Autoxidation of Fatty Acid Monolayers Adsorbed on Silica Gel. IV. Effects of Antioxidants

GUEY-SHUANG WU, ROBERT A. STEIN, and JAMES F. MEAD, Laboratory of Nuclear Medicine and Radiation Biology, 900 Veteran Avenue, University of California, Los Angeles, California 90024, and Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, California 90024

ABSTRACT

Inclusion of α- or γ-tocopherol in linoleic acid monolayers deposited on a silica gel surface introduced a definite induction period before the initiation of rapid autoxidation, as measured by the disappearance of linoleic acid. The length of the induction period was found to be proportional to the amounts of tocopherol incorporated at the concentration range tested, and the protection was generally efficient with little loss of linoleic acid to autoxidation. At the onset of the rapid autoxidation, approximately 12% of the tocopherol remained, and most of this was destroyed after an additional short period. γ-Tocopherol, at the same concentration, was found to be 1.4 times as effective as the α-isomer. 3-(ω-Carboxynonyl)-4-methoxy-5-pentylphenol, the synthesis of which is described here, did not introduce a detectable induction period, but rather reduced the overall rate of autoxidation throughout a very long period.

INTRODUCTION

Studies of membrane-related phenomena, including peroxidation, are often hampered by the complexity of the living systems used. To overcome this difficulty, artificial mono- and bilayers are frequently used as admittedly oversimplified models for such systems but aid in rational approaches to their understanding.

Several aspects of membrane autoxidation have recently been studied using lipid monolayers adsorbed on a silica gel surface. The extent of the surface coverage has been correlated to the rate of autoxidation and the combined effects of tocopherol, metals and acid synergists have been examined (3). The effective adsorption sites on the silica gel were shown to be the isolated, nonhydrogen-bonded hydroxyl groups on the surface (4). The autoxidation of linoleic acid monolayers has been found to be entirely different from that of bulk phase both in the rate and the mechanism of the major reaction (5,6). Other important membrane constituents, such as saturated fatty acids and cholesterol, have been incorporated into the linoleic acid monolayers, and their effects on the rates and products of autoxidation have been investigated (7).

The effects of antioxidants on monolayer autoxidation have also been investigated by others (6,8,9). By measuring the disappearance of oxygen in the headspace, Porter et al. showed that autoxidation of silica gel-supported linoleic acid monolayers containing 0.05 mole % of α-tocopherol was preceded by an induction period of ca. 80 min, which could be lengthened by the removal of iron from the silica gel (3).

As part of our continuing effort to clarify various important aspects of membrane autoxidation by using the unsaturated fatty acid-silica gel system, we studied the effect of α- and γ-tocopherol as well as a synthetic antioxidant, 3-(ω-carboxynonyl)-4-methoxy-5-pentylphenol, on the rate of autoxidation of linoleic acid monolayers. The relationship between the length of the induction period and the concentration of the antioxidants was established, and the disappearance of the tocopherols during the induction period was measured. The results and our interpretations are presented in this paper.

EXPERIMENTAL PROCEDURES

Materials and Methods

Linoleic and palmitic acids (99+% pure), purchased from Applied Science Laboratories, Inc., were used directly after a purity check with thin layer chromatography (TLC) and gas liquid chromatography (GLC). Both d-α-tocopherol (Eastman Organic Chemicals) and dl-γ-tocopherol (a gift from Dr. H.S. Olcott, University of California, Davis) were found to be pure on TLC and gave UV absorption maxima at 292 nm (ε 3,430) and 297 nm (ε 4,570), respectively. Bathophenanthroline purchased from Sigma Chemical Company and analytical grade ferric chloride from Mallinckrodt Chemical Works were used directly. 3-(ω-Carboxynonyl)-4-methoxy-5-pentylphenol was synthesized in this laboratory (see below). The pertinent physical characteristics of the Silica Gel G used have been reported (4).

