Mem. Fac. Fish., Kagoshima Univ.
Vol. 28 pp. 21~26 (1979)

Biosynthesis of Fatty Acids from Acetate in the Prawns, *Penaeus monodon* and *Penaeus merguiensis*

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Abstract

The biosynthesis of fatty acids from acetate-1-^{14}C was examined on the prawns, *Penaeus monodon* and *Penaeus merguiensis*. After injection of acetate-1-^{14}C, polar lipids (PL) and neutral lipids (NL) were isolated from the whole body of the prawns, and the proportional radioactivity of individual fatty acids constituting PL and NL was investigated by using argentation thin-layer chromatography and preparative gas-liquid chromatography on 10% DEGS followed by radioactive measurements of the trapped samples.

In both *P. monodon* and *P. merguiensis*, radioactivity was mainly associated with palmitic acid (16: 0), palmitoleic acid (16: 1), stearic acid (18: 0), and oleic acid (18: 1 ω9) but scarcely or slightly with linoleic acid (18: 2 ω6), linolenic acid (18: 3 ω3), eicosapentaenoic acid (20: 5 ω3), and docosahexaenoic acid (22: 6 ω3). These results suggest that 18: 2 ω6, 18: 3 ω3, 20: 5 ω3, and 22: 6 ω3 may be essential for *P. monodon* and *P. merguiensis*.

Nutritional studies on lipids have demonstrated that several crustaceans require essential fatty acids (EFA) for their normal growth, and also that the nutritive values of lipids for crustaceans as well as fish are highly affected by the dietary content of EFA[1]. Concerning the prawn, *Penaeus japonicus*, we have shown the presence of EFA by both feeding trials[2~4] and tracer experiments using radioactive acetate[5], palmitic acid (16: 0)[6], and linolenic acid (18: 3ω3)[7].

On the other hand, *Penaeus monodon* and *Penaeus merguiensis* are prawns inhabiting mainly in tropical countries such as Malaysia, Philippines, Indonesia, Thailand, etc., and the development of the culture of these prawns under artificially controlled conditions is being watched with keen interest in the above countries. However, there is little information available at present on the nutritional requirements of *P. monodon* and *P. merguiensis*. In the present study, hence, we investigate the *de novo* synthesis of fatty acids, especially ω3-series of fatty acids, from acetate-1-^{14}C as parts of clarifying EFA in these prawns.

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Materials and Methods

Prawns and Injection of Acetate-1-^{14}C

The prawns, *P. monodon* and *P. merguiensis*, were hatched in the Phuket Marine Fisheries Station, Phuket Province, Thailand, transported to this laboratory by air November 19th, 1977, and maintained on a commercial diet for the prawn, *P. japonicus* (Evian; Kyowa-Hakko Kogyo Co. Ltd., Japan) until use. As shown in Table 1, *P. monodon* and *P. merguiensis* were injected with sodium acetate-1-^{14}C (specific activity, 59.1 mCi/mmol; Radiochemical Centre, Amersham, England) dissolved in small amounts of 0.6% sodium chloride solution, and held in a closed container as mentioned previously[7]. During the holding period, expired carbon dioxide was collected by the method of DALL[8]. The molting stage of the prawns used was determined by the method of DRACH and TCHERNIGOVTZEFF[9].

Table 1. Injection of acetate-1-^{14}C into the prawns, *P. monodon* and *P. merguiensis*

Dosage and holding period	*P. merguiensis*	*P. monodon*	*P. monodon*
Number of prawns	2	3	2
Molting stage	D_1–D_2	D_0	D_0
Fresh weight (g)	18.0	18.6	9.9
Acetate-1-^{14}C injected (μCi)	9.0	9.0	5.0
Holding period (hr)	24	12	24
Holding temperature	25°C	25°C	25°C

Extraction of Lipids and Separation of Neutral and Polar Lipids

Lipids were extracted with chloroform-methanol-water (2: 2: 1) by the method of BLIGH and DYER[10] and then separated into neutral lipids (NL) and polar lipids (PL) by column chromatography on Kieselgel 60 (70–230 mesh, Merck, West Germany) as follows. In a typical case, 1 g of lipids was loaded on a column packed with 13 g of Kieselgel 60, and NL and PL were eluted with 500 m*l* of chloroform-methanol (98: 2) and with 350 m*l* of methanol, respectively.

Separation of Individual Fatty Acids

NL and PL were saponified with 10% methanolic potassium hydroxide, and then the fatty acids so obtained from NL and PL were converted to methylesters. The fatty acid methylesters (FAME) were separated into several fatty acid fractions with different numbers of double bonds by thin-layer chromatography (TLC) on 9.3% (w/w) silver nitrate-Kieselgel G with hexane-ether-acetic acid (94: 4: 2) (twice developments) according to the method of DUDLEY *et al.*[11]. The FAME fractions so obtained were further subjected to preparative gas-liquid chromatography (GLC) on 10% DEGS[7], and then the radioactivity of the trapped samples corresponding to individual FAME was measured with a Beckman liquid scintillation counter.

