Characterization of the yolk protein lipovitellin and its developmental fate in embryos and larvae of winter flounder, Pleuronectes americanus

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Abstract

Lipovitellin, the predominant yolk protein of vertebrate eggs, is a mixture of heat-stable and heat-labile molecules in mature winter flounder eggs. The heat-stable lipovitellin fraction, purified from extracts of unfertilized eggs by brief heat treatment and gel permeation chromatography, contains a single 94 kDa polypeptide. Native lipovitellin also possesses several smaller polypeptides, suggesting that heat-labile lipovitellin contains proteolytic cleavages of the 94 kDa polypeptide which destabilize its structure. The Stokes radii of native, heat-stable and heat-labile lipovitellin are 4.50 nm, 4.26 nm and 5.17 nm, respectively. ^ A polyclonal antiserum raised against heat-stable lipovitellin binds a 175 kDa polypeptide in vitellogenic female winter founder serum, but does not bind any component of male serum. An ELISA constructed from this antiserum identifies serum vitellogenin as a single gel permeation peak with a Stokes radius of 6.70 nm and confirms that vitellogenin is a dimer, while lipovitellin from mature eggs is a monomer. ^ During embryogenesis, lipovitellin is cleaved from a 94 kDa polypeptide to 67 kDa and 26 kDa polypeptides. Proteolytic processing is initially slow, but becomes more rapid between days eight and 12 post fertilization in embryos reared at 4°C–5°C, approaching 50% completion at day ten (tail-bud stage). Processing is essentially complete three days before hatching; nevertheless, major degradation of the lipovitellin polypeptides only occurs in larvae. The Stokes radius of lipovitellin decreases from 4.50 nm in unfertilized eggs to 4.19 nm in late embryos and newly hatched larvae, while processed lipovitellin retains its heat stability relative to other yolk polypeptides. However, 49.2% of the lipid moiety is released from lipovitellin concomitant with cleavage of the 94 kDa polypeptide. Lipovitellin processing may thus render a portion of its stored lipids more accessible to the embryo; alternately, removal of lipid may heighten proteolytic vulnerability of the polypeptide. In either case, only a portion of the lipovitellin particle plays a significant nutritive role for the embryo, while most of the molecule, including its protein component, is reserved for larval use. ^

Subject Area

Cellular biology|Animal Physiology|Biochemistry

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