Myosin light chain 2 in gilthead sea bream (Sparus aurata): a molecular marker of muscle development and growth

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Introduction

White muscle is the final product of aquaculture and counts for up to 70% of the fish body mass. The mechanisms of its development and growth are of great physiological significance for the optimization of swimming performance, feeding capacity and overall larval quality and growth potential. Myosin light chain 2 (MLC2) is a component of the myosin molecule. In gilthead sea bream (*Sparus aurata* L.), an important species for the Mediterranean aquaculture, two isoforms of MLC2, A and B, have been isolated and characterized. Their expression differentiate throughout development and MLC2A expression marks the phases in white muscle development of at the molecular level (Moutou et al.,2001; Sarropoulou et al., 2006). The purpose of the present study was to further investigate the potential of MLC2 as a molecular marker of muscle development and growth in sea bream. To that direction, we investigated by the means of real-time RT-PCR a) the levels of expression of MLC2 transcripts throughout the crucial stages of development, before and after hatch and up to completion of metamorphosis; b) the regulation of expression of MLC2 transcripts during myoblast proliferation and differentiation in primary muscle cell cultures of sea bream; c) the effect of growth hormone at variable doses *in vivo* on the expression of MLC2 transcripts in white muscle.

Materials and Methods

1. *The expression of myosin light chain 2 during development:* Sea bream eggs and larvae were collected at 24, 27, 36, 43 hpf and on 1, 4, 15, 20, 24, 46 and 64 dph.
2. *The expression of myosin light chain 2 during myoblast proliferation and differentiation:* Sea bream primary muscle cell cultures were established according to Montserrat et al (2007). Cells were harvested at 24, 36, 48 hrs and on 3, 4, 8, 13 days after establishment.
3. *The effect of growth hormone on the expression of MLC2 transcripts in white muscle:* Four groups of sea bream of 57.3g mean weight were administered ovineGH (NIADDK-oGH-15) at 0, 0.1, 1.0 and 10.0 μg/g BW, respectively as a single i.p injection. White muscle was sampled on days 1, 2, 4 and 7 after oGH administration.

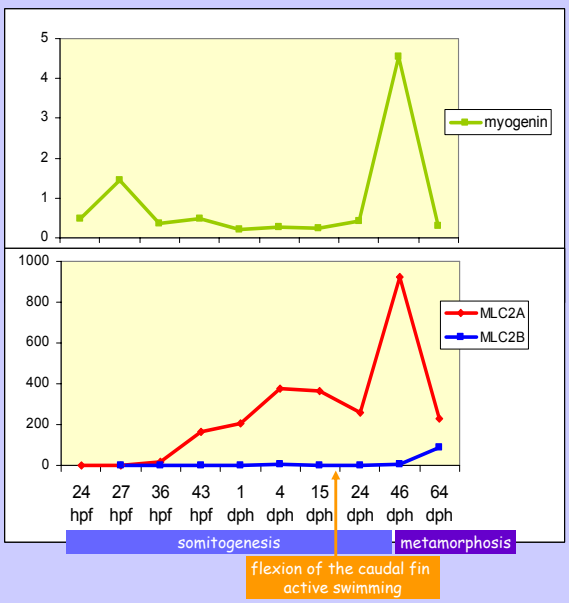
The expression levels of MLC2 transcripts were determined by real time RT-PCR method. In experiments 1 and 2, all samples were run in parallel with EF-1a for normalization of cDNA loading (Nowell et al., 2000). Relative mRNA expression was determined using the ΔCt (Livak & Schmittgen, 2001). In order to distinguish the differentiation phase, myogenin expression, was included in the analysis (Codina et al., 2008).

In experiment 3, expression levels were normalized against two housekeeping genes, EF1a and RPS18, using geNorm.

Results

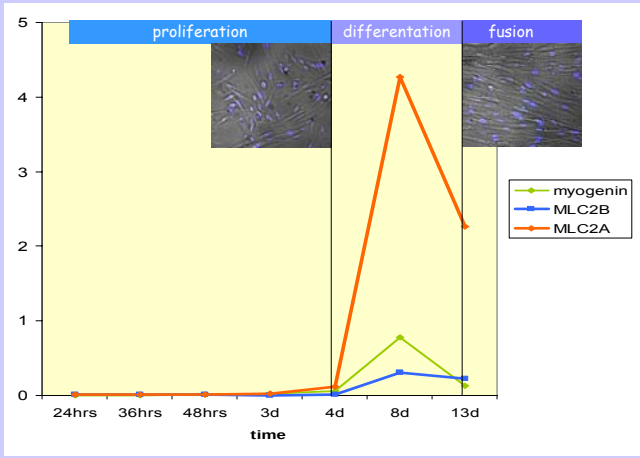
1. The expression of myosin light chain 2 during during development

MLC2A expression increased gradually from 24hpf up to metamorphosis, when it peaked to decline afterwards. MLC2B expression remained at steadily low levels throughout development and increased slightly only after metamorphosis. MLC2A appeared earlier in development, and its expression pattern followed that of myogenin. Its transcription marked the hyperplastic phase of muscle development and decreased post-metamorphosis.



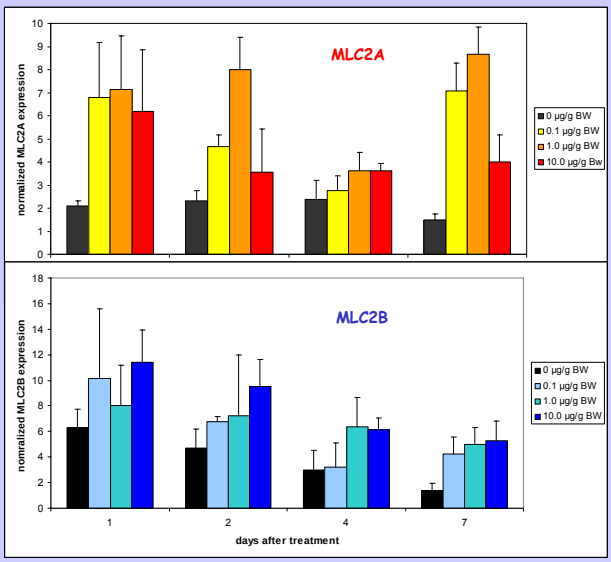
2. The expression of myosin light chain 2 during myoblast proliferation and differentiation

MLC2A expression followed myocyte differentiation pattern, marked by a gradual increase in myogenin expression. Transcript levels remained low in the first three days in culture. From day 4 onwards, MLC2A expression increased significantly and peaked on day 8. MLC2B expression also increased during differentiation, yet the expression levels were very low.



3. The effect of growth hormone on the expression of MLC2 transcripts in white muscle

Expression levels of MLC2A were significantly elevated in the white muscle of sea bream on day 1 following GH admnistration. The effect was not dose dependent. On the contrary, GH did not elicit a significant effect on MLC2B expression



Conclusions

Overall, the results of the present study are supportive of the validity of MLC2A as a marker of newly-formed muscle fibers and its potential use for the study of the effect of different physiological factors on muscle development in gilthead sea bream.

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