Isolation and partial characterization of Asian sea bass (Lates calcarifer) vitellogenin

ABSTRACT

A study was conducted to isolate, partial characterize Asian sea bass (Lates calcarifer) vitellogenin (vtg). Two-year-old juvenile L. calcarifer (n = 10) were given three intraperitoneal injections of 17-ɓ estradiol (E2) at a dose of 2 mg/kg body weight to induce vitellogenesis. Blood was collected 3 days after the last injection, and plasma was purified through gel filtration chromatography. A broad single symmetrical peak consisting of vtg molecule was produced. Protein concentration was 0.059 mg/ml as determined by Bradfrod assay using bovine serum albumin as a standard. The protein appeared as one circulating form in Native PAGE considering the dimeric form of putative vtg with molecular weight of 545 kDa. In SDS-PAGE under reducing conditions, two major bands appeared at 232.86 and 118.80 kDa and minor bands at 100.60, 85.80 and 39.92 kDa, respectively. The purified vtg was used to generate a polyclonal antibody, and the specificity of antibody was assessed by Western blot analysis. Two major bands were immunoreacted, but no cross-reactivity was observed with plasma from non-induced males. The protein was characterized as phosphoglycolipoprotein as it positively stained for the presence of lipid, phosphorus and carbohydrate using Sudan Black B, methyl green and periodic acid/Schiff reagent solution, respectively. The amino acid composition was analyzed by high sensitivity amino acid analysis that showed high percentage of non-polar amino acids (~48 %). The results suggest the potential utilization of vtg as a basis tool to further study about reproductive physiology of this important economical species.

Keyword: Amino acid analysis; Chromatography; Lates calcarifer; Phosphoglycolipoprotein; Vitellogenin; Western blot