Catalysts of Lipid Peroxidation in Meats

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ABSTRACT

Hemoprotein and non-heme iron components are active catalysts of lipid peroxidation. The behavior of these two catalysts under a number of conditions was compared as a basis for a study of their activities in meats. In model systems, MetMb accelerated linoleic acid peroxidation in a pH range from 5.6 to 7.8; it catalyzed especially rapidly at higher pH. A complex of ferrous ion [Fe(II)] and EDTA, a non-heme iron model, in a 1:1 ratio accelerated peroxidation at lower pH; no catalysis took place above pH 6.4. Most chelating agents eliminated Fe(II)-EDTA catalysis, but had no effect on MetMb catalysis. Reducing agents, on the other hand, accelerated Fe(II)-EDTA catalysis but inhibited MetMb catalysis. In model systems in which fresh dilute (1.2%, w/v) meat homogenate was the catalyst, the effect of the heme predominated. An exception was ascorbic acid; it accelerated oxidation at pH 5.6. The pattern of linoleate peroxidation catalyzed by heme-free (H2O2-treated) beef homogenate and shrimp homogenate was similar to that in the Fe(II)-EDTA model system. Again, ascorbic acid accelerated the catalysis and the acceleration could be eliminated by adding chelating agents. The presence of a non-heme iron catalyst in meat is thus indicated. Evidence is presented for both types of catalytic activity in meats. In cooked meats, heme was the dominant catalyst, but significant lipid oxidation, apparently catalyzed by a non-heme iron-type catalyst, occurred in cooked meats in which the heme had been destroyed by H2O2. In raw meats, lipid oxidation was inhibited at high pH because of removal of oxygen by enzymatic reducing systems. Both heme and non-heme iron were active at lower pH values. EDTA inhibited lipid oxidation during storage, presumably by its demonstrated effect on non-heme iron catalysis. Ascorbic acid also inhibited lipid oxidation, probably indirectly by keeping the heme pigment in the catalytic inactive ferrous state.

INTRODUCTION

Although the catalytic effect of hemoglobin (Hb) and other iron porphyrins on lipid oxidation is a generally accepted phenomenon, it is still not certain that this is the main oxidative catalyst in meat. Hematin-catalyzed lipid peroxidation has been demonstrated as an important deterioration reaction in unsaturated fat (1), precooked meats (2) and dehydrated foods (3). However, inorganic ferrous ion has also been demonstrated to be a catalyst of unsaturated lipid peroxidation in mitochondria (4) and microsome (5) as well as in pure unsaturated lipids (6). The pro-oxidant contribution of metals in meat and meat products has not been widely investigated.

Wills (7) presented evidence that both types of catalysts hemoprotein and inorganic iron, functioned in rat tissue. The two types could be differentiated by their relative activities at different pH and in the presence of chelating agents, ascorbic acid and thiol compounds. Barber (8) demonstrated that the catalytic role of iron and ascorbic acid was an important nonenzymic mechanism for lipid oxidation in tissue. He also indicated that sufficient iron was present in tissue particulates to bring about such catalysis. Lipid peroxidation could be inhibited by EDTA. Robinson (5) tried several different metals and found that only iron was capable of increasing lipid peroxidation with ascorbate or cysteine in microsomal suspension. Both heme and non-heme iron-containing enzymes present in the microsomal fractions could catalyze lipid peroxidation if the iron were made accessible through some change in tertiary structure (9).

It has been suggested that oxidative deterioration of the lipids in meat has been caused by the catalysis of hematin compounds, namely, MetMb. Metals have been investigated as pro-oxidants in meats. Moskovits and Kielsmeier (10) demonstrated that the contaminating iron in sausages exerted a powerful pro-oxidant effect. Moskovits and Castell (11) have found that trace amounts of iron or copper ion added to whole or blended cod fillets produced a serious off-odor ranging from “seaweedly” to that of strongly rancid fish oil. However, their work did not try to assess the catalytic activity of the metal originally present in tissue.

Another factor which influences the oxidative rancidity in meat is pH. Keskinel et al. (12) indicated that there was an inverse relationship between the pH of meat samples and the thiobarbituric acid (TBA) number. Stewart et al. (13) also demonstrated that the enzymatic reducing activity of MetMb (MRA) increased with pH from 5.1 to 7.1. There is a highly negative correlation between the MRA and malonaldehyde (MA) formation in raw meats (14). Presumably, at higher pH, the reducing enzymes are in a much more active state. Oxygen is utilized by way of the electron transport system and any MetMb present is reduced. Emanuel and Lyaskovskaya (15) found that a mixture of tocopherol with ascorbic acid and citric acid was 80 times more effective than ascorbate alone or in combination with citric acid in the tissue fats of fish. Several condensed phosphates were found to have antioxidative activity in cooked meat (16). Ascorbic acid again acted synergistically with phosphates to protect against rancidity in meats. The inhibition mechanisms of the chelating agents citric acid and phosphates and the reducing agent ascorbic acid have not been discussed.

Addition of free amino acids to blended cod muscle affected the subsequent development of rancidity (17). The aliphatic amino acids and cysteine showed strongly pro-oxidant activity. They did not explain the mechanism of the catalysis; however, the rancidity was found to be inhibited by adding the chelating agent EDTA and enhanced by adding ascorbic acid. It is thus probable that non-heme iron present in tissue catalyzes the catalysis by complexing with cysteine or ascorbic acid and inhibits by combining with EDTA.

EXPERIMENTAL PROCEDURES

Before undertaking the investigation of lipid peroxidation in meats, the properties of both types of catalysts in model systems and semimodel systems should be studied systematically.

