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PURIFICATION OF ACUTE PHASE PROTEIN FROM RAINBOW TROUT

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C-reactive protein(CRP)-like protein was purified from the acute phase serum of rainbow trout inoculated with Turpentine oil by using affinity chromatography coupled with streptococcal C-polysaccharide(CPS), DE-52 anion exchange chromatography and gel filtration on Sephacryl S-300. The purified CRP-like protein showed a broad band in disc slab gel electrophoresis(PAGE). In 12.5% SDS-PAGE, non reduced protein showed two main bands, which reacted with CPS, that had apparent molecular weights of 43,700 and 26,600, and the molar ratio of the components was 1: 1. After reduction with 2-mercaptoethanol, the CRP-like protein showed a single band with an apparent molecular weight of 26,600 in the same electrophoresis. The molecular weight of CRP-like protein was estimated as 81,400 from sedimentation equilibrium analysis by ultracentrifugation. These observations indicated that the CRP-like protein of rainbow trout was a trimer, which was composed of one monomer subunit and one disulfide-linked dimer. The CRP-like protein may be a glycoprotein in nature, since it was sensitive to the treatment with sodium meta-periodate. The isoelectric point(pI) was estimated as 4.74 by column isoelectric focusing.

Sandwich enzyme-linked immunosorbent assay(ELISA) and CPS-ELISA were used for detection of rainbow trout CRP-like protein. The minimum amount of detectable CRP-like protein was about 3.2ng per ml by both methods. The level of CRP-like protein in serum was measured by CPS-ELISA in the rainbow trout inoculated experimentally with virulent *Vibrio anguillarum* strain NCMB 571. While the level of CRP-like protein in normal serum was about 38 μ g/ml serum, the level in the inoculated fish serum increased remarkably 48 hrs after the inoculation. The increase of CRP-like protein level correlated to the increase of number of the viable bacteria in the liver. These results indicated that the measurement of the level of CRP-like protein in rainbow trout serum could be used for screening of health conditions and bacterial infection in fish.