Fatty Acid Metabolism in Young Oysters, *Crassostrea gigas:* Polyunsaturated Fatty Acids

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ABSTRACT

Tetraselmis suecica and *Dunaliella tertiolecta* were grown for 24 hr in the presence of ^{14}C sodium bicarbonate and then fed separately to batches of juvenile oysters, *Crassostrea gigas,* for 3 days. *D. tertiolecta* contained fatty acids no longer than C_{18}; 22:6ω3 was absent in *T. suecica.* Analysis of the oyster fatty acids by radio gas chromatography (GC) showed that oysters were able to incorporate some of the dietary ^{14}C label into long-chain fatty acids not supplied in the diet, e.g., C_{20} and C_{22} mono- and polyunsaturated fatty acids, and particularly 20:5ω3. However, the low ^{14}C incorporation into fatty acids longer or more unsaturated than those supplied in the diet suggests that elongation and desaturation activity in young oysters is not sufficient to sustain optimum growth.
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INTRODUCTION

Dietary ω3 long-chain polyunsaturated fatty acids (PUFA), e.g., 20:5ω3 and 22:6ω3, are essential for the optimum growth of several marine fish (1-4), and for penaeid prawns (5-9). However, freshwater fish, e.g., rainbow trout and carp, can biosynthesize the long-chain C_{22} PUFA from shorter chain precursors and may be cultured successfully on diets containing C_{18} ω3 and ω6 fatty acids (10-12). Further studies on fish and prawns (13,14) have confirmed that many freshwater species have active fatty acid elongation and desaturation systems, whereas marine species, especially those high in the food chain, e.g., turbot and plaice (13), have a reduced ability to synthesize, from shorter chain precursors, the important C_{22} ω3 long-chain fatty acids by desaturation and elongation.

In a series of studies on the bivalve clam, *Mesoderma mactroides,* Moreno et al. (15,16) have shown that linoleic acids, 18:2ω6, and α-linolenic acid, 18:3ω3, can be desaturated and to some extent elongated to higher homologues, e.g., 20:2ω6, 18:4ω3 and 20:3ω3, but no biosynthesis of the longer and more unsaturated acids such as 20:4ω6, 20:5ω3 and 22:6ω3 occurred.

In this laboratory, the studies on juveniles of another bivalve, the Pacific oyster, *Crassostrea gigas,* have shown that dietary requirements exist for ω3 long-chain PUFA when animals are actively growing (17). Thus, when 20:5ω3 and 22:6ω3 were absent in the algal diets, the growth of juvenile oysters was poor and endogenous PUFA were rapidly depleted. Growth was improved when the algal diets were supplemented with microencapsulated ω3 C_{22} PUFA. Whether or not the ability to elongate and desaturate dietary C_{18} fatty acids was low or totally absent was not conclusive.

Because bivalves can directly incorporate all labeled algal fatty acids into tissue lipids (15) and because, in this laboratory, routine cultures of *Dunaliella tertiolecta* contain fatty acids no longer or more unsaturated than 18:3ω3 and cultures of *Tetraselmis suecica* contain no trace of 22:6ω3, this provided the basis for an in vivo study on the capability of young oysters to synthesize C_{20} and C_{22} ω3 PUFA from dietary algal fatty acids. *T. suecica* and *D. tertiolecta*, preincubated with ^{14}C bicarbonate to label the fatty acids, were fed to juvenile *Crassostrea gigas* at a stage when young oysters are still actively accumulating lipid in the body tissues (18) and the incorporation of radiolabel into tissue fatty acids was determined by radio gas chromatography (GC).

MATERIALS AND METHODS

Materials

^{14}C labeled sodium bicarbonate was obtained from Amersham International, Amersham, U.K.

Labeling Algal Fatty Acids

Nonaxenic cultures of *D. tertiolecta* and *T. suecica* were grown in 20 l borosilicate flasks under continuous illumination (from 10,000 lux, daylight fluorescent lamps at 18 C) in filtered seawater, salinity 32‰, enriched with Conway medium (19). The cultures were gently agitated by aeration and harvested during the growth phase on a semicontinuous basis.

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Two hundred ml of each algal culture were taken during the growth phase and placed in 2 l conical flasks containing 200 ml filtered, UV-irradiated seawater, salinity 32‰, enriched with Conway medium, and 100 μCi of ^{14}C sodium bicarbonate was added to the flask and mixed by gentle swirling. The algae were cultured at 15 C under continuous illumination from 10,000 lux daylight fluorescent lamps. Each culture was left for 24 hr with occasional swirling and then fed to the spat. Samples of the labeled algae were filtered off and taken to determine the level of ^{14}C incorporation by scintillation counting.

