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

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Summary: 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 EST 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 (ORF) shared high similarity with the elongases of *C. elegans* and human. When expressed in *Saccharomyces cerevisiae,* the zebrafish ORF conferred the ability to lengthen the chain of a range of C_{18}, C_{20} and C_{22} polyunsaturated fatty acids, indicating that biosynthesis of 22:6n-3 from 18:3n-3 via a 24-carbon intermediate is not only feasible, but 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.

INTRODUCTION

The long-chain highly unsaturated fatty acids (HUFA) 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 (Mcmurchie, 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, that modulate many physiological processes including reproduction, haemostasis and inflammation (Weber, 1990). Increased dietary levels of the n-3HUFA, 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 (Anon., 1992).

The only major source of n-3HUFA for the human diet is fish and seafood and generally declining catches from 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 the predominant protein and lipid sources (Sargent and Tacon, 1999). However, stagnation in the industrial fisheries, along with the 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 which are rich in C_{18} polyunsaturated fatty acids (PUFA) such as

18:2n-6 and 18:3n-3, but devoid of the n-3 HUFA 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 synthesised from 18:3n-3 by desaturation at the Δ6 position, followed by a 2-carbon elongation, that is in turn followed by a further desaturation at the Δ5 position (Fig.1) Cook, 1996). Synthesis of DHA requires further elongation and desaturation of EPA, that 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 synthesise 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 polypetide 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 (Aki et al., 1999; Cho et al., 1999a,b; Leonard et al., 2000a; Michaelson et al., 1998; Napier et al., 1998; Watts and Browse, 1998).

Elongation of fatty acid, whether saturated, monounsaturated or polyunsaturated, involves four enzymic steps (Fig. 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 three 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 catalysing 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 biosynthesise DHA, and thus to thrive on a diet largely devoid of HUFA (Tocher et al., 2002). 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 programme. 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., Palo Alto, USA). PCR fragments were cloned into the Bluescript SKII vector, and the sequences were determined by standard dye terminator chemistry (Perkin Elmer, 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 (CGG AAT TCA AGC TT**A AGA TG**G AGA CGT TTA GTC A) contains an EcoRI site (underlined) and a Kozak translation initiation sequence (boldface). The antisense primer ZfEloXB (CTC TAG ACT CGA GTC AAT CTG CTC GTG CTT TTC T) contains an *XhoI* site (underlined). PCR was performed using high fidelity DNA polymerase mix (Roche Diagnostics Ltd., Lewes, UK) following the manufacturer's instruction. After PCR the DNA fragments were restricted with *EcoRI* and *XhoI*, ligated into a similarly treated yeast expression vector pYX222 (R&D Systems, Abingdon, UK), 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$^{-histidine}$(SCMM^{-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 min, the pellets 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 (FAME) 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 for 1 hour at 85^{o}C. After incubation, FAME 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 min 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 evaporated under a stream of OFN. The FAME were purified by thin-layer chromatography and then resuspended in hexane, all as described previously (Tocher and Harvie, 1988). FAME 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., London) 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 ºC to 180 ^{o}C at 40 ºC/min, and then to a final temperature of 235 ^{o}C at 2 ºC/min. Individual FAME 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). FAME were

quantified using a directly linked PC operating Chrom-Card Software (Thermo-Quest Italia S.P.A., Milan, Italy). 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 (GC-MS). 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, Crawley, U.K.). The 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, Macclesfield, U.K.) and used helium as carrier gas. Samples were applied using on-column injection with the oven temperature programmed to rise from 80 ^{o}C to 250 ^{o}C at 40 ^{o}C per minute.

