Fatty Acid Metabolism in the Calanoid Copepod *Paracalanus parvus:* 1. Polyunsaturated Fatty Acids

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ABSTRACT

The metabolic fate of radioactive linoleate and α-linolenate administered to the South Atlantic copepod *Paracalanus parvus* was studied. The wild copepod was able to incorporate the labeled acids dissolved in seawater. The radioactive linoleate was elongated to 20:2ω6 and 22:2ω6 and desaturated by a Δ6 desaturase to 18:3ω6. α-Linolenate was also desaturated by a Δ6 desaturase to 18:4ω3 and elongated to 20:3ω3. The copepod was able to convert α-18:3 to 20:5ω3 and 22:6ω3.

INTRODUCTION

Phytoplankton is the first link of the food chain in the sea. Some algae are able to synthesize de novo the polyunsaturated acids 20:5ω3 and 22:6ω3 (1,2) that are preferentially found in marine animals instead of 20:4ω6 acid that is found predominantly in land animals. We have shown in previous work (3) that the phytoplanktofagous mollusc *Mesodesma mactroides* is not only able to incorporate polyunsaturated acids of phytoplankton, but also to convert linoleic, and α-linolenic acid to higher members of the families. Therefore, these animals and others may contribute to modify the primary fatty acid composition synthesized by the phytoplankton.

Zooplankton is an important link in the marine food chain and calanoid copepods especially are crucial, since they are phytoplanktofagous and the major food of a variety of fish. For this reason, we have decided to study the capacity of *Paracalanus parvus* to convert linoleic and α-linolenic acids to highly polyunsaturated acids. *P. parvus* is a cosmopolitan calanoid copepod, abundant in Argentine sea waters. It largely modifies the amount and composition of the fatty acids with the ecological conditions (4).

MATERIALS AND METHODS

Materials

The acids [1-^{14}C] linoleic (56 mCi/mmol) and [1-^{14}C] α-linolenic (57 mCi/mmol) were provided by Amersham-Searle (Amersham, England). The radiochemical purity was higher than 99%.

Organisms

Calanoid copepods *P. parvus* of 0.75-0.80 mm were collected in September, in shore, in Mar del Plata, Buenos Aires Province, Argentina, with zooplankton nets (160-180 μ mesh). In the laboratory they were suspended in fresh, sterile sea water and filtered through a thicker sieve to eliminate larger organisms than this copepod. They were concentrated on the surface of the water by light attraction, filtered through a 160-180 μ mesh and washed with sterile sea water. The sample of *P. parvus* so obtained was more than 90% pure. The organisms were then transferred to the incubation solution.

Incubation

The incubation solution was prepared with sterile synthetic sea water and the ammonium salt of the radioactive fatty acids. The concentration of the fatty acids in the medium was 0.06-0.10 μM.

One gram (wet weight) of the wild *P. parvus* was incubated in 2400 ml of incubation solution at 15 ± 2 C. A stream of air was gently passed through the solution. After determined periods of time, the copepods were collected in a net and repeatedly washed with fresh synthetic sea water until the washings were free of radioactive material. The copepods were then dried on filter paper and the lipids extracted with chloroform-methanol (2:1 v/v) (5). The lipids were weighed and the radioactivity counted in a Packard scintillation counter. They were saponified, the nonsaponifiable extracted, the aqueous-alcoholic solution acidified and the free acids were taken in petroleum ether. The free acids were esterified with

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TABLE I

Radioactivity Incorporated in the Lipids of *P. parvus* after Incubation with [1-^{14}C] Linoleic and [1-^{14}C] α-Linolenic Acids[a]

Substrate	Radioactivity			
	Fatty acids	Nonsaponifiable	Water soluble products	Nonidentified
[1-^{14}C] 18:2ω6	74.8	18.2	1.0	6.0
[1-^{14}C] 18:3ω3	73.2	18.5	1.2	7.1

[a]The copepods were incubated during 5 hr. Results represent the percent distribution of the radioactivity in the total lipids extracted with $CHCl_3$-CH_3OH (2:1 v/v). They are the mean of two incubations of ca. 1 g copepods.

