Isolation of peroxisomal enoyl-CoA hydratase in rainbow trout and immunochemical identification with the bifunctional enzyme

Linda A. Baldwin¹, Edward J. Calabrese¹, Paul T. Kostecki¹ and Jae-Ho Yang²

¹Environmental Health and Sciences, School of Public Health, University of Massachusetts, Amherst, MA 01003, and ²Institute of Chemical Toxicology, Detroit, MI 48201, U.S.A.

Keywords: rainbow trout, peroxisome, enoyl-CoA hydratase, peroxisomal bifunctional protein, PPA-80, beta oxidation

Abstract

Peroxisomes are the sites for β-oxidation of long-chain fatty acids. The peroxisomal bifunctional enzyme (PBE) enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase catalyzes the second and third reactions of the β-oxidation system. Originally termed PPA-80 for peroxisome-proliferation associated 80,000 MW polypeptide, PBE levels are monitored to measure peroxisome proliferation in rodents and other species. The quantity of a 79,000 MW polypeptide in the light mitochondrial fraction of the liver, as analyzed by SDS-PAGE, increases when rainbow trout are exposed to peroxisome proliferating agents. This correlates with increases in acyl-CoA oxidase activity and peroxisome volume density. In the present study, peroxisomal enoyl-CoA hydratase was purified from trout liver and analyzed by immunoblotting with anti-PBE. A positive reaction with the 79,000 MW polypeptide band was observed providing strong evidence that this is the bifunctional enzyme.

Introduction

Induction of an 80,000 MW polypeptide designated PPA-80 (peroxisome-proliferation-associated polypeptide, approximate MW 80,000) by a wide variety of peroxisome proliferating agents has been well documented in rodents and other species, including humans (Reddy and Lalwani 1983; Reddy et al. 1984; Chen et al. 1987). PPA-80 was identified in rat liver as the heat-labile enoyl-CoA hydratase located in peroxisomes, but not in mitochondria (Reddy et al. 1981). Similar findings were reported in the mouse liver and renal cortex (Lalwani et al. 1981). This peroxisomal enzyme is a bifunctional protein exhibiting both enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities (Osumi and Hashimoto 1979). These activities catalyze the second and third reactions of the β-oxidation pathway (Lazarow and de Duve 1976). Rat liver peroxisomal and microsomal enoyl-CoA hydratase bifunctional enzymes were found to be identical (Cook et al. 1987) and the peroxisome was determined to be the sole origin (Ghesquier et al. 1987). Hartl and Just (1987) provided further data specifically locating the bifunctional enzyme in the peroxisome. Due to this definitive localization and function, quantification of PBE is an important indicator of enhanced β-oxidation.

We are currently investigating xenobiotic-induced peroxisome proliferation in fish to determine their potential as a model for epigenetic carcinogens. Identification of PBE in trout provides a sen-
sitive and valuable parameter to assess induction of peroxisomal β-oxidation.

Materials and methods

Chemicals

Crotonoyl Coenzyme A, hexamethylphosphoric triamide, 2-mercaptoethanol, nitroblue tetrazolium chloride, 5-bromo-4-chloro-indoylphosphatase p-toluidine salt, ovalbumin, bovine serum albumin and ammonium sulfate were purchased from Sigma Chemical Company (St. Louis, MO). Acrylamide, N-N'-methylene-bis-acrylamide, SDS-PAGE molecular weight standards, carboxymethyl cellulose, phosphoryl cellulose and nitrocellulose paper were obtained from Bio Rad Laboratories (Richmond, CA). All other chemicals were of the highest analytical grade.

Antibody sources

Rabbit anti-rat peroxisomal bifunctional enzyme (PBE) was a kind gift from Dr. Janardan K. Reddy (Northwestern University Medical School, Chicago, IL). Alkaline phosphatase conjugated goat anti-rabbit IgG was purchased from Sigma Chemical Company (St. Louis, MO).

Animals

Rainbow trout (Salmo gairdneri), weighing 300–350 g, were obtained from the Massachusetts Division of Fisheries and Wildlife fish hatchery in Sunderland, MA.

Subcellular-fractionation

A light mitochondrial fraction containing peroxisomes was prepared according to the procedure of Small et al. (1985). The livers were homogenized in 4 volumes of 3 mM imidazole buffer containing 10% sucrose. The homogenate was centrifuged at 6,000 × g for 10 min at 4°C. Supernatant was saved and the pellet resuspended in the imidazole buffer. This suspension was centrifuged at 6,000 × g for 10 min at 4°C. Supernatants were pooled and centrifuged at 30,000 × g for 30 min at 4°C. The pellet containing peroxisomes, mitochondria and lysosomes was resuspended in 10 mM potassium phosphate containing 2 mM 2-mercaptoethanol and 0.1% hexamethylphosphoric triamide.

Enoyl-CoA hydratase assay

Enoyl-CoA hydratase activity was measured by the method of Steinman and Hill (1975) in 0.3 M Tris-HCl, pH 7.4, containing 5 mM EDTA, 0.05 mg/ml ovalbumin and 200 μM crotonoyl-CoA. To determine the heat-labile peroxisomal enoyl-CoA hydratase activity the enzyme preparations were diluted with 50 mM potassium phosphate, pH 7.0 and heated at 57°C for 5 min. The decrease in absorbance was recorded at 280 nm. Protein concentration was measured using a Sigma protein assay kit based on the method of Lowry et al. (1951) or a BCA protein assay kit (Pierce Chemical Company, Rockford, IL) based on the method of Smith et al. (1985).

Purification of peroxisomal enoyl-CoA hydratase

Peroxisomal enoyl-CoA hydratase was purified using the method of Osumi and Hashimoto (1979). The light mitochondrial fraction from 20 g of trout liver, suspended in 10 mM potassium phosphate, pH 7.0, containing 2 mM 2-mercaptoethanol and 0.1% hexamethylphosphoric triamide (all buffers in this procedure contained these reagents), was applied to a phosphocellulose column (100 ml bed volume, equilibrated with 50 mM potassium phosphate, pH 7.0). The column was eluted with a linear gradient system from 50–500 mM phosphate buffer in a total volume of 400 ml. Fractions with enoyl-CoA hydratase activity were pooled, treated with ammonium sulfate and protein precipitated between 200 and 400 g/l was dissolved in a minimal volume of 50 mM potassium phosphate, pH 7.0. Ammonium sulfate was removed by passage