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%) (Fig. 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 plasmid, the identity of the CDS was reconfirmed 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 (Fig. 4A). Several PUFA were subsequently investigated as potential substrates, with all six 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 (Figs. 4A-C). The identities of elongation products were confirmed by comparison with known standards and gas chromatography/mass spectrometry of picolinyl esters. For example, the mass spectra for the products of elongation of C_{22} PUFA are shown in Fig.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 (Fig.5) (Christie, 1998). The EI spectra of the additional fatty acid in ZfELO-pYX222 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* (Fig. 5A). The initial interval of 15 (451-436) represented the terminal methyl and was

followed by four intervals of 14 (436-380) indicating four methylene groups. The intervals of 26 (380-354, 340-314, 300-274 and 260-234) denoted the positions of four double bonds, indicating that this fatty acid is $^{\Delta 18,15,12,9}$24:4 = 24:4n-6 (Fig.5A). 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 (449-434) represented the terminal methyl and was followed by an interval of 14 (434-420) indicating one methylene group. The intervals of 26 (420-394, 380-354, 340-314, 300-274 and 260-234) denoted the positions of five double bonds, confirming this fatty acid as $^{\Delta 21,18,15,12,9}$24:5 = 24:5n-3 (Fig.5B).

Generally, the efficiency of zfELO for the different PUFA substrates, as measured by the relative conversion, decreased with the substrate chain length, that is, $C_{18} > C_{20} > C_{22}$ (Fig.6). In addition, n-3 PUFA were more efficiently converted than the n-6 PUFA of the same chain length. Thus, the zebrafish elongase converted 90% of 18:4n-3 to 20:4n-3 compared to 60% 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 fresh water fish species. The cDNA encodes a protein that is very 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 characterisation has previously been reported for PUFA elongases of nematode (*C. elegans*), fungus, (*M. alpina*), rat and human with all four 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 90% conversion of 18:4n-3 substrate and 60% of 18:3n-6. Due to the very high activity of the zebrafish elongase towards 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 towards C_{20} PUFA (Parker-Barnes et al., 2000; Beaudoin 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, and so its production by the action of the zebrafish elongase on either EPA or 22:5n-3 is significant. Secondly, however, the zebrafish elongase is very active towards 18:4n-3, and so it is possible that only this single elongase enzyme is required to perform all the elongation steps required 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., in press). Therefore, zebrafish would appear to be able to perform all six desaturation/elongation reactions necessary for the production of DHA from 18:3n-3 using just two gene products. This is in contrast to all other vertebrate and eukaryotic systems studied in which desaturase activities are encoded by disparate genes (Aki et al., 1999; Cho et al., 1999a,b; Leonard et al., 2000a; Michaelson et al., 1998; Napier et al., 1998; Watts and Browse, 1998).

Very recently, two mammalian genes have been cloned and characterized, a further human elongase (ELOVL2) and a mouse elongase (elovl2), both of 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 towards C_{20} and C_{22} PUFA (Leonard et al., 2002). Neither the elovl2 or ELOVL2 clones showed any significant activity towards 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 very little activity towards C_{18} PUFA, but no activity towards C_{20} and C_{22} PUFA (Inagaki et al., 2002).

Thus, vertebrate fatty acid elongases display quite 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 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(s) of this

gene in zebrafish, although the very high activity towards C_{18} PUFA suggests that its primary role is in PUFA metabolism. However, the existence of more than one PUFA elongase in rat and human suggests that other elongases with differing substrate specificities may yet be isolated in zebrafish.

The cloning and characterisation 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 inter-species 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 are 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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Legends to Figures

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).

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. Step 2-4 are acyl reductive processes.

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 grey. The threshold for similarity shading was set at 75%.

Figure 4. Identification of fatty acid elongation products in transgenic yeast (*Saccharomyces cerevisiae*). Fatty acids were extracted from yeast transformed with ZfELO-pYX222 grown in the presence of either A: 18:4n-3, B: 20:5n-3 or C: 22:5n-3. The first four peaks in panels A-C 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 additional 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).

Figure 5. Mass spectra of fatty acids (peaks) produced by yeast transformed with the ZfELO-pYX222 plasmid and grown in the presence of either A: 22:4n-6, or B: 22:5n-3. 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 **A**: 24:4n-6, and **B**: 24:5n-3.

Figure 6. Relative activity of zebrafish elongase (ZfELO) towards 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 0.5mM of γ-linolenic acid (GLA), stearidonic acid (STA), arachidonic acid (AA), eicosapentaenoic acid (EPA), docosatetraenoic acid (DTA) and docosapentaenoic acid (DPA) respectively. The conversion rate was calculated as 100 × [product area/ (product area + substrate area)].

