Relative Susceptibility of Microsomes from Lung, Heart, Liver, Kidney, Brain and Testes to Lipid Peroxidation: Correlation with Vitamin E Content

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ABSTRACT

Rates of in vitro lipid peroxidation of microsomes and homogenates were found to vary widely among different tissues and species. In rats and rabbits, lung microsomes peroxidized at a 25- to 50-fold lower rate than liver, kidney, testes and brain microsomes. Heart microsomes peroxidized at a rate slightly greater than, but most similar to, lung microsomes. Comparison of tissue homogenates also revealed the unique resistance of lung and heart to lipid peroxidation. The ratio of vitamin E to peroxidizable polyunsaturated fatty acids in lung and heart microsomes was several-fold higher than in microsomes from the other tissues studied, which accounted for the relative resistance of lung and heart to lipid peroxidation. Liposomes of extracted rat lung microsomal lipid were also resistant to peroxidation and the amount of vitamin E contained in the lung lipid extract was sufficient to confer the same degree of resistance when incorporated into an equivalent amount of rat liver lipid. Higher rates of peroxidation in mouse lung microsomes relative to rabbit, rat and human lung microsomes were similarly correlated with a lower ratio of vitamin E to peroxidizable fatty acids in mouse lung microsomes. These data provide strong support for the role of vitamin E as the major cellular antioxidant, especially in the highly oxygenated tissues of heart and lung, and demonstrate the utility of the microsomal system in characterizing tissue differences in susceptibility to peroxidative membrane decomposition.

INTRODUCTION

Intracellular lipid peroxidation has been often hypothesized as a mechanism of action of toxic agents (1) and has been implicated as a degenerative mechanism underlying cellular aging (2) and certain disease states (3). Liver microsomes or reconstituted systems containing extracted liver lipid plus purified liver microsomal NADPH-cytochrome P-450 reductase have been most frequently employed in investigations of the mechanism of peroxidation of the fatty acyl moieties of membrane phospholipids (4,5). Little information is available, however, regarding the relative susceptibility of membranes from various tissues to undergo lipid peroxidation. The peroxidizability of lung is of particular interest since it is the point of entry for oxidant gases contaminating the atmosphere. Roback observed that over 50 times more thiobarbituric acid-reactive material was produced during incubation of liver homogenate than during incubation of lung or spleen homogenates (6). Willis and Recknagel recently reported lung microsomes were only 4% as active as liver microsomes in producing malondialdehyde, although the basis for the low activity of lung microsomes was not identified in that study (7).

The biological antioxidant, vitamin E, has been shown to afford protection against the injurious effects of nitrogen dioxide and ozone on experimental animals (8). In addition, numerous studies have indicated susceptibility to lipid peroxidation is greatly influenced by tissue levels of vitamin E (9). Bieri and Anderson (10) demonstrated that the ability of tissue homogenates to undergo lipid peroxidation in vitro was inversely related to the dietary vitamin E status of the animal. Similarly, other investigators have shown that liver microsomes (11-13) or mitochondria (14) isolated from vitamin E-deficient animals peroxidize at a faster rate in vitro than fractions from control animals. Peroxidation of liver microsomes was inhibited by dietary supplement of vitamin E (12,13) or when vitamin E was added directly to liver microsomal suspensions (11,15), which support the role of vitamin E as a membranous antioxidant.

Taylor et al. (16) have shown there is a high degree of variation in the content of vitamin E in subcellular fractions from different tissues, which indicates there may be considerable differences among tissues with respect to protection against peroxidative reactions. In these investigations, we have measured rates of in vitro lipid peroxidation in microsomes and homogenates from several different tissues and have compared the variation in these rates with the microsomal vitamin E content. Microsomes and homogenates from lung and heart showed a low rate of in vitro peroxidation compared to
the other tissues studied. This resistance of lung and heart to lipid peroxidation can be explained by relatively high levels of microsomal vitamin E in these tissues.

