to those observed with unsaturated fats and heme compounds and the mechanisms may be related.

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