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Development of a Quantitative Enzyme-Linked Immunosorbent Assay for Vitellin and Vitellogenin of the Blue Crab Callinectes Sapidus

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Abstract

Vitellin (Vn) and vitellogenin (Vg) were detected in the blue crab Callinectes sapidus by nondissociating polyacrylamide gel electrophoresis (PAGE). Vn was purified by gel filtration chromatography followed by PAGE, and anti-Vn immune serum was raised in rabbits. A slot blot analysis indicated that anti-Vn immune serum reacted with the ovarian homogenate from which Vn was isolated. In a double immunodiffusion test, single precipitin lines were found between the center well containing immune serum and surrounding wells containing purified Vn, ovarian homogenate, purified Vg, or female hemolymph. The precipitin lines were continuous, suggesting that the immune serum precipitated proteins of complete identity. Finally, a Western blot analysis revealed that immune serum specifically bound Vn and Vg. The IgG fraction was purified from anti-Vn immune serum and used to develop a quantitative enzymelinked immunosorbent assay (ELISA). The Vn standard curve was linear over the range of 62.5-1,500 ng Vn. The sensitivity of the ELISA was 148 ng/ml. Male hemolymph yielded background optical density values, confirming the specificity of the assay. Serial dilutions of ovarian homogenate or female hemolymph produced sample titration curves that were parallel to the Vn standard curve. Thus, the ELISA is suitable for quantification of Vn and Vg in ovary and hemolymph, respectively.