Food Research International 48 (2012) 346–352

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of a membrane permeable metal chelator on iron and hemoglobin-mediated lipid oxidation in washed fish muscle

Priya Kathirvel *, Mark P. Richards

Meat Science and Muscle Biology Laboratory, Department of Animal Sciences, University of Wisconsin-Madison, 1805 Linden Drive West, Madison, WI 53706, USA

ARTICLE INFO

Article history:
Received 20 December 2011
Accepted 11 April 2012

Keywords:
TPEN
Washed cod
Lipid oxidation
Hemoglobin
Metal chelators
Microsomes

ABSTRACT

The effect of a membrane permeable metal chelator, N′,N′,N′,N′-tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN) on iron and hemoglobin-mediated lipid oxidation in washed cod muscle was evaluated. TPEN stimulated added iron-mediated lipid oxidation in washed cod muscle (pH 6.3) while ethylenediaminetetraacetic acid (EDTA) did not. TPEN delivered iron to cellular membranes of cod muscle more effectively compared to EDTA ($p<0.05$). Fe–TPEN remained intact in the presence of excess EDTA which indicated that TPEN had a relatively high affinity for iron. TPEN also promoted lipid hydroperoxide (LOOH) formation in washed cod alone, presumably by chelating and thereby activating endogenous metals. LOOH and hydrogen peroxide formation due to Fe–TPEN may partly explain the observation that TPEN accelerated trout hemoglobin-mediated lipid oxidation in cod muscle. Peroxides accelerate oxidation of hemoglobin (Hb). Oxidized trout Hb readily releases hemin that efficiently decomposes LOOH to free radicals that, in turn, incur oxidation of polyunsaturated fatty acids. These studies indicate that TPEN activates low molecular weight metals that are endogenously present in washed cod and facilitates transport of metals to cellular membranes which exacerbates the ability of hemoglobin to promote lipid oxidation.

1. Introduction

Ferrous (Fe^{2+}) iron is generally considered more capable of promoting lipid oxidation compared to ferric (Fe^{3+}) iron (Chaiyasit, Elias, Mc Clements, & Decker, 2007). This is due to higher rate constants of Fe^{2+} reacting with pre-formed lipid hydroperoxides to produce free radicals compared to Fe^{3+} (Halliwell & Gutteridge, 1999). Most metal chelators are water-soluble and cause the chelated iron to partition nearly completely into the aqueous phase. N′,N′,N′,N′-tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN) (Fig. 1) is unique as an iron complexing agent because it has been described as membrane permeable (Arslan, Di Virgilio, Beltrame, Tsien, & Pozzan, 1985).

Iron has poor solubility when unbound. This is the reason that in biological systems, iron mostly exists as complexes to nucleotides, phospholipid head groups, amino acids and proteins (Hultin, 1994). The membrane permeability of TPEN provides an interesting possibility that TPEN can inhibit metal-mediated lipid oxidation in muscle tissues via chelating iron specifically at the site of cellular membranes which contain labile phospholipids. It is also possible that TPEN can increase the ability of endogenous metals to promote lipid oxidation. TPEN is noted for having a high affinity for Fe^{2+}, $10^{14.61}$ M^{-1} (Arslan et al., 1985). This suggests that TPEN may displace some of the iron from complexing agents that are endogenously present in tissues.

There have been no reports to our knowledge describing the ability of TPEN to affect iron-mediated lipid oxidation in muscle foods. The primary objective of this work was to examine the ability of TPEN to affect lipid oxidation in a model system comprised of washed cod muscle with and without added oxidants. The oxidants examined were iron and hemoglobin. Another objective was to examine the ability of TPEN to affect partitioning of added iron in muscle tissue.

