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Study on isolation of vitellin-like protein from the Pacific oyster *Crassostrea gigas*.

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Vitellins or so named yolk proteins are stored in yolk granules of oocytes. They play major role in providing energy and nutrient for developing embryo. Vitellins are very diverse group of chemically uncharacterized complexes of glyco-, lipo-, phosphoproteins and proteoglycans. Isolation of vitellins and production of antibodies specific to them would be useful for studying the physiology of yolk formation and developmental immunological techniques for investigation reproduction for commercially important marine molluscs.

In present study vitellin-like proteins specific for eggs of the Pacific oyster *Crassostrea gigas* have been identified using immunological methods. One of them, termed as oyster egg specific protein (OESP), was purified by a combination of ammonium sulphate precipitation, ion-exchange chromatography on DEAE-Sephadex and gel-filtration on Sephadex G-200. Control for purification and homogeneity of the OESP was carried out with help both polyspecific antiserum obtained to whole oyster eggs extract and specific antibodies received after immunoadsorption. Cross-reacting antibodies were removed on the immunosorbent obtained by glutaric dialdehyde polymerization of whole extract of oysters collected in winter season. The purified OESP gives single precipitation line in immunodiffusion and immunoelectrophoresis using polyspecific antiserum and specific antibodies demonstrating immunochemical homogeneity. It is possible isoelectric point of OESP is in the range from 8 to 9 because experimentally OESP shows lack of electrophoretic mobility. SDS-PAGE electrophoresis under non reducing condition revealed three main protein bands with molecular masses about of 300, 200 and 58 kDa and three minor bands 105, 87, 76 kDa. Treatment of OESP by β -mercaptoethanol gives rise to eight main protein bands with molecular masses of 105, 87, 76, 64, 62, 53 and 42 kDa. It is possible that under physiological conditions exist as high molecular complex consisting from molecules with different weight. It may be possible that these difference

is due to variation in glycosylation or lipids content.

As was shown in immunohistological experiments specific antibodies interest only with cytoplasmic components of eggs. In contrast, cross-reacting antibodies react not only with yolk but also with another tissues. In our opinion, biosynthesis of vitellins occurs not only autosynthetically in oocytes but also heterosynthetically. It is possible, cross-reacting antibodies react with precursors of vitellins which are transported via open circulation system or hemolymph and later are used as building block for synthesis of yolk proteins.