M.S.106. MATHEW, P.T.–Purification and Characterization of Glucose 6–Phosphate Dehydrogenase in Fish–1989– Dr. K. Gopakumar.

The thesis concerned with a detailed study of a purification characterization and immobilization of glucose 6–phosphate dehydrogenase in pearl spot. *(Etroplus suratensis)* Studies on carbohydrate metabolism is very important in plants, animals and other living organisms. Tricarboxylic acid cycle or kreb's cycle is the most important sequence of reactions by which glucose is metabilized. Hexose monophosphate shunt is a sequence of reaction, other than tricarboxylic acid cycle, by which carbohydrateis metabilized in biological systems including plants and microorganisms. Glucose 6–phosphate dehydrogenase is the key regulatory enzyme in thios pathway. One of the main function of it is to supply

reducing equivalents in the form of NADPH. Hepatic glucose 6–phosphate dehydrogenase has been shown to be under both nutritional and hormonal long term regulation.

There are very few reports about the study of hexose monophosphate shunt and glucose 6–phosphate dehydrogenase in fish. Immobilization of enzymes has become very important in recent times. Various materials has been tried for immobilizing glucose 6–phosphate dehydrogenase from fish. Fishes, *Etroplus suratensis* (pearl spot), *Otolithus argentneus* (Tigertooth croacker) *Tilapia mossambica* (Tilapia), *Labeo rohita* (Rohu). *Catla catla* (Catla), *Mugil cephalus* (Mullet), *Sciaena aneus* (Bigeye croaker), *Chanos chanos* (Milk fish) and *Meretrix casta* (Back water clam) were analysed for the glucose 6–phosphate dehydrogenase activity. Muscle, liver and intestine were separately examined for glucose 6–phosphate dehydrogenase activity. It was observed that glucose 6–phosphate dehydrogenase activity was present in the liver of pearl spot to a significant level.

Liver of mature fresh pearl spot was used in the purification of the enzyme. It was homogenized in the buffer at pH 7.0 and proteins were precipitated using ammonium sulphate. Precipitated proteins were made free of ions by gel filtration on Sephadex G–25. It was further purified by ion exchange chromatography on DEAE cellulose in a gradient elution method. Proteins thus obtained were subjected to gel filtration on Sephadex G–100. Purified homogeneous active enzyme protein was obtained. 141 fold purification was effected by the above method.

Homogenity of enzyme was verified by gel filtraion on sephacryl S–200. It was further confirmed by poly-acrylamide gel electrophoresis. Absorption spectrum of purified glucose 6–phosphate dehydrogenase was also found out. Ratio of absorption at 280 nm to 260 nm was 1.71.

Subunit molecular weight of purified glucose 6–phosphate dehydrogenase was determined using sodium-dodecylsulphate polyacrylamide gel electrophoresis. It was found as 60,000. No activity was observed with the glucose 6–phosphate dehydrogenase protein separated on SDS polyacrylamide gel electrophoresis. So monomer was found to be an inactive form. Subunit molecular weight was similar to that of other glucose 6–phosphate dehydrogenase purified from other sources. Molecular weight of purified glucose 6–phosphate dehydrogenase from liver of pearl spot was further determined by gel filtration on sephadex G–100 as 118,000. Investigations showed that nature protein was a dimer of two subunits. So it was proved that active form of glucose 6–phosphate dehydrogenase purified from pearl spot was a dimer.

Amino acid composition of purified glucose 6–phosphate dehydrogenase was determined. Amino acid pattern of the enzyme was similar to that 6–phosphate dehydrogenase isolated from other sources.

Thermal stability studies and pH stability studies were also carried out. pH optimum was found to be pH 8.0. Glucose 6–phosphate dehydrogenase was having maximum activity in Tris HCl buffer. Enzyme was stable between the pH 6.5 to 8.5. It was stable between the temperatures 20°C to 50°C.

Glucose 6–phosphate dehydrogenase purified from pearl spot was specific for the substrate glucose 6–phosphate and coenzyme NADP. It was found to be an NADP specific enzyme. Lineweaver–Burk plots were also drawn. Km values were 105 uM and 50 uM with respect to glucose 6–phosphate and NADP respectively.