Effects of Growth Temperature on the Fatty Acid Composition of the Free-Living Nematode Caenorhabditis elegans

Tamotsu Tanaka*a, Kouji Ikitaa, Tetsuji Ashidaa, Yoshiaki Motoyamaa, Yasunori Yamaguchib, and Kiyoshi Satouchia

aDepartment of Food Science and Technology and bDepartment of Biotechnology, Fukuyama University, Fukuyama 729-02, Japan

ABSTRACT: The effects of growth temperature on the fatty acid compositions of the phosphatidylcholine (PC), phosphatidylethanolamine (PE), and total lipid (TL) fractions of the free-living nematode Caenorhabditis elegans were investigated. A reduction in growth temperature from 25 to 15°C caused the proportions of eicosapentaenoic acid (20:5n-3) to increase from 23.6 to 32.5% in the PC, from 7.4 to 10.8% in the PE, and from 12.9 to 19.9% in the TL fractions. Conversely, the levels of dihomoy-linolenic acid (20:3n-6) and arachidonic acid (20:4n-6) in these phospholipid fractions and the TL fraction both decreased with decreasing growth temperature. Analysis of the positional distribution of fatty acids in the PC fraction revealed that the change in the composition of C20 polyunsaturated fatty acid was obvious in position sn-2. Lowering the growth temperature induced an increase in the level of the diacyl subclass of PE from 58% at 25°C to 71% at 15°C, with a concomitant decrease in the levels of the alkylacyl and alkenylacyl subclass of PE of C. elegans. These changes observed in the phospholipids of C. elegans might be one mechanism for adaptation to low temperature.


The free-living nematode Caenorhabditis elegans has been the subject of a number of recent biochemical studies such as investigations of behavioral genetics and experimental gerontology. Because of its small size and the fact that it is a self-fertilizing hermaphrodite, C. elegans can be easily grown under laboratory conditions with Escherichia coli as food. Using monoxenic culture conditions, there is no possibility for involvement of intermediate organisms between the nematodes and their food. This offers an advantage in the study of lipid metabolism of this multicellular organism.

Previously, we examined the phospholipid composition and fatty acid compositions of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of C. elegans and found that the nematode contained both n-3 and n-6 series polyunsaturated fatty acid (PUFA), and that eicosapentaenoic acid (EPA) (20:5n-3) was the most abundant fatty acid in the PC fraction (1). The abundance of EPA in the total lipids of C. elegans was also shown by Hutzell and Krusberg (2). In addition, the free-living nematode species Turbatrix aceti and the entomopathogenic nematode Steinernema carpocapsae have been shown to contain EPA and to have the ability to synthesize PUFA de novo (3-5).

Several lower classes of organisms have been reported to produce EPA de novo and accumulate a large amount of EPA to adapt to a decrease in environmental temperature. Shimizu et al. (6) showed that several fungal microorganisms produced a large amount of EPA only when grown at low temperature. The increased level of EPA at lower growth temperature was also observed in the Vibrio species of bacterium isolated from fish intestine (7) and the nematode S. carpocapsae (5).

In the present study, we examined the effects of temperature on the fatty acid compositions and subclass compositions of phospholipids of C. elegans. The results showed that the levels of EPA, the most abundant and the most unsaturated fatty acid in C. elegans, were increased in the PC, PE, and total lipid (TL) fractions when the nematodes were cultured at low temperature. Furthermore, lowering the growth temperature induced an increase in the level of the diacyl subclass of PE of C. elegans.

MATERIALS AND METHODS

Materials. Agar, peptone, tryptone, and yeast extract were obtained from Difco Laboratories (Detroit, MI). Glycerol and precoated thin-layer plates (Silica gel 60) were from Merck (Darmstadt, Germany). Silic AR (100-200 mesh) was the product of Mallinckrodt (Paris, KY). Phospholipase A2 (EC 3.1.1.4, from Crotaulus adamanteus venom) was from Sigma Chemical Co. (St. Louis, MO).

Growth of E. coli and lipid extraction. Escherichia coli (OP50 strain) was grown in a phosphate buffer (pH 7.4) with tryptone, glycerol, and yeast extract at 37°C. Escherichia coli from late-logarithmic cultures was centrifuged at 5,000 × g for 10 min. The pellet of E. coli was resuspended in S. medium (8) and stored at 4°C until use. For lipid analysis,
E. coli which had been stored at 4°C was homogenized by grinding in a mortar with a pestle in a small amount of chloroform/methanol mixture (1:2, vol/vol). The lipids were extracted from the homogenate according to the method of Bligh and Dyer (9).