TABLE II

Radioactivity Distribution in *P. parvus* Fatty Acids after Incubation with [1-^{14}C] Linoleic Acid[a]

Fatty acids	Time of incubation (hr)		
	1 hr	5 hr	12 hr
14:0	---	0.6 ± 0	---
16:0 + 16:1	0.8 ± 0.1	4.0 ± 0.6	3.3 ± 0.1
18:0 + 18:1	2.3 ± 0.2	0.8 ± 0.2	1.5 ± 0.1
18:2ω6	86.5 ± 0.5	85.3 ± 1.0	86.1 ± 0.9
18:3ω6	1.5 ± 0.1	1.8 ± 0.4	1.6 ± 0.3
20:0	1.3 ± 0.1	---	---
20:1	0.9 ± 0.1	---	1.7 ± 0.2
X	1.9 ± 0.1	---	---
20:2ω6	1.0 ± 0.1	4.6 ± 0.8	4.1 ± 0.1
20:3ω6	1.5 ± 0.1	0.7 ± 0.1	---
22:2ω6	2.3 ± 0.5	2.1 ± 0.3	1.7 ± 0.2
Total incorporation in the lipids (%)	4.1 ± 0.5[b]	9.3 ± 0.6	12.2 ± 1.8

[a]Results represent the present distribution of the radioactivity in the fatty acids. They are the mean of the analysis of two samples of 1 g copepods ± extreme values. The chromatogram was run until 22:6 ω3 peak.

[b]The radioactivity measured in the medium before incubation was considered 100%.

methanol and sublimated (6).

The distribution of radioactivity in the fatty acid methyl esters was determined by gas liquid radiochromatography in a Pye apparatus equipped with a proportional counter as described previously (3).

RESULTS AND DISCUSSION

Radioactivity Incorporation in the Lipids

The incorporation of the radioactivity of the fatty acids in the lipids of *P. parvus* is summarized from Tables I to III. After 1 hr incubation, 4.1 to 4.2% of the radioactivity of [1-^{14}C] linoleic and [1-^{14}C] α-linolenic acids was incorporated in the lipids of *P. parvus*. These figures increased with time, reaching 12.2% and 13.2%, respectively, after 12 hr. Therefore, the wild copepod is able to absorb fatty acids dissolved in the sea water and incorporate them to the lipids. Table I shows that after 5 hr, more than 70% of the incorporated radioactivity was present in the fatty acids and the rest in the nonsaponifiable, water soluble compounds and unidentified products. Therefore, although the majority of the labeling remained in the substrate and the fatty acids derived from the substrate, part of the linoleic and α-linolenic acids was metabolized. A substantial proportion of the [^{14}C] of both substrates went to the nonsaponifiable fraction. Unfortunately, this fraction was lost, and we could not determine if the radioactivity was present in wax esters, alcohols or other similar products that have been found in copepods (7). However, previously we found that samples of *P. parvus* collected in July and October contained little wax esters (4). Therefore, the labeled nonsaponifiable might represent another lipid, but this is improbable.

Biosynthesis of Fatty Acids of Linoleic Acid Family

The distribution of the radioactivity in the

TABLE III

Labeling Distribution in *P. parvus* Fatty Acids after Incubation with [1-^{14}C]α-Linolenic Acid[a]

