α2-Macroglobulin is Cleaved at a Novel Site by ADAMTS-4 and ADAMTS-5 and Represents an Endogenous Inhibitor of These Enzymes

* Malfait, AM; *Arner, EC; +*Tortorella, M
++ Pharmacia Co, Skokie, IL.

Introduction

Alpha-2-Macroglobulin (α2M) is a noncovalent tetramer, consisting of two 370 kDa disulfide-linked homodimers, that circulates in blood at concentrations of approximately 0.6-2 mg/mL and is found in the synovial fluid of arthritic joints at similar concentrations. Each subunit of the α2M molecule contains a region referred to as the "bait region"; a short stretch of amino acids that is very susceptible to proteolytic cleavage. When cleaved by an enzyme the α2M changes shape in such a way that it traps the proteinase and thus inhibits its activity by preventing substrate access to the active site. Although α2M is a general endoproteinase inhibitor inhibiting a variety of proteinases from all classes. Therefore, we evaluated whether the cartilage aggrecanases, ADAMTS-4 and ADAMTS-5, which have been shown to cleave only chondroitin-sulfate proteoglycans, including aggrecan, brevican, and versican, can cleave and be inhibited by α2M.

Materials and Methods

ADAMTS-4 or ADAMTS-5 (0.5nM) were preincubated with α2M (0.1-100 nM) for 1 hour at 37°C. Following the pre-incubation, the respective enzymes were tested for their ability to cleave bovine aggrecan in vitro. Aggrecan was added to a final concentration of 500 nM and the reactions incubated for 2 hours at 37°C. The aggrecan products were analyzed by Western blot analysis for fragments generated by specific aggrecanase-mediated cleavage at the Glu498/Gly499 bond using a GEL238 neoepitope antibody. To determine the site of cleavage, α2M at a concentration of 1 μM was incubated with ADAMTS-5 and ADAMTS-4 at a concentration of 448 nM or 200 nM, respectively, for 4 hours at 37°C. Following incubation, the products were separated by SDS-PAGE (10% acrylamide) and transferred to PVDF membranes. The proteins were detected by staining with Coomassie Brilliant Blue R-250 and the bands of interest were excised, placed onto a polystyrene-membranes. The proteins were detected by staining with Coomassie Brilliant

Results

α2M (0.5 nM) inhibited the activity of both ADAMTS-4 (Fig.1) and ADAMTS-5 in a concentration-dependent manner, showing a 1:1 stoichiometry.

The mechanism of α2M inhibition of proteinases is by physical entrapment of the proteinase upon being cleaved within the "bait" region by the enzyme. This process is blocked by the treatment of α2M with methylamine, which inactivates the "trapping" mechanism. Therefore, to determine whether this mechanism is involved in the inhibition of ADAMTS-4 and ADAMTS-5 by α2M, native α2M and α2M treated with methylamine were analyzed by their ability to inhibit ADAMTS-4 and ADAMTS-5 cleavage of aggrecan by active-site titration. The inability of the methylamine-treated α2M to inhibit ADAMTS-4 and ADAMTS-5 suggests that the inhibition of these two proteinases by α2M is triggered by proteolysis of the "bait" region.

N-terminal sequencing of the bands that were generated when α2M was incubated with ADAMTS-4 or ADAMTS-5 for 4 hours, revealed that cleavage of α2M by ADAMTS-4 or ADAMTS-5 resulted in the generation of a new Nterminus, GRGHLRVHEEP, which was found to be a sequence within the bait region of α2M. The site of cleavage, which was located between Met690 and Gly691 was compared with sites of cleavage generated by other proteinases, including collagenase, stromelysin and trypsin and was found to be unique.

Discussion

These results demonstrate that α2M inhibits both ADAMTS-4 and ADAMTS-5, and may thus play a role as an endogenous inhibitor of the cartilage aggregcanses present in synovial fluid. Interestingly, both aggregcanases cleave α2M between Met690 and Gly691. This is the first cleavage site, described for these enzymes, where the amino acid in the P1 position is not Glu, and suggests that these enzymes may have other, as yet unidentified, substrates. Because α2M is present in synovial fluid and plasma, the aggregcanase-generated neoepitopes in the bait region may be useful as biomarkers for aggregcanase activity.