Culture and ^{14}C Labeling of C. gigas Juveniles

Young oysters were provided by the Fisheries Experiment Station, Conway, N. Wales, U.K. In 2 separate experiments, 2 batches of young oysters were maintained in filtered and UV-irradiated sea water at a salinity of 25‰ and at 24 C ± 1 C. One batch was fed on nonlabeled *D. tertiolecta* and the other on *T. suecica* for 1 week before the labeling experiments. Algae were added at a concentration of 10^6 cells per mg of live fresh weight of oyster per week as described by Walne (20). The spat were fed twice daily and the water changed every other day. The cultures were agitated by gentle aeration (100 ml filtered air/min) to keep the algal cells in suspension. The batches of oysters were then fed on the appropriate single species of radiolabeled algae in the same way for 3 days, followed by 1 day of feeding on nonlabeled algae, to ensure that all the radiolabel was absorbed from the gut and incorporated into the body tissues. Finally, the oysters were starved for 24 hr to clear the gut of any algae. Samples of oysters were then taken from each batch, briefly washed in chloroform/methanol (2:1, v/v) to remove lipids from any attached algae on the outside of the shell and digested in a N.C.S. (Amersham International, U.K.) tissue solubilizer. The total incorporated ^{14}C radioactivity was then determined by scintillation counting using a Tracerlab Services scintillation counter.

The remainder of the batches were briefly washed in chloroform/methanol (2:1, v/v) and then homogenized for 3 min in a minimum volume of seawater in a top-drive macerator. Total lipid was extracted according to the procedure of Folch et al. (21) with 20 vol chloroform/methanol (2:1, v/v) containing 0.005% w/v of the antioxidant 2,6-di-tert-butyl-p-cresol, and finally stored in chloroform/antioxidant. The extracted lipids were then methylated with BF_3 and methanol (22). Impurities were removed from the fatty acid methyl esters by thin layer chromatography (TLC) on Merck precoated silica gel plates in a continuous elution system. Plates were developed for 90 min with petroleum ether (60-80 C)/diethyl ether/glacial acetic acid (85:15:1) at room temperature (ca. 22 C). Separation of the methyl esters and determinaton of ^{14}C incorporation was carried out on a Pye 104 gas liquid chromatograph coupled to an E.S.I. Nuclear radio gas detector. The conventional 5′ × ¼″ i.d. glass column was packed with 5% SP 1000 on Chromasorb W, AW DMCS 80-100 mesh. Argon was used as the carrier gas and the temperature program was 130-200 C at 2 C/min. Analysis of nonlabeled methyl esters was carried out using a Carlo Erba 4160 fitted with a 20 m × 0.5 mm i.d. glass capillary column coated with SP 1000. Hydrogen was used as the carrier gas and the temperature program was 130-200 C at 2 C/min.

RESULTS AND DISCUSSION

The levels of incorporation of the ^{14}C radiolabel into the dietary algae and into oyster lipid is shown in Table 1. After 3 days, ca. 10% of the ^{14}C activity in oyster tissues was in the lipid fraction.

Table 2 shows the distribution of ^{14}C label

TABLE 1

Assimilation of Radioactivity from ^{14}C Labeled *D. tertiolecta* and *T. suecica* into *C. gigas* Spat

Diet	Experiment	Total ^{14}C in the diet (dpm)	Total ^{14}C recovered in each spat (dpm)	^{14}C in oyster lipid (dpm)	Percent of total ^{14}C label incorporated by the oysters as lipid
D. tertiolecta	1	4.06×10^6	3.98×10^5	4.34×10^4	10.90
	2	6.30×10^5	1.56×10^4	1.33×10^3	8.52
T. suecica	1	6.90×10^6	2.68×10^6	2.75×10^5	10.26
	2	9.59×10^5	1.55×10^5	2.04×10^4	13.20

TABLE 2

Labeling Distribution in *C. gigas* Fatty Acids after Feeding for 3 Days on a Single Species of ^{14}C Labeled Algae

Diet	Experiment	Major fatty acid or group of fatty acids											
		14:0 14:1	16:0 16:1	16:2 16:3 16:4	18:0 18:1	18:2 18:3 18:4	20:1 20:2	20:3 20:4	20:5ω3	22:1	22:2 22:3 22:4	22:5ω3	22:6ω3
D. tertiolecta	1	3.9	21.7	11.0	19.8	28.0	6.2	6.7	0.5	1.6	nd	nd	0.3
	2	6.0	24.2	1.8	20.7	26.1	7.0	6.7	0.9	4.3	nd	nd	0.9
T. suecica	1	0.5	19.8	6.9	17.2	18.1	10.4	2.6	14.5	5.7	2.2	0.9	nd
	2	2.1	20.1	2.5	13.7	16.4	13.4	2.9	24.8	0.5	1.0	1.2	0.5

nd = not detected.
The results are expressed as the percentage of total recovered ^{14}C radiolabeled fatty acids.