Measurement of Radioactivity

Radioactivity was measured with a Beckman liquid scintillation counter LS-230 during this study. Radioactivity of expired carbon dioxide was determined as barium carbonate by the method of STEVENSON and TUNG[12] as follows: 15 m*l* of the scintillator (0.3% PPO+0.05% POPOP/toluene), 600 mg of Cab-O-Sil^R (Eastman Kodak Co.), and 40 mg of non-radioactive barium carbonate were added to radioactive samples and subjected to radioactive measurements. Radioactivity of aqueous samples was measured by adding 12 m*l* of the scintillator 0.5% PPO+0.03% POPOP/toluene-Triton X-100 (2: 1) to 1.5 m*l* of sample. Radioactivity of lipids was measured by using the scintillator (0.6% PPO/toluene).

Results and Discussion

Table 2 shows the radioactivity recovered in the expired carbon dioxide and lipid fractions 12 or 24 hr after injection of acetate-1-^{14}C into the prawns, *P. monodon* and *P. merguiensis*. About 60% or more of the injected radioactivity was recovered as expired carbon dioxide in 12 or 24 hr after injection. In *P. monodon*, radioactive lipids were present as PL rather than NL, and the ratio of radioactive PL/NL was increased after 24 hr. In *P. merguiensis*, radioactivity was also associated with PL rather than NL.

Table 3 shows the proportional radioactivity of the fatty acid fractions with different numbers of double bonds in *P. monodon* and *P. merguiensis* after injection of acetate-1-^{14}C. In *P. monodon*, more than 80% of radioactive fatty acids was present as the saturates and monoenes not only in PL but also in NL after 12 and 24 hr. Concerning *P. merguiensis*, similar results were obtained on the

Table 2. Radioactivity recovered in the expired carbon dioxide and lipid fractions 12 or 24 hr after injection of acetate-1-^{14}C into *P. monodon* and *P. merguiensis*

Fraction	Weight (mg)			Radioactivity (dpm × 10^4)		
	P. monodon (12 hr)	*P. monodon* (24 hr)	*P. merguiensis*(24 hr)	*P. monodon* (12 hr)	*P. monodon* (24 hr)	*P. merguiensis*(24 hr)
Expired carbon dioxide	—	—	—	402(69%)*1	763(70%)	1000(64%)
Rearing water	—	—	—	46(8%)	66(6%)	167(11%)
Aqueous extract*2	—	—	—	73(13%)	191(18%)	260(17%)
Total lipids (TL)	240	103	220	58(10%)	71(7%)	125(8%)
Neutral lipids (NL)	71	28	62	20(3%)	12(1%)	19(1%)
Polar lipids (PL)	169	75	158	38(7%)	59(6%)	106(7%)
Saponifiable matters	43	16	20	17(3%)	11(1%)	17(1%)
Ratio of TL/CO_2	—	—	—	0.14	0.09	0.12
Ratio of PL/NL	2.4	2.7	2.6	1.9	4.9	5.6

*1 % Distribution of radioactivity

*2 Radioactivity in an aqueous layer after extraction of lipids from the whole body of prawns by BLIGH and DYER method using chloroform-methanol-water (2 : 2 : 1)

Table 3. Distribution of radioactivity in fatty acids with different degrees of unsaturation in polar and neutral lipid fractions isolated from the prawns 12 or 24 hr after injection of acetate-1-^{14}C

Fatty acid fraction	% Distribution of radioactivity*					
	Polar lipid			Neutral lipid		
	P. monodon (12 hr)	*P. monodon* (24 hr)	*P. merguiensis* (24 hr)	*P. monodon* (12 hr)	*P. monodon* (24 hr)	*P. merguiensis* (24 hr)
Saturates	55	29	74	48	28	66
Monoenes	38	58	19	34	55	22
Dienes	1.0	2.0	6.0	11	3.3	4.2
Trienes	0.5	1.3	0.2	0.1	0.8	0.5
Tetraenes	3.9	7.0	0.2	0.5	1.9	0.6
Pentaenes	0.6	1.5	0.3	3.5	6.3	3.8
Hexaenes	1.0	1.2	0.3	2.9	4.6	2.8

* % of total radioactive fatty acids

proportional radioactivity of fatty acid fractions.

Table 4 shows the distribution of radioactivity in the individual fatty acids constituting PL and NL from *P. monodon* and *P. merguiensis*. As for *P. monodon*, in both PL and NL radioactivity was exclusively associated with palmitic acid (16: 0), palmitoleic acid (16: 1), stearic acid (18: 0), and oleic acid (18: 1ω9) but slightly with C_{18}–C_{22} acids with double bonds at ω3 and ω6 positions 12 hr after injection of acetate-1-^{14}C. The proportional radioactivity in both PL and NL from *P. monodon* decreased in 16: 0 and 18: 0 and slightly increased in 16: 1, 18: 1ω9, 20: 1ω9, 22: 1ω9, 20: 2ω6, 20: 4ω3+20: 3ω3, 20: 5ω3, and 22: 6ω3 between 12 and 24 hr after injection. As for *P. merguiensis*, the labelling pattern of individual fatty acids constituting PL and NL was similar to that in *P. monodon*. The results on *P. monodon* and *P. merguiensis* obtained in the present study essentially agreed with those on *P. japonicus*[5] injected with acetate-1-^{14}C.