2. Materials and methods

2.1. Materials

Fresh cod fillets were obtained from a local fish market in Madison, WI that utilizes overnight shipping from the New England area. Blood used in the preparation of hemoglobin (Hb) was obtained from rainbow trout (*Onchorynchus mykiss*) raised at the Water Science and Engineering Laboratory (University of Wisconsin-Madison). Sealable, polyethylene bags (10 cm × 15 cm) and vacuum pouches (3 mil standard oxygen barrier) were purchased from Koch Supplies (Chicago, IL). Distilled, deionized water was collected using Milli-Q plus (Millipore, Billerica, MA). Ethanol (absolute, 200 proof) was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, Kentucky). Tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN), 2-thiobarbituric acid, disodium ethylenediaminetetraacetic acid, ferrous sulfate, ferrous chloride, sodium ascorbate, potassium chloride, histidine, Proteinase, HEPES buffer (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid buffer hemisodium salt) were procured from

* Corresponding author at: 985 Brussels St, #16, Halifax, Nova Scotia, Canada B3H 2S9. Tel.: +1 902 817 5328; fax: +1 902 893 1404.
E-mail address: priyakathirvel@hotmail.com (P. Kathirvel).

0963-9969/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2012.04.016

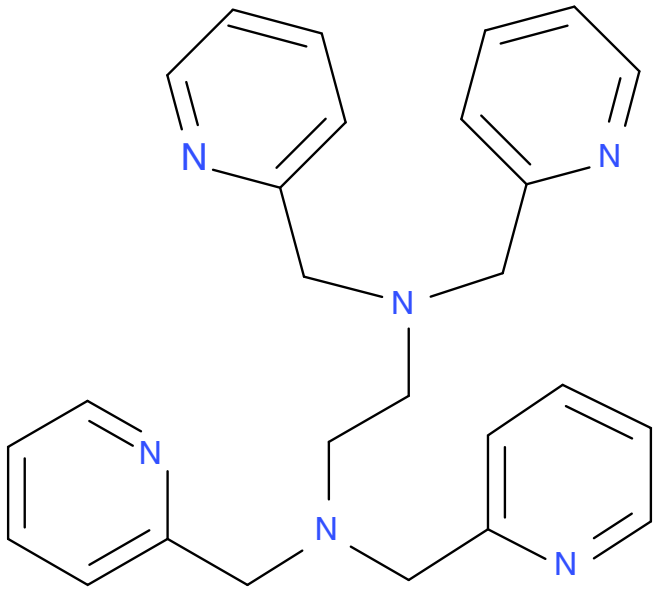

Fig. 1. Structure of N′,N′,N′,N′-tetrakis (2-pyridylmethyl)-ethylenediamine (TPEN).

Sigma A/S (St. Louis, MO). Ammonium thiocyanate, barium chloride, hydrochloric acid, sulphuric acid, trichloroacetic acid, sodium carbonate, methanol, 1-butanol and chloroform were purchased from Fisher Scientific (Chicago, IL). The solvents used were of HPLC grade.

2.2. Preparation of washed cod muscle

Cod fillets were considered of excellent quality based on odors that ranged from sea-like (very fresh) to minimal overall odor. Fillets were trimmed to remove all bones and dark muscles and cut into small pieces. The light muscles were ground in a KS M90WW mincer (Kitchen Aid Inc., St. Joseph, MI) (plate diameter, 5 mm). The mince was washed three times with 50 mM sodium phosphate buffer (pH 6.3) at a 1:3 mince:water ratio (w/w) by stirring with a heavy glass rod for 2 min. The mince-buffer mixture was homogenized (rheostat set to 30) using a Polytron Type (PT) 10/35 (Brinkmann Instruments, Westbury, NY). The homogenate was centrifuged (15,000 *g* for 20 min at 4 °C) using a Beckman L8-70M ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA). The resulting pellet was stored at −80 °C in vacuum sealed bags until use.

2.3. Determination of moisture content and pH in washed cod

The moisture content of washed cod was determined according to the AOAC method (AOAC, 1990). The moisture content of washed cod ranged from 84.75%–85.05%. pH of washed cod was measured by adding 10 volumes of water and homogenizing the mixture for 20 s using a Polytron Type PTA 20/2W probe (Brinkmann Instruments, Westbury, NY). pH of washed cod was 6.3.

