RESEARCH ARTICLE

Desaturase Enzyme Activity in the Red Tilapia (*Oreochromis* hybrid) and Catfish (*Clarias gariepinus*)

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Abstract

Objective—To determine the Δ6 desaturase enzyme activity in the red tilapia and the catfish.

Materials and Methods—Six one month old red tilapia (*Oreochromis* hybrid) and six one month old catfish (*Clarias gariepinus*) were used in this experiment. Δ6 desaturase enzyme activity was measured from the liver microsomes of these two species, by using radioactive labeled linoleic acid [1-^{14}C]. The radioactivity of samples was measured with a LSC (Liquid Scintillation Counter).

Results—The percentage desaturation of [1-^{14}C] - linoleic acid recorded in the 60 min incubation in the red tilapia and the catfish were 3.55% and 3.07%, respectively. The absolute activities in these fish were 1.19 and 1.02 pmol lionleic acid metabolized/min/mg microsomal protein, respectively. % Desaturation of [1-^{14}C] - linoleic acid and Δ6 desaturase activities were not significant in two fish were observed.

Conclusion—The percentage desaturation of [1-^{14}C] - linoleic acid and Δ6 desaturase activities were higher in the red tilapia although it was not significant.

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Keywords: Δ6 desaturase; Liver microsomes; radioactive; LSC; PUFAs; Catfish; Red Tilapia

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Introduction

The long chain polyunsaturated fatty acids (PUFAs) are synthesized by desaturation and chain elongation enzyme systems which are present in the microsomes of most animal tissues, mainly liver [1] by biosynthetic pathways. The desaturase and chain elongase activities vary with different tissue [2]. Fatty acid desaturases are responsible for the introduction of specific double bond in higher animals, yeast, protozoa, red algae, blue-green algae and in certain bacteria [3]. Both Δ5 and Δ6 desaturases are important in the metabolism of essential fatty acid (EFA). Δ6 desaturation is considered to be rate

limiting enzyme (4, 5). Marine fish have only a very limited desaturase activity [6-8]. Unlike marine fish, freshwater fish are able to desaturate and elongate larger quantities of dietary C18 n-6 and n-3 PUFA to C20 and C22 desaturates [6,8-10].

The lipid of fish in general are characterized by high contents of eicosapentaenoic acid (EPA 20:5 n-3) and especially docosahexaenoic acid(DHA 22:6 n-3) [11].Vertebrate animals are incapable of synthesizing these PUFA *de novo* although they can, depending on species and to greater or lesser extents, convert 18:3 n-3 to 20:5 n-3 and then to 22:6 n-3. Because 22:6 n-3 has important roles in animal health and development, its rate and mechanism of formation from 18:3 n-3 and 20:5 n-3 have been investigated in several systems including isolate rat hepatocytes [12] and rod outer segment of frog retinas [13]. Δ6 desaturase also known as linoleoyl-CoA desaturase (EC1.14.19.3). Linoleoly-CoA, hydrogen-donor:oxygen oxidoreductase is key enzyme localized in the endoplasmic reticulum [14]. Δ6 desaturase reaction is the rate limiting step in the conversion of linoleic acid and alpha linolenic acids to the longer, more highly unsaturated members of the n-6 and n-3 PUFA [15] and required for the conversion of dietary linoleic acid to arachidonic acid [16]. The present study is to determine the Δ6 desaturase enzyme activity in the red tilapia and the catfish.