**Growth of C. elegans.** The standard wild-type strain N2 of C. elegans was grown at temperatures (±0.5°C) of 15, 20, and 25°C on 90-mm nematode growth medium (NGM) agar plates seeded with E. coli as described previously (1). NGM agar plates were prepared as follows: NaCl, 3 g; agar, 17 g; peptone, 5.0 g; cholesterol (5 mg/mL in ethanol), 1 mL and H2O, 975 mL were mixed, then autoclaved, and 1 M CaCl2, 0.5 mL; 1 M MgSO4, 1 mL and 1 M potassium phosphate (pH 6.0), 25 mL were mixed. The CaCl2, MgSO4, and potassium phosphate solutions were autoclaved separately before addition. Upon observation under the microscope, the worms on plates rich with adult worms were washed off the plates with M9 buffer (8). The suspension of bacteria and nematodes was centrifuged at 170 × g for 2 min to pellet the nematodes. The supernatant solution was discarded and the pelleted nematodes were repeatedly washed with M9 buffer. The packed worms were suspended with a small amount of M9 buffer, layered over 35% sucrose solution and centrifuged at 1,800 × g for 5 min. Escherichia coli and dead worms were sedimented, whereas live C. elegans floated on the sucrose layer. The live C. elegans was withdrawn and repeatedly washed with M9 buffer to remove sucrose. In another experiment, C. elegans was cultured at 25°C until the plates were rich with adult worms on NGM agar plates with E. coli. Half of the nematodes were then exposed to 15°C for 5 h before harvesting, and the other half of the nematodes were harvested without low temperature treatment. This shift in temperature was achieved within 30 min.

**Lipid extraction and fractionation.** Purified live C. elegans was homogenized by grinding in a mortar with a pestle in a small amount of chloroform/methanol mixture (1:2, vol/vol). TL were extracted from the homogenate according to the method of Bligh and Dyer (9) as described previously (1). The lipid extracts were fractionated into the neutral lipid fraction and the phospholipid fraction using silic AR column chromatography (1). The triglyceride (TG) from the phospholipid fraction was prepared from the neutral lipid fraction by preparative thin-layer chromatography (1). The PC and PE fractions were prepared by eluting with small amount of chloroform/methanol mixture (1:2, vol/vol). The lipids were extracted from the homogenate according to the method of Bligh and Dyer (9) as described previously (1).

**Fatty acid analysis.** Fatty acyl residues of PC, PE, and TG of the C. elegans were converted to fatty acid methyl esters with 5% methanolic HCl and analyzed by GC (Shimadzu 14A; Shimadzu, Kyoto, Japan) equipped with a capillary column fused with a 0.25 μm film of polar CBP20 (0.22 mm × 30 m; Shimadzu). The temperature of both the injector and the flame-ionization detector was 250°C. The initial column temperature was set at 170°C and then raised to 225°C at 5°C/min. The fatty acid methyl esters obtained from the total lipid extracts of C. elegans were purified before GC by TLC using the solvent system petroleum ether/diethyl ether/acetic acid (80:30:1, by vol) to remove unsaponifiable materials. Fatty acid methyl esters were identified by comparing their retention times with those of authentic standards as described previously (1).

**RESULTS**

**Effects of growth temperature on fatty acid composition of C. elegans.** Because the standard wild-type strain N2 of C. elegans can be grown at temperatures between 12 and 25°C monoxenically on E. coli as a food source (8), we chose temperatures of 15, 20, and 25°C for growth of C. elegans in this study. The culture times of nematodes were 9–10 d, 7–8 d, and 6–7 d at 15, 20, and 25°C, respectively. The fatty acid compositions of the PC, PE, and TL fractions of C. elegans grown at these temperatures are shown in Table 1. Growth temperature affected the proportions of C20 PUFA in the PC, PE, and TL fractions of C. elegans. In the PC fraction, the level of EPA was increased from 23.6% at 25°C to 32.5% at 15°C. Conversely, dihomo-γ-linolenic acid (DGLA) (20:3n-6) and arachidonic acid (AA) (20:4n-6) in the PC fraction of C. elegans were both decreased with lowered growth temperature. Although the extent of the changes was smaller than that in the PC fraction, the increased level of EPA and the decreased level of AA were also observed in the PE and