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Biosynthesis of Fatty Acids in *Tilapia zillii* and the Puffer Fish

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Abstract

The biosynthesis of fatty acids from acetate-1-^{14}C, linoleic acid-1-^{14}C, and linolenic acid-1-^{14}C was examined on *Tilapia zillii*. Also, the bioconversion of linoleic acid-1-^{14}C to highly unsaturated fatty acids (HUFA) was investigated on the puffer fish, *Fugu rubripes*. After injection of the radioactive precursors, the proportional radioactivity was measured on each fatty acid which was isolated by means of thin-layer chromatography on $AgNO_3$-Kieselgel G and preparative gas-liquid chromatography.

T. zillii was found to synthesize palmitic acid (16: 0), palmitoleic acid (16: 1), stearic acid (18: 0), and oleic acid (18: 1ω9) from acetate but not HUFA such as eicosapentaenoic acid (20: 5ω3) and docosahexaenoic acid (22: 6ω3). Also, *T. zillii* converted linolenic acid to 20: 5ω3 and 22: 6ω3, but the fish slightly metabolized linoleic acid to ω6 series of HUFA. The puffer fish converted linoleic acid to eicosadienoic acid (20: 2ω6) and arachidonic acid (20: 4ω6). These results were discussed in relation to the essential fatty acid requirements for *T. zillii* and the puffer fish.

Many investigations have been presented on the requirements of essential fatty acids (EFA) for a variety of species of fish[1-3], indicating the necessity of ω3 series of fatty acids such as linolenic acid (18: 3ω3), eicosapentaenoic acid (20: 5ω3), and docosahexaenoic acid (22: 6ω3) for their growth. Our previous study[4] has shown that *Tilapia zillii* requires ω6 fatty acids such as linoleic acid (18: 2ω6) and arachidonic acid (20: 4ω6) rather than ω3 fatty acids such as 18: 3ω3, 20: 5ω3, and 22: 6ω3. Thus, the EFA requirements of *T. zillii* have been demonstrated to be quite different with those of other aquatic animals reported so far[1-3,5,6].

The purpose of the present study is to obtain the knowledge on the biosynthesis of fatty acids, especially EFA, in *T. zillii*, in relation to the EFA requirements. To this approach, the incorporation of radioactive acetate, 18: 2ω6, and 18: 3ω3 into the individual fatty acids was investigated by using the fingerlings of *T. zillii*. Since the effect of dietary 18: 3ω3 as EFA on the fish and crustaceans was shown to be closely related to the capacity for bioconversion of 18: 3ω3 to ω3-highly unsaturated fatty acids (HUFA)[7], the incorporation of radioactive 18: 2ω6 to ω6-HUFA in the puffer fish, *Fugu rubripes*, was also examined as a part of understanding the EFA requirements of this fish. The present paper deals with these results and discussion.

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Materials and Methods

Injection of radioactive precursors and extraction of lipids The specimens of the puffer fish and *T. zillii* were injected with the radioactive precursors and then maintained in the aquaria at 24–25°C for 24 hours as shown in Table 1. Twenty-four hours after injection of the radioactive precursors, the fish was killed by freezing at −20°C and lipids were extracted with chloroform-methanol-water[8] from the whole body.

Table 1. Administration of the radioactive precursors to the fishes and the holding conditions.

Remark	Precursor injected*1			
	Acetate-1-^{14}C	18: 2ω6-1-^{14}C	18: 3ω3-1-^{14}C	18: 2ω6-1-^{14}C
Fish	*Tilapia*	*Tilapia*	*Tilapia*	Puffer fish
Number of fish	3	3	3	1
Total body weight (g)	10.9	12.1	12.1	0.02
Dosage (μCi)	124	5.0	5.0	20.0
Water temperature (°C)	24–25	24–25	24–25	24–25
Holding time (hr)	24	24	24	24

*1 Radioactive precursors were obtained from the Radiochemical Centre, Amersham, England, and had the following specific activity (μCi/mmol): sodium acetate-1-^{14}C, 59.1; 18: 2ω6-1-^{14}C, 50.0; 18: 3ω3-1-^{14}C, 49.8. Acetate-^{14}C and radioactive fatty acids were dissolved into 5 μl of distilled water and 10 μl of ethanol, respectively, and injected into the muscle of the fishes.

