PURIFICATION AND PARTIAL CHARACTERIZATION OF *AEROMONAS HYDROPHILA* HEMOLYSIN

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SUMMARY

1. Aeromonas hydrophila isolated from infected fish has been cultured. Conditions for culture have been established under which hemolytic activity of 68.79 HU/ml of culture has been observed

2. The hemolysin in the cell free culture medium has been shown to be thermo-labile with half life at 0°C for about 6 days. Evidence has been presented to show that the toxin polymerizes when lyophilized. In addition, it undergoes " activation " when heated at 10-37°C for 30 minutes.

3. The hemolysin has been purified to homogeneity using chromatography on DEAE-cellulose with salt gradient elution. The specific activity of the product is 121.2 HU/mg protein while overall recovery is 39.6 % with 14 folds of purification.

4. The hemolysin has been shown to contain one single polypeptide with molecular weight 54-55 Kdal. It is active in monomeric form.

5. The purified hemolysin has been subjected to amino acid analysis and shown to contain, excluding tryptophan, 502 residues. The extinction coefficient

$E^{1\%}_{280}$ is 4.92 while the molar extinction coeffient is 1.3519×10^{5}

6. The pH activity curve was bimodal with optima at 6.0 and 7.5

7. A number of multivalent ions and reducing agents have been shown to inhibit the activity of the purified hemolysin.