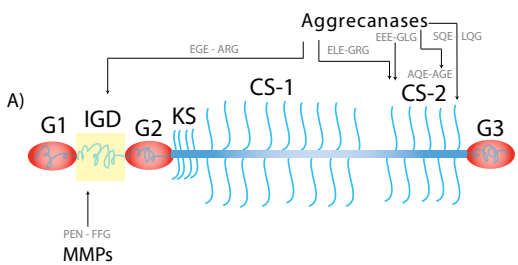
ELISA to Measure the Proteolytic Degradation of Aggrecan: Hallmark in the Pathology of Arthritis

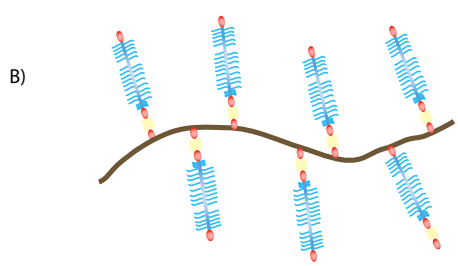
Aggrecan is a large aggregating proteoglycan of articular cartilage [1], making up 10% of the dry weight. It is responsible for hydrating cartilage, giving it compressibility and a resilience during joint loading, thereby playing a major role in the normal function of cartilage. Depletion of glycosaminoglycan bearing aggrecan fragments from articular cartilage is one of the earliest events in cartilage destruction.

Aggrecan monomers consist of a 250 kDa core protein and three globular domains, G1, G2 and G3 [2]. With the attachment of a chondroitic sulfide (CS) chain at the c-terminus and a keratin sulfide (KS) chain at the n-terminus, the monomer exists as a 1000-2000 kDa molecule. It is through the G1 domain and hyaluron, resulting in a large aggregate containing up to 100 aggrecan monomers, which is weaved into the collagen network [1, 3].

Proteolytic cleavage of its interglobulin domain (IGD) results in release of aggrecan fragments from tissue, which eventually leads to loss of joint function. This cleavage has been attributed to metalloprotease activity. Members of the matrix metalloprotease (MMP) family that are present in cartilage (MMP-2, -3, -7, -8, -9, -13 and -14) are capable of degrading



A) aggrecan monomer showing proteolytic cleavage sites.



B) aggregate showing aggrecan monomers bound to hyaluronic acid.

aggrecan between the Asn341 and Phe324 amino acids within the IGD, while members of the ADAMTS family (ADAMTS4 and ADAMTS-5/11 referred to as aggrecanase-1 and -2 respectively) are capable of degrading aggrecan at the Glu373 and Ala374 amino acids [4-7]. In addition ADAMTS4 also cleaves the relevant aggrecanase sites within the CS2 domain [4]. The major portion of aggrecan released from tissue appears to be cleaved by aggrecanases [2], and this release eventually leads to loss of joint function in diseases such as rheumatoid arthritis and osteoarthritis.

Enzymatic activity of aggrecanases has been analyzed with isolated aggrecan preparations, recombinant aggrecan fragments, and a 41-residue peptide immobilized onto streptavidin-coated microplates. An ELISA method for aggrecanase activity provides an improved and ready-to-use method for sensitive determination of aggrecanase activity and can be used to screen and characterize aggrecan inhibitors.

Aggrecanase Activity Assay

Aggrecanase Module: Proteolysis of aggrecan-IGD by aggrecanase. A recombinant fragment of human aggrecan-IGD is first digested with aggrecanase, and proteolytic cleavage releases an aggrecan peptide with the N-terminal sequence ARGSVIL (ARGSVIL Peptide). Samples of unknown aggrecanase activity would also be incubated with aggrecanase and the amount of ARGSVIL-peptide would be compared to the recombinant aggrecan-IGD standard.

ELISA Module: Aggrecan peptide ELISA. The ARGSVIL-peptide resulting from the proteolytic degradation from the recombinant aggrecan-IGD and the unknown aggrecanase in the sample is then quantified with two monoclonal antibodies using an ELISA format. The amount of ARGSVIL-peptide measured from both proteolytic degradations is correlated to the ARGSVIL-peptide standard provided to determine the amount of aggrecanase activity in the sample.

Protocol for Testing potential Aggrecanase Inhibitors using the Aggrecanase Activity Assay:

1. Prepare the diluted ADAMTS-4 standard from stock solution.
2. Prepare reaction mixture:
   10 µL aggrecan-IGD
   10 µL Pefabloc (inhibitor)*
   'x' µL inhibitor test sample
   Bring to 95 µL with reaction buffer
3. Preheat reaction mixture to 37 °C
4. Start reaction by adding 5 µL ADAMTS-4
5. Incubate for 15 min. at 37 °C
6. Stop reaction with 150 µL EDTA solution
7. Assay 100 µL of reaction for ARGSVIL-peptide by ELISA as described above

*Pefabloc is a serine protease inhibitor used to inhibit proteases found in the test sample and does not affect aggrecanase activity. If the test sample is thought to be free of protease activity, Pefabloc can be excluded. Depending on the mode of action of an inhibitor, an ADAMTS-4/test inhibitor pre-incubation step (30 min at 37 °C) may be necessary. After the pre-incubation, the reaction will be started with the addition of aggrecan-IGD. ■

References:

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