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Zebrafish cDNA Encoding Multifunctional Fatty Acid Elongase Involved in Production of Eicosapentaenoic (20:5n-3) and Docosahexaenoic (22:6n-3) Acids

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Abstract: Enzymes that increase the chain length of fatty acids are essential for biosynthesis of highly unsaturated fatty acids. The gLELO gene encodes a protein involved in the elongation of polyunsaturated fatty acids in the fungus *Mortierella alpina*. A search of the GenBank database identified several expressed sequence tag sequences, including one obtained from zebrafish (*Danio rerio*), with high similarity to gLELO. The full-length transcript ZfELO, encoding a polypeptide of 291 amino acid residues, was isolated from zebrafish liver cDNA. The predicted amino acid sequence of the open reading frame shared high similarity with the elongases of *Caenorhabditis elegans* and human. When expressed in *Saccharomyces cerevisiae*, the zebrafish open reading frame conferred the ability to lengthen the chain of a range of C_{18}, C_{20}, and C_{22} polyunsaturated fatty acids, indicating not only that biosynthesis of 22:6n-3 from 18:3n-3 via a 24-carbon intermediate is feasible, but also that one elongase enzyme can perform all three elongation steps required. The zebrafish enzyme was also able to elongate monounsaturated and saturated fatty acids, and thus demonstrates a greater level of promiscuity in terms of substrate use than any elongase enzyme described previously.

Key words: PUFA-elongase, saturated, monounsaturated, polyunsaturated fatty acids, zebrafish.

INTRODUCTION

The long-chain highly unsaturated fatty acids (HUFAs) arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) are essential for normal cellular function, being important constituents of membrane phospholipids, affecting membrane fluidity, and the activity of membrane proteins involved in transport, signal transduction, and various enzymic reactions (Macmurchie, 1988). DHA and AA have important roles in the development and function of neural tissues (Innis et al., 1999), and EPA and AA are precursors of eicosanoids, which modulate many physiological processes including reproduction, hemostasis, and inflammation (Weber, 1990). Increased dietary levels of the n-3HUFAs, EPA and DHA, have significant beneficial health effects in relation to cardiovascular, immune, and inflammatory conditions (Knapp, 1999, 2001), and the importance of maintaining a balanced dietary intake of n-6 and n-3 HUFA for human health is well established (British Nutrition Foundation, 1992).

The only major source of n-3HUFA in the human diet is fish and seafood, and generally declining catches from

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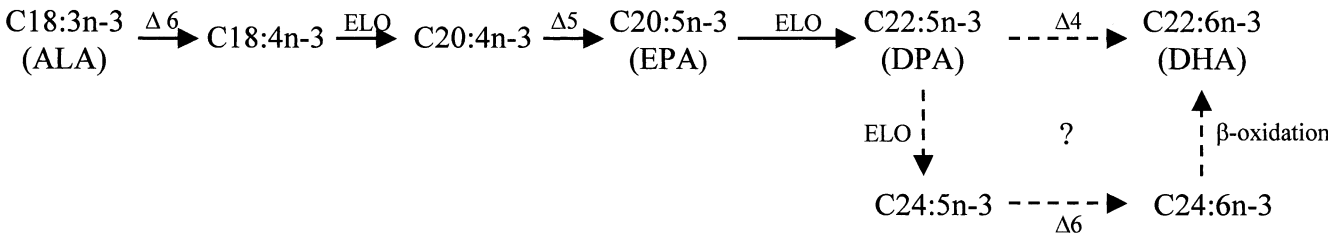

Figure 1. Schematic of DHA (22:6n-3) biosynthesis from α-linolenic acid (18:3n-3; ALA) in vertebrates. DHA is biosynthesized by sequential desaturation and elongation (ELO) of ALA. Δ4, Δ5, and Δ6 are desaturations at the respective chain positions. The broken lines represent the alternative routes to DHA postulated by Sprecher et al. (1995).

wild fisheries have resulted in an increasing proportion of fish for human consumption being provided by aquaculture (Tidwell and Allan, 2002). However, much aquaculture, including salmonid (salmon and trout) culture, is itself dependent upon wild capture fisheries for the provision of fish meals and oils that have traditionally been predominant protein and lipid sources (Sargent and Tacon, 1999). However, stagnation in industrial fisheries, along with increased demand for fish oils, has dictated that alternatives to fish oil must be found if aquaculture is to continue to expand and supply more of the global demand for fish (Barlow, 2000). The only sustainable alternative to fish oils are plant (vegetable) oils that are rich in C_{18} polyunsaturated fatty acids (PUFAs), such as 18:2n-6 and 18:3n-3, but devoid of the n-3HUFAs abundant in fish oils (Sargent et al., 2002).