Fig.1.

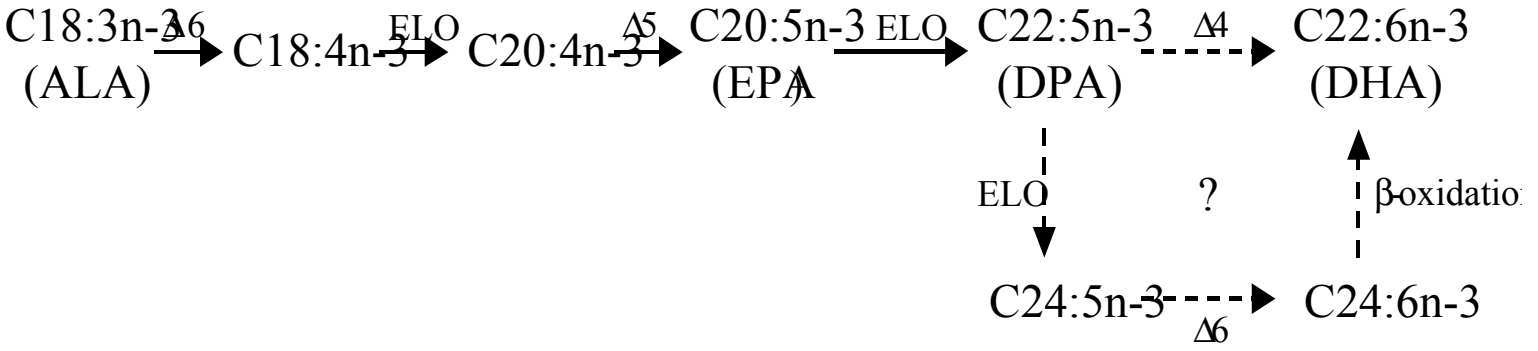

C18:3n-3
(ALA)
Δ6
C18:4n-3
ELO
C20:4n-3
Δ5
C20:5n-3
(EPA)
ELO
C22:5n-3
(DPA)
Δ4
C22:6n-3
(DHA)
ELO
?
β-oxidation
C24:5n-3
Δ6
C24:6n-3

Fig.2.

