M.S.30. MUKUNDAN,M.K.–Studies on Fish Lipases –1983 –Dr. K. Gopakumar

Oil sardine contains significant amounts of lipase distributed in its body organs, of which hepatopancreas has the maximum concentration viz. 126 units per gm wet weight of hepatopancreas.

A method to purify lipase from the hepatopancreas of oil sardine has been worked out. Pure sardine lipase was prepared at a yiels of 35% at a specific activity of 940 units. The method consists of four major steps, which are Steps 1. Defatting hepatopancreas. Step 2. Ammonium sulphate fractionation. Step 3.

DEAE sephadex ion exchange chromatography; Step 4. Sephadex G-100 column chromatography.

The sardine lipase protein upon chromatography on sephacryl S-200 gave only one peak. Further on polyacrylamide gel electrophoresis it gave only one band. Molecular weight estimation by gel filtration on sephadex G-100 and SDS acrylamide electrophoresis gave a value of 54500. Carbohydrate analysis of sardine lipase showed the presence of 6.1% carbohydrate. The carbohydrates present in sardine lipase were glucose, arabinose and xylose in 1:4.8:4.2 proportion. Amino acid analysis data revealed that sardine lipase protein do not contain any sulphur amio acids. Sardine lipase is not an SH. enzyme.

Sardine lipase had pH and temperature optima at pH 8 and 37 °C respectively. The enzyme was stable up to a temperature of 45°C. It was also stable in the pH range 5 to 9.5.

A variety of molecules and ions were inhibitors of sardine lipase.

Studeis with various substrates showed that sardine lipase hydrolyses a variety of substrates like various triglycerides, 1-mon-glycerides and diglycerides.

Lipolysis of the neutral triglycerides of sardine oil and coconut oil by sardine lipase showed that lauric acid was released preferentially at a higher rate than the next higher acids and decanoic acid ($C_{10:0}$). Further the acids with odd carbon number viz. $C_{15:0}$ and $C_{17:0}$ were released at substanitially higher rates than the fatty acids $C_{14:0}$, $C_{16:0}$ etc with even number of carbon atoms.

Saridine lipase was found to have triacetin and 12×10^{-6} for tributrin as substrates.

Sardine lipase is specific for 1 ester bond and the lipolysis observed with 2-monocaprylin is due to the hydrolysis of 1 monocaprylin formed as a result of acyl migration of the acyl group from 2-position to 1-position.

Sardine lipase was immobilised in polyacrylamide gel. The immoblised enzyme showed 65% to 70% of the activity of the native enzyme. It was observed that immobilised sardine lipase was more stable than the native enzyme that it can be stored at 0°C for 4 months without any loss in activity.