INSIGHTS INTO MECHANISMS OF POLYUNSATURATED FATTY ACID BIOSYNTHESIS IN PARALARVAE OF THE COMMON OCTOPUS (Octopus vulgaris)

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INTRODUCTION AND OBJECTIVE
Culture of Octopus vulgaris is hindered by massive mortalities during early life stages (paralarvae). Polyunsaturated fatty acids (PUFA) have been identified as essential compounds for paralarvae, but precise biosynthetic capabilities and dietary requirements have not been yet determined.

The present study gathers all the existing information to provide a comprehensive account of the PUFA biosynthetic capabilities of O. vulgaris that ultimately determine the essential fatty acid requirements in this species.

MATERIALS AND METHODS

Molecular cloning
Four genes encoding desaturases and elongases involved in PUFA biosynthesis were targeted. Specifically, we studied a fatty acyl desaturase (Fad), two elongases of very long-chain fatty acids (Elovl) and a stearoyl-CoA desaturase (Scd). Total RNA from adult tissues was extracted by homogenisation in Tri Reagent (Sigma-Aldrich) and cDNAs were subsequently synthesised. In order to isolate the first fragment of Scd, Fad and Elovl, degenerate primers were designed in conserved regions deduced from the alignment of homologous proteins from a variety of organisms. The PCR fragments were sequenced and specific primers were designed to produce the full-length cDNA by 5’ and 3’ rapid amplification of cDNA ends (RACE) PCR.

Functional characterisation in yeast
In order to functionally characterise the enzymes, their coding sequences, cloned into pYES2 (Invitrogen), were expressed in Saccharomyces cerevisiae (INVSc1 strain, Invitrogen) and grown in presence of potential fatty acid (FA) substrates: 18:3n-3, 18:2n-6, 20:3n-3, 20:2n-6, 20:4n-3, 20:3n-6, 22:5n-3 and 22:6n-3 for Fad; 18:3n-3, 18:2n-6, 18:4n-3, 18:3n-6, 20:5n-3, 20:4n-6, 22:5n-3, 22:4n-6 and 22:5n-3 and 24:5n-3 for Scd; and 18:3n-3, 18:2n-6, 18:4n-3, 18:3n-6, 20:3n-3, 22:5n-3 and 22:4n-6; for Elovl. To establish the role of Scd and Δ5 Fad in the biosynthetic pathways of Δ5,9 (or Δ5,11) non-methylene interrupted fatty acids (NMI FA), yeast expressing the octopus Scd, were grown in the presence of exogenously added 5-eicosenoic acid (20:1n-5 or Δ5 20:1), and yeast expressing the octopus Δ5 Fad were grown in the presence of 11-eicosenoic acid (20:1n-9 or Δ11 20:1). After 2 days, transgenic yeast samples were collected for further FA analysis.

“In vivo” fatty acid incorporation, esterification and elongation-desaturation
O. vulgaris hatchlings were incubated in 10 ml of filtered sea water, in cell culture plates, with horizontal stirring, during 6 hours, at 21 °C. To each incubation chamber, 0.3 micromoles (μM) of radiolabelled 18:1n-9, 18:2n-6, 18:3n-3, 20:4n-6, 20:5n-3 and 22:6n-3 were individually added as potassium salts bound to bovine serum albumin. This type of experiment allowed us to verify “in vivo” the metabolism of each FA in separate without influence of other FA or nutrients.

RESULTS

Model of biosynthetic pathways of unsaturated fatty acids in O. vulgaris. Enzymatic activities predicted from heterologous expression of fatty acyl desaturases and elongases in yeast.

Desaturases found: Scd (∆9 activity); Fad (∆5 activity).

Elongases found: Elovl 2/5 (elongation of C18, C20); Elovl 4 (elongation=C22).

NMI FA synthesis occurs only via ∆9, ∆5.

CONCLUSIONS

• 22:6n-3 is an essential fatty acid for O. vulgaris.
• The essentiality of 20:4n-6 and 20:5n-3 is conditioned by the dietary availability of 18:3n-6 and 18:4n-3.
• 20:4n-6 is preferentially incorporated into TL.
• 20:4n-6 and 20:5n-3 are preferentially esterified into PE, and 22:6n-3 into PC.

“In vivo” Incorporation of [1-14C] fatty acids: 20:4n-6 is preferentially incorporated into total lipids (TL), and especially esterified in Phosphatidylethanolamine (PE), as is 20:5n-3, whereas 22:6n-3 shows preferential esterification into Phosphatidylcholine (PC).

To note that only elongations were recorded “in vivo”.

Full information on these results in:
• Reis et al. 2014: Aquaculture 431: 28–33.
• Reis et al. 2015: Aquaculture Nutrition 21: 797-806

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