Materials and Methods

Preparation of Liver Microsomes

Six one month old red tilapia (*Oreochromis* hybrid), mean body weight ± SD, 104.9 ± 15 g; mean standard length ± SD, 18.1 ± 0.7 cm and six one month old catfish (*Clarias gariepinus*), mean body weight ± SD, 294.5 ± 19.1 g; mean standard length ± SD, 36.2 ± 4.1 cm were used. Fresh livers were removed immediately after the fish were sacrificed. They were cut with scissors into pieces in three volumes of ice-cold 0.25M sucrose solution and drained. After that they were homogenized in extraction buffer (25mM Sucrose in 10 mM HEPES, pH 7.4) and centrifuged in 10,000 g, for 60 min, at 4˚C. The supernatant was discarded and the pellet was dissolved in the extraction buffer and centrifuged in 100,000 g, for 60 min at 4 ˚C again. The microsomal pellet was resuspended in the buffer solution. The protein content was measured by the method of Lowry [17]. The Lowry method combines the biuret reaction with the reduction of the Folin-Ciocalteu phenol reagent (phosphomolybdic-phosphotungstic acid) to heteropolymolybdenum blue by tyrosine and tryptophan residues in the proteins. The bluish colour developed was read at 750 nm.

Incubation

Radioactive labeled linoleic acid [1-^{14}C] (Amersham Biosciences UK) was kept in the freezer in radioactive control room before being used. The stock solution was made with petroleum ether to the concentration of (1 µCi/mL) under the fume hood. The stock solution was labeled, stored and refrigerated at -20˚C.

To three mL of [1-^{14}C] - linoleic acid was added 0.2 mL of unlabelled linoleic acid (1.232 mg/

mL) and the mixture was dried under N_2 (99.999%, MOX) and redissolved in 1 mL of 0.1% (w/v) K_2 CO3 (Hamburg). The mixture was placed in a 50 mL tube and preincubated at 37˚C for five min with shaking. The microsomal suspension (0.2 mL of buffer solution; 5 mg protein) was added and the incubation was continued for 1 hr at 37˚C with shaking.

Extraction of Lipids

The reaction was stopped by addition of 0.25 mL of 4N KOH (Hamburg), 125 µg each of unlabelled linoleic, gamma-linolenic and arachidonic acid (Sigma) were added to act as carriers followed by five mL of methanol. The mixture was warmed at 50˚C for 60 min with shaking, acidified with 0.2 mL of 9N HCL (M&B) and 1 mL of distilled deionised water was added. This was followed by ten mL of chloroform (Ri). The mixture was shaken and let to stand overnight at 4˚C. The upper aqueous phase was removed while the lower phase was dried under N_2. It was transferred with chloroform into a 15 mL methylating tube and trans-methylated with boron trifluoride (Sigma) as in preparation of fatty acid methyl esters (FAME).

Separation of FAME by Argentation-TLC

Glass thin–layer plates (20 x 20 cm) were coated manually with 0.25 mm layer of silica gel G. Silica gel contained gypsum, calcium sulfate hemihydrate, which has been added as a binder to aid the adherence of the silica gel to the support. Silica gel was impregnated with 7.5% silver nitrate (Sigma) prepared from a slurry containing 0.53 g of silver nitrate, seven g of silica gel G (Fluka chemic AG Ch-9470 Buchs) and 16.5 mL of distilled deionized water. The plates were air dried and activated for one hr at 110˚C, cooled and stored in a desiccator until used.

The FAME mixture was dried under N_2 and redissolved in 100 µL of petroleum ether (BDH) and spotted as a thin band about 6 cm long about 2.5 cm from the bottom edge of the plate. About 250 µg of standard were spotted along side the sample. The plate was developed in petroleum ether- diethyl ether (BDH)- methanol (Merck)-glacial acetic acid (BDH)(40:10:1:1, v/v) for six hours. After development and staining with 2', 7'-dichlorofluorescein (Sigma), the FAME components were located under an ultraviolet light source and individual fatty acids were marked on the plate. Starting from the top of the plate and proceeding towards the origin, horizontal four cm by three mm fractions of the silica gel were scraped directly into 20 mL scintillation vials. Ten milliliters of scintillation fluid, which consisted of 4 g of 2,5-diphenyloxazole (PPO) (Sigma) and 0.2 g of 1,4-bis-[2-(5-phenyloxazoly)]-benzene (POPOP) (Sigma) dissolved in a litre of toluene (M & B), were added to each of the scintillation vials carrying the scraped silica gel fractions, the mixture shaken vigorously and then counted for radioactivity.