Fatty acids	Time of incubation (hr)	
	1 hr	5 hr
14:0	---	0.9 ± 0
16:0 + 16:1	0.7 ± 0.3	5.9 ± 0.5
18:0	0.3 ± 0.1	0.9 ± 0.1
18:1	0.2 ± 0.1	1.6 ± 0.2
18:2ω9	0.2 ± 0.1	---
18:3ω3	90.2 ± 1.1	78.2 ± 1.2
18:4ω3	2.4 ± 0.1	2.1 ± 0.1
X	1.7 ± 0.1	---
20:3ω3	3.4 ± 0.5	5.7 ± 0.3
20:4ω3	0.9 ± 0.1	0.6 ± 0.1
20:5ω3	---	1.9 ± 0.3
22:3ω3	---	0.8 ± 0.2
22:4ω3	---	0.5 ± 0.1
22:5ω3	---	0.3 ± 0
22:6ω3	---	0.6 ± 0.1
Total incorporation in the lipids (%)	4.2 ± 0.2[b]	10.4 ± 1.5

[a]Results represent the percent distribution of the radioactivity in the fatty acids. They are the mean of the analysis of two samples of 1 g copepods ± extreme values. The chromatogram was run until 22:6ω3 peak.

[b]The radioactivity measured in the medium before incubation was considered 100%.

fatty acids of *P. parvus* after the incubation of the copepod with [1-^{14}C]linoleic acid is illustrated in Table II. The total radioactivity incorporated in lipids increased with the incubation time, but most of this increase occurred between 1 and 5 hr. Therefore, since the percent distribution in the fatty acids remained rather constant from 1 to 12 hr incubation, it means that the radioactivity increased steadily in all the fatty acids of Table II. The wild organism was able to elongate and desaturate the fatty acids, but since small amounts of labeling were also found in the fatty acids: 14:0, 16:0, 16:1, 18:0, 18:1, 20:0 and 20:1, we may admit that part of the substrate was oxidized and labeled acetate so formed was used in de novo synthesis of fatty acids.

Labeled linoleic acid was converted to 18:3ω6. Therefore, we must conclude that a Δ6 desaturase is present. In addition, 20:2ω6, 20:3ω6 and 22:2ω6 acids were also labeled, suggesting the existence of elongating enzymes. Both types of enzymes are present in land animals and, together with a Δ5 desaturase that converts 20:3ω6 to 20:4ω6, contribute to the synthesis of arachidonic acid (8). However, even after 12 hr incubation, no labeling in arachidonic acid was detected. Similar results were also obtained by us when [1-^{14}C]linoleic acid was administered to the marine mollusc *Mesodesma mactroides* (3). The difficulty in synthesizing arachidonic acid in both marine animals could be attributed to the absence or very low activity of the Δ5 desaturase, since the desaturation does not go farther than 20:3ω6. However, the absence of the enzyme is apparently not admissible, since [1-^{14}C]α-linolenic acid was converted by the copepod to 20:5ω3 acid (Table III). Besides, it is known (9) that the same Δ5 desaturase converts 20:3ω6 to 20:4ω6 and 20:4ω3 to 20:5ω3. Therefore, it would be easier to admit that the absence or very low synthesis of arachidonic acid, when compared to the synthesis of 20:5ω3 shown in Table III, is apparently the consequence of low Δ5 desaturase activity and preferential inhibition of the Δ5 desaturation of fatty acid of linoleic acid family. Note that the activity of the Δ5 desaturase is lower in the fish *Pimelodus maculatus* than in the rat, whereas the activity of the Δ6 desaturase is higher (9).

Biosynthesis of Fatty Acids of α-Linolenic Family

The distribution of the radioactivity of [1-^{14}C]α-linolenic acid in the fatty acids of *P. parvus* is shown in Table III. Again the amount of the labeled acid incorporated increased with the incubation time. Some oxidation of the substrate and de novo synthesis of fatty acids took place apparently, since the radioactivity was also detected in 14:0, 16:0, 16:1, 18:0, 18:1 and 18:2ω9 peaks.

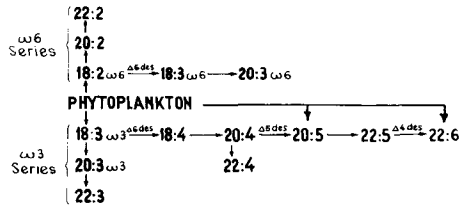

FIG. 1. Possible biosynthetic chains of the fatty acids of ω6 and ω3 families in *P. parvus*.