TABLE 3

Fatty Acid Composition of Total Lipids of *D. tertiolecta* and *T. suecica*

Fatty acids	*D. tertiolecta*	*T. suecica*
14:0	2.1	4.2
14:1	0.2	trace
15:0	0.7	0.5
16:0	18.4	21.9
16:1ω9	0.3	2.2
16:1ω7	1.8	2.0
16:1ω5	0.5	nd
16:2ω6	1.3	0.3
16:3ω6	1.0	trace
16:3ω3	3.1	0.7
16:4ω3	13.1	13.1
17:0	0.5	nd
18:0	0.9	2.2
18:1ω9	5.0	20.8
18:1ω7	0.6	trace
18:1ω5	trace	nd
18:2ω6	7.1	1.6
18:3ω6	4.0	trace
18:3ω3	36.2	8.2
18:4ω3	nd	3.1
20:1	nd	3.0
20:2ω6	nd	trace
20:3ω3	nd	nd
20:4ω6	nd	0.8
20:4ω3	nd	1.0
20:5ω3	nd	12.6
22:1ω11+ω9+ω7	nd	nd
22:3ω3	nd	nd
22:4ω6	nd	nd
22:5ω3	nd	nd
22:6ω3	nd	nd

nd = not detected.
The results are expressed as the percentage of total fatty acids present.

in the fatty acids of juvenile oysters after feeding ^{14}C labeled *D. tertiolecta* or *T. suecica* for 3 days.

The highest proportion of ^{14}C in oyster fatty acids was in fatty acids that are also present in the dietary algae (Table 3) and were presumably incorporated directly into the lipid reserves of the oysters in a largely unaltered form, as dietary algal lipids are in the clam *M. mactroides* (15). In oysters fed *D. tertiolecta*, 84.4% and 78.8% of the ^{14}C label in fatty acids were recovered in fatty acids less than C_{18} in chain length, and only 18.4% and 15.3% in the fatty acids longer or more unsaturated than C_{18}. In oysters fed *T. suecica*, 91.3% and 98.9% of the label in fatty acids were present in fatty acids up to 20:5ω3 in chain length. Longer chain fatty acids contained only 8.8% and 3.2% of the ^{14}C label. Because *D. tertiolecta* contains fatty acids no longer or more unsaturated than 18:3ω3 and *T. suecica* contains fatty acids no longer or more unsaturated than 20:5ω3

(Table 3), the ^{14}C label in the C_{20} and C_{22} fatty acids in oysters fed *D. tertiolecta* and the C_{22} fatty acids in oysters fed *T. suecica* must have been formed by modifying dietary precursor fatty acids within the tissues of the oysters or by de novo synthesis in the case of nonessential fatty acids. Chain elongation could account for the ^{14}C label in the nondietary C_{20} and C_{22} fatty acids with the exception of 20:5ω3 and 22:6ω3 in oysters fed *D. tertiolecta* and 22:6ω3 in oysters fed *T. suecica*. Although elongation of 20:5ω3 could account for the label in 22:5ω3 in oysters fed *T. suecica*, elongation cannot account for the ^{14}C label in 20:5ω3 or 22:6ω3 when these acids are absent in the diet as a desaturation step is necessary in the biosynthesis. Less than 1% of the ^{14}C label was found in 20:5ω3 and 22:6ω3 when these fatty acids were omitted from the diet, indicating that desaturation activity is low. The improvement of oyster growth with the addition of PUFA such as 20:5ω3 and 22:6ω3 in the diet (17) does suggest that the activities of desaturation enzymes in the oyster are too low to sustain optimum growth. Young oysters grow much better on a diet of *T. suecica*, which contains appreciable levels of the PUFA 20:5ω3, one of the ω3 PUFA so important in marine animals, than on *D. tertiolecta* (17), which contains no trace of the ω3 C_{20} or C_{22} PUFA. The fatty acid composition of the algae is shown in Table 3.

In mature oysters, *Crassostrea virginica*, Trider and Castell (23) found that better weight gain was achieved by feeding cod-liver oil containing ω3 fatty acids than by feeding ω6 fatty acids in the form of corn oil and concluded that oysters appear to have a major dietary requirement for ω3, and possibly a minor requirement for ω6, PUFA. However, they did not indicate whether or not these fatty acids should be C_{18} or C_{20}/C_{22} PUFA.

In Moreno's experiments with copepods (24), after 5 hr incubation with [1-^{14}C] α-linolenic acid, the ^{14}C label in 20:5ω3 and 22:6ω3 was only 1.9% and 0.6%, respectively, of the total label recovered in the fatty acids; other ω3 PUFA contained significantly less than 1% of the ^{14}C label. These figures are comparable with those in Table 2 for ^{14}C labeled 20:5ω3 and 22:6ω3 in oysters fed *D. tertiolecta*, i.e., synthesized from shorter chain precursors. On the other hand, in the clam, *M. mactroides*, Moreno et al. (15) detected no ^{14}C activity at all in 20:5ω3 or 22:6ω3 after administering 18:3ω3 for 12 hr. In Moreno's experiments, the clams were placed in a synthetic seawater medium containing dissolved radiolabeled [1-^{14}C] linoleic and [1-^{14}C] α-linolenic acid, supplying the ^{14}C label directly in the diet for 72 hr as in the experiments described in this paper, may provide a reason for the differences between clams and oysters. Nevertheless, the oyster, *C. gigas*, has some ability to elongate and desaturate ω3 fatty acids to produce 20:5ω3 and 22:6ω3, whereas this activity is lacking in the clam, *M. mactroides*. All this points the way for further studies on fatty acid desaturase and elongation enzymes in bivalves and marine invertebrates in general.

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