Bovine Follicular Fluid and Serum Share a Unique Isoform of Matrix Metalloproteinase-2 That Is Degraded by the Oviductal Fluid

Minjung Kim, Minjeong Hong, Jisoo Kim, Haekwon Kim, Seung-Jae Lee[1], Sung Goo Kang[2] and Dong Jae Cho[3]

Laboratory of Developmental Biology, Seoul Womens Univ., Seoul, 139-774

Mirae & Heemang Ob/Gyn Clinic[1], Seoul, 135-120

Department of Biology[2], College of Natural Sciences, Inje Univ., Kimhae 621-749

Department of Ob/Gyn[3], College of Medicine, Younsei Univ., Seoul, 135-270

Introduction

At the time of ovulation in mammals, a part of follicular fluid as well as cumulus-oocytes complex is known to enter into the oviduct even in those species that lack ovarian bursa (Hunter, 1988). Thus it appears that the environment for fertilization and embryonic development consists of follicular fluid, oviductal fluid and possibly resulting products of the mutual interaction between two fluids (McNutt and Killian, 1991). However, there has not been any report regarding to the molecular nature of the interaction. Matrix metalloproteinases (MMPs) are a group of enzymes known to play important roles in the remodeling of almost tissues and thereby participates in the differentiation and organogenesis (Werb, 1997). To date, more than 20 enzymes are identified among which gelatinase A (MMP-2) is most widely distributed one throughout body tissues. After its synthesis as an inactive proform by the cell, proMMP-2 is secreted into the extracellular space where it is believed to become active MMPs by proteases. However, molecules responsible for the activation are poorly understood. Ovarian follicular fluids of mammals have also been reported to contain gelatinases, yet unidentified, which is involved in the cyclic remodeling of ovarian tissues such as folliculogenesis and luteolysis (Ichikawa et al., 1983). In this study, the presence of MMP-2 and its isoforms in bovine follicular (bFF) and oviductal fluids (bOF) and sera (bS) was firstly investigated. Secondly. the possible interaction between body fluids was examined by observing the gelatinase profiles in zymograms of body fluids before and after mixing.

Materials and Methods

BFF and bOF were prepared from ovaries and oviducts collected from a local abattoir. BS was also donated from the animals and FBS was purchased from Sigma. To examine gelatinase profiles in body fluids, zymogram using gelatin-gel SDS PAGE was used and to identify the type of MMP, immunoblotting experiments using anti-human MMP-2 and anti-human MMP-9 antibodies was done.

Results and Discussion

BFF consistently exhibited 110 kDa (GA110), 92 kDa, 84 kDa and 62 kDa gelatinases of which activities varied depending on the individual (Fig. 1). Surprisingly, when bFF was mixed with bOF and then incubated, GA110 but not other gelatinases of bFF disappeared even 30 min after mixing (Fig. 2). To see it gelatinolytic proteins were indeed gelatinases, gelatin gels after SDS PAGE were incubated in the presence of proteinase inhibitors. As seen in Fig. 3, EDTA and phenanthroline that are known to be
MMP inhibitors blocked most gelatinases of bFF except 84 kDa while PMSF failed to inhibit the development of enzymatic activities. These results demonstrate that GA110 and 62 kDa are indeed gelatinases. When similar zymographic analyses were done using bS and FBS, they also gave both GA110 and 62 kDa gelatinases (Fig. 4). Interestingly, EDTA treatment to bFF and bS resulted in the enhanced enzymatic ctivity of GA110. Other gelatinase activities were not affected by EDTA. To identify the nature of gelatinases of both GA110 and 62 kDa present in bFF and bS, immunoblotting experiments were done. As seen in Fig. 5, anti-human MMP-2 antibody recognized both GA110 and 62 kDa gelaitnase bands of bFF and bS. EDTA-treated bFF and bS showed enhanced immunoreactivity and bOF treatment to these fluids abolished the reactivity. Anti-human MMP-9 antibody failed to react with GA110 or 62 kDa proteins. Based upon these results, it is concluded that bFF and serum share a unique isoform of MMP-2, GA110. Deduced from other MMP isoforms, GA110 is suggested to be

produced by the interaction of MMP-2 with other protein(s) via covalent bonding. BOF, on the other hand, possesses a proteolytic activity which specifically acts on GA110, suggesting that the mutual interaction between bFF and bOF could indeed occur.

Abstract

Gelatin zymograms of bFF and bS showed GA110 and 62 kDa gelatinses in adsition to several minor ones. Of these, GA110 gelatinase was abolished by treating bFF or bS with bOF and interestingly, its enzymatic activity was enhanced by adding EDTA to bFF or bS before zymographic analyses. Experiments using specific inhibitors of MMPs indicated that GA110 and 62 kDa proteins were indeed gelatinases. Immunoblotting experiments using an antibody against human MMP-2 showed that both GA110 and 62 kDa were an MMP-2 isoform and active MMP-2, respectively. The results suggest that the interaction between bFF and bOF can occur at the time of fertilization.

Fig. 1

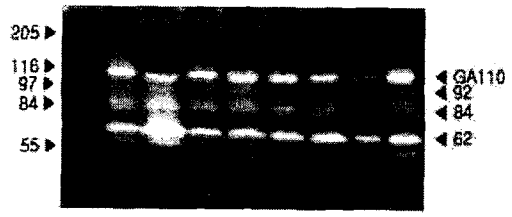


Fig. 2

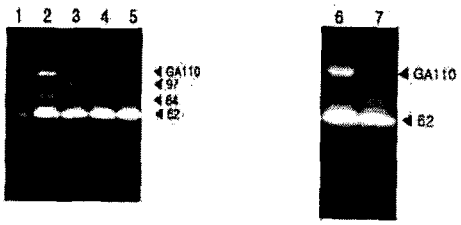


Fig. 3

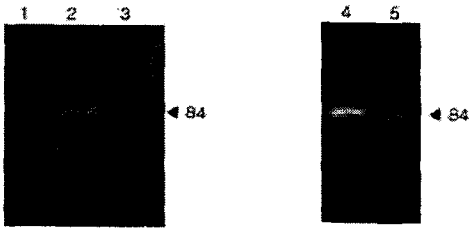


Fig. 4

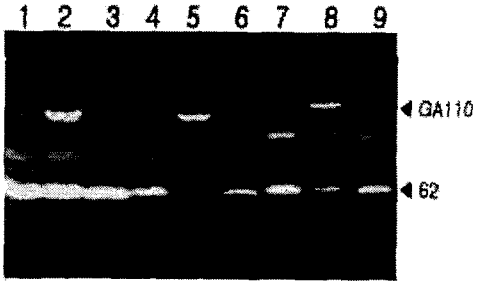
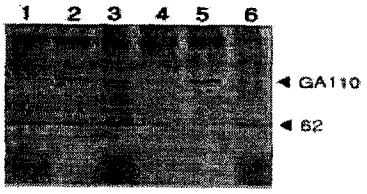


Fig. 5



References

Hunter, RHF. The fallopian tubes. New York: Springer-Verlag. 1988: 12-29.

Ichikawa S, Morioka H, Ohta M, Oda K, Murao S. Effect of various proteinase inhibitors on ovulation of explanted hamster ovaries. J Reprod Fertil 1983; 68:407-412.

McNutt TL, Killian GJ. Influence of bovine follicular and oviduct fluids on sperm capacitation in vitro. J Androl 1991; 12:244-252.

Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. Cell 1997; 91:439-442.