Pharmaceutica Analytica Acta
ISSN: 2153-2435

Shintani, Pharmaceut Anal Acta 2013, 4:8
DOI: 10.4172/2153-2435.1000e152

Editorial Open Access

Fluorescent Products

Hideharu Shintani*

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

Introduction

The reaction of lipid peroxidation products, such as malonaldehyde, with amino groups of proteins, free amino acids, aminophospholipids, or nucleic acid bases, produces fluorescent lipid-peroxidation products known as conjugated Shiff bases with the general structure RN=CHCH=CHNHR' (N,N'-disubstituted 1-amino-3-iminopropenes) [1]. When these products are excited at 360 nm the fluorescence maximum is in the region 430-440 nm.

Protocol

Spectrofluorimetric analysis of fluorescent lipid peroxidation products is covered in detail elsewhere [2,3]. The method described here is a simple procedure for detection of lipid-soluble and water-soluble fluorescent products formed during lipid peroxidation of biological membranes.

Lipid-Soluble Fluorescent Products

1. Place membrane sample (e.g. oxidized tissue homogenate, plasma, mitochondria, microsomal suspension, or lipoproteins; 0.5 mL) and ethanol-ether (3:1, v/v, 1.5 mL) in screw-capped tubes (1.3×10 cm, Pyrex).
2. Mix on a vortex mixer for 1 min.
3. Centrifuge for 10 min at 3,000 rpm (4°C).
4. Wash the sediment at the bottom of tube twice with ethanol-ether (3:1, v/v, 2 mL) repeating assay steps 2 and 3.
5. Pipette the solution (1.5 mL) into a 1-mL quartz cuvette.
6. Measure fluorescence (emission) and excitation spectra by spectrofluorimetry. (The fluorescent lipid-peroxidation products have usually an excitation maximum in the region of 355—365 nm and an emission maximum at 430-440 nm).

Water-Soluble Fluorescent Products

1. Dissolve sediments from assay of lipid-soluble fluorescent products (above) in 2.0 mL 15% SDS-PBS (phosphate-buffered saline, pH 7.4) on a vortex mixer for 5 min.
2. Centrifuge for 10 min at 3,000 rpm (room temperature).
3. Pipette the solution (1.5 mL) into a 1-mL quartz cuvette.
4. Measure fluorescence (emission) and excitation spectra by spectrofluorimetry.

Calculation

The ftuorescence intensity of quinine sulfate (1 mg mL^{-1}) in H_2SO_4 solution (0.05 M) is the standard for the relative ftuorescence intensity of the sample [2]. Quinine sulfate in the solution has a ftuorescence maximum at 457 nm when excited at 360 nm.

Comments

Fluorescent products which bind to proteins are insoluble in common organic solvents and their structures (ftuorophores) can be disrupted, in part, during extraction of lipids with 2:1 (v/v) chloroform-methanol. They are, however, stable in 3:1 (v/v) ethanol-ether, as described elsewhere [4]. It should be noted that the lipid extracts from biological membranes and tissue homogenates contain retinol as one of interfering ftuorescent compounds. Retinol, a lipid-soluble compound, has a ftuorescence maximum at 478 nm when excited at 335 nm [5] and its ftuorescence can be removed from the lipid extracts by exposing it to high-intensity ultraviolet light for 30 s [2].

References

1. Tappel AL (1980) Free radicals in Biology.(4thedn), WA Pryor. Academic Press, New York: 1-47.
2. Dillard CJ, Tappel AL (1984) Fluorescent damage products of lipid peroxidation. Methods Enzymol 105: 337-341.
3. Shimasaki H (1994) Assay of fluorescent lipid peroxidation products. Methods Enzymol 233: 338-346.
4. Shimasaki H, Ueta N, Privett OS (1982) Covalent binding of peroxidized linoleic acid to protein and amino acids as models for lipofuscin formation. Lipids 17: 878-883.
5. Shimasaki H, Ueta N, Privett OS (1980) Isolation and analysis of age-related fluorescent substances in rat testes. Lipids 15: 236-241.

***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

Received August 24, 2013; **Accepted** August 27, 2013; **Published** August 31, 2013

Citation: Shintani H (2013) IFluorescent Products. Pharmaceut Anal Acta 4: e152. doi:10.4172/2153-2435.1000e152