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Characteristics of fish yolk proteins and a method for inducing vitellogenin

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Abstract

Teleosts are one of the most diverse groups of vertebrates. They utilize a wide array of reproductive strategies and tactics to overcome the challenges of the many ecological niches they inhabit. The most common reproductive method for teleosts is oviparity. Oviparous animals lay eggs with little or no embryonic development from the mother. The embryos are supplied with nutrition via yolk. Vitellogenesis is the process of the ovary sequestering yolk. It is regulated by exogenous environmental cues that act on the hypothalamus-pituitary-gonad axis. Through a series of hormonal controls, the liver produces the yolk precursor, vitellogenin. Vitellogenin is secreted by the liver and absorbed by the growing oocyte by receptor mediated endocytosis. There it is cleaved into the two main yolk proteins which are subsequently used by the growing embryo. The biggest source of nutrition is the yolk protein lipovitellin which also plays a key role in marine teleosts' ability to osmoregulate their eggs. Lipovitellin is a large glyco-phospho-lipo-protein ca. 200 kDa. Large proteins usually denature easily. However, prior evidence shows that fish lipovitellins are thermally stable. Using differential scanning calorimetry, I quantify lipovitellin's thermostability amongst four right-eye flounders (Pleuronectidae: winter flounder, American plaice, witch flounder, and yellowtail flounder). Differential scanning calorimetry allows direct interpretation of all thermodynamic properties; however, Lipovitellin was too large and precipitated before other thermodynamic properties could be determined. Pleuronectid lipovitellins all showed high melting points indicative of high thermostability. This shows that despite differing life histories, lipovitellin is conserved.

Presence of the pre-cursor, vitellogenin in male or juvenile fish is used as a biomarker for xenoestrogens, a type of endocrine disrupting chemicals that blocks or mimics natural estrogens. They are known to disrupt aquatic life by interfering with natural development and reproduction. A major biological side effect of xenoestrogens is the accumulation of vitellogenin. This effect has made vitellogenin a useful biomarker for monitoring levels of contamination. Unfortunately, vitellogenin can vary greatly in its immunological and structural characteristics, which means that species-specific assays are necessary. This study took the first step in developing an immunoassay for bluefish (Pomatomus saltatrix). Vitellogenin was induced by injecting a group of bluefish with an estrogen, estradiol, and the resulting vitellogenin was isolated from the serum of males. The protein was characterized as vitellogenin by determining its large Stokes radius in gel permeation chromatography combined with its characteristic peptide molecular weight in sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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