ISOLATION AND IDENTIFICATION OF "AGGRECANASE": A NOVEL CARTILAGE AGGRECAN-DEGRADING-METALLOPROTEASE (ADMP)

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RELEVANCE TO MUSCULOSKELETAL DISEASE

Joint destruction in arthritic diseases involves the loss of articular cartilage aggrecan. These studies purify and identify the "aggrecan-degrading-metallo-protease" (ADMP), thought to play a key role in the degradation of cartilage aggrecan.

INTRODUCTION

The degradation of aggrecan, which provides normal cartilage with its properties of compressibility and elasticity, is a key factor in the gradual erosion of articular cartilage that leads to a loss of joint function in arthritis. Matrix metalloproteinases (MMPs) have been shown to cleave *in vitro* at the Asn^{341}-Phe^{342} bond of aggrecan, however, an "aggrecan-degrading-metallo-protease" (ADMP), or aggrecanase, can cleave aggrecan between amino acid residues Glu^{373}-Ala^{374} to release large C-terminal fragments which have been identified in synovial fluids from patients with arthritis and joint injury [1,2]. We have purified ADMP to homogeneity from bovine nasal cartilage conditioned media, cloned its complementary DNA and identified this novel protein.

METHODS

Aggrecanase was generated by stimulating bovine nasal cartilage explants with IL-1 (500 ng/ml) first to deplete the matrix of proteoglycan and then allowing secreted aggrecanase to accumulate in the media. ADMP was purified from IL-1-stimulated bovine nasal cartilage conditioned media by following its activity with an assay for detection of specific cleavage at the Glu^{373}-Ala^{374} bond using a neoepitope antibody, BC-3, which recognizes the new N-terminus, ARGS, on fragments formed by ADMP-mediated cleavage [3]. Bovine cartilage conditioned media was passed through a 1.2 micron filter, and loaded onto a Macro S column. ADMPs were eluted from the column with 1 M NaCl and the eluted material was then loaded onto a gelatin-agarose column. Material from this column, containing the ADMP activity, was collected and concentrated 6 to 7-fold. The protein was further purified by either affinity-purification using bovine TIMP-1 and an ADMP-inhibitor resin or by phenyl-sepharose and CM chromatography followed by size exclusion using Sephacryl S-200 and by HPLC separation on a C4 alkylsilane-derivatized silica column. Based on amino-terminal sequence and sequence of internal fragments from a tryptic digestion of the purified protein, its complementary DNA was cloned and expressed.

RESULTS AND DISCUSSION

ADMP is a member of the adamalysin family of zinc-binding metalloproteinases. Structural comparisons with other disintegrin-containing proteases indicate that ADMP is unique. ADMP exhibits a zinc-binding domain similar to those found in several MMPs, and an aspartic acid residue following the third conserved histidine similar to that found in the adamalysin family. It contains a probable cysteine switch and a potential furin cleavage site, which precedes the catalytic domain suggesting that, like the MMPs, ADMP is synthesized in the inactive pro form and is cleaved to remove the cysteine in the propeptide domain and generate the mature, active enzyme. This is supported by data demonstrating that a compound which interferes with normal proMMP activation through a cysteine switch mechanism inhibits IL-1-induced cleavage of aggrecan at the Glu^{373}-Ala^{374} bond in cartilage organ cultures [4,5]. Expression of recombinant human ADMP (rADMP) results in production of a functional enzyme which cleaves aggrecan as expected at the Glu^{373}-Ala^{374} bond and not at the Asn^{341}-Phe^{342} bond where MMPs preferentially cleave aggrecan. The pattern of aggrecan fragments generated by digestion of aggrecan with purified native bovine ADMP or recombinant human ADMP is indistinguishable from that observed during aggrecan degradation mediated by the endogenously generated ADMP in stimulated cartilage cultures. This activity was inhibited by several hydroxamates which are effective in blocking cleavage of cartilage aggrecan at the Glu^{373}-Ala^{374} bond by endogenously generated ADMP. In addition, the rank order of potency of these compounds was similar against the bovine ADMP activity in cartilage conditioned media and the recombinant human ADMP.

CONCLUSIONS

The isolation of this novel protease which appears to play an important role in cartilage degradation should facilitate the development of potential therapeutics for cartilage protection in arthritis that target inhibition of aggrecan degradation.

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