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Biosynthesis of Fatty Acids from Acetate in the Prawn, *Penaeus japonicus*

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Abstract

The biosynthesis of fatty acids from acetate-1-^{14}C was investigated in a prawn, *Penaeus japonicus*, at molting stages C, D_0 and D_1'. After injection of acetate-^{14}C, saturated and unsaturated fatty acid fractions were isolated from the whole body of prawn, and the proportional radioactivity of individual fatty acids was measured by using preparative gas-liquid chromatography on 10% DEGS.

In every stage, radioactivity was mainly associated with palmitic (16: 0), stearic (18: 0), palmitoleic (16: 1), oleic (18: 1ω9), and 20: 1ω9 acids, whereas linoleic (18: 2ω6), linolenic (18: 3ω3), eicosapentaenoic (20: 5ω3) and docosahexaenoic (22: 6ω3) gave extremely low radioactivity. These results suggest that linoleic, linolenic and ω3-long chain polyunsaturated acids may be essential for the prawn, *P. japonicus*.

During the course of investigation of the nutritional lipid requirements of a prawn, *Penaeus japonicus*, the authors have demonstrated that the addition of linoleic (18: 2ω6) and linolenic (18: 3ω3) acids to a basal diet containing oleic acid (18: 1ω9) as a sole lipid source, improved the growth of the prawn[1]. Also, feeding trials to examine the nutritive values of various lipids have shown that lipids containing large amounts of ω3-series of polyunsaturated fatty acids give a high body-weight gain[2]. These results suggest that the variable effects of lipid supplementation in the diet for *P. japonicus* are due mainly to the difference in the fatty acid composition of dietary lipids, and also that linoleic, linolenic, eicosapentaenoic (20: 5ω3) and docosahexaenoic (22: 6ω3) acids may not be formed from lower compounds such as acetate in this prawn.

The purpose of the present study is to clarify the types of fatty acids originating by *de novo* synthesis. In this approach, the incorporation of acetate-1-^{14}C into the individual fatty acids was examined by using prawns at several molting stages.

Materials and Methods

Prawn, molting stage and injection of acetate-1-^{14}C The prawns, *P. japonicus*, 8.0–9.8 g in body weight, were divided into three groups according to the molting stages determined by the methods of DRACH and coworker[3] and COGNIE[4]:

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group 1, stage C (intermolt); group 2, stage D_0 (the beginning of premolt); group 3, stage D_1' (early premolt). The prawns in each group were injected with an aqueous solution of sodium acetate-1-^{14}C (40–60 mCi/mmol; Radiochemical Centre, Amersham, England) into the muscle at the base of the legs, and then maintained in an aquarium at 20°C for 24 hr as shown in Table 1.

Table 1. Injection of acetate-1-^{14}C into the prawn.

Dosage and holding period	Molting stage		
	C	D_0	D_1'
Number of prawns	2	5	2
Fresh weight (g)	19.6	40.5	15.9
Acetate-1-^{14}C injected (μCi)	10.0	22.0	10.0
Holding period (hr)	24	24	24

Extraction of lipids and separation of fatty acids into saturated and unsaturated fatty acids From the prawns injected with acetate-^{14}C, lipids were extracted with chloroform-methanol-water[5], saponified with 10% potassium hydroxide in ethanol at 80°C for 1 hr, and then fatty acids were obtained from the saponification mixture in the usual manner. The fatty acids were converted to fatty acid methylesters with 3% hydrogen chloride in methanol, and the fatty acid methylesters were separated into saturated and unsaturated fractions with mercuric acetate according to the method of GOLDFINE and BLOCH[6].

Measurement of proportional radioactivity in the individual fatty acids The saturated and unsaturated fatty acid methylesters were separately subjected to preparative gas-liquid chromatography (GLC)[7] under the following conditions: column, a stainless-steel column (3 m×4 mm i.d.) packed with 10% DEGS on 60–80 mesh Shimalite W; column temperature, 190°C; flow rate of nitrogen, 40 ml/min. For measurements of radioactivity present in the individual fatty acid methylesters after separation by GLC, the effluent was subdivided (1: 10) and samples were trapped in counting vials with chloroform at room temperature. Radioactivity was measured with a Beckman liquid scintillation counter LS-230 using a toluene solution of 0.6% PPO as a scintillator.

Results and Discussion

The literature shows that crustaceans are capable of synthesizing some fatty acids and unsaponifiable matter from acetate, although they lack the ability for sterol synthesis[8]. Also, O'CONNOR and GILBERT[9] have pointed out that lipid metabolism in crustaceans varies with the molting stages. However, most investigations on fatty acid synthesis in crustaceans have been carried out using individuals without speci-

Table 2. Weight and radioactivity of the lipid fractions isolated from the prawns after injection of acetate-l-^{14}C.

Lipid fraction	Weight (mg)			Radioactivity (cpm × 10^4)		
	Molting stage			Molting stage		
	C	D_0	D_1'	C	D_0	D_1'
Total lipids	172	408	132	137	270	138
(% Incorporation)				(6.85)	(6.13)	(6.90)
Saturated fatty acid ME*	38.8	55.1	23.4	28.7	56.5	19.0
(% of total fatty acid ME)				(76.9)	(55.9)	(64.6)
Unsaturated fatty acid ME	57.0	93.1	38.4	8.6	44.5	10.4
(% of total fatty acid ME)				(23.1)	(44.1)	(35.4)

* Methylesters

fying the molting stage, and results only contain limited information concerning the types of fatty acids derived from *de novo* synthesis.

