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ISOLATION AND PURIFICATION OF TWO ACID PROTEINASES FROM PORCINE OVARIAN TISSUES

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An acid proteinase, cathepsin D derived from various human cell cultures has been shown to be responsible for proteolytic processing of insulin like-growth factor binding protein-3 (IGFBP-3). Importance of IGFBPs lies in their potential to modulate the diverse growth promoting effects of insulin like-growth factors (IGFs) in tissues including the ovary. Therefore acid proteinases might play an important role in ovarian homeostasis. Hence it is very important to study on acid proteinases of ovary to identify their role. However there are no reports available on attempts made on purification of acid proteinases from mammalian ovaries. Previously we have reported the purification and characterization of acid proteinases from porcine ovarian follicular fluid. In this study two acid proteinases were isolated and purified from porcine ovarian tissues.

Forty six porcine ovaries were sliced in phosphate buffered saline and the ovarian follicular fluid contents were removed by separating the supernatants by centrifugation. Whole procedure was carried out at 4°C. Remaining pellet was used to extract the ovarian tissue content. The pellet was homogenized using phosphate buffered saline. Ovarian tissue extract (OTE) was obtained by centrifuging at 15,000 rpm for 1 hr and dialysing the supernatant in 20 mM phosphate buffer at pH 7.5. Acid proteinase activity was detected at pH 3, using bovine haemoglobin as the substrate. Total protein content of the pooled fractions was detected using Lowry method. Acid proteinases in the crude extract were purified by DEAE cellulose chromatography, ammonium sulphate precipitation, Sephacryl S-200 chromatography and pepstatin Sepharose chromatography. Polyacrylamide gel electrophoresis (PAGE) under non-denaturing conditions was performed and followed by incubation in the presence of haemoglobin and staining with amido black to identify acid proteinases. Effect of pepstatin on proteinase activity was tested.

Two types of acid proteinases DE-unbound (fraction which was not bound to DEAE cellulose) and DE-bound (fraction which was bound to DEAE cellulose) were identified and purified to near homogeneity. Purification fold and yield of DE-unbound proteinase obtained was 388 fold with a 16.2% recovery and that of DE-bound proteinase was 399 fold with an 11.5% recovery. Both proteinases showed a molecular weight of approximately 40 kDa based on Sepacryl S-200 chromatography. Two to three isoenzymes of both DE-unbound and DE-bound proteinases were suggested to be present based on the results of PAGE under non-denaturing conditions. Both DE-unbound and DE-bound proteinases were suggested to be aspartic proteinases based on their complete inhibition in the presence of 0.1 μM pepstatin.

Further studies are in progress to identify the N-terminal amino acid sequencing and cleavage specificity to identify their important role in IGFBP processing.