Analysis of Linoleic Acid Peroxidation Products by GC/MS

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Proposed Scheme for Linoleic Acid Oxidation

Abstract

This project utilized gas chromatography – mass spectrometry (GC-MS) to analyze the lipid peroxidation products of linoleic acid, an essential ω-6 fatty acid, and its reaction with oxygen. Optimum reaction conditions were identified by testing various combinations of variables: buffer, pH, oxidant, and time. With treatment of optimum conditions, several products were identified including, 2,4-decadienal, 4-hydroxy-2-nonenal (HNE), and 4-oxo-2-nonenal (ONE). The mechanisms behind the formation of these products have been proposed by several researchers. It was determined that varying oxidant concentration, pH, or time greatly impacted the obtained concentrations of final oxidation products. Additionally, beta-carotene and Vitamin E were tested as antioxidant assays to assess their effectiveness in preventing oxidation. Tested assays were successful, with beta-carotene reducing the concentration of oxidation product the most.

Background

A lipid, or fatty acid, is one of the main components of the cell membrane. Lipid peroxidation is a process in which free radicals such as the hydroxyl radical (HO·) or the dicalcium of oxygen gas (O₂) abstracts a hydrogen atom from a lipid. Lipid peroxidation processes and their importance and consequences in biological systems have been reviewed. When peroxidation occurs, the lipid breaks down into smaller molecules that can lead to cell death and has been implicated in a variety of inflammatory diseases and cancers, including Alzheimer’s disease and atherosclerosis.

A previous study has shown that the linoleic acid peroxidation products can be easily formed using Fenton chemistry, where the lipid is reacted with the hydroxyl radicals (HO·) that are produced by Fe²⁺ ions and hydrogen peroxide (H₂O₂). These products were monitored and quantified using liquid chromatography / mass spectrometry (LC/MS). Based on this study, we have developed a protocol to perform Fenton oxidation of linoleic acid and analyze the oxidation products by GC/MS, using methanol chemical ionization.

To demonstrate the effect of antioxidants, β-carotene and Vitamin E (alpha-tocopherol) were added to the reaction. These antioxidants are expected to decrease the amount of oxidation products produced.

A series of lipid oxidation reactions and control experiments were performed. The resulting products were analyzed by GC/MS to determine the concentration of products. Authentic samples of 2,4-decadienal, HNE, and ONE were used to validate the GC/MS assignments of the peroxidation products. Two internal standards were used to normalize the concentration of the products after extraction and the second, a Biphenyl solution, was used as an internal GC/MS standard.

Materials

Materials: 4-Hydroxy-2-nonenal (HNE), 4-oxo-2-nonenal (ONE), 13 (5) HPDE, and 15 (8) HPDE were purchased from Sigma Aldrich, (An Arbor, MI). 2,4-Decadienal, Linoleic acid were purchased from Sigma Aldrich, and Vitamin E was purchased from Aldrich.

Methods

100 mM MOPS Buffer: 5.63g/l dissolved for final volume of 250mL metal-ion free water and addition of 1.366g NaCl.
193mM Linoleic Acid: 60mg of Linoleic Acid in 1 mL Hexanes
7.2 mM Ferrous Ammonium Sulfate Hexahydrate: 0.1990 g Ferrous Sulfate Heptahydrate for final volume of 100 mL metal-ion free water.
1000µg Vitamin E: 0.4303 g in hexanes to a final volume of 1 mL, 1000µg β-carotene: 0.0495 g in hexanes to a final volume of 1 mL.
64.835 mM Biphenyl Standard: 0.1000g biphenyl in hexanes to a final volume of 10 mL.
78.021 mM Naphthalene Standard: 0.1000g biphenyl in hexanes to a final volume of 10 mL.

GC Method

Samples were analyzed on a Varian VMS-5 column; 1 µL sample injected; injection temp 250° C; flow rate 1 mL He / min; column program 40° C for 2 min, heat to 140° C at 10 °C/min, heat to 260° C at 20° C/min, hold 3 min, total 21 min per analysis; ion trap MS parameters – chemical ionization using methanol from 4 to 17 min; electron impact from 17 to 21 minutes.

Data Analysis

All reactant and product retention times and mass spectra were authenticated using standards. An automated analysis method was used to integrate the resulting GC chromatograms. After integration of all peaks in the chromatogram, the integration data for all reactants and products were normalized using the naphthalene internal standard, followed by normalization using the naphthalene to correct for loss of product(s) in the extraction process.

Results

Oxidation products in MOPS pH 5 with Fe²⁺ oxidant

Conclusions

• The most easily detected oxidation product is 2,4-decadienal
• More acidic buffer conditions led to more oxidation
• Iron (II) mediated oxidation resulted in higher concentrations of peroxidation products
• β-carotene was not an effective antioxidant
• Vitamin E was an effective antioxidant at higher concentrations

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References