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Lipid Peroxides

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Introduction

Lipid peroxides are major primary products of lipid peroxidation. Measurement of lipid peroxides in *in vitro* experiments or biological samples is often required to assess the extent both of oxidative damage and of changes in antioxidant activity. Various methods have been proposed and used, but each method has both merits and demerits. There still is no single method which is specific, quantitative and sufficiently accurate that can be applied widely.

Numerous kinds of lipid peroxide can be formed during lipid peroxidation and these subsequently undergo secondary reactions. Hence, measurement of lipid peroxides might not always reflect the extent of lipid peroxidation. There are, furthermore, several types of lipid peroxide, e.g. hydroperoxides, dialkyl-peroxides and cyclic peroxides, and it is important to appreciate what is measured by the method employed.

Iodometric method

Introduction

This method is based on the capacity of a wide range of hydroperoxides to react with iodide to liberate iodine. Its advantage is that the reaction is quantitative, exactly stoichiometric, rapid, and reproducible; a disadvantage is that the sensitivity is often not high enough for biological samples. 1n the presence of excess iodide, iodine gives triiodide 1_3^-, which can be measured by titration with thiosulfate, or spectrophotometrically or ftuorometrically [1].

Protocol

1. Prepare methanol-acetic acid (2:1 vJv) containing KI (6% wJv) and EDTA (1 mM) and deoxygenate with a slow stream of inert gas (nitrogen or argon).
2. Place KI solution (2 mL) in a cuvette and purge for 5 min with either nitrogen or argon.
3. Introduce a sample (up to 100 µl) containing hydroperoxide (between 1 and 100 nmol) into the cuvette and leave to react with the KI.
4. Record the absorption of the solution at 358 nm until any further change is small.
5. Calculate the hydroperoxide concentration using the molar extinction coefficient $\varepsilon = 2.97\times10^4\ M^{-1}\ cm^{-1}$ at 358 nm.

Comments

The measurement is performed in an anaerobic cuvette with continuous monitoring at 358 nm in a UV spectrophotometer. Any cuvette which can be made anaerobic can be used.

TBA assay

Introduction

This TBA method is based on the acid-catalysed decomposition of lipid hydroperoxides to malondialdehyde (MDA), which reacts with thiobarbituric acid (TBA), to form a red chromogen. This is quite a sensitive method, but its major disadvantage is that different lipid hydroperoxides produce MDA in different yields and some potentially significant lipid hydroperoxides, e.g. cholesterol hydroperoxide, are not detected by this method.

Protocol

1. Place TBA reagent (2 m l) and sample (1 ml) in a tube (10 mL) with a cap.
2. Add a solution of BHT in ethanol (1% v/v, 50 mM; final BHT concentration 500µM).
3. Heat at 100°C in a water bath for 15 min.
4. Cool in ice-cold water.
5. Centrifuge at 3000 rpm for 10 min.
6. Measure the absorbance of supernatant at 535 nm.
7. Calculate the hydroperoxide concentration using the molar absorption coefficient $\varepsilon = 1.56\times0^5\ M^{-1}\ cm^{-1}$ at 535 nm.

Comment

It should be appreciated that different hydroperoxides and aldehydes give different yields of TBA-reactive substances (TBARS), making this method less quantitative.

High-performance liquid chromatography (HPLC)

Introduction

The major advantage of this HPLC method is that, when applied appropriately, different classes of lipid hydroperoxide (for example, hydroperoxides of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cardiolipin, and free fatty acids) can be analysed and quantified separately with high sensitivity. Cholesterol ester hydroperoxide, which has different fatty acid moieties and oxidation products from free cholesterol, can be separately measured. Furthermore, regio, stereo, and even optical isomers can be analysed after reduction and transesterification. The separated hydroperoxides can be measured by chemiluminescence [2,3], fluorescence [4], and by electrochemical detection. HPLC with post-column detection with diphenyl-l-pyrenylphosphine as a fluorescence reagent [4] is probably the most sensitive method.

The post-column HPLC-chemiluminescence method is described briefly. After separation by HPLC, lipid hydroperoxides are

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decomposed by metal ion-containing enzymes such as microperoxidase and cytochrome-c and the resulting lipid alkoxyl radical reacts with isoluminol or luminol, resulting in emission of light. A schematic diagram of the HPLC-chemiluminescence system is shown in Figure 1.

