Biosynthesis of Fatty Acids from Palmitic Acid in the Prawn, Penaeus japonicus

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Biosynthesis of Fatty Acids from Palmitic Acid in the Prawn, *Penaeus japonicus*[*1]

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Abstract

The biosynthesis of fatty acids from palmitic acid (16: 0) was examined in the prawn, *Penaeus japonicus*, at the intermolt stage. After injection of 16: 0-1-^{14}C, polar lipids (PL) and neutral lipids (NL) were isolated from the whole body of *P. japonicus*, and the proportional radioactivity of individual fatty acids constituting PL and NL was determined by preparative gas-liquid chromatography followed by radioactive measurements of the trapped samples.

In PL and/or NL, radioactivity was mainly associated with 12: 0, 14: 1, 16: 0, 18: 1ω9, and 20: 1ω9 but not or scarcely with 18: 2ω6, 18: 3ω3, 20: 5ω3, and 22: 6ω3. These results suggest that 18: 2ω6, 18: 3ω3, 20: 5ω3, and 22: 6ω3 may be essential for the prawn, *P. japonicus*.

Feeding trials using the artificial diets have shown that supplementation with linoleic acid (18: 2ω6)[1,2], linolenic acid (18: 3ω3)[1,2], eicosapentaenoic acid (20: 5ω3)[3], and docosahexaenoic acid (22: 6ω3)[4] improved the weight gain of the prawn, *Penaeus japonicus*. These foregoing studies suggest that crustaceans such as *P. japonicus* lack the ability for *de novo* synthesis of 18: 2ω6, 18: 3ω3, 20: 5ω3, and 22: 6ω3. In fact, we have demonstrated that the prawn, *P. japonicus*, incorporated the injected acetate-1-^{14}C into palmitic acid (16: 0), stearic acid (18: 0), palmitoleic acid (16:1), oleic acid (18: 1ω9), and 20: 1ω9 but not 18: 2ω6 and the ω3 series of highly unsaturated fatty acids (HUFA)[5]. These results indicate that the prawn, *P. japonicus*, lacks the capacity to introduce double bonds into the ω6 and ω3 positions. In the present study, we intend to clarify further the biosynthesis of fatty acids in the prawn using 16: 0-1-^{14}C.

Materials and Methods

Injection of 16: 0-1-^{14}C *and Extraction of Lipids*

Five specimens of the prawn, *P. japonicus*, 8.84 g in average body weight, at intermolt period, were injected with 2.5 μCi of 16: 0-1-^{14}C (Specific acitivity, 50

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mCi/mmol; Radiochemical Centre, Amersham, England) as reported previously[5] and maintained in the aquaria at 22–23°C. Lipids were extracted with chloroform-methanol-water[6] from the whole body of prawns 24 h after injection of 16: 0-^{14}C and then separated into neutral lipids (NL) and polar lipids (PL) by column chromatography on silicic acid[7].

Radioactive Measurements of Individual Fatty Acids

The incorporation of radioactivity into the individual fatty acids constituting NL and PL was examined by the same methods as described in the previous paper[5]. The fatty acids from NL and PL were converted to methylesters, and the methylesters were separated into saturated and unsaturated fatty acid methylesters with mercuric acetate[8]. The proportional radioactivity in the individual fatty acid methylesters was determined by using preparative gas-liquid chromatography (GLC)[9] on 10 % DEGS followed by radioactive measurements of the trapped samples with a Beckman liquid scintillation counter LS-230.

Results and Discussion

Table 1 shows the incorporation of radioactivity into the lipid fractions. Radioactivity was incorporated into PL highly more than into NL. In PL radioactive fatty acids were present as saturated acids rather than as unsaturated acids. On the contrary, in NL radioactive unsaturated acids were more abundant than saturated acids. Table 2 shows the proportional radioactivity in the individual fatty acids constituting NL and PL. In PL, about 80 % of radioactivity was associated with 12: 0, 16: 0, 14: 1, 16: 1, and 18: 1ω9, and no or extremely low radioactivity was recovered in 18: 2ω6, 18: 3ω3, 20: 5ω3, 22: 6ω3, and other C_{20} and C_{22} acids except 22: 5ω3. In NL, radioactivity was also associated mainly with 16: 0, 14: 1, 18: 1ω9, and 20: 1ω9 but not or scarcely with 18: 2ω6,

Table 1. Incorporation of radioactivity into the lipid fraction after injection of palmitic acid-1-^{14}C

Lipid fraction	Weight (g)	Radioactivity (dpm×10^3)	% Incorporation*
Fresh weight of prawns	44.2	—	—
Total lipids	0.420	4760	86.5
Polar lipids (PL)	0.270	3660	66.5
Saturated fatty acids	—	2210	40.2
Unsaturated fatty acids	—	1440	26.2
Neutral lipids (NL)	0.150	1100	20.0
Saturated fatty acids	—	392	7.1
Unsaturated fatty acids	—	708	12.9

* Five prawns were injected with palmitic acid-1-^{14}C (0.5 μCi/5 μl of ethanol×5). Lipid fractions were isolated from the whole body of prawns 24 h after injection.

