Matrix Metalloproteinase in Mammary Gland
Remodeling-Modulation by Glycosaminoglycans

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Mammary gland which undergoes proliferation, differentiation and involution in adult life is a useful model system to study the role of extracellular matrix (ECM) in regulating tissue-specific functions. The involution that follows weaning results in the suppression of casein gene expression, collapse of alveolar structures and degradation of basement membrane as evidenced by biochemical analysis of matrix components like proteoglycans and collagen. Differential expression of three different MMPs viz. 130 K, 68 K and 60 K with varying specificity to Col IV of basement membrane and Col I of stroma, their selective inhibition by TIMP and proteoglycans and modulation by estrogen highlight the importance of these in the remodeling of the ECM in the mammary gland. The inhibition of these MMPs by glycosaminoglycans, particularly CS and change in the concentration of CS at different stages of mammary gland development suggests the existence of a novel mechanism for the regulation of the activity of MMPs at extracellular sites.

KEY WORDS: Mammary gland remodeling; MMPs; GAG changes; inhibition by GAG.

ABBREVIATIONS: Matrix metalloproteinases (MMPs), Glycosaminoglycans (GAG), Chondroitin sulfate A (CSA); Chondroitin sulfate C (CSC), Hyaluronic acid (HA); Heparin (H); Dermatan sulfate (DS); Extracellular matrix (ECM); Tissue inhibitor of metalloprotease (TIMP).

INTRODUCTION

Mammary gland undergoes developmental changes during adult life and expresses tissue specific cell function in a cyclic manner. In vitro studies using cultures of mammary epithelial cells on isolated matrix macromolecules, have demonstrated the role of extracellular matrix in regulating tissue specific functions. Involution of the mammary gland after weaning results in a gradual loss of tissue specific functions which is accompanied by the drastic degradation of the extracellular matrix components. ECM degradation is brought about largely by the action of a group of neutral matrix metalloproteinase (MMP). Three different MMPs viz. 130, 68 and 60 K gelatinases have been found to be involved in the degradation of ECM in rat mammary gland [1, 2]. The 68 K gelatinase is a constitutive enzyme while the 130 K that appeared during the early involutary phase and 60 K that appeared during the late

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involuntary phase were inducible. A temporal relationship found between the appearance of 60 K gelatinase activity and loss of tissue specific differentiated function such as casein production [3] and morphological changes in the mammary gland suggest that MMPs, particularly 60 K gelatinase which appears at the later phase of involution has important role in remodeling of ECM in mammary gland [4]. The regulation of the activities of these MMPs is therefore crucial to the control of the nature of the cellular microenvironment which is important in the regulation of tissue specific functions.

MMPs are known to be subject to regulation by transcriptional control, growth factors, hormones and specific inhibitors such as TIMP [5–7]. A reciprocal relation between the expression of TIMP and the activity of certain MMPs in mammary gland has been reported [3]. Although 68 K gelatinase which is a constitutive enzyme in mammary gland is regulated by TIMP, it is not known how its activity is regulated when TIMP expression is low/absent [3]. Similarly, 130 and 60 K are not subject to TIMP regulation. Although 60 K is regulated by β estradiol, 130 and 68 K are not affected by β estradiol [4]. In ECM, collagen components are present in association with a number of other macromolecular components such as glycoproteins and proteoglycans (PG). The possibility of other components particularly polyanionic glycosaminoglycans and PGs which are capable of binding to cations, influencing the action of these matrix metalloproteinase at the extracellular sites was examined and the results are presented here.

MATERIALS AND METHODS

Mammary tissues were collected from rat involuting glands. 130 K gelatinase was prepared from the 2nd day involuting gland by gel filtration as described before [8]. 68 and 60 K gelatinase were prepared from the mammary gland on the 6th day of involution by gelatin sepharose affinity chromatography as described earlier [8]. Zymographic assays of purified enzymes in gelatin impregnated gels in multiwells were carried out [9]. The effect of various GAGs on the purified enzyme was tested by preincubating the enzyme with the various GAGs and developing zymograms. It was also tested by using radioiodinated gelatin as substrate and measuring the TCA soluble radioactivity. The amount of different GAGs in the mammary gland at different stages of development was quantified by chondroitinase digestion, nitrous acid degradation and ion exchange chromatography as described earlier [10].

RESULTS AND DISCUSSION

1. Effect of Glycosaminoglycans on MMP Activity

The possibility of PGs influencing MMP activity was examined in vitro by measuring the activity of different gelatinase of mammary gland pretreated with different GAGs, viz. Hyaluronic acid (HA), Heparin (H), Chondroitin sulfate A (CSA) and Chondroitin sulfate C (CSC). Addition of HA or heparin did not alter the ability of gelatinase to degrade gelatin. But as reported earlier [8], CSA and CSC inhibited the ability of all the three gelatinases. Since the CS showed inhibitory effect, it was