The extent to which animals, including fish, can convert C_{18} PUFA to $C_{20/22}$ HUFA varies with species and correlates with their complement of active microsomal fatty acyl desaturase and elongase enzymes. Thus, EPA is synthesized from 18:3n-3 by desaturation at the Δ6 position, followed by a 2-carbon elongation, which is in turn followed by a further desaturation at the Δ5 position (Figure 1) (Cook, 1996). Synthesis of DHA requires further elongation and desaturation of EPA, which has been suggested to proceed via a C_{24} intermediate (Sprecher et al., 1995). However, there is variation among vertebrate species in the ability to synthesize HUFA from the C_{18} PUFA. In herbivores, whose diet is essentially deficient in HUFA, requirements for HUFA must be met by endogenous biosynthesis via desaturation and elongation of the copious C_{18} PUFA in their plant diet. Carnivores, however, are generally dependent on a dietary supply of HUFA, which may be a response to a diet rich in HUFA but relatively poor in C_{18} PUFA (Rivers et al., 1975, 1976). This spectrum of HUFA biosynthesis ability may be reflected in, and possibly caused by, genetic variation in structure and function of genes encoding the key enzymes in the HUFA biosynthesis pathway. Such variation certainly occurs, as exemplified by the fact that both Δ5 and Δ6 fatty acid desaturase activities in zebrafish (*Danio rerio*) are properties of a single polypeptide product of one gene (Hastings et al., 2001). This is unlike the case in other systems studied in which the two desaturase activities are encoded by disparate genes (Michaelson et al., 1998; Napier et al., 1998; Aki et al., 1999; Cho et al., 1999a, 1999b; Watts and Browse, 1999; Leonard et al., 2000a).

Elongation of fatty acid, whether saturated, monounsaturated, or polyunsaturated, involves 4 enzymic steps (Figure 2). The first step essentially effects chain elongation by condensation of the activated fatty acid with malonyl-CoA (Cinti et al., 1992). This step is rate-limiting, and operates on substrates specified by a putative elongase polypeptide. The subsequent 3 steps are in essence the reverse of β-oxidation. Biochemical studies have suggested that different elongase enzymes are involved in the elongation of saturated and unsaturated fatty acids (Sprecher, 1974; Prasad et al., 1986) and that there may be different enzymes catalyzing the elongation of $C_{18/20}$ PUFA and C_{22} PUFA (Luthria and Sprecher, 1997). Recently, enzymes catalyzing the elongation of C_{18} PUFA have been cloned from the fungus *Mortierella alpina* (Parker-Barnes et al., 2000), the nematode *Caenorhabditis elegans* (Beaudoin et al., 2000), and humans (Leonard et al., 2000b). Variation in HUFA biosynthesis may also operate at the elongation steps, as supported by the low $C_{18/20}$ elongase activity in the fish species turbot (*Scophthalmus maximus*) (Ghioni et al., 1999), a carnivorous marine teleost that requires a dietary supply of HUFA for normal growth (Bell et al., 1985).

In this report we describe an elongase (zfELO), cloned on the basis of sequence similarity to existing PUFA elongases, from zebrafish (*Danio rerio*), a model fish species that we have previously shown to possess a high capacity to biosynthesize DHA, and thus to thrive on a diet largely

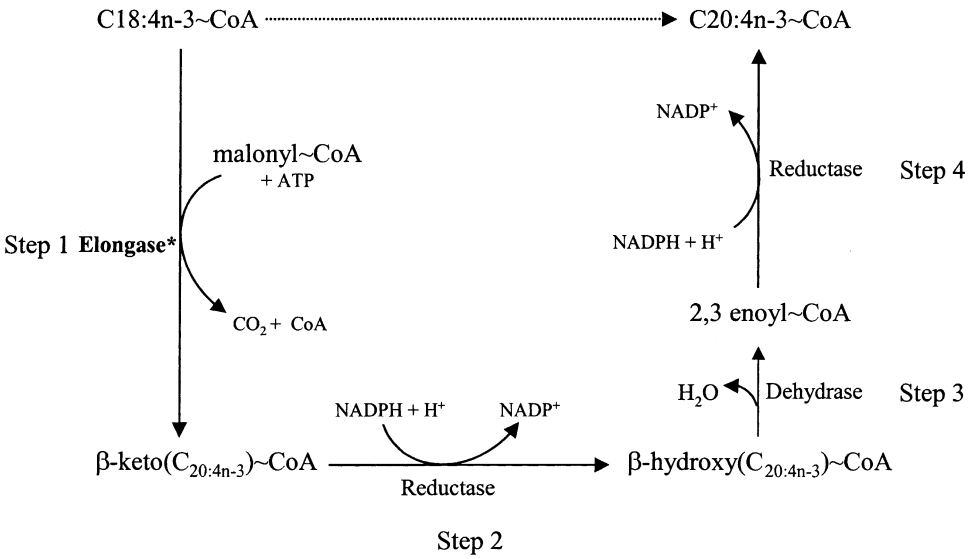

Figure 2. Microsomal elongation of PUFA. PUFA elongation is a multistep process. Step 1, which effects chain elongation through the condensation of a fatty acyl-CoA with malonyl-CoA, is rate-limiting and substrate-specific as directed by the elongase polypeptide. Steps 2, 3, and 4 are acyl-reductive processes.

devoid of HUFA (Tocher et al., 2001). The zebrafish enzyme showed broad substrate specificity, elongating C_{18}, C_{20}, and C_{22} PUFA, indicating that only one elongase enzyme is necessary for the production of DHA in zebrafish. The zebrafish enzyme also elongated both saturated and monounsaturated fatty acids.

Materials and Methods

Cloning of the PUFA Elongase cDNA

The PUFA elongase sequence (AF206662) of *M. alpina* was used to query the GenBank EST database (at NCBI) for homologues using the tblastn program. Several vertebrate ESTs, including one from zebrafish (GeneBank accession number BF157708), were identified that had high similarity to the query sequence. The zebrafish EST sequence was used to design primers for 5′ RACE and 3′ RACE cloning of the full-length transcript from zebrafish liver cDNA using the SMART RACE system (Clontech Laboratories Inc.). PCR fragments were cloned into the Bluescript SKII vector, and the sequences were determined by standard dye terminator chemistry (PerkinElmer, Applied Biosystems) following the manufacturer's protocols. Sequence analysis was performed using the EBI and NCBI suites of software.