Measurement of Radioactivity

The radioactivity of samples was measured with a LSC (Tri-carb 2700TR, Liquid Scintillation Analyzer, Packard, A Packard Bioscience Company) in two min using the preset ^{14}C energy regions. Background counts of sample were automatically subtracted and samples were compensated for quenching using the automatic efficiency control (AEC), facility available in machine. Background IPA

data processed was Bkg (0-18.6 keV) = 12.45 cpm and (0-156 keV) = 22.73 cpm. Quench indicator: tSIE, Ext Std Terminator: 5 sec. The counting efficiency was at least 96.21%.

Determination and Expression of Enzyme Activity

Delta-6-desaturase activity was calculated using the following equation, and was converted to pmol of linoleic acid metabolized per min per mg microsomal protein.

$$\% \text{ desaturation} = \frac{\text{Total cpm in [Trienoic + Tetraenoic fractions]}}{\text{Total cpm in [Dienoic + Trienoic + Tetraenoic fractions]}} \times 100$$

The data were analysed using the Independent t- test by the functions of SPSS version 10.0 [18].

Results

The percentage desaturation of [1-^{14}C] - linoleic acid recorded in the 60 min incubation in the red tilapia and the catfish are 3.55% and 3.07% respectively. The absolute activities in these fish are 1.19 and 1.02 pmol lionleic acid metabolized/min/mg microsomal protein. % Desaturation of [1-^{14}C] - linoleic acid and Δ6 desaturase activities were not significant in two fish were observed (**Table 1**).

Table 1. Distribution of Radioactivity from [1-14C] - Linoleic Acid and Δ6 Desaturase Activities in Red Tilapia and Catfish Liver Microsomes

	(Mean ± SE) (n = 6)	
	Red Tilapia	**Catfish**
% Desaturation of [1-^{14}C] - linoleic acid ^{ns}	3.55 ± 0.2	3.07 ± 0.2
Δ6 desaturase activity ^{ns} pmol/min/mg protein)	1.19 ± 0.1	1.02 ± 0.1

Abbreviations: SE = standard error; ns = not significant at $p < 0.05$.

Discussion

Percentage (%) desaturation of [1-^{14}C] - linoleic acid in red tilapia is 3.55 ± 0.2 and it was 3.07 ± 0.2 in catfish. Δ6 desaturase activity (pmol/min/mg protein) was 1.19 ± 0.1 in red tilapia and in catfish it was 1.02 ± 0.1. Both % desaturation of [1-^{14}C] - linoleic acid and Δ6 desaturase activity were higher in the red tilapia although it was not significant. It was noted that red tilapia desaturated about 2/3 of the initial substrates to the tetraenoic fraction, whereas in the catfish there was the trienoic fraction only. It showed that the red tilapia can desaturase the linoleic acid (18:2 n-6) to γ linolenic acid (18:3 n-6) by Δ6 desaturase and through elongation to become dihomo-γ linolenic acid (20:3 n-6). Tetraenoic arachidonic acid (20:4 n-6) was formed from 20:3 n-6 by the Δ5 desaturase enzyme. In the catfish only dienoic and tetraenic fractions were observed. Linoleoyl-COA (Δ6 desaturase) and eicosatrienoyl-COA (Δ5

desaturase) activity were seen in the herbivorous red tilapia, whereas only linoleoyl-COA (Δ6 desaturase) was observed in the omnivorous catfish. There was loss of Δ6 desaturase activity in carnivores [19]. In our study, % desaturation and Δ6 desaturase activity were slightly higher in the herbivorous red tilapia when compared to the omnivorous catfish. The essential fatty acid (EFA) requirements of freshwater and marine fish have long been known to differ qualitatively. The 18:3 n-3 and /or 18:2 n-6 can satisfy the EFA requirements in freshwater fish, whereas marine species require the longer-chain highly unsaturated fatty acids (HUFA) 20:5 n-3 and 22:6 n-3 to be supplied by the diet for optimal growth [20,21].

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