The results of the present study indicated that 18: 2ω6, 20: 2ω6, 20: 5ω3, 22: 5ω3, and 22: 6ω3 from PL and NL gave low but significant radioactivity in both *P. monodon* and *P. merguiensis*. Accordingly, it comes into question whether these ω3 and ω6 fatty acids are synthesized *de novo* in these prawns or not. ZANDEE[13,14] has shown that the crayfish, *Astacus astacus*, and the lobster, *Homarus gammarus*, were incapable of incorporating acetate-1-^{14}C into 18: 2 and 18: 3. MORRIS and SARGENT[15] have indicated that the mysid, *Gnathophausia* sp., the decapod, *Acanthephyra purpurea*, and the euphausid, *Nematobrachion sexpinosus*, incorporated 16: 0-1-^{14}C slightly into 20: 5ω3 and 22: 6ω3 besides 16: 0+16: 1 and 18: 0+18: 1. In the prawn, *P. japonicus*[6], we have also observed a similar aspect of fatty acid biosynthesis from 16: 0-1-^{14}C to that observed in the above crustaceans by MORRIS and SARGENT[15]. The above-mentioned information suggests

Table 4. Proportional radioactivity in the individual fatty acids constituting polar and neutral lipids 12 or 24 hr after injection of acetate-1-^{14}C

Fatty acid	% Distribution of radioactivity*					
	P. monodon (12 hr)		*P. monodon* (24 hr)		*P. merguiensis* (24 hr)	
	PL	NL	PL	NL	PL	NL
14: 0	0.4	2.4	0.2	2.6	2.4	3.7
15: 0	0.3	0	0.8	0.3	0.1	0.7
16: 0	19.3	31.3	13.6	16.4	50.0	43.3
17: 0	1.1	1.2	1.4	2.1	1.2	0.3
18: 0	24.4	11.0	11.2	6.6	18.5	16.4
20: 0	4.9	1.7	0.3	0	2.0	1.0
22: 0	0	0	2.0	0	0	0
14: 1	0	0.4	0	0.6	0	0.1
16: 1	7.9	4.7	9.1	8.9	6.1	5.6
17: 1	0.6	1.2	1.8	0	0.1	1.2
18: 1ω9	29.2	22.2	37.3	31.2	10.2	14.0
20: 1ω9	0.2	3.4	9.7	10.9	2.6	1.2
22: 1ω9	0	2.0	0	3.6	0	0
18: 2ω6	0.6	1.6	0.1	1.3	0.3	0.2
20: 2ω6	0.4	9.6	1.2	2.7	5.9	4.1
20: 3ω6	0	0	1.1	0	0	0.3
20: 4ω6 20: 3ω3	0.4	0.6	7.8	0.8	0.1	0.4
22: 4ω6	2.7	0.2	0	0.6	0	0
22: 5ω6	0.2	0.5	0.4	0.5	0	0.1
18: 3ω3	0	0	0	0	0	0
18: 4ω3	0	0	0	0	0	0
20: 4ω3	0.8	0.2	0.2	0.5	0	0
20: 5ω3	0.1	2.0	0.5	2.9	0.1	3.0
22: 5ω3	0.1	1.0	0.7	3.0	0.1	0.7
22: 6ω3	0.9	2.9	1.1	4.6	0.2	2.8

* Fatty acids from neutral lipid (NL) and polar lipid (PL) fractions were subjected to argentation TLC as methylesters, and then the methylesters of saturated, monoene, diene, triene, tetraene, pentaene, and hexaene fatty acids were subjected to preparative GLC on 10% DEGS followed by radioactive measurements of trapped samples.

that ω3 highly unsaturated fatty acids might be synthesized from 18: 3ω3 by chain-elongation and successive dehydrogenation. In addition, we have shown by a series of feeding trials that the weight gain of the prawn, *P. japonicus*, was improved by the dietary supplementation of 18: 2ω6, 18: 3ω3, 20: 5ω3, and 22: 6ω3[2~4]. Considering these results, we suspect that the radioactive ω6 and ω3 fatty acids detected in the present study might be formed by the addition of radioactive C_2 units to pre-existing ω6 and ω3 acids by a mechanism similar

to that which had been reported in fish[16~19].

The present study indicates that the prawns, *P. monodon* and *P. merguiensis* lack the ability for *de novo* synthesis of 18: 2ω6 and 18: 3ω3 from acetate. Therefore, it is suggested that *P. monodon* and *P. merguiensis* propably require 18 : 2ω6, 18: 3ω3, 20: 5ω3, or 22: 6ω3 for their normal growth as does in the prawn, *P. japonicus*, and other crustaceans.

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