Heterologous Expression of Elongase ORFs in Yeast

The open reading frame (ORF) was amplified from zebrafish liver cDNA. The sequence of the sense primer ZfEloXA (CGGAATTCAAGCTT**AAGATG**GAGACGTTTAGTCA) contains an *Eco*RI site (underlined) and a Kozak translation initiation sequence (boldface). The antisense primer ZfEloXB (CTCTAGACTCGAGTCAATCTGCTCGTGCTTTTCT) contains an *Xho*I site (underlined). Polymerase chain reaction (PCR) was performed using high-fidelity DNA polymerase mix (Roche Diagnostics Ltd.) following the manufacturer's instruction. After PCR the DNA fragments were restricted with *Eco*RI and *Xho*I, ligated into a similarly treated yeast expression vector pYX222 (R & D Systems), which was then used to transform Top10 *E. coli* competent cells. Transformation of yeast with recombinant ZfELO-pYX222 plasmids, yeast culture, and fatty acid analysis were performed as described previously (Hastings et al., 2001). Triplicate cultures of recombinant yeast were grown in *Saccharomyces cerevisiae* minimal medium$^{-\text{histidine}}$ (SCMM$^{-\text{his}}$) supplemented with one of the following PUFA substrates: stearidonic acid (18:4n-3), γ-linolenic acid (18:3n-6), 20:5n-3, 20:4n-6, 22:5n-3, and 22:4n-6. Approximately equal amounts of yeast cells were transferred into glass conical test tubes after determination of culture densities at OD_{600}. The cells were collected by centrifugation at 500*g* for 2 minutes, and the pellets were washed twice with 5 ml of ice-cold Hanks balanced salt solution and dried under a stream of oxygen-free nitrogen (OFN).

Fatty Acid Analysis

Fatty acid methyl esters (FAMEs) were prepared by incubating the dried yeast cells directly with 1 ml of methylation reagent containing 10% (v/v) concentrated HCl, 5% (v/v) 2,2-dimethoxypropane, and 85% (v/v) dry methanol

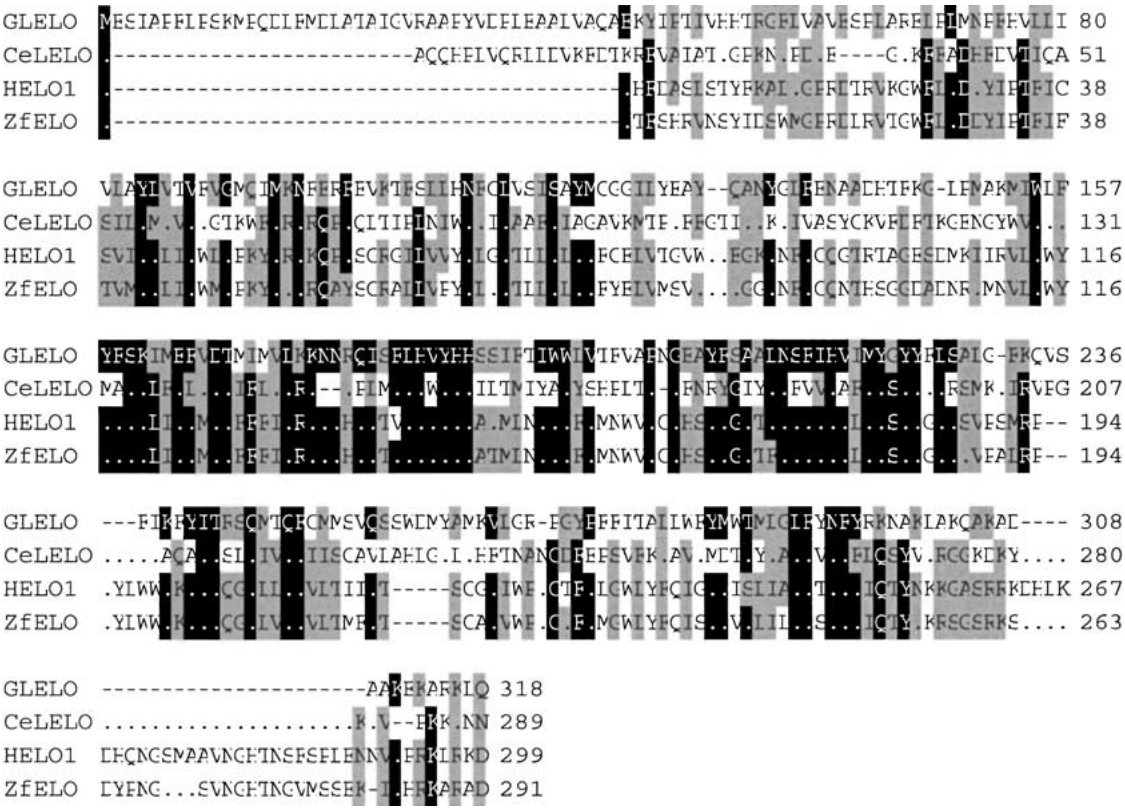

Figure 3. Comparison of the amino acid sequences of PUFA elongases cloned from *Mortierella alpina* (gLELO), *Caenorhabditis elegans* (CeLELO), *Homo sapiens* (HELO1), and zebrafish (*Danio rerio*) (ZfELO). Identical residues are shaded black and similar residues are gray. The threshold for similarity shading was set at 75%.

