EFFECT OF OVARIAN ASPARTIC PROTEINASES IN MODULATING IGFBP-3

H.K.I. Perera[1], Senarath B.P. Athauda[1] and T. Takahashi[2]

[1]Department of Biochemistry, Faculty of Medicine, University of Peradeniya.
[2]Laboratory of Molecular Cellular interactions, Faculty of Advanced Life Science, University of Hokkaido, Japan

Introdution

Although ovarian functions are primarily controlled by the hypothalamus-pitiutary-axis, it is well understood that a microenvironment specific for each individual ovarian follicle and other ovarian structures exists. Autocrine and paracrine factors that act alone or modulate gonadotropin actions are of paramount importance in regulating ovarian functions.

The existence of insulin-like growth factor (IGF) system in the ovary has been well documented in many species. The concentration of free IGF is controlled by the local balance of IGF binding proteins (IGFBP) and proteinase activities that degrade IGFBP. In the ovary, proteinases, specifically cleave IGFBPs into smaller molecular weight forms that have a reduced or no activity (Spicer, 2004). Thus, the proteolytic activity may be an important mechanism by which bioactive IGFs are made availble to ovarian tissues. Different groups of proteinases are shown to be involved in the proteolysis of IGFBP in ovary. However, the studies conducted on aspartic proteinases in the ovary are scarce. Previously we have established a purification procedure to isolate two aspartic proteinases from porcine ovary (Perera, 1999). Objective of the present study is to study the effect of the purified ovarian aspartic proteinases on IGFBP-3 which is one of the most predominant ovarian IGFBPs.

Materials and Methods

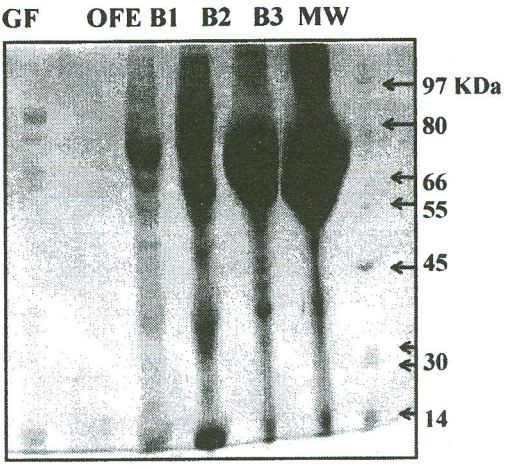
Ovaries were collected (n = 200) from pigs around 6-8 months of age, from abattoir. Recombinant human IGFBP-3 and IGFBP-3 antibody were from Genzyme/Techene, U.S.A.

Ovarian fluid extracts were obtained. This extract was used to purify aspartic proteinases by the method established by us previously (Perera, 1999). Four chromotography steps used were anion exchange, gel filtration, affinity and fast protein liquid chromatography. Purity was detected by analysing specific activity of fractions obtained and using SDS PAGE.

Purified proteinases were used to study their effect on IGFBP-3. IGFBP-3 (250 ng) was incubated with purified proteinases (100 ng) at pH 5, 5.5, 6, 6.5 and 7 (0.1 M formic or phosphate buffer) at 37°C. Aliquots of the reaction mixture were obtained at 1.5, 3, 6, 12 and 24 hrs and analysed using SDS PAGE, Western blotting and immuno detection techniques. Control series was conducted for comparison, by incubating IGFBP-3 without proteinases for 24 h.

Results

Aspartic proteinases were purified to near homogeneity in two fractions, namely DE-unbound (DE-U) fraction and DE-bound (DE-B) fraction based on their binding ability to the anion exchange column (DE-52 cellulose) at pH 8.5. Specific activities of two fractions were 746 U/ mg and 525 U/ mg respectively. Some of the fractions obtained during purification were analysed using SDS PAGE and the results are shown in Figure 1.



OFE- ovarian fluid extract (5 µl), B1- DE-bound pool 1 –high activity pool used for further purification (15 µl), B2- DE bound pool 2 (15 µl), B3- DE- bound pool 3 (15 µl), GF- gel filtration high activity pool (15 µl), MW- molecular weight markers

Figure 1. SDS PAGE analysis of fractions obtained during early purification steps

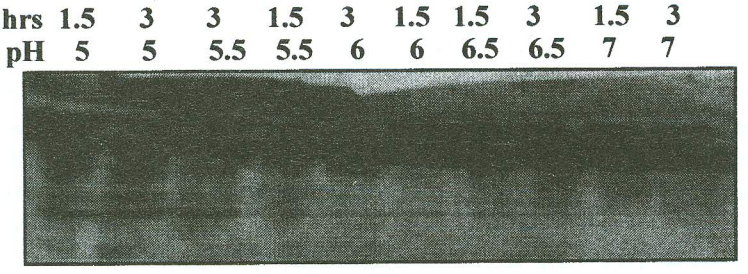


Figure 2. Effect of DE-bound proteinase on IGFBP-3 at pH 5- 7 for 1.5 or 3 h incubation. Cleaved IGFBP-3 is detectable in first 3 lanes (arrow). Other lanes show uncleaved IGFBP-3. Top most band is bovine serum albumin (BSA).

