MULTIPLE VITELLOGENIN YOLK PRECURSORS IN EUROPEAN SEA BASS (*DICENTRARCHUS LABRAX)*

Yilmaz, O.[(1)], Prat, F.[(2)], Ibáñez, A.J.[(3)], Amano, H.[(4)], Koksoy, S.[(1)], and Sullivan, C.V.[(5)]
[(1)]INRA LPGP, Rennes Cedex, France; [(2)]Instituto de Ciencias Marinas de Andalucía, CSIC, SPAIN; [(3)]Instituto de Acuicultura de Torre de la Sal, CSIC, SPAIN (Present address: University of Valencia, SPAIN); [(4)]Kitasato University, JAPAN; [(5)]North Carolina State University, USA (Present address: Carolina AquaGyn, USA).
E-mail: ozlem.yilmaz@rennes.inra.fr

Introduction

We characterized three deduced sea bass vitellogenin (Vtg) polypeptides with regard to linear and 3-dimensional (3-D) structural and functional features, verified the presence and relative contribution to the yolk of each form of Vtg, and assessed maturational degradation and potential contribution of each product lipovitellin (Lv) to the free amino acid (FAA) pool driving oocyte hydration and supporting early embryonic nutrition.

Methods

Three complete *vtg* cDNAs were assembled from contiguous partial sequences obtained by RT-PCR. Deduced Vtg proteins were classified using BLAST, revealing homologies across a broad array of taxa. ClustalW alignments of Vtg sequences revealed conservation of cysteine (C), proline (P) and glycine (G) residues by Vtg-type. Residue positions were localized in the 3-D structure of a lamprey Lv template using Cn3D and mapped to phylogenetically-conserved functional surfaces using EvoTrace. Relative concentrations of each Vtg or their yolk protein (YP) products in postvitellogenic female liver, plasma and ovary were measured by nanoLC-MS/MS as ProteoIQ-normalized spectral counts. Maturational degradation of each type of Lv was detected by Western blotting using antisera raised against purified grey mullet (*Mugil cephalus*) Lvs.

Results and Discussion

Homology analyses definitively identified sea bass VtgAa, VtgAb and VtgC. The VtgC lacks two C-terminal domains ($\mathcal{B}$'-component, $\mathcal{B}$'c; C-terminal component, Ct) and its N-sheet, which bears the functional surface of the classical Vtg receptor (Vtgr), has undergone extensive substitutions at C, P and G residues likely to result in massive alteration of its structure and Vtgr-binding properties. VtgAb tryptic peptide spectra were generally several fold more abundant than for the other Vtgs, and VtgC spectra were very limited, except in ovary. Comparison of contiguous tryptic peptides detected in plasma versus ovary indicate that most Ct is degraded just after VtgA uptake by oocytes, whereas Lv degradation is minor and $\mathcal{B}$'cs are not degraded. Western blotting revealed limited degradation of all three forms of Lv during oocyte maturation, unlike the case in other marine pelagic spawners. Virtually identical Vtgs and patterns of YP degradation during oocyte growth and maturation in sea bass and *Moronidae* that spawn in freshwater (striped bass, pelagic; white perch, demersal) indicate that Vtg system structure and function cannot be inferred solely from reproductive life history.

Conclusions

The multiple Vtg system of D. labrax was characterized in detail using some novel technical approaches, setting the stage for studies of Vtg involvement in determining egg quality in this premier aquaculture species.