for 1 hour at 85°C. After incubation, FAMEs were extracted by the addition of 1 ml of 1% NaCl solution and 0.5 ml of hexane containing 0.01% butylated hydroxytoluene as antioxidant. The mixture was vigorously mixed and centrifuged at 600*g* for 5 minutes to promote phase separation. The top phase was carefully removed and filtered through Whatman No. 1 filter paper into a clean glass test tube, and the solvent was evaporated under a stream of OFN. The FAMEs were purified by thin-layer chromatography and then resuspended in hexane, all as described previously (Tocher and Harvie, 1988). They were separated in a Fisons GC8160 gas chromatograph equipped with a chemically bonded CP Wax 52CB fused silica wall coated capillary column (30-m × 0.32-mm i.d., Chrompack U.K. Ltd.) with an on-column injection system and flame ionization detection. Hydrogen was used as carrier gas with an oven thermal gradient from an initial 50°C to 180°C at 40°C/min, and then to a final temperature of 235°C at 2°C/min. Individual FAMEs were identified by comparison with known standards, with a well-characterized fish oil, and by reference to published data, as described previously (Tocher and Harvie, 1988). FAMEs were quantified using a directly linked PC operating Chrom-Card Software (Thermo-Quest Italia S.P.A.). The proportion of substrate fatty acid converted to the longer chain fatty acid product was calculated from the gas chromatograms as 100× [Product Area/(Product Area + Substrate Area)]. All solvents contained 0.01% butylated hydroxytoluene as an antioxidant.

Gas Chromatography–Mass Spectrometry

The identities of fatty acids and positions of their double bonds were confirmed by subjecting the picolinyl esters to electron ionization (EI) GC-MS. Free fatty acids were prepared from FAME by alkaline hydrolysis as described by Christie (1982). Picolinyl esters were prepared by the method of Balazy and Nies (1989). This involves activating the free fatty acid by reaction with 1,1′-carbonyldiimidazole to form the imidazolide, which then reacts with 3-(hydroxymethyl)pyridine under basic conditions to form the picolinyl ester. GC-MS of the picolinyl esters was performed using a Fisons GC8000 gas chromatograph coupled to an MD800 mass spectrometer (Fisons Instruments). The