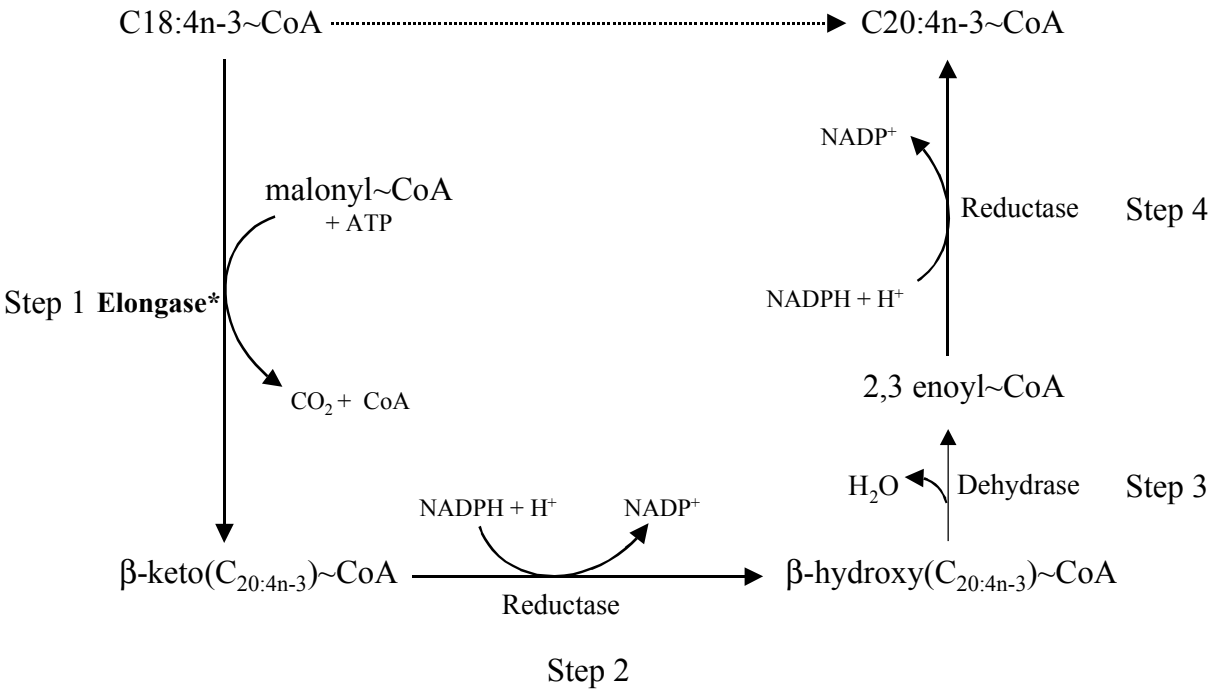

C18:4n-3~CoA
C20:4n-3~CoA
malonyl~CoA
+ ATP
Step 1 Elongase*
CO2 + CoA
NADP+
Reductase
Step 4
NADPH + H+
2,3 enoyl~CoA
H2O
Dehydrase
Step 3
NADPH + H+
NADP+
β-keto(C20:4n-3)~CoA
β-hydroxy(C20:4n-3)~CoA
Reductase
Step 2

Fig.3.