EXPERIMENTAL PROCEDURE

Materials

Distilled water was filtered and deionized in a system custom designed by W.E. Chaffee Co., Inc., an affiliate of Continental Water of Buffalo, NY. NADPH, ascorbic acid, FeSO₄, EDTA, cytochrome c and D,L-α-tocopherol were obtained from Sigma Chemical Company, St. Louis, MO. Thiobarbituric acid was a product of Eastman Chemicals, Rochester, NY. Fatty acid methyl ester standards were purchased from Nu-Chek-Prep, Elysian, MN. Rats were male Long Evans (200-300 g), mice were male BALB/c (20-30 g) and rabbits were male New Zealand albino (2-3 kg).

Methods

All animals received Agway laboratory chow (Rochester, NY) ad libitum until the time of sacrifice. Animals were sacrificed by intraperitoneal (ip) injection of sodium pentobarbital, the chest was opened and a cannula was inserted into the trachea. Heart, lungs and liver were perfused by injecting ice-cold 0.15 M NaCl into the right ventricle of the heart while simultaneously ventilating the lungs through the tracheal cannula. Lungs were excised and the parenchyma was separated from the visible bronchi and blood vessels and minced thoroughly with scissors. Hearts, livers, kidneys, brains and testes were removed and similarly minced. The minced tissues were washed several times in ice-cold buffered potassium chloride (0.15 M KCl, 5 mM Tris-maleate, pH 7.4) containing 1 mM EDTA and homogenized in the same, using a teflon-glass homogenizer. The homogenate was centrifuged successively at 300, 1600, 8000 and 30,000 x g for 10 min at each speed and the pellets discarded. The “cell-free homogenate” refers to the supernatant after centrifugation at 300 x g. Microsomes were obtained from the 30,000 x g supernatant by centrifugation at 100,000 x g for 1 hr. The microsomal pellet was washed by suspension in and resedimentation from buffered potassium chloride with no EDTA, resuspended in the same type of solution and stored in liquid nitrogen. Lipid peroxidation in these EDTA-treated microsomes has been shown to be absolutely dependent on the concentration of added free ferrous iron (17). Protein concentrations were determined by the Lowry method (18); NADPH-cytochrome c reductase was assayed spectrophotometrically, as described by Williams and Kamin (19) and the initial rates measured were linearly proportional to time of incubation and amount of protein added over the ranges used.

Lipid peroxidation was measured by quantitation of malondialdehyde formed during the incubations. Microsomes (50-80 µg of protein) were incubated at 37 C for the specified lengths of time with 40 mM Tris-maleate buffer (pH 7.4) and either 3.0 µM FeSO₄ plus 250 µM NADPH or 1.0 µM FeSO₄ plus 500 µM ascorbate in a total volume of 0.5 ml. Peroxidation was terminated by rapid addition of 20% trichloroacetic acid (0.15 ml), 0.05 M thiobarbituric acid (0.3 ml) and 0.2% butylated hydroxytoluene (50 µl). Bovine serum albumin (0.5 mg) was added to facilitate precipitation of protein during a 10 min centrifugation; the resulting clear supernatant was removed and delivered to glass test tubes which were then tightly capped and boiled for 8 min. The amount of colored product was measured spectrophotometrically as described by Buege and Aust (4).

Lipid peroxidation was expressed in terms of nmol malondialdehyde (MDA)/mg protein or as “percent peroxidation” which is simply the percentage of maximal MDA which would be produced by complete peroxidation of the peroxidizable polyunsaturated fatty acids (PUFA) present in the tissue fractions. PUFA includes all of the detectable polyunsaturated fatty acids except linoleic acid (18:2), which we have demonstrated, in agreement with others (20,21), to be relatively resistant to peroxidation and is not believed to evolve malondialdehyde (22). Maximal MDA formation per mg of protein was routinely determined for each liver microsomal preparation by incubating with a sufficient amount of FeSO₄ and ascorbate over time until MDA formation reached a maximal value. We have previously shown that all of the liver microsomal PUFA has reacted at this point and that the percentage of the maximal MDA formation obtained during a given incubation closely correlates with the percentage depletion of peroxidizable lipid substrate (17).

In separate experiments, the yield of MDA/mol PUFA was found to be similar for all tissues studied. Thus, in these studies, the determination of the maximal MDA formation in lung, heart, kidney, brain and testes microsomes was obtained simply by multiplying the value for the yield of MDA/mol PUFA from liver microsomes by the PUFA content of the other tissues as determined by phospholipid (23) and fatty acid analysis by gas liquid chromatography (GLC) (24). The amount of