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- The nature of reactions leading to production of protein-centered free radicals upon exposure to peroxidizing lipids;
ABSTRACT CONTINUED:

The amino acids residues most susceptible to react under those conditions and the nature of changes occurring in them.

Effects of environmental factors, especially water activity.

Mechanisms of antioxidant action in above systems.
THE VIEW, OPINIONS, AND/OR FINDINGS CONTAINED IN THIS REPORT ARE THOSE OF THE AUTHOR(S) AND SHOULD NOT BE CONSTRUED AS AN OFFICIAL DEPARTMENT OF THE ARMY POSITION, POLICY, OR DECISION, UNLESS SO DESIGNATED BY OTHER DOCUMENTATION.
I. Statement of Problem:

The overall purpose of this study was the elucidation of interactions between proteins and peroxidizing lipids in foods and related systems, including:

- The nature of reactions leading to production of protein-centered free radicals upon exposure to peroxidizing lipids.
- The amino acids residues most susceptible to react under those conditions and the nature of changes occurring in them.
- Effects of environmental factors, especially water activity.
- Mechanisms of antioxidant action in above systems.

II. Summary of Most Important Results:

Results were obtained in the following model systems which represent typical interaction situations between peroxidizing lipids, and proteins in various types of foods (dried, emulsified, and at natural water contents).

1) Lysozyme and peroxidizing methyl linoleate in an aqueous emulsion.

2) Lysozyme and peroxidizing methyl linoleate in a freeze-dried system.

3) Lecithin liposomes in the presence and absence of iron and of proteins.

4) Aqueous dispersion of erythrocyte ghosts.

5) Freeze-dried erythrocyte ghosts.

Detailed results are reported in six research papers, one review paper and one graduate thesis, which are listed in this report. A brief summary of these results is given below:

1) Reported in Funes et al., 1982. The influence of concentration of protein and lipid on peroxidative damage to lysozyme was studied. Lysozyme polymerization and loss of biological activity are promoted by higher protein-lipid concentration and higher degree of lipid unsaturation. Freeze-drying promotes protein polymerization in aqueous emulsions containing lysozyme and peroxidizing methyl fatty acid esters.
and decreases lipid hydroperoxide and malondialdehyde concentrations. These results suggest that reactant concentration induced by freeze-drying promotes hydroperoxide decomposition and facilitates free radical transfer reactions between lipids and proteins. However, freeze-drying does not affect the rate of loss of enzyme activity of the protein.

2) Reported in two papers by Leake and Karel, 1984. Exposure of protein to oxidizing lipid may cause polymerization of the protein. The mechanisms of this crosslinking were investigated in a freeze-dried emulsion of chicken egg lysozyme and methyl linoleate. Covalent oligomers of lysozyme were found in this system by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and this crosslinking was attributed to three different types of reactions: intermolecular disulfide formation, detected by electrophoresis with and without disulfide-breaking reagents; condensation of dicarbonyl breakdown products of the lipid with protein amines, detected by ion exchange chromatography of reduced protein hydrolysates; and other crosslinking reactions including radical-initiated reactions which were studied by gas chromatography-mass spectroscopy. In the samples tested, 2-18% of the crosslinking was due to disulfide formation, 15-40% from Schiff base reactions, and the remaining 40-85% was due to other crosslinking reactions. There was a minimum of three different condensation crosslinks, and at least three but probably more than ten different radical-induced crosslinks.

Exposure of protein to oxidizing lipid induced fluorescence with excitation wavelengths of 350-360 nm and emission wavelengths of 420-430 nm. This fluorescence was studied in freeze-dried emulsions of methyl linoleate with chicken egg lysozyme and other proteins, and in solution with amino acids and several aldehydes. Generation of this fluorescence was found to depend on the reaction of amines with 2,4-decadienal or one of its breakdown products. Evidence was found suggesting that Schiff base formation of protein lysyl amines with conjugated dicarbonyls other than malonaldehyde is responsible for the fluorescence generated in the lysozyme-methyl linoleate system.

3) Reported in Weiss et al., 1983. The effects of the three-dimensional arrangement of the constituents on the interactions of the peroxidizing lipids with proteins were studied by using large oligolamellar vesicles prepared by the reverse-phase technique.
Egg lecithin liposomes with hen-egg lysozyme localized either inside or outside the vesicles were oxidized in the presence of ferric iron. Protein dimerization, TBA values, and permeation of [14C]glucose and of protein; as well as vesicle structure were monitored during oxidation. Lysozyme polymerization, measured by protein dimer appearance, and lipid oxidation (TBA) are not greatly affected by initial protein location. The presence of protein outside the vesicles exerts a slight retardation of oxidation. α-Tocopherol inhibits lecithin oxidation and lysozyme polymerization. Changes in the vesicle structure as shown by electron micrographs and significant protein leakage are caused by vesicle oxidation. The presence of cholesterol increases TBA values without affecting protein polymerization.

4) Reported in Funes and Karel, 1984. The effects of molecular organization on the interaction of peroxidizing lipids with proteins were studied by using human red cell membranes. Intact and chloroform-disrupted membranes were oxidized. Disruption of membranes was achieved by treatment with chloroform and subsequent elimination of the solvent. Lipid oxidation rates and SDS-electrophoretic protein patterns in the two types of membranes were compared. Specific 3H-DIDS label of band 3 allowed the study of the role of intrinsic proteins in the aggregation process. Lipid oxidation rate is not greatly affected by chloroform disruption although disrupted membranes show higher rate of protein aggregation. α-Tocopherol decreases lipid oxidation and protein aggregation rates. Band 3 protein dimers (188,000 M.W.) appear earlier than the higher molecular weight protein aggregates (400,000 M.W.). The results suggest that native membrane organization prevents protein damage upon lipid peroxidation.

5) Reported in Bouzas and Karel, 1984. In this study freeze-dried, human red cell membranes were used as a model system to investigate free radical transfer reactions between peroxidizing lipids and proteins focusing on the effects of lipid protein organization on such interaction.

Oxidation of membrane lipids and oxidation-induced polymerization of membrane proteins were affected by disruption of membrane structure. Oxidation resulted in formation of covalently bound protein polymers. Acetyl choline esterase, an externally oriented enzyme, lost all its activity when treated with chloroform while retaining most of it when
membranes were only freeze-dried or sonicated and freeze-dried. During storage no further changes were observed in activity of this enzyme. Enzymes bound to the inner membrane surface and oriented toward the cytosol showed an entirely different behavior. Upon storage there was a gradual decrease in activity. These protein changes show both oxidative and non-oxidative mechanisms of damage.

III. Publications:

A. List of papers published under the contract #DAAG-29-81-K-0081:


B. List of papers accepted for publication:


C. List of papers submitted for publication:


D. Graduate theses produced:

Thesis Title: Crosslinking and damage in proteins exposed to oxidizing methyl linoleate.

IV. Personnel participating in this project. (All in the Department of Nutrition and Food Science, Massachusetts Institute of Technology):

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Ms. Zahra Nakhost, Research Specialist

Dr. Jorge Funes, Research Associate

Dr. Jorge Bouzas, Visiting Scientist

Mr. Luther Leake, Graduate Student
(received Ph.D. degree in May 1984)