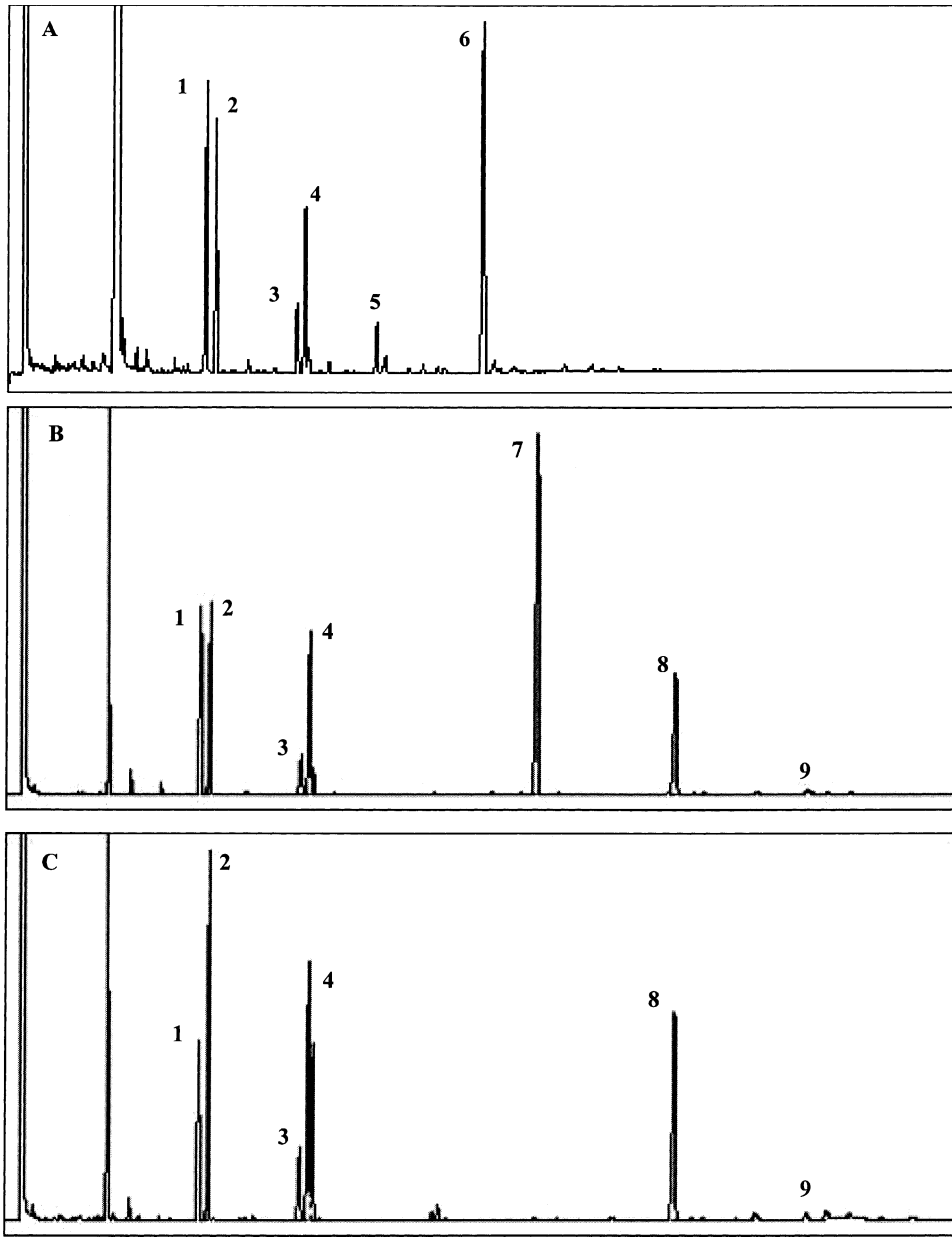

Figure 4. Identification of fatty acid elongation products in transgenic yeast (*Saccharomyces cerevisiae*) by gas chromatography. The x-axis represents retention time (full scale = 65 minutes) and the y-axis represents detector response in arbitrary units. Fatty acids were extracted from yeast transformed with ZfELO-pYX222 grown in the presence of 18:4n-3 (**A**), 20:5n-3 (**B**), 22:5n-3 (**C**). The first 4 peaks in the panels are the main endogenous fatty acids of *S. cerevisiae*, namely, 16:0 (1), 16:1n-7 (2), 18:0 (3), and 18:1n-9 (4). The other peaks in each panel are the exogenously added substrate fatty acids and the resultant elongated products, namely, 18:4n-3 (5), 20:4n-3 (6), 20:5n-3 (7), 22:5n-3 (8), and 24:5n-3 (9).

gas chromatograph was equipped with a fused silica capillary column (60-m × 0.32-mm i.d, 0.25-mm internal film thickness) coated with Zebron ZB-Wax (Phenomenex) and used helium as carrier gas. Samples were applied using on-column injection with the oven temperature programmed to rise from 80°C to 250°C at 40°C/min.

Results

The translated amino acid sequence of *M. alpina* elongase was used to interrogate the GenBank EST nucleotide sequence database. This identified homologous sequences involved in PUFA chain elongation from mammals (*Homo sapiens* and *Mus musculus*), birds (*Gallus domesticus*), amphibians (*Xenopus* spp.), and fish (*Danio rerio*). The full-length PUFA elongase cDNA sequence from zebrafish (zfELO; GenBank accession number AF532782) translated to a polypeptide of 291 amino acid residues, the sequence of which showed varying degrees of similarity with the sequences of elongases of other organisms, e.g., *C. elegans* (41%), *M. alpina* (48%), and *Homo sapiens* (87%) (Figure 3).

High-fidelity PCR was used to amplify the coding DNA sequence (CDS) of the elongase gene using zebrafish liver cDNA as template. After cloning into the pYX222