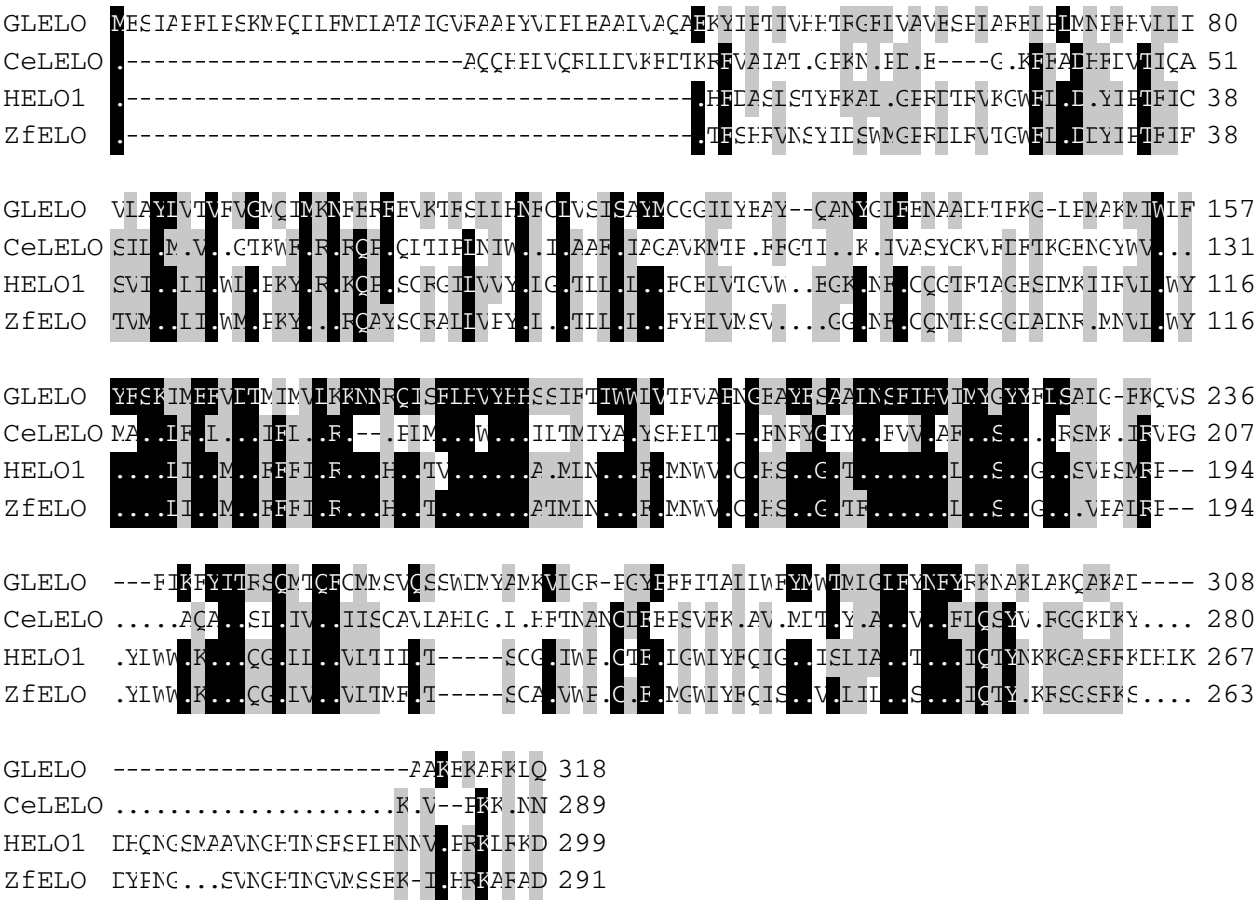


Fig.4.