In developing a model system, MetMb (6x10^-7M) was used to represent the catalyst for heme-iron component,
which would produce moderate oxidation with $7 \times 10^{-9} M$ linoleic acid emulsion in 0.1 M phosphate buffer. The non-heme iron model developed for this study consisted of a mixture of Fe(II) and EDTA. Each, present at a concentration of $1.5 \times 10^{-4} M$, was found to produce a constant catalytic rate in 0.05 M phosphate buffer linoleic acid emulsion. Such iron chelates of EDTA have been used to produce free radicals in various oxidative reaction (18-21). Thioli, ascorbic acid and chelating agents were expected to have different effects on hemoprotein vs. non-heme iron catalysis. Addition of these compounds therefore might help to differentiate the two systems.

Homogenates of rat liver, spleen, heart and kidney can actively catalyze peroxide formation in emulsions of linoleic or linolenic acid (7). Observation on a semimodel system, utilizing tissue as catalyst, was considered desirable. In this study, beef muscle homogenate is assumed to contain both heme and non-heme iron components. To differentiate non-heme iron from hemoprotein, Wills (7) gave the tissue a prior treatment with H$_2$O$_2$ to destroy all the catalytic functions of hemoprotein and liberate the inorganic iron, so that non-heme iron would be the only catalyst. Shrimp tissue contains no hemoprotein other than cytochromes. Shrimps and H$_2$O$_2$-treated beef muscle were, therefore, selected as heme-poor sources to be compared with the heme-rich sources, i.e., beef. Effects of various treatments on these three types of catalyzed linoleate peroxidation may, therefore, shed light on the catalytic systems in these foods.

Meat is more complex and difficult to study than either model or homogenate systems. In homogenate systems, excess of the linoleate substrate was added to the catalysts, and the rate of oxygen uptake, recorded directly during the reaction, was a measure of lipid oxidation. The conditions of oxidation in meat are quite different. The catalysts may not be able to make complete contact with the intramuscular lipid, so that the catalytic rates are different from that in model systems, and the extent of lipid oxidation can not be determined directly, since a number of enzymatic oxygen-consuming reactions occur in meat.

Some lipid oxidation product, rather than oxygen uptake, must be measured. The TBA test which measured the pink color produced by reacting TBA with MA has been widely used for measurement of rancidity in foods. The TBA method may be an unreliable test for MA under test conditions. However, a UV spectrophotometric method developed by Kwon and Watts (23) can be successfully applied to the beef and shrimp from rancid foods. Although its sensitivity is only about 40% of the TBA test, it is more specific.

**METHODS**

In both model and semimodel systems, emulsions of linoleic acid were used as substrates for peroxidation studies by a slight modification of Surrey’s method (24). One gram of linoleic acid was added drop by drop to 20 ml water in which 1 ml Tween 20 was dissolved. The contents were thoroughly mixed to disperse the acid into a fine emulsion. Then, 1 N KOH was added and the mixture once again agitated with a magnetic stirrer until a clear transparent solution was obtained. To this solution, 200 ml of 0.2 M phosphate buffer were added. A few drops of concentrated HCl were used to adjust to the desired pH. The final volume was made up to 400 ml with H$_2$O. The resulting solution contained approximately $9 \times 10^{-3} M$ linoleic acid in 0.1 M phosphate buffer.

To 20 ml of the emulsion were added the catalysts and test solutions desired and the total volume made up to 25 ml in which the concentration of linoleic acid was $7 \times 10^{-3} M$. Changes in oxygen tension were recorded, using the Beckman Oxygen Analyzer 777 calibrated at 160 mm Hg, the partial pressure of oxygen in air. The rates were linear after the first few minutes. The oxygen utilization was then calculated from the recording by measuring the linear slope over a 3 min period and expressing the values as mm Hg pO$_2$/min.

In semimodel systems, homogenates from beef and shrimp were used as the catalysts. Eye of the round (semimendous) beef and shrimp were purchased at local retail markets. Beef was trimmed, ground and mixed thoroughly. Ground beef (50 g) was homogenized for 2 min in a Virtis blender with 100 ml 0.25 M cold sucrose solution, then filtered through four layers of cheese cloth. The filtrate was collected and stored in the refrigerator not longer than 30 min. It was brought to room temperature before using. Shrimp homogenate was prepared in the same way, except that less tissue (10 g) was used to prevent bubble formation.

To separate the non-heme iron, beef homogenate was treated drop by drop with 30% H$_2$O$_2$ until the pigment was decolorized. The treated tissue showed no absorption peaks in the range 400-700 mm when analyzed by reflectance spectrophotometry. One milliliter (1.2% w/v) of the homogenate was added to the linoleic acid emulsion and additives.

All reagents were of standard quality. The water used for the preparation of all solutions was passed through a deionizing column.

In meat study, beef round was used. The meat was trimmed of visible fat, ground and mixed thoroughly. Three milliliters of 0.1% chlorotetracycline-HCl was added to every 100 g meat in order to prevent spoilage by bacteria during the storage period (13). After the addition of various test solutions or water for the control, an amount equivalent to 50 g of meat was placed in a polyethylene bag and stored in the refrigerator at 3 C until analysis.

The extent of lipid oxidation was determined by distilling MA from the meat sample as described by Tarladgis et al. (22). The distillate was then analyzed for MA by UV spectrophotometry (23). To convert to mg MA per 1000 g meat, the UV absorbance was multiplied by the factor 18. The meat to be analyzed by reflectance spectrophotometry for MetMb formation must be quickly stirred to a uniform color before the determination, and the percentage of the pigment present as MetMb was derived from the assumed linearity of the ratio, K/S 572nm/K/S 525nm (25).