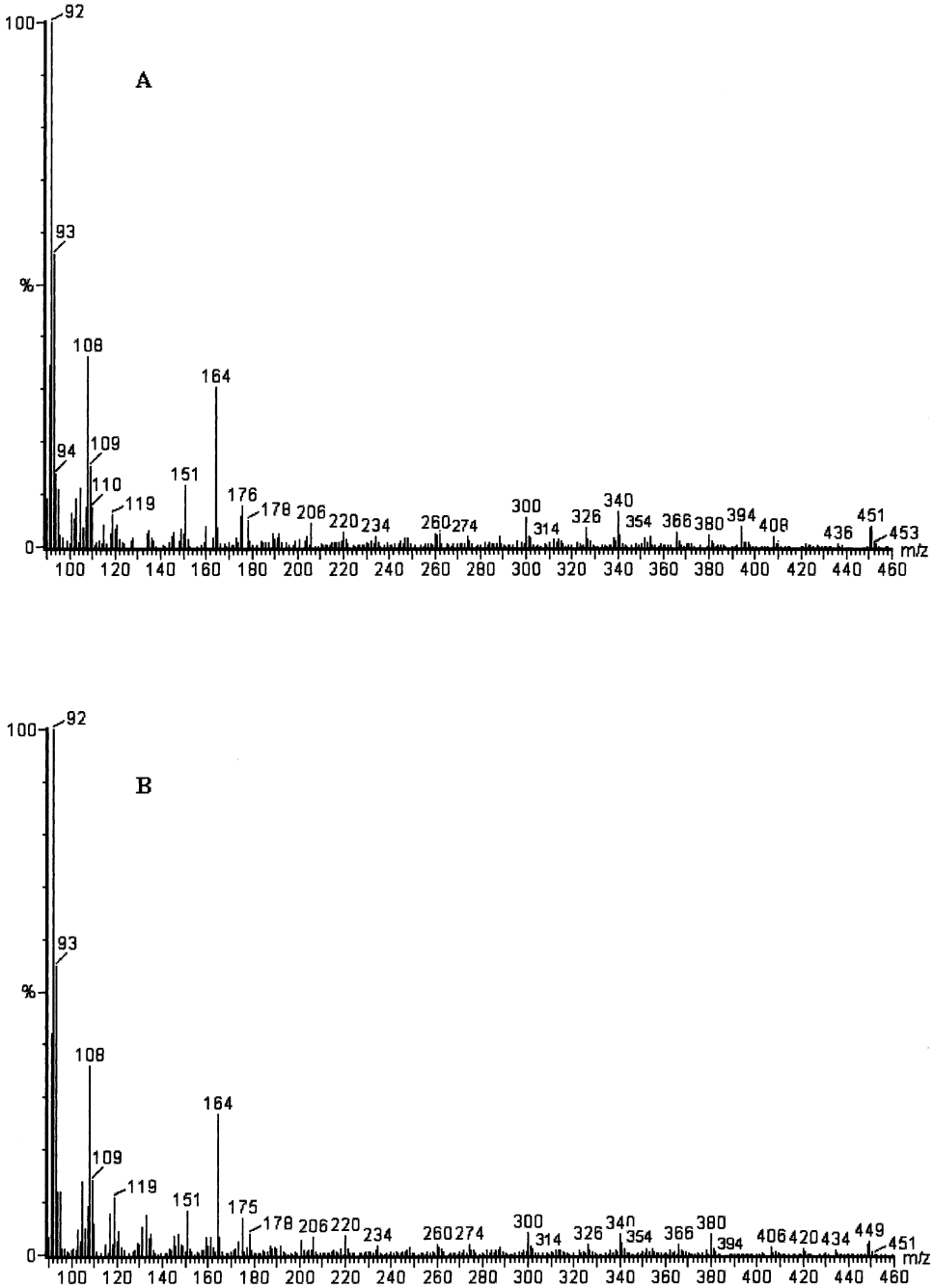

Figure 5. Mass spectra of fatty acids (peaks) produced by yeast transformed with the ZfELO-pYX222 plasmid and grow in the presence 22:4n-6 (**A**) or 22:5n-3 (**B**). The x-axis represents mass (*m*/*z*) and the y-axis represents detector response as a percentage of total response. Picolinyl esters were prepared from fatty acid methyl esters derived from fatty acids extracted from the yeast and analyzed by GC-MS as described in the "Methods" section. The mass spectra confirmed the product fatty acid identities as 24:4n-6 and 24:5n-3.

plasmid, the identity of the CDS was confirmed by sequencing before transforming yeast. Heterologous expression of the zebrafish elongase ORF in *S. cerevisiae* in the presence of exogenous 18:4n-3 confirmed that the cDNA encoded a polypeptide that is a component of the PUFA chain elongation system of zebrafish (Figure 4, A). Several PUFAs were subsequently investigated as potential substrates, with all 6 fatty acids tested converted to the respective elongation products by recombinant yeast as follows: 18:4n-3 → 20:4n-3, 18:3n-6 → 20:3n-6, 20:4n-6 → 22:4n-6, 20:5n-3 → 22:5n-3, 22:5n-3 → 24:5n-3, and 22:4n-6 → 24:4n-6 (Figure 4, A–C). The identities of elongation products were confirmed by comparison with known standards and GC-MS of picolinyl esters. For example, the mass spectra for the products of elongation of C_{22} PUFA are shown in Figure 5. The samples all showed prominent ions at *m*/*z* = 92, 108, 151, and 164, which are characteristic of picolinyl esters representing fragments about the pyridine ring (Figure 5) (Christie, 1998). The EI spectra of the additional fatty acid in ZfELO-pYX222-

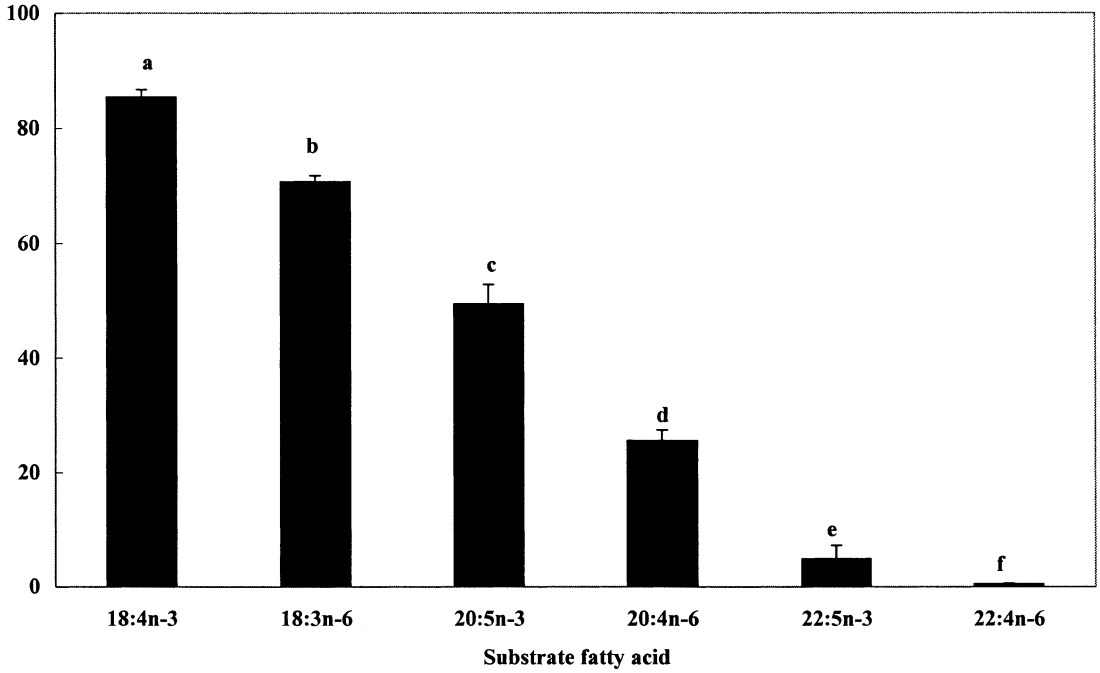

Figure 6. Relative activity of zebrafish elongase (ZfELO) toward various polyunsaturated fatty acids. The activity of ZfELO was assessed by gas chromatographic analysis of the fatty acid composition of yeast (*S. cerevisiae*) cells containing the ZfELO-pYX222 plasmid grown in the presence of 0.5 mM 18:4n-3, 18:3n-6, 20:5n-3, 20:3n-6, 22:5n-3, and 22:4n-6. The conversion rate was calculated as 100 × [Product Area/(Product Area + Substrate Area)]. Results are expressed as means ± SD ($n = 4$). Different letters indicate that means are significantly different as determined by 1-way ANOVA followed by Tukey's multiple comparison test.

