EXTRACELLULAR MATRIX PROTEINS COMP AND FIBRONECTIN ARE SUBSTRATES FOR HTRA, A NOVEL SERINE PROTEASE UPREGULATED IN OSTEOARTHRITIC CARTILAGE

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**INTRODUCTION**

Osteoarthritic cartilage has been shown by differential display analysis to contain increased mRNA levels of a serine protease similar to bacterial stress response protein designated HtrA (1). Although HtrA does not cleave aggregan or type II collagen, recent work has shown that it degrades fibromodulin (2), a small proteoglycan found in cartilage. The role of HtrA in the pathophysiology of osteoarthritis is not known, but identification of additional HtrA substrates could potentially contribute to elucidating this issue. Here we report that recombinant human HtrA enzyme degrades both purified bovine cartilage oligomeric matrix protein (COMP, 435 kd) and purified human plasma fibronectin (Fn, 440 kd)

**METHODS**

Full-length recombinant human HtrA was produced in 293 cells as previously described (1), and its truncated form containing the protease domain was expressed in and purified from E. coli. Recombinant human stromelysin (MMP-3) was prepared in-house, and it was activated with APMA prior to use. Bovine COMP was purified from articular cartilage isolated from bovine hoof (3). Purified plasma fibronectin was purchased from (Gibco/BRL). Trypsin inhibitor, 1-anti-trypsin was purchased from (Sigma Chem. Co., St. Louis, MO)

*Analysis of COMP degradation by HtrA: Purified bovine COMP was incubated with truncated HtrA, full-length HtrA, or recombinant human stromelysin for 18 h at 37 °C. The fragments of COMP were then analyzed by Western blot analysis using an antibody to the C-terminal domain of COMP (4). Analysis of fibronectin degradation by HtrA: Plasma fibronectin was incubated with truncated HtrA or recombinant human stromelysin for 4 h at 37 °C, and the products were analyzed by SDS-PAGE after staining with Coomasie blue, and also by Western analysis using rabbit anti-human fibronectin

**RESULTS**

Human recombinant truncated or full-length HtrA degraded purified COMP to produce a fragment of molecular weight ~31 kd which was detectable only under reducing conditions. Under non-reducing conditions, SDS-PAGE showed a minor shift in the molecular weight of native COMP (Fig. 1a). The fragmentation by HtrA was inhibited by 1-anti-trypsin, a known inhibitor of HtrA. In contrast, metallo- proteinases such as stromelysin (MMP-3) cleaved COMP to generate fragments which were visible under non-reducing conditions. Stromelysin did not produce the 31 kd fragment.

Western analysis of fibronectin digested with HtrA showed the degradation of native Fn to form fragments migrating from 34.8 to 180 kd (Fig. 1b). Under the experimental conditions, stromelysin digestion of fibronectin produced fragments of different molecular weight than those produced by HtrA.

**DISCUSSION:**

Our results show that HtrA is capable of degrading COMP and fibronectin, two key ECM components of cartilage. COMP consists of five 89 kd monomeric chains containing several disulfide bonds, and most likely HtrA cleaved COMP within a disulfide loop towards the C-terminal domain of COMP monomer. A metalloproteinase, stromelysin, did not produce this fragment. We have previously shown that arthritic synovial fluid contains COMP fragments of mol. wt. 31-20 kd that are detectable only under reducing conditions (5). Additional experiments are in progress to determine whether the synovial fluid fragments are formed by the action of HtrA on COMP.

The digestion of plasma fibronectin by HtrA produced fragments in the range of 180-29 kd. Fibronectin fragments have previously been shown to stimulate catabolic as well as anabolic activity in chondrocytes (6). Therefore, HtrA may contribute to cartilage homeostasis by modulating the formation of fibronectin fragments. Thus, based on earlier work and the work described here, HtrA may contribute to cartilage destruction not by degrading aggregan or type II collagen but by degrading other cartilage proteins such as COMP, fibronectin, and fibromodulin.