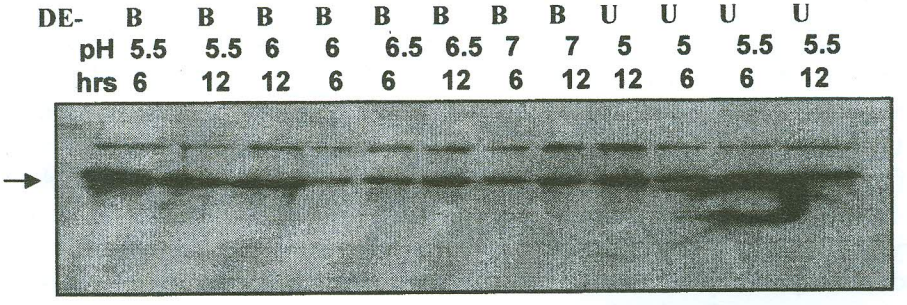


Figure 3. Effect of DE-B and DE-U proteinases on IGFBP-3 at pH 5- 7 for 6 or 12 h incubation. Cleaved IGFBP-3 is shown (arrow). Top band is BSA.

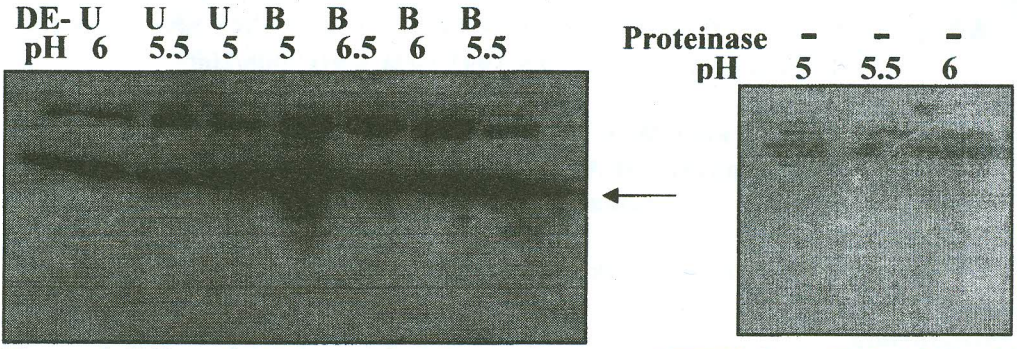


Figure 4. Effect of DE-U and DE-B proteinases on IGFBP-3 at pH 5- 6.5 for 24 h incubation. Cleaved IGFBP-3 with a lower molecular mass is shown (arrow). Top band is BSA. Control reaction without proteinase is shown at right side (BSA and IGFBP-3 are not apart).

Effect of purified aspartic proteinases on IGFBP-3: DE-bound and unbound proteinases showed a specific cleavage pattern on IGFBP-3 at pH 5 to 7 (Figure 3 and 4). Cleavage seems to occur faster towards lower pH (Figure 2).

Discussion

Regulatory effects of IGF in ovarian functions such as enhancement of cell proliferation, aromatase activity, and progesterone biosynthesis are documented. It is known that proteinases modulate IGF action by hydrolysing IGFBPs (Spicer, 2004). IGFBP-3 is the most predominant IGFBP present in the follicular fluid. IGFBP-3 is reported to have an inhibitory role in the porcine ovarian granulosa cells.

Hence, we have analysed the effect of the two novel aspartic proteinases purified from porcine ovary on IGFBP-3. Our results provide evidence to suggest that IGFBP-3 is specifically cleaved by the two novel aspartic proteinases. Cleavage was faster towards lower pH values. Hypovascularity and anaerobic glycolysis have been observed in the ovary during late folliculogenesis, early CL development and regression, where pH becomes acidic. Therefore, it is likely that these aspartic proteinases have a role in regulating the bioavailability of IGF in the ovary.

Conclusion

Purified aspartic proteinases were able to specifically cleave IGFBP-3. Hence, aspartic proteinases may regulate IGFBP-3 concentration in the ovary and thereby the free IGF concentration.

References

Perera, H.K.I. (1999). M. Phil. Thesis. Isolation, purification and characterization of acid proteinases from porcine ovary.

Spicer, L.J. (2004). Proteolytic degradation of insulin-like growth factor binding proteins by ovarian follicles: a control mechanism for selection of dominant follicles. Biol Reprod, 70(5):1223-30.