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HPLC Analysis of Phospholipids and their Hydroperoxides with Chemiluminescence Detection

Hideharu Shintani*

Chuo University, School of Science, 1-13-27, Kasuga Bunkyo 112-0003 Tokyo, Japan

Introduction

Biomembranes contain phosphatidylcholine, phosphatidylethanolamine, phos.phatidylserine, and phosphatidylinositol as major phospholipids. Here we describe the separation of these phospholipids by HPLC, and the simultaneous detection of their primary oxidation products, hydroperoxides, by means of hydroperoxide-specific chemiluminescence detection [1-9].

Protocol

- Prepare tissue homogenate. For example, homogenize minced rat liver (0.5 g) in Chelex^{R} 100-treated Tris-HCI (5 mM, pH 7.4, 5 ml) containing sucrose (0.25 M) by means of a Teflon^{R} homogenizer.
- Discard solids after centrifugation at 3000 rpm for 10 min.
- Extract oxidized and unoxidized phospholipids with 2 vol. chloroform.methanol (2:1, v/v) containing 2,6-di-tert-butyl-4-methylphenol (100 µM; to prevent oxidation).
- Centrifuge at 12,000 rpm for 3 min.
- Analyse aliquots (20 µL) of the organic phase by HPLC equipped with UV and chemiluminescence detection. Column: 250 mm × 4.6 mm i.d., 5 µm, aminopropylsilyl (Supelco) with a 20 mm × 4.6 mm i.d., 5 µm, silica gel guard column (Supelco): Mobile phase: 6:3:1 (v/v) methanol.tert-butyl alcohol-aqueous monobasic sodium phosphate (40 mM) at a flow rate of 1.0 ml/min. Chemiluminescence reagent: aqueous borate buffer (100 mM; 38.14 g sodium tetraborate decahydrate/L) is prepared, and the pH is adjusted to 10 with sodium hydroxide. lsoluminol (177.2 mg, final concentration 1 mM) is dissolved in methanol (500 mL) and borate buffer (500 ml), and microperoxidase (5 mg) is added. The flow rate for the chemiluminescence reagent is 1.5 ml/min. The UV detector is operated at 210 nm.

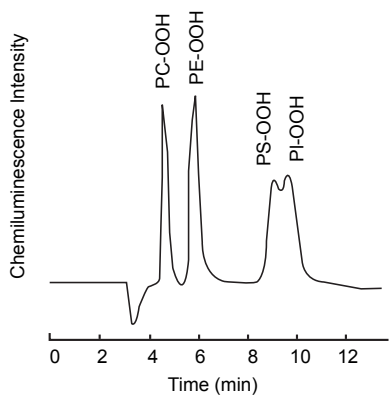

Figure 1: Typical chromatogram of the hydroperoxides detected by chemiluminescence.

- Analyse aliquots (10 µL) of methanolic phosphatidylcholine hydroperoxide (PC-OOH: standard, 10 µM).

Results and Calculations

Figure 1 depicts the separation and detection of 10 pmol each of PC-OOH, phosphatidylethanolamine hydroperoxide (PE-OOH), phosphatidylserine hydroperoxide (PS-OOH), and phosphatidylinositol hydroperoxide (PI-OOH) [3].

The concentration (µM) of hydroperoxides in the homogenate can be calculated by use of the equation:

[hydroperoxide] = [(100/20 × 3 × 0.46) × peak area for hydroperoxide] (peak area for standard)

because the organic and aqueous phases are present in the ratio 46:54 (v!v), respectively.

Discussion

- Levels of phospholipids can be estimated from the absorption at 210 nm.
- PC-OOH and PE-OOH were the major oxidation products of rat liver homogenate [3].
- PC-OOH and PE-OOH can be baseline separated. However the separation of PS-OOH and PI-OOH was not satisfactory and in this status determination cannot be attained. In that meaning further HPLC analytical condition must be studied by changing constituent of mobile phase, selection of column, choice of gradient elution and/or use of solid phase extraction and so on.

References

1. Yamamoto Y, Brodsky MH, Baker JC, Ames BN (1987) Detection and characterization of lipid hydroperoxides at picomole levels by high-perform. ance liquid chromatography. Anal Biochem 160: 7-13.
2. Yamamoto Y (1994) Chemiluminescence-based high-performance liquid chroma.tography assay of lipid hydroperoxides. Methods Enzymol 233: 319-324.
3. Kambayashi Y, Yamashita S, Niki E, Yamamoto Y (1997) Oxidation of rat liver phospholipids: Comparison of pathways in homogeneous solution, in liposomal suspension and in whole tissue homogenates. J Biochem 121: 425-431.

***Corresponding author:** Hideharu Shintani, Chuo University, School of Science, 1-13-27, Kasuga Bunkyo 112-0003 Tokyo, Japan, Tel: +81425922336; E-mail: shintani@mail.hinocatv.ne.jp

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4. Hui SP, Chiba H, Sakurai T, Asakawa C, Nagasaka H (2007) An improved HPLC assay for phosphatidylcholine hydroperoxides (PCOOH) in human plasma with synthetic PCOOH as internal standard. J Chromatogr B Analyt Technol Biomed Life Sci 857: 158-163.

5. Yamamoto Y, Kambayashi Y, Ueda T (1998) Assay of phospholipid hydtoperoxides by chemiluminescence-based high-performance liquid chromatography. Methods Mol Biol 108: 63-70.

6. Yasuda M, Narita S (1997) Simultaneous determination of phospholipid hydroperoxides and cholesteryl ester hydroperoxides in human plasma by highperformance liquid chromatography with chemiluminescence detection. J Chromatography B Biomed Sci Appl 693: 211-217.

7. Miyazawa T, Fujimoto K, Oikawa S (1990) Determination of lipid hydroperoxides in low density lipoprotein from human plasma using high performance liquid chromatography with chemiluminescence detection. Biomed Chromatogr 4: 131-134.

8. Miyazawa T, Saeki R, Inaba H (1989) Detection of chemiluminescence in lipid peroxidation of biological systems and its application to HPLC. J Biolumin Chemilumin 4: 475-478.

9. Miyazawa T (1989) Determination of phospholipid hydroperoxides in human blood plasma by a chemiluminescence-HPLC assay. Free Radic Bioi MeD 7: 209-217.