Table 2. Proportional distribution of radioactivity in the individual fatty acids constituting polar and neutral lipids

Fatty acid	Lipid fraction			
	Polar lipid		Neutral lipid	
	% Distribution	(dpm×10³)*	% Distribution	(dpm×10³)*
12: 0	20.6	(799)	1.7	(18.7)
14: 0	0.3	(11.6)	0.9	(10.0)
15: 0	2.7	(104)	1.9	(20.6)
16: 0	30.3	(1174)	22.6	(251)
17: 0	0.3	(13.4)	4.5	(49.8)
18: 0	2.7	(103)	3.7	(41.3)
14: 1	5.2	(202)	29.1	(324)
16: 1	14.5	(562)	—	
16: 2	3.2	(142)	4.7	(52.8)
18: 1ω9	10.2	(394)	7.2	(79.6)
18: 2ω6	0.1	(0.9)	0.2	(2.0)
18: 3ω3	0		0	
18: 4ω3 20: 0	3.0	(118)	2.3	(25.3)
20: 1ω9	0.1	(0.3)	4.8	(53.3)
20: 2ω6 20: 3ω9	0.2	(7.7)	8.3	(92.6)
20: 3ω3 20: 4ω6	0.1	(3.6)	4.4	(48.4)
20: 5ω3	0.6	(22.5)	1.1	(11.9)
22: 1ω9	0.1	(2.2)	0.4	(4.2)
22: 4ω6	0		0	
22: 4ω3	0		0	
22: 5ω6	0		0	
22: 5ω3	5.4	(209)	1.3	(14.6)
22: 6ω3	0.2	(8.1)	1.0	(11.2)

* Roman numerals in parentheses indicate total radioactivity recovered in each fatty acid.

18: 3ω3, 20: 5ω3, and 22: 6ω3. In PL and/or NL, low but significant radioactivity was detected in the ω3 HUFA such as 20: 3ω3+20: 4ω6, 20: 5ω3, 22: 5ω3, and 22: 6ω3. We assume that the radioactive ω3 HUFA might be formed by the addition of radioactive C_2-units, which were produced during β-oxidation, to pre-existing C_{18} and C_{20} acids with double bonds at the ω6 and ω3 positions by the same mechanism as demonstrated in fish[10~13] and crustaceans[14]. Also, the conglomerates of 18: 4ω3+20: 0 from NL and PL and 20: 2ω6+20: 3ω9 from NL gave some radioactivity. The radioactive labelling of these fatty acid fractions is probably due to 20: 0 and 20: 3ω9. However, there is a small

possibility that the pre-existence of ω3 HUFA in the prawn tissues may have inhibited the conversion of 16: 0-^{14}C to ω3 HUFA, and also that the *de novo* synthesis of ω3 HUFA may take place in the prawn at other molting stages. In the previous study[5], we have shown that the fatty acid-synthesizing ability from acetate in the prawn, *P. japonicus*, did not vary markedly. Also, feeding experiments on the prawn, *P. japonicus*, and other crustaceans have revealed that the absence of 18: 2ω6[1], 18: 3ω3[1,15,16], 20: 5ω3[3], and 22: 6ω3[4] resulted in the reduction of weight gain. Considering these results, we conclude that the prawn, *P. japonicus*, elongates and/or desaturates 16: 0 to 16: 1, 18: 0, 18: 1ω9, 20: 0, and 20: 1ω9 but almost lacks the ability to introduce double bonds into the ω6 and ω3 positions of C_{18}, C_{20}, and C_{22} acids. Essentially similar results have also been shown for the *de novo* synthesis of fatty acids from acetate-1-^{14}C in the crayfish, *Astacus astacus*[17], the lobster, *Homarus gammarus*[18], and the prawn, *P. japonicus*[5].

In connection with the results of feeding trials and tracer experiments, 18: 2ω6, 18: 3ω3, 20: 5ω3, and 22: 6ω3 are deduced to be essential for the prawn, *P. japonicus*, and probably for other crustaceans as well.

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