transformed yeast incubated with 22:4n-6 showed a fragmentation pattern with a mass ion of 451 *m/z* and peaks at 436, 380, 354, 340, 314, 300, 274, 260, and 234 *m/z* (Figure 5, A). The initial interval of 15 (451 to 436) represented the terminal methyl and was followed by 4 intervals of 14 (436 to 380), indicating 4 methylene groups. The intervals of 26 (380 to 354, 340 to 314, 300 to 274, and 260 to 234) denoted the positions of 4 double bonds, indicating that this fatty acid is $^{\Delta 18,15,12,9}$24:4 = 24:4n-6 (Figure 5, A). The EI spectra of the additional fatty acid from cells incubated with 22:5n-3 showed a mass ion of 449 *m/z* and fragments at 434, 420, 394, 380, 354, 340, 314, 300, 274, 260, and 234 *m/z*. The initial interval of 15 (449 to 434) represented the terminal methyl and was followed by an interval of 14 (434 to 420), indicating one methylene group. The intervals of 26 (420 to 394, 380 to 354, 340 to 314, 300 to 274, and 260 to 234) denoted the positions of 5 double bonds, confirming this fatty acid as $^{\Delta 21,18,15,12,9}$24:5 = 24:5n-3 (Figure 5, B).

Generally, the efficiency of zfELO for the different PUFA substrates, as measured by the relative conversion, decreased with the substrate chain length: that is, $C_{18} > C_{20} > C_{22}$ (Figure 6). In addition, n-3 PUFAs were more efficiently converted than the n-6 PUFAs of the same chain length. Thus, the zebrafish elongase converted 85% of 18:4n-3 to 20:4n-3 compared to 70% of 18:3n-6 converted to 20:3n-6.

By comparing the fatty acid profiles of the recombinant yeast carrying the elongase with that of yeast carrying only the pYX222 plasmid, it was clear that endogenous fatty acids in the yeast were also elongated as follows: 16:0 → 18:0; 16:1n-7 → → → 18:1n-7 → 20:1n-7; and 18:1n-9 → 20:1n-9 (Table 1). This indicates that the zebrafish elongase is also active on saturated and monounsaturated fatty acid substrates, albeit with lower efficiency than with PUFA substrates. Significant activity was also observed on 18:3n-3, which was converted to eicosatrienoic acid (20:3n-3), a so-called dead-end product (data not shown).

Discussion

Here we report the sequence and function of a cDNA, zfELO, derived from a gene that is responsible for the chain elongation of a range of PUFA in zebrafish, a model freshwater fish species. The cDNA encodes a protein that is

Table 1. Fatty Acid Compositions (percentage of total fatty acids by weight) of *S. cerevisiae* Transformed with Zebrafish Elongase (ZfELO) or Empty Vector (control)[a]

Fatty acid	Control	ZfELO
14:0	1.0 ± 0.6	1.3 ± 0.4
15:0	0.6 ± 0.4	0.7 ± 0.1
16:0	25.3 ± 1.7	22.0 ± 1.2*
18:0	9.5 ± 0.4	14.8 ± 3.1*
Total saturated	36.3 ± 2.2	38.8 ± 2.6
16:1n-7	35.2 ± 1.5	25.3 ± 4.0*
18:1n-9	27.2 ± 3.6	27.0 ± 1.4
18:1n-7	1.1 ± 0.3	8.2 ± 1.5*
20:1n-9	ND	0.5 ± 0.2*
20:1n-7	ND	0.4 ± 0.2*
22:1n-9	ND	ND
22:1n-7	ND	ND
24:1n-9	ND	ND
Total monoenes	63.2 ± 2.0	61.3 ± 2.6
18:2n-6	Trace	Trace

[a]Results are means ± SD ($n = 4$). Where indicated (*), mean values for fatty acids in yeast transformed with ZfELO are significantly different from control as determined by the Student t test ($P < 0.05$). ND, not detected; trace, < 0.05%.

similar to mammalian elongases, and has all the main structural characteristics possessed by elongases derived from other systems. These include the predicted transmembrane domains, the so-called histidine box (HXXHH) and the canonical C-terminal endoplasmic reticulum (ER) retention signal.

Functional characterization has previously been reported for PUFA elongases of nematode (*C. elegans*), fungus (*M. alpina*), rat, and human, with all 4 enzymes being predominantly active on C_{18} PUFA (Beaudoin et al., 2000; Leonard et al., 2000b; Parker-Barnes et al., 2000; Inagaki et al., 2002). This was also the case with zebrafish elongase, the enzyme achieving 85% conversion of 18:4n-3 substrate and 70% of 18:3n-6. Because of the high activity of the zebrafish elongase toward C_{18} PUFA, the highest activity observed, we suggest that the primary role of zfELO is as a PUFA elongase involved in the production of HUFA from C_{18} PUFA. The zebrafish enzyme also had substantial C_{20} PUFA elongase activity, converting some 46% and 26% of 20:5n-3 and 20:4n-6, respectively, to the respective C_{22} products. This is similar to human (ELOVL5) and rat elongases (rELO1), which also have high activity on 20:5n-3 and 20:4n-6 (Leonard et al., 2000b; Inagaki et al., 2002), but in contrast to the elongases of *M. alpina* and *C. elegans*, which show virtually no activity toward C_{20} PUFA (Beaudoin et al., 2000; Parker-Barnes et al., 2000). However, in contrast to the previously reported human and rat elongases described above, the zebrafish elongase also displayed the capacity to elongate C_{22} PUFA, converting about 5% of 22:5n-3 to 24:5n-3 in the recombinant yeast system studied.