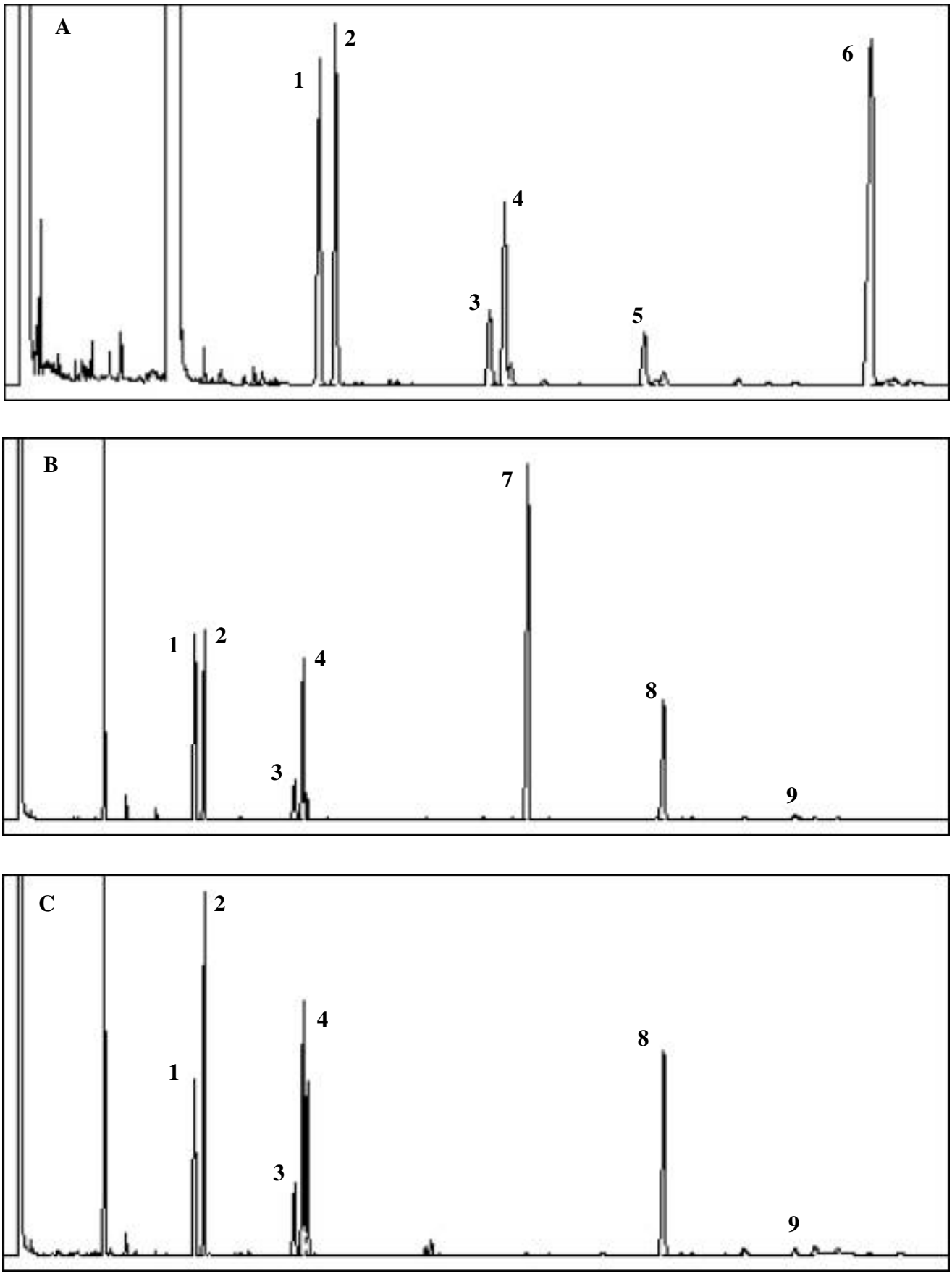
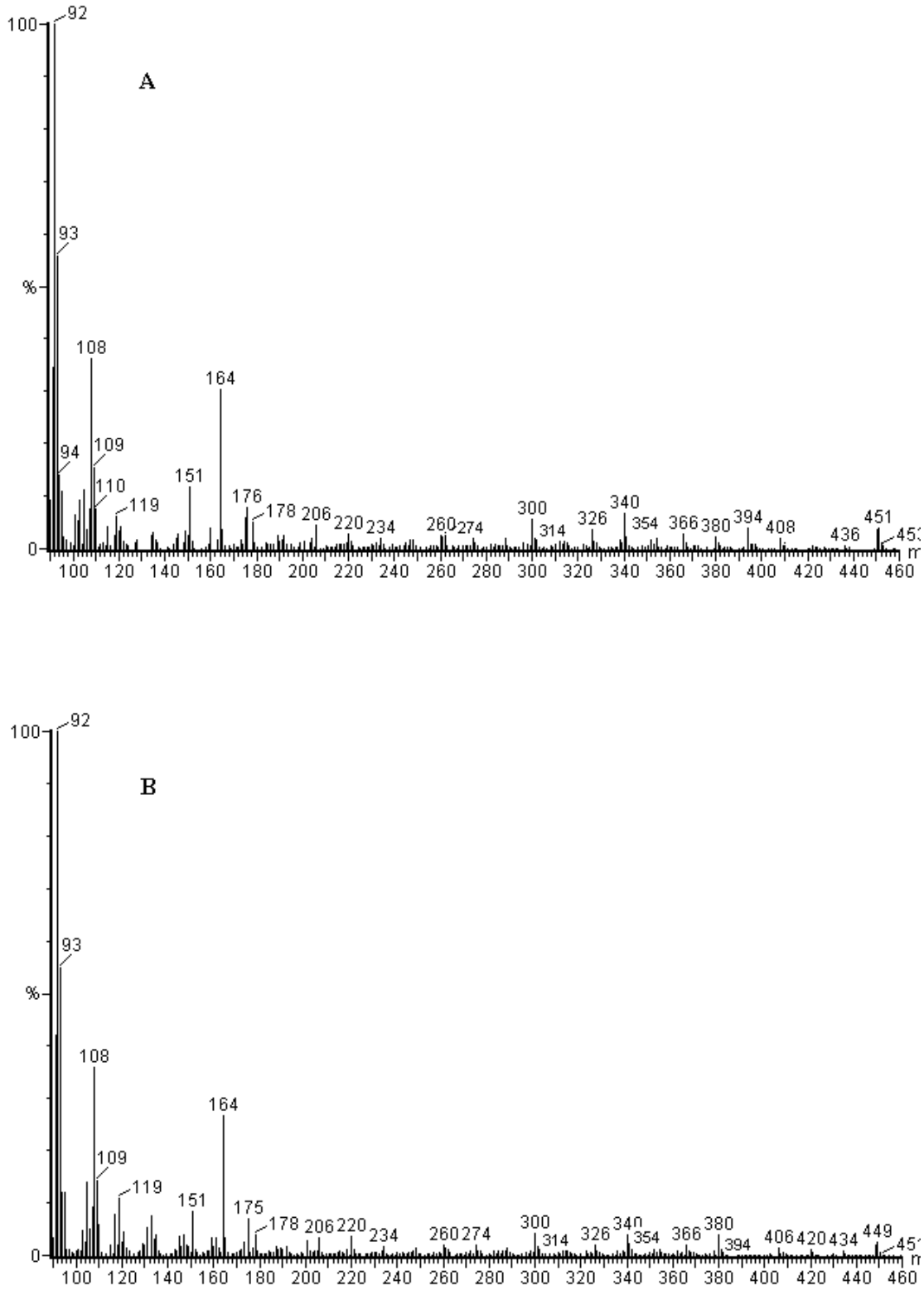


