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ISOLATION, PURIFICATION AND CHARACTERIZATION OF ACID PROTEINASES FROM PORCINE OVARY

By

Handunge Kumudu Irani Perera

Department of Biochemistry,

Faculty of Medicine,

University of Peradeniya

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Faculty of Medicine,

University of Peradeniya

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Abstract

Isolation, purification and characterization of acid proteinases from porcine ovary

Acid proteinases are suggested to play an important role in the ovarian folliculogenesis, through their proteolytic action on insulin-like growth factor binding protein-3 (IGFBP-3). Cathepsin D, derived from a variety of human cell lines, has been identified as a proteinase responsible for the proteolytic processing of IGFBP-3 that can alter the structure and presumably the function of IGFBP-3, which modifies the diverse growth promoting activities of the insulin-like growth factors (IGFs). Therefore cathepsin D has been suggested to play a significant role in regulation of cellular IGF action. IGF system appears to play a role in ovarian folliculogenesis, and act locally on the follicular cells to modulate the timing and direction of their differentiation. However, there are no reports available on attempts to purify acid proteinases from mammalian ovary, although several purification studies with various other tissues, including ovaries of non-mammals have been reported. Therefore the objective of this study was to extract, purify and characterize acid proteinases of porcine ovary, which is essential for future studies on the identification of their function in the ovary.

In this study, two types of acid proteinases, DE-unbound (proteinase which was not bound to DEAE cellulose) and DE-bound (proteinase which was bound to DEAE cellulose) present in the porcine follicular fluid extract (OFFE), and ovarian tissue extract (OTE)

were purified to near homogeneity. Purification steps used were DEAE cellulose chromatography, ammonium sulphate precipitation, Sephacryl S-200 chromatography, Q Sepharose chromatography, pepstatin Sepharose chromatography and Mono Q chromatography using fast protein liquid chromatography system. Proteins obtained at each step of purification were analysed using SDS PAGE and PAGE under non-denaturing conditions. Western blotting was done using cathepsin D antibody. Enzymatic properties of purified proteinases such as time dependency, enzyme concentration dependency, pH dependency, temperature dependency, effect of proteinase inhibitors and stability at different temperature and pH were studied.

Purification fold and yield obtained after the pepstatin Sepharose chromatography step, were 1411 times and 18.9% with OFFE, DE-unbound proteinase, 1851 times and 10.7% with OFFE, DE-bound proteinase, 388 times and 16.2% in OTE, DE-unbound proteinase, and 399 times and 11.5% with OTE, DE-bound proteinase.

Based on characteristics of proteinases it was suggested that both ovarian follicular fluid and ovarian tissue have same type of acid proteinases. Purified proteins were suggested to be of aspartic proteinases based on the complete inhibition of proteolytic activity by 0.01μM pepstatin in the reaction mixture. They were not inhibited by phenyl methane sulphonyl fluoride and soy bean trypsin inhibitor. Molecular weight of both types was around 40 kDa, based on Sepahcryl S-200 chromatography. All 4 fractions of proteinases purified showed 64, 54, 32 and 30 kDa bands on SDS PAGE, which are suggested to be products of the same protein. Results of the PAGE suggested the presence of 5

isoenzymes of one acid proteinase in both OFFE and OTE. Fifty four kDa protein is suggested to be cathepsin D or a cathepsin D-like proteinase based on immuno precipitation with rat cathepsin D antibody. Purified enzymes had an optimum pH of around 2.8 with 2% denatured haemoglobin as substrate. Optimum temperature of purified enzymes were found at 39°C. Thermal stability of DE-unbound proteinase was lower than that of DE-bound proteinase. Stability of both enzymes was highest at 37 to 40 °C. Maximum stability of the proteinases were found between pH 6.5 and 7.5. The purified acid proteinases may have an important role in ovarian homeostasis. Further, studies with the purified proteinsases are necessary to identify their role in the regulation of IGF action via degradation of IGFBPs.