That zebrafish elongase can chain elongate C_{18}, C_{20}, and C_{22} PUFA is significant for two reasons. The first is simply the fact that 22:5n-3 is elongated to tetracosapentaenoic acid (24:5n-3). Direct synthesis of DHA from 22:5n-3 would require desaturation at the Δ4 position. While a Δ4 desaturase has been described in the marine microheterotroph *Thraustochytrium* sp. (Qui et al., 2001), desaturation of PUFA at the Δ4 position has not been conclusively demonstrated in any vertebrate system. Moreover, a search of the human genome sequence using the sequence of the *Thraustochytrium* sp. Δ4 desaturase as query returned no positive result. The work of Sprecher and colleagues indicated that synthesis of DHA in rat liver proceeded via C_{24} PUFA intermediates through two sequential elongations of 20:5n-3 to produce 24:5n-3, which is then desaturated to 24:6n-3 before being chain shortened to 22:6n-3 (Sprecher et al., 1995). Therefore, 24:5n-3 is an important intermediate in the biosynthesis of DHA in vertebrates, so its production by the action of the zebrafish elongase on either EPA or 22:5n-3 is significant.

The second reason is the zebrafish elongase is very active toward 18:4n-3, so it is possible that only this single elongase enzyme is required to perform all the elongation steps necessary for the synthesis of DHA from 18:3n-3. Furthermore, the desaturase we cloned previously from zebrafish expressed both Δ5 and Δ6 fatty acid desaturase activities (Hastings et al., 2001) and is also capable of desaturating 24:5n-3 to 24:6n-3 (Tocher et al., 2003). Therefore, zebrafish would appear to be able to perform all 6 desaturation and elongation reactions necessary for the production of DHA from 18:3n-3 using only two gene products. This is in contrast to all other vertebrate and eukaryotic systems studied, in which desaturase activities are encoded by disparate genes (Michaelson et al., 1998; Napier et al., 1998; Aki et al., 1999; Cho et al., 1999a,b; Watts and Browse, 1999; Leonard et al., 2000a).

Recently, two mammalian genes have been cloned and characterized, a further human elongase (ELOVL2) and a mouse elongase (elovl2), which were able to elongate 22:5n-3 and 22:4n-6 to 24:5n-3 and 24:4n-6, respectively

(Leonard et al., 2002). Like the zebrafish enzyme, the mouse elovl2 clone was also able to elongate C_{18} and C_{20} PUFA, whereas the human ELOVL2 clone was only active toward C_{20} and C_{22} PUFA (Leonard et al., 2002). Neither the elov12 nor the ELOVL2 clones showed any significant activity toward saturated or monounsaturated fatty acids (Leonard et al., 2002). However, the previously cloned human elongase ELOVL5 elongated monounsaturated fatty acids in addition to C_{18} and C_{20} PUFA (Leonard et al., 2000b). Thus, the two human elongases display different but overlapping substrate specificities. Similarly, the two rat elongases display overlapping substrate preferences, with rELO1 displaying a substrate specificity similar to that of human ELOVL5 (monounsaturated fatty acids and PUFA), whereas rELO2 elongated saturated and monounsaturated fatty acids with little activity toward C_{18} PUFA, but no activity toward C_{20} and C_{22} PUFA (Inagaki et al., 2002).

Thus, vertebrate fatty acid elongases display broad substrate specificities depending upon both chain length and degree of unsaturation. The zebrafish elongase is entirely consistent in this respect, but appears to be unique in its capacity to elongate saturated and monounsaturated fatty acids, and PUFA with chain lengths from C_{18} up to and including C_{22}. Whether or not this is a reflection of the position of zebrafish in evolutionary history and hierarchy in comparison to mammals is unclear. However, it is noteworthy that the situation described with the fatty acid elongase of zebrafish is to some degree analogous to the previously described situation with the zebrafish fatty acid desaturase. Indeed as zfELO also elongates saturated and monounsaturated fatty acids, it is interesting to speculate on the precise role of this gene in zebrafish, although the high activity toward C_{18} PUFA suggests that its primary role is in PUFA metabolism. The existence of more than one PUFA elongase in rat and human suggests, however, that other elongases with differing substrate specificities may yet be isolated in zebrafish.

The cloning and characterization of the zebrafish PUFA elongase, along with the other elongases cloned from fungus, worm, rat, and human, prompts the question of how the structural differences in elongase contribute directly to the interspecies variation in substrate specificity? Clearly, the above discussion indicates that the primary structures of elongases must influence the range of fatty acid substrates accepted by the fatty acid chain elongation complex. The number of different elongases with different and overlapping substrate specificities is now such that it may be possible to infer structure–function relationships, at least at the level of primary structure. For instance, the sequences of human and fish elongases are very similar, but the differences that occur clearly affect the substrate range of the enzymes. This presents an opportunity to study the structural features that have shaped fatty acid elongase substrate specificity during the evolution of vertebrates, and the possibility of artificially selecting for elongases with superior specification.

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