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Biological Antioxidant Protection against Lipid Peroxidation Damage[1]

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VITAMIN E AND SOME OTHER BIOLOGICAL antioxidants and reducing compounds stabilize polyunsaturated lipids and minimize lipid peroxidation damage. Although such relationships are very complex, nonbiological relationships between oxidant-labile olefinic compounds and antioxidant protection systems are well known (1–3) and provide valuable background for biological studies. In vivo lipid peroxidation has been identified as a basic deteriorative reaction in cellular mechanisms of aging processes (4, 5), in some phases of atherosclerosis (6, 7), in chlorinated hydrocarbon hepatotoxicity (8), in ethanol-induced liver injury (9), and in oxygen toxicity (10). For human nutrition the greatest impact may come from increasing knowledge of lipid peroxidation aging processes. These processes may be a universal disease the chemical deteriorative effects of which might be slowed by use of increased amounts of antioxidants (11, 5).

As a biochemical model of these deteriorative reactions, we have studied lipid peroxidation damage to proteins and enzymes. Some of the oxidized lipid–protein reaction products were characterized as protein–protein cross-linked polymers, and the polymerization mechanism was characterized as a free-radical chain polymerization (12). The pattern of damage to proteins induced by peroxidizing lipid is similar to that observed in radiation damage; lipid peroxidation is about one-tenth as damaging as radiation. Among the most labile amino acids in a number of proteins are methionine, histidine, cystine, and lysine (13).

Recent studies (14, 15) of quantitative enzyme inactivation by lipid peroxidation showed that sulfhydryl enzymes are most susceptible to inactivation. Oxidation products of polyunsaturated lipids also inactivate nonsulfhydryl enzymes; ribonuclease A was used as an experimental model. Concomitant with the loss of ribonuclease activity is the appearance of fluorescence in the enzyme-lipid reaction mixture. Inactivated ribonuclease shows fluorescent monomer, dimer, and higher molecular weight species in a gel filtration fractionation. The fluorescence maximum is 470 mμ with excitation maximum at 395 mμ. Ribonuclease, inactivated by malonaldehyde, has fluorescence and a gel filtration pattern similar to those of the enzyme inactivated by polyunsaturated lipids. Malonaldehyde from peroxidizing lipids is probably the reactant for the intra- and intermolecular cross-linking of ribonuclease. The fluorescence produced from the cross-linking is attributed to the conjugated imine structure formed in protein between 2 ϵ-amino groups and malonaldehyde. The structure of the fluorescent chromophore was determined; 1 mole of malonaldehyde reacts with 2 moles of amino compound to yield *N*,*N*′-disubstituted 1-amino-3-iminopropenes. The fluorescent chromophore, R—N=CH—CH=CH—NH—R, develops from the cross-linking reaction of malonaldehyde with many biologically important amines including RNA, DNA, and phospholipids.

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The American Journal of Clinical Nutrition

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Fluorescent products form in many biological systems, including mitochondria, microsomes, and lysosomes, when they undergo lipid peroxidation in vitro. The chromophore may be identified qualitatively as molecular damage in lipofuscin, which accumulates in tissues, especially the brain and heart of animals, as a function of age (16). Age pigments have the same characteristic fluorescence spectra as those found in model systems, with a fluorescence maximum at 470 $m\mu$ when excited at 365 $m\mu$.

Present knowledge indicates that lipid peroxidation aging processes would develop in a time sequence of damaging reactions as follows:

Polyunsaturated lipid peroxidation causes molecular damage through its free-radical intermediates. Free-radical damage, analogous to radiation effects, includes reactions such as cross-linking of body proteins. Membranes and subcellular organelles are structurally damaged. Digestion of these disintegrating cell parts would be the normal role of the cell's lysosomes, which contain all necessary hydrolytic enzymes. The damaged cell parts are not completely hydrolyzed, and the congested lysosomes accumulate and become lipofuscin age pigments.

As reactions that produce the molecular damage of age pigments have been identified, and as sensitive fluorescent measurements of this aging reaction are now possible, research on antioxidant nutrients as possible inhibitors should accelerate.

Vitamin E, the major biological antioxidant, shows complex relationships with other antioxidants and reducing compounds (17). These relationships are appropriately described in the classification of the following reactions: The major biological function of vitamin E may be described as radical chain breaking in its inhibition of lipid peroxidation. Small amounts of ubiquinol can, as an ancillary function, react as a chain-breaking lipid antioxidant in a synergistic relationship with vitamin E. Vitamin C can act as a synergist for vitamin E. Small amounts of sulfhydryl compounds—mainly glutathione, sulfhydryl proteins, and cysteine—apparently react as free radical scavengers and peroxide decomposers as do small amounts of methionine as well as selenoamino acids, which are powerful catalysts of sulfhydryl-disulfide exchange (18).

The reactions of vitamin E and several biological antioxidants with lipid hydroperoxyl radicals were explored (19–21). In reactions of methyl linoleate hydroperoxides at 37 C, the antioxidants and their relative rates were: ubiquinol-6, 4.5; α-tocopherol hydroquinone, 2.9; ubichromenol-6, 1.1; ubichromenol-10, 1.0; and α-tocopherol, 1.0. Concentrations of ubiquinol, α-tocopherol, and polyunsaturated fatty acids present in the mitochondrial membrane are in the range for reasonable protective influence by biological antioxidants. Other research (22) indicated that small amounts of ubiquinol may function as an antioxidant in the mitochondrion. These reactions of ubiquinols and related compounds have been found effective in the relief of certain vitamin E-deficiency syndromes in some species (23).

Maximum protection against oxidant and lipid peroxidation damage is to be achieved by optimum concentrations of biological antioxidants, most of which are dietary components. To acquire knowledge of the integrated qualitative and quantitative functions of the biological antioxidants is a major challenge of future research.

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