Incorporation of radioactivity into the individual fatty acids Lipids were separated into neutral (NL) and polar (PL) lipid fractions by column chromatography on Kieselgel 60[9]. The individual fatty acids constituting NL and PL were separated into the components by thin-layer chromatography (TLC) on $AgNO_3$-Kieselgel G (1: 10, w/w)[10] with *n*-hexane-ether-acetic acid (94: 4: 2) and preparative gas-liquid chromatography (GLC) on 10% DEGS as reported previously[7,11]. Radioactivity was measured with a liquid scintillation counter, Beckman LS-230, using PPO (0.6%) in toluene as a scintillator.

Results and Discussion

To investigate the biosynthesis of fatty acids in *T. zillii* and the puffer fish, acetate-1-^{14}C, 18: 2ω6-1-^{14}C, or 18: 3ω3-1-^{14}C was injected to the fishes and the incorporation of radioactivity into the lipid fractions and the individual fatty acids was examined. The results were given in Tables 2, 3, and 4.

In the *Tilapia* injected with the radioactive precursors, radioactivity was recovered in NL more than in PL. In PL isolated from the *Tilapia* received acetate-^{14}C, radio-

Table 2. Incorporation of radioactivity into neutral and polar lipid fractions after injection of the radioactive precursors.

Fish	Precursor injected	Lipid fraction	Weight (mg)	Radioactivity (dpm × 10^3)	Specific activity (dpm × 10^3/mg)	Incorporation (%)*1
Tilapia	Acetate-^{14}C	NL	381.4	3,950	7.1	16.5
		PL	102.0	2,420	19.0	10.0
	18: 2ω6-^{14}C	NL	555.2	670	1.0	25.2
		PL	128.3	290	4.2	10.9
	18: 3ω3-^{14}C	NL	356.3	585	1.6	22.0
		PL	116.4	310	2.7	11.6
Puffer fish	18: 2ω6-^{14}C	NL	666.2	6,690	10.0	15.2
		PL	250.8	1,130	4.5	2.5

*1 Incorporation of the injected radioactive precursors into neutral lipid (NL) and polar lipid (PL) fractions

activity was mainly associated with palmitic acid (16: 0) and stearic acid (18: 0). In NL, a high radioactivity was recovered in palmitoleic acid (16: 1) and oleic acid (18: 1ω9) besides 16: 0 and 18: 0. However, only an extremely low radioactivity was detected in ω6- and ω3-HUFA such as 20: 4ω6, 20: 5ω3, and 22: 6ω3. These data show that *T. zillii* is capable of synthesizing 16: 0, 16: 1, 18: 0, and 18: 1 from acetate but incapable of introducing the double bonds into the ω6- and ω3-positions. The detection of small amounts of radioactive HUFA could be explained by the addition of radioactive C_2-units from β-oxidation to the preexisting ω6- and ω3-acids as also observed in other fish[12-15]. Therefore, 18: 2ω6 and 18: 3ω3 are likely to be essential for growth of *T. zillii*. In fact, our previous study[4] has revealed by the feeding trials that the supplement of either 18: 2ω6 or 18: 3ω3 to the diet containing lauric acid (12: 0) improved the weight gain of *T. zillii*, indicating that the growth-promoting effect of 18: 2ω6 was superior to that of 18: 3ω3.

Next, our purpose was aimed to clarify whether *T. zillii* possesses the ability for formation of ω6- and ω3-HUFA, especially 20: 4ω6, 20: 5ω3, and 22: 6ω3, from 18: 2ω6 and 18: 3ω3. In both PL and NL, most of radioactivity in the fatty acids was associated with the unchanged radioactive precursors in the *Tilapia* injected with 18: 2ω6-^{14}C and 18: 3ω3-^{14}C. In the *Tilapia* injected with 18: 3ω3-^{14}C, however, some radioactivity was detected in the conglomerate of 20: 3ω3+20: 4ω6, 20: 5ω3, and 22: 6ω3. The results suggest that *T. zillii* is capable of converting exogenous 18: 3ω3 to ω3-HUFA such as 20: 5ω3 and 22: 6ω3 to some extent. Whereas, *T. zillii* slightly incorporated the injected 18: 2ω6-^{14}C into ω6-HUFA. As shown in Table 3, the conglomerate of 20: 3ω3+20: 4ω6 from PL gave a low radioactivity (2.9% of total radioactive fatty acids of PL). Accordingly, *T. zillii* was suspected to convert slightly exogenous 18: 2ω6 to 20: 4ω6.

