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Purification of two acid proteinases from procine ovarian follicular fluid and their enzymatic properties

Insulin-like growth factors (IGFs0 play an important role in the stimulation of folliculogenesis and their binding proteins (IGFBPs) have the ability to modify the diverse metabolic and mitogenic effects of IGFs. IGFBp availability and bioactivity is determined not only by gene expression but also by the regulated proteolytic processing of the protein. An IGFBP -3 processing proteinase derived from media of variety of human cell lines is identified as cathepsin – D like acid proteinase.

Therefore, its is very important to purify the acid proteinases present in the mammalian ovary inorder to study their important role in mammalian ovarian physiology. Last year we have reported the isolation and preliminary characterization of acid proteinases from porcine ovarian follicular fluid. In this report purification procedure of isolated two acid proteinases from porcine follicular fluid and their enzymatic properties will be presented.

Two types [DE (DEAE cellulose0-nbound (55%) and DE-bound type (30%)] of acid proteinases were purified to apparent homogeneity by using successive chromatographies on DEAE cellulose -52, (70% ammonium sulphate saturation), Sephcryl S -200, Q sepharose. Molecular weight of both types were around 40,000 D (dalton) based on gel filtration. Specific activity of DE-unbound proteinase was increased by 743 times with around 10% yields and DE-bound proteinase by 410 times with around 5% yield after the affinity chromatography step.

Major difference between the two types was the ability of their binding to the DEAE -52 cellulose at pH 8.7 indicating a difference in net charge. They showed a liner increase of proteolytic activity over increasing incubation time from 15 to 150 min and increasing enzyme concentration from 0.167 to 2 µg in the reaction mixture. They showed rather high at activity pH2.5 to 3.5 and the optimum pH was around 2.8. They had no detectable activity at pH 1.8. Both enzymes showed an optima activity at 37 to 40 ^{o}C. Stability of the DE- unbound from at higher temperatures was much lower than that of DE-bound from. DE-bound enzyme was relatively more stable at pH 6 than that of pH 2, 4 and 8.7. Proteolytic activity of both enzymes were inhibited completely at 0.01 µM pepstatin and not inhibited by 1mM PMSF and 1mM soy bean trypsin inhibitor suggesting that they belong to the class of aspartic acid proteinases. Further studies are on progress to identify their role on IGFBp processing.