GLC was carried out using either a Varian
Aerograph Model 2100 coupled with an electronic integrator (Infotronics Corp., Model CRS-11 HBS) or a Hewlett Packard Gas Chromatograph Model 5830 A. The GLC columns used were an 0.20 x 183 cm glass U-tube containing 3% OV-101 on 100/120 mesh Gas Chrom Q, and an 0.20 x 300 cm metal coiled column packed with 10% Silar 10 C on 100/120 mesh Gas Chrom Q. TLC was carried out using precoated Silica Gel G plates (0.25 mm thick, Analtech, Inc.) with the solvent system — petroleum ether/diethyl ether/acetic acid, 80:20:1 — and precoated alumina sheets (Brinkmann Instruments, Inc). with the solvent system — benzene/diethyl ether, 50:50. Visualization of spots in both cases was carried out by spraying with 3% cupric acetate solution in 8.5% phosphoric acid with subsequent charring at 140 C. Ultraviolet and visible spectra were recorded using 1 cm path quartz cells in a Cary Model 14 Spectrophotometer. Elemental analysis was performed at Elek Microanalytical Laboratories, Torrance, CA.

Preparation and Autoxidation of Tocopherol-Containing Monolayers

A detailed description of our preparation and autoxidation of monolayers has been published (5). In a typical preparation, a solution of 0.522 g (1.86 mmole) of linoleic acid and 5 ml of α-tocopherol stock solution (1.35 x 10^{-4}M in hexane) in a total of 44 ml of hexane was stirred under nitrogen at room temperature with 2.013 g of Silica Gel G for 1 hr. After the adsorption, the supernatant was withdrawn and freed from solvent to give 0.141 g of linoleic acid and 58.2 μg of α-tocopherol. The amount of α-tocopherol remaining in the supernatant was determined by the bathophenanthroline-ferric chloride color reaction (see below). It was noted that α-tocopherol was adsorbed preferentially from the hexane solution. The ratio of α-tocopherol to linoleic acid in the initial solution before adsorption was 0.036 mole % and the ratio adsorbed was 0.040 mole %. The dry, coated silica, ca. 0.220 g portions, were incubated at 60 C for the desired length of time and then extracted with methanol. The unchanged acid was methylated and quantitated by GLC as previously described. The α-tocopherol in the control and incubated samples was extracted with acetone and assayed by the bathophenanthroline-ferric chloride reaction.

Monolayers coated with α-tocopherol alone were prepared using the same proportions as in the above run but omitting the linoleic acid.

For the preparation of linoleic acid-palmitic acid-α-tocopherol monolayers, the procedure previously described was adapted (7), but α-tocopherol was included in the coating solution to give an adsorbed concentration close to 0.04 mole % of the linoleic acid.

The γ-tocopherol-linoleic acid monolayers were prepared in a manner similar to that for the α-tocopherol-containing monolayers. The partition coefficient for γ-tocopherol between silica gel and hexane was also slightly higher than for linoleic acid, so that from a solution of 0.018 mole % of γ-tocopherol, the final adsorbed concentration was 0.020 mole %.

Determination of Tocopherols Using Bathophenanthroline

The method used is basically that of Emmerie and Engel except that bathophenanthroline (4,7-diphenyl-1,10-phenanthroline) was used in place of 2,2'-bipyridine to improve the sensitivity (10). The tocopherol-containing solution was usually freed from solvent and was made up to 3 ml with ethanol. To this solution, 0.5 ml of bathophenanthroline (0.09% in ethanol) and 0.5 ml of ferric chloride (0.03% in ethanol) were added. The absorbance of the solution at 534 nm (ε 42,950) was measured with a Cary Model 14 spectrophotometer.

Preparation of 3-(ω-Carboxynonyl)-4-Methoxy-5-Pentylphenol

To a solution of 10 g (26 mmole) 2-(ω-carboxynonyl)-6-pentyl-4-nitrophenol (I) (11) in 50 ml of anhydrous acetic acid was added 11.0 g (80 mmole) potassium carbonate and 9.5 g (75 mmole) dimethyl sulfate dissolved in 25 ml acetic acid. The reaction vessel was fitted with a reflux condenser protected by a drying tube and a magnetic stirrer. The mixture was refluxed and stirred for 10 hr. At the conclusion of the reaction, the product was poured into 200 ml water and extracted with ether, and the extract washed well with water. The ether was evaporated, and the residue was dissolved in 150 ml methanol and then mixed with 8 g (0.2 mole) sodium hydroxide in 50 ml water. The mixture was warmed until complete solution was achieved and then allowed to stand at room temperature over night. A solution of 50 ml concentrated hydrochloric acid in 200 ml water was added to the alkaline mixture. The oil which separated was extracted with ether, the extract washed well with water and dried over MgSO₄. Removal of the ether left an oil that...