During 1 hr incubation, the 18:3ω3 acid apparently followed two independent biosynthetic pathways. On one side, it was elongated to 20:3ω3 and, on the other, it was converted to 18:4ω3 by a Δ6 desaturase. After this time, the biosynthetic route only reached 18:4ω3 acid step. After 5 hr incubation, the biosynthesis went farther on synthesizing the acids of ω3 family: 20:5, 22:3, 22:4, 22:5 and 22:6. Therefore, it is possible to deduce that the wild *P. parvus* has the full array of enzymes, Δ6, Δ5 and Δ4 desaturases as well as the elongating systems, recognized in the mammals to synthesize 20:5ω3 and 22:6 ω3 from 18:3ω3 (Figure 1). However, it was apparently some catabolism of administered [1-^{14}C]α-linolenic acid to [1-^{14}C]acetate which, in turn, was used to synthesize 14:0, 16:0, 18:0, and 18:1 acids. Therefore, it could be expected that labeling of polyunsaturated fatty acids of 20 and 22 carbons could be produced by biosynthesis from this [^{14}C]acetate. The same reasoning could be applied to the polyunsaturated acids 20:2ω6, 20:3ω6, and 22:2ω6 biosynthesized when [1-^{14}C]linoleate was administered. However, the different labeling patterns produced from [1-^{14}C]linoleate compared to [1-^{14}C]α-linolenate seem to indicate the desaturation and elongation of exogenous linoleate and α-linolenate. This would confirm the lack of label in the polyunsaturated fatty acids of 20 and 22 carbons following administration of [1-^{14}C]acetate (10).

The biosynthetic route outlined in Figure 1 has been also suggested for other marine animals. Morris et al. (11) showed the biosynthesis of 20:5ω3 and 22:6ω3 in *Neomysis integer* after the administration of labeled 18:3ω3 acid. Moreno et al. (3) also showed that the clam *M. mactroides* converts 18:3ω3 to 18:4ω3 and 20:3ω3 acids. Kayama et al. (12), after [1-^{14}C]18:3ω3 injection to the fish *Paralabrax clathratus* suggested that 20:5ω3 and 22:6ω3 biosynthesis was produced by the same pathways found in the rat (8,13,14). Besides, the properties of the Δ6 desaturase in the fish *P. maculatus* and the conversion of 18:3ω3 to 18:4ω3 and 18:2ω6 to 18:3ω6 acids have been carefully studied (9,15). Therefore, in spite of the scarce information available and the large variety of marine life, it is possible to consider that at least in many sea animals the biosynthetic chain of polyunsaturated fatty acids is the same recognized in mammals.

Since all the experiments shown in this work were not performed with axenic cultures, but with wild copepods with less than 100% purity, it is possible to discuss if the biosynthetic pathways outlined in Figure 1 correspond to *P. parvus* or if they are the joined contribution of *P. parvus* and other microorganisms. This last possibility is rather unlikely since the purity of the sample was higher than 90%, and the biosynthetic routes found did not deviate from the "normal" pathways typical of animals. Therefore, if some other microorganisms also contributed to the results, this contribution would be very small, and it would not invalidate the conclusions.

The presence of 18:3ω3 in diatoms and other components of phytoplankton constituting *P. parvus* food is not very high, whereas 20:5ω3 and 22:6ω3 acids are rather abundant in the same food sources (1,2,4). Therefore, although the copepod has the capacity to synthesize its own 20:5ω3 and 22:6ω3 acids from α-linolenate, the predominance of these fatty acids in the copepod composition (4) is apparently mainly due to the food composition (Fig. 1). In the last instance, some unicellular components of phytoplankton for still unknown reasons, require and synthesize de novo 20:5ω3 and 22:6ω3 acids.

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