Fig.5.


A
100
%
0
92
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326
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354
366
380
394
408
436
451
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460
B
100
%
0
92
93
108
164
109
119
151
175
178
206
220
234
260
274
300
314
326
340
354
366
380
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406
420
434
449
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460

Fig.6.

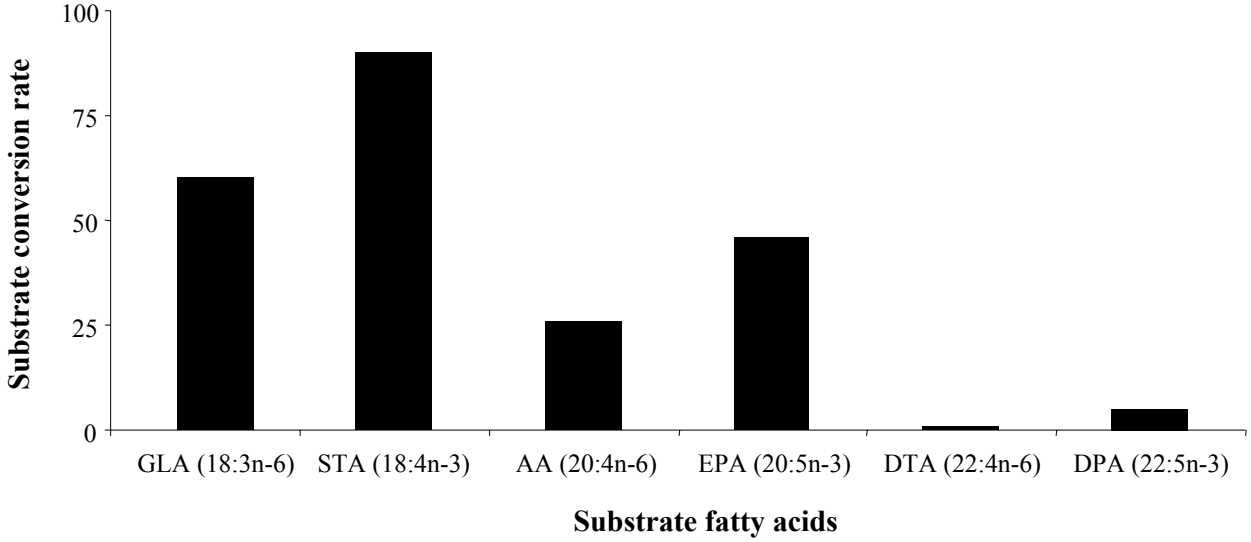
100
75
50
25
0
Substrate conversion rate
GLA (18:3n-6)
STA (18:4n-3)
AA (20:4n-6)
EPA (20:5n-3)
DTA (22:4n-6)
DPA (22:5n-3)
Substrate fatty acids

Table 1. Fatty acid compositions (percentage of total fatty acids by weight) of *S.cerevisiae* transformed with zebrafish elongase (ZfELO) or empty vector (control). Results are means ± SD (n=4). Where indicated (*), mean values for fatty acids in yeast transformed with ZfELO are significantly different to control as determined by the Student t-test ($p < 0.05$). nd, not detected; trace, < 0.05%.

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	