2.4. Preparation of trout hemoglobin and quantification of hemoglobin concentration

The methods described by Richards and Dettmann (2003) were followed. Trout were anesthetized with ethyl 3-aminobenzoate (0.5 g/L) and blood was removed from the caudal vein via syringe (25 G 1 in. needle) preloaded with heparin solution (150 units/mL) containing 150 mM NaCl. Four volumes of ice-cold 1.7% NaCl in 1 mM Tris, pH 8.0, were added to heparinized blood and centrifuged (700 *g* for 10 min at 4 °C) in a Beckman J-6B centrifuge (Beckman Instruments, Inc., Palo Alto, CA). After removal of the plasma, the red blood cells were washed by being suspended three times in 10 volumes of the above buffer. Cells were lysed in 3 volumes of 1 mM Tris, pH 8.0 for 1 h. One-tenth of the volume of 1 M NaCl was then added to aid in stromal removal before ultracentrifugation (28,000 *g* for 15 min at 4 °C) using a Beckman L8-70 ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA). Hemoglobin solutions were stored at −80 °C prior to use.

Trout hemoglobin was diluted in 50 mM Tris pH 8.0 (final volume 1.5 mL). Approximately 1 mg of sodium dithionite was dissolved in the diluted solution. The solution was then bubbled with carbon monoxide gas (Badger Welding, Madison, WI) for 30 s. The solution was scanned from 440 to 400 nm in a model UV-2401 dual beam spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD) using 50 mM Tris pH 8.0 as the reference. Calculation of the concentration of heme protein on a heme basis was performed using the peak absorbance occurring near 420 nm and a standard curve generated from bovine hemoglobin.

2.5. Lipid oxidation studies in washed cod

Washed cod stored at −80 °C was thawed overnight at refrigerated conditions (0–4 °C) on ice before use. On the next day, washed cod was transferred to a plastic beaker on ice and mixed for 15 min with a plastic spatula to break the cod muscle pellet into small pieces. It was then ground three times for 5 s at a medium setting of 15 in a Hamilton Beach Custom Grind Type CM04 coffee grinder (Hamilton Beach/ Proctor Silex, Inc., Southern Pines, NC). The final moisture content was made up to 90% using distilled water. Streptomycin sulfate (200 ppm) was added to inhibit microbial growth. Contents were then mixed with a plastic spatula for 2 min. All the mixings were done on ice. This process remained the same for all the studies detailed below.

The first study examined the effect of TPEN alone on lipid oxidation in washed cod. TPEN at 500 μmol/kg washed cod was added and mixed for 2 min with a plastic spatula. A control sample without TPEN was also prepared. For determining the effect of TPEN and EDTA on iron-mediated lipid oxidation, ferrous chloride, TPEN or EDTA were added to washed cod at 40 μmol/kg washed cod respectively and mixed well for 2 min with a plastic spatula. The effect of TPEN on hemoglobin-mediated lipid oxidation in washed cod was also examined. TPEN was added at 40 μmol/kg washed cod and mixed well with a spatula followed by addition of hemoglobin (Hb) to washed cod. Hemoglobin was added to washed cod so that a final concentration on a heme basis was 40 μmol heme/kg washed cod. A control sample without TPEN was also prepared. All samples were then transferred to amber colored bottles (30 mL capacity) that blocks light and stored at 2 °C and progress of lipid oxidation was followed. Two independent determinations were made for each treatment in all the studies. Ethanol was used as a carrier solvent at 1% of muscle weight in trials utilizing chelators. Since EDTA was not soluble in ethanol, it was first dissolved in water before adding to washed cod. To account for the amount of water added to samples containing EDTA, equal amount of water was also added to samples containing TPEN. pH of the samples was also determined.

2.6. Determination of thiobarbituric acid reactive substances (TBARS)

The progress of lipid oxidation in washed cod was followed by periodically removing samples and quantifying TBARS formed by the method described by Lemon (1975) with slight modification. Approximately, 1 g of the sample was mixed with 6 mL of trichloroacetic acid (TCA) solution (7.5% TCA, 0.1% disodium ethylenediamine tetraacetic acid [EDTA], 0.1% propyl gallate) and homogenized with a Polytron Type (PT) 10/35 (Brinkmann Instruments, Westbury, NY) for 30 s. The homogenate was filtered through Whatman no. 1 filter paper. One milliliter of the filtrate was then mixed with 1 mL of thiobarbituric acid (TBA) (0.02 M) in a screw cap tube and incubated at 100 °C for 40 min. After cooling the reaction mixture in cold water for 5 min and centrifuging