Protocol

1. Dissolve microperoxidase (250 mg) in water (30 mL) and add methanol (70 mL). This can be stored as a stock solution in an ice box.
2. Dissolve isoluminol (177.2 mg) in a mixture of borate buffer (pH 10.0, 100 mM, 500 mL) and methanol (500 mL) and add microperoxidase solution (2 mL).
3. Place plasma (0.5 mL) in a test tube with a cap.
4. Add methanol (2 mL) and mix with vortex mixer,
5. Add hexane (10 mL) and shake the test tube vigorously.
6. Centrifuge at 1,000 g for 10 min.
7. Take the upper hexane layer (9 mL) and evaporate to dryness by rotary evaporation or under a gas flow.
8. Add methanol-tert-butyl alcohol (1:1 v/v, 0.45 mL) and mix with a vortex mixer.
9. Analyse 50 µL by HPLC-chemiluminescence. Column: 4.6 mm i.d. × 250 mm octylsilyl-LC-8; mobile phase: 95:5 (v/v) methanol-tert-butyl alcohol; flow rate 1.0 mL min^{-1}; flow rate of chemiluminescence reagent: 1.5 mL min^{-1}.

Comments

This HPLC-chemiluminescence method is not specific to hydroperoxides. Ubiquinol gives a positive peak and tocopherols give negative peaks. These antioxidants should be separated from the lipid hydroperoxides on the HPLC column.

An inherent drawback of the HPLC-chemiluminescence method is that only a portion of the light emitted is actuaIIy measured; this depends on the type of hydroperoxide, the reaction conditions, and the apparatus used [5]. A standard calibration curve should, therefore, be prepared for each hydroperoxide under the analytical conditions employed.

F2-Isoprostanes

Introduction

A series of prostaglandin F2-1ike compounds termed F2-isoprostanes is produced *in vivo* in man by non-cycJooxygenase free-radical-mediated peroxidation of arachidonic acid. It has been suggested that quantitation of F2-isoprostanes in plasma and urine is a reliable and useful non-invasive approach for assessing lipid peroxidation and oxidative stress *in vivo* [6,7]. Both free F2-isoprostanes and isoprostanes esterifd to phospholipids are present *in vivo*. The esterified isoprostanes should first be extracted and then subjected to alkaline hydrolysis to release the free form. The protocol for analysis of urine is given below.

Protocol

1. Acidify urine (1 mL) to pH 3 with HCI (1 M).
2. Add [2H4]$PGF_{2\alpha}$(0.2-1µg) as internal standard.
3. Perform solid-phase extraction on C_{18} and silica Sep-Pak cartridges.
4. Perform TLC of F2-isoprostanes as free acids.
5. Convert F2-isoprostanes to pentafluorobenzyl esters by treatment with a mixture of pentafluorobenzyl bromide and *N,N*-diisopropylethyl.amine.
6. Convert to trimethylsilyl (TMS) ether derivatives.
7. Perform quantitative analysis by gas chromatography-negative ion chemical ionization mass spectrometry (GC-NICIMS).

Comment

F2-isoprostanes can be generated when samples containing arachidonate are left standing at room temperature or even during storage at -20°C. Samples should, therefore, either be processed immediately or stored at -70°C.

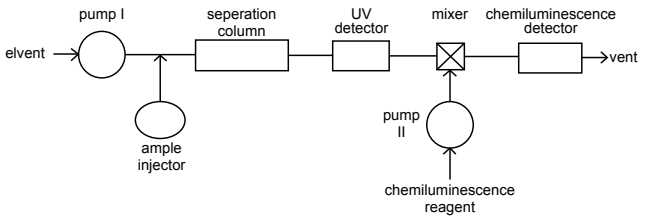

Figure 1: The HPLC-chemiluminescence system.

References

1. Jessup W, Dean RT, Gebicki JM (1994) Iodometric determination of hydroperoxides in lipids and proteins. Methods Enzymol 233: 289-303.
2. Yamamoto Y (1994) Chemiluminescence-based high-performance liquid chromatography assay of lipid hydroperoxides. Methods Enzymol 233: 319-324.
3. Miyazawa T, Fujimoto K, Suzuki T, Yasuda K (1994) Determination of phospholipid hydroperoxides using luminol chemiluminescence--high-performance liquid chromatography. Methods Enzymol 233: 324-332.
4. Meguro H, Akasaka K, Ohrui H (1990) Determination of hydroperoxides with fluorometric reagent diphenyl-1-pyrenylphosphine. Methods Enzymol 186: 157-161.
5. Noguchi N, Niki E (1995) Dynamics of free radical formation from the reaction of peroxides with haemproteins as studied by stopped-flow chemiluminescence. Free Radic Res 23: 329-338.
6. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, et al. (1990) A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A 87: 9383-9387.
7. Morrow JD, Roberts LJ 2nd (1994) Mass spectrometry of prostanoids: F2-isoprostanes produced by non-cyclooxygenase free radical-catalyzed mechanism. Methods Enzymol 233: 163-174.