Table 4 shows the proportional radioactivity in the individual fatty acids of the

Table 3. Proportional radioactivity in the individual fatty acids of polar and neutral lipids isolated from *T. zillii* after injection of acetate-l-^{14}C, 18: 2ω6-l-^{14}C, and 18: 3ω3-l-^{14}C.

Fatty acid	Distribution (%)*1 of radioactivity and precursors					
	Acetate-^{14}C		18: 2ω6-^{14}C		18: 3ω3-^{14}C	
	PL	NL	PL	NL	PL	NL
12: 0	0	0	0	0	0	0
14: 0	0.8	4.2	4.6	16.8	2.2	0
16: 0	73.1	11.8	0	1.2	0	0.7
17: 0	0.6	0	0	0	0	0
18: 0	11.4	12.4	0	1.2	0	0
14: 1	0.7	4.8	0	0	0	0
16: 1	4.1	31.1	5.2	0.5	0	0
18: 1ω9	4.1	28.8	0	9.6	0	0
20: 1ω9	2.5	1.1	0	0	0	0
18: 2ω6	0.9	1.0	85.6	70.7	1.3	1.1
20: 2ω6	0	0	0	0.1	0	0
18: 3ω3	0.1	0.3	0.1	0	63.0	87.7
20: 3ω6	0.4	1.6	0	0	1.0	0.8
18: 4ω3			0	0.1	1.2	0.5
20: 3ω3 + 20: 4ω6			2.9*2	0.3*2	9.5*3	1.8*3
22: 4ω6	1.2		0.1	0	0.1	0.5
22: 5ω6			0.2	0	0	0
20: 5ω3			0.1	0	4.7	1.0
22: 5ω3			0.2	0.1	2.9	1.2
22: 6ω3			1.0	0	2.2	4.0

*1 Proportional radioactivity in each fatty acid

*2 Radioactivity was suspected to be due to 20: 4ω6.

*3 Radioactivity was suspected to be due to 20: 3ω3.

puffer fish injected with 18: 2ω6-^{14}C. In both NL and PL, most of radioactivity was recovered in the unchanged 18: 2ω6-^{14}C. However, a substancial radioactivity was detected in the conglomerate of 20: 3ω3+20: 4ω6 in both NL and PL and in 20: 2ω6 from NL. We assumes that radioactivity in the conglomerate of 20: 3ω3+20: 4ω6 is mainly due to 20: 4ω6. These results indicate that the puffer fish, *F. rubripes*, is capable of converting exogenous 18: 2ω6 to 20: 2ω6 and 20: 4ω6. As for the EFA requirements of the puffer fish, no data are available on the feeding experiments at the present.

Table 4. Proportional radioactivity in the individual fatty acids of polar and neutral lipids isolated from the puffer fish after injection of 18: 2ω6-^{14}C.

Fatty acid	Distribution (%) of radioactivity	
	PL	NL
14: 0	0	0
16: 0	1.3	1.2
18: 0	0	0
14: 1	0	0
16: 1 18: 1ω9	1.8	1.6
20: 1	0	0
16: 2	1.2	0.3
18: 2ω6	84.3	70.8
20: 2ω6	0	8.8
18: 3ω3	2.9	3.0
20: 3ω6	0.2	0
20: 3ω3 20: 4ω6	7.0*1	10.5*1
20: 5ω3	0.4	3.1
22: 5ω3	0.3	0.8
22: 6ω3	0.1	0.1

*1 Radioactivity was suspected to be associated mainly with 20: 4ω6.

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