DEGRADATION OF PRG4/SZP BY MATRIX PROTEASES

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INTRODUCTION: PRG4, also known as articular cartilage superficial zone proteoglycan (SZP), lubricin and megakaryocyte stimulating factor (MSF) precursor, is a ~345kDa mucinous glycoprotein/proteoglycan that has been detected in a variety of tissues including cartilage, tendon, bone, heart and liver [1]. In the synovial joint, PRG4 is specifically synthesised by chondrocytes located in the superficial zone of articular cartilage and by some surface lining cells of the synovium [2]. Unlike most cartilage proteoglycans it is not retained in the cartilage matrix. Sequence analyses have indicated that PRG4 is a multifunctional domain protein that has the potential for a number of functions including cell proliferation, cytoprotection, lubrication and self-aggregation/matrix binding [3]. The occurrence of PRG4 splice variants in diseased cartilage and its differential expression in the presence of cytokines and growth factors suggest that it may prevent cellular adhesion in normal cartilage metabolism [4]. Lower molecular weight forms of PRG4 have been found in synovial fluid [2], and catabolism of the molecule may affect its potential functionality. Thus, the objective of this study was to determine the susceptibility of PRG4 to degradation by a variety of matrix proteases which may be present within the synovial joint.

METHODS: Human recombinant PRG4 was obtained from the culture medium of a PRG4-expressing CHO cell line (Wyeth Research). Protein was purirified using heparin agarose and peanut agglutinin (PNA) lectin agarose affinity columns. Aliquots of PRG4 (40 micrograms in 100 microliters) were incubated for 16 h at 37°C with the following: human leukocyte elastase (Sigma) at 0.2U/ml; calpain (Calbiochem) at 1µg/ml; cathepsins B and D (Sigma) at 0.5U/ml; plasmin (Sigma) at 0.2U/ml; ADAMTS-4 (Aggrecanase-1; Wyeth Research) at 9.3µg/ml; matrix metalloproteinases (MMPs) –1 and –7 (Paul Cannon, Roche Bioscience, Palo Alto, CA) at 5µg/ml and 10µg/ml respectively. The buffers used were as follows: 100mM Tris-HCl, 100mM $CaCl_2$, pH8.8 for elastase; 110mM imidazole, 1mM EGTA, 5mM β-mercaptoethanol, pH7.5 for calpain; 0.2M NaCl, 1mM EDTA, 10mM DTT, 0.25M Na-Acetate, pH5.5 for cathepsins B and D; 0.15M NaCl, 20mM Tris-HCl, pH7.4 for plasmin; 0.1M Tris-HCl, 50mM NaCl, 10mM $CaCl_2$, 10mM $MgCl_2$, pH 7.4 for ADAMTS-4; 100mM Tris-HCl, 50mM NaCl, 1mM $CaCl_2$, 1mM APMA, pH 7.5 for MMPs 1 and 7. Digestion products were separated on 4-12% SDS-PAGE gradient gels and visualised by silver staining or Western immunoblotting blotting using rabbit anti-human PRG4 polyclonal antibody 06A10 (Wyeth Research).

RESULTS: Digestion of PRG4 with MMP-7 (Fig. 1, lane 7) generated a catabolite of ~275kDa, leaving no intact protein and resulting in loss of antibody binding (Fig. 1). MMP-1 digestion as seen in lane 8 generated a product of ~320kDa whilst leaving most of the PRG4 intact. Cathepsin B (lane 3) appeared to completely digest PRG4, while limited digestion occurred following incubation with Cathepsin D (lane 4). Plasmin and ADAMTS-4 (lanes 5 and 6) generated catabolites with a slightly reduced molecular weight and reduced antibody reactivity. Limited digestion was observed with elastase in the digest shown, although in other similar experiments complete digestion occurred (data not shown). The protein species observed in the calpain digestion (~300 to 150 kDa) was also seen in a reaction performed without PRG4 substrate (data not shown).

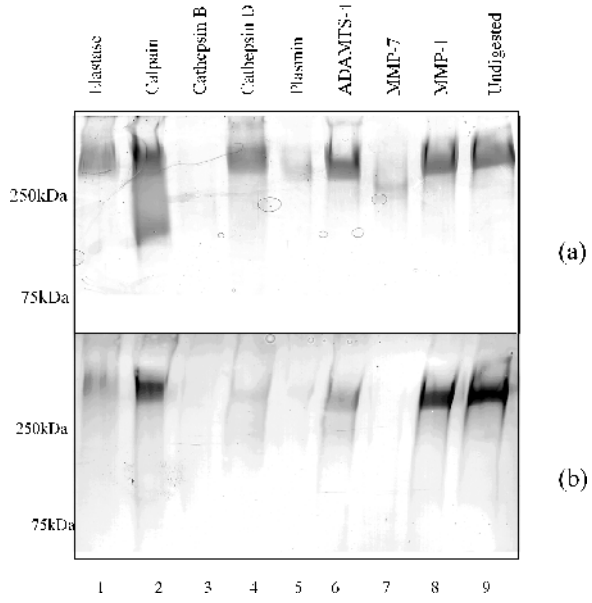

Figure 1. Gel electrophoresis of PRG4 protease digests visualised by (a) silver stain and (b) Western blot using antibody 06A10. Migration positions of molecular mass standards are shown on the left.

DISCUSSION: Our results demonstrate that PRG4 is susceptible to proteolytic digestion by matrix metalloproteases (MMP-1 & MMP-7), serine proteases (elastase, plasmin) and cysteine proteases (cathepsin B). MMP-7 is secreted by macrophages and is present in the synovial joint, and has been shown to be upregulated in osteoarthritic cartilage [5]. Cathepsin B has been implicated as potentially playing a role during cartilage proteoglycan breakdown [6]. We have shown that these enzymes also appear capable of degrading PRG4, implicating PRG4 catabolism as a possible event during the degeneration of articular cartilage associated with arthritic diseases. Matrix protease-mediated digestion of PRG4 could affect the lubricating and cytoprotective properties of the molecule, thereby compromising the integrity and normal function of articular cartilage.

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