In the present study, the biosynthesis of fatty acids from acetate was investigated by using prawns at specified stages in the molting cycle. Table 2 shows the incorporation of injected acetate-^{14}C into the lipid fractions. Acetate-^{14}C was incorporated into the lipids in every stage examined, and the percentage incorporation of acetate-^{14}C into the lipids did not vary so markedly with the molting stages. The radioactive lipids were found to be present as saturated fatty acids rather than unsaturated ones in every stage, but the ratio of both the fatty acid fractions differed with the molting stages; the formation of unsaturated fatty acids appeared to proceed more actively at stages D_0 and D_1' than at stage C.

Table 3 shows the proportional radioactivity of individual fatty acids constituting the saturated and unsaturated fatty acids. In the saturated fatty acids, more than 95% of radioactivity was associated with palmitic (16: 0) and stearic (18: 0) acids in every stage, and no significant radioactivity was detected in C_{20} and C_{22} acids. In the unsaturated fatty acids, palmitoleic (16: 1), oleic (18: ω9) and 20: 1ω9 acids were the prominent acids in every stage, whereas linoleic (18: 2ω6), linolenic (18: 3ω3), eicosapentaenoic (20: 5ω3) and docosahexaenoic (22: 6ω3) acids gave extremely low radioactivity.

In the unsaturated fatty acids from the prawns at stage C, the fraction corresponding to 20: 2ω6 and 20: 3ω9 in the GLC revealed significant radioactivity, and the authors suggest that radioactivity might be associated with 20: 3ω9 acid. In addition, linoleic and linolenic acids and the conglomerate of 20: 4ω6 and 20: 3ω3 from the prawns at some stages revealed low but substantial radioactivity and this suggests the possibility of *de novo* synthesis of these acids from acetate. However, radioactive labelling of the above fatty acids may be derived from the addition of C_2 units, originating from radioactive fatty acids such as 16: 0 and 18: 0, to pre-existing C_{18} and C_{20} acids with double bonds at ω6 and ω3 positions as pointed out in the fish[10-13]

Table 3. Proportional radioactivity in the individual fatty acids constituting the saturated and unsaturated fatty acids.

Fatty acid	% Distribution of radioactivity		
	Molting stage		
	C	D_0	D_1'
Saturated acid			
14: 0	3.2	2.6	2.4
15: 0	0.6	0.2	1.1
16: 0	64.5	72.1	71.2
17: 0	0.9	0.2	1.4
18: 0	30.7	25.0	23.9
Unsaturated acid			
14: 1 / 15: 1	12.2	0.7	6.5
16: 1	16.6	27.9	53.2
16: 3	2.4	4.2	9.5
18: 1ω9	37.6	47.1	9.9
18: 2ω6	0.9	1.8	4.7
18: 3ω3	1.6	0.9	0.1
18: 4ω3	—	0.8	—
20: 1ω9	14.4	5.6	9.4
20: 2ω6 / 20: 3ω9	7.1	2.3	1.3
20: 4ω6 / 20: 3ω3	2.6	2.4	1.3
20: 5ω3	0.9	1.2	0.9
22: 1ω9	0.7	2.0	0.4
22: 4ω6	0.9	0.9	0.9
22: 4ω3	0.2	—	—
22: 5ω6	0.4	0.5	0.6
22: 5ω3	0.8	0.7	0.6
22: 6ω3	0.7	0.7	0.2

Each fatty acid was isolated by preparative GLC and radioactivity was measured with a liquid scintillation counter. The purity (%) of isolated fatty acids determined by the GLC was as follows: 16 : 3, 94; 18: 1ω9, 89; 18: 2ω6, 88; 18: 3ω3, 92; 20: 5ω3, 90; 22: 6ω3, 92.

and crustaceans such as the mysid, *Gnathophausia* sp.[14], decapod, *Acanthephyra purpurea*[14], and euphausid, *Nematobrachion sexspinosus*[14]. However, this assumption should be confirmed by appropriate experiments in the future.

In addition, the present study shows that the proportional radioactivity of individual fatty acids making up the unsaturated fatty acids, especially 16: 1 and 18: 1ω9, varies with the molting stage, indicating that *de novo* fatty acid-synthesizing ability in the prawn, *P. japonicus*, differs with the molting stage.

Considering the above data, we conclude that in the prawn, *P. japonicus,* fatty acid synthesis from acetate proceeds up to C_{20} monoenoic (probably $\omega 9$) acid, and that the successive introduction of double bonds into the $\omega 6$ and $\omega 3$ positions hardly occurs. ZANDEE has also obtained essentially the same results on the fatty acid synthesis from acetate-^{14}C in the crayfish, *Astacus astacus*[15], and the lobster, *Homarus gammarus*[16]. Therefore, these results taken in conjunction with the feeding trials for *P. japonicus* suggest that linoleic, linolenic, and $\omega 3$-long chain polyunsaturated fatty acids may be essential for marine crustaceans.

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