C1-inhibitor potentiation by glycosaminoglycans

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Chapter 1

Introduction
INFLAMMATION

The defence of the human body against infection or injury involves several pathways to restore homeostasis. The complement, coagulation and fibrinolytic pathways all serve a specialised function in the inflammatory process, but these pathways must be tightly regulated because excessive activation can lead to unwanted tissue damage. One important regulator of inflammatory proteases is C1-Inhibitor (C1-Inh).

C1-INHIBITOR FUNCTION

The plasma protein C1-Inh inhibits several inflammatory pathways. The classical and mannose-binding-lectin pathways of complement and the contact system of coagulation are all inhibited by C1-Inh. Patients with hereditary angioedema, who are deficient for C1-Inh, suffer from recurrent episodes of acute, local, circumscribed oedema of skin or mucosa. C1-Inh purified from plasma has been used for treatment of these attacks since the 1970's. Because it inhibits several pathways of inflammation the application of C1-Inh in other inflammatory diseases has been studied. It has been shown that C1-Inh treatment reduces inflammation in conditions like sepsis, acute myocardial infarction, and the vascular leakage syndrome (reviewed by Caliezi et al.).

SERPINS

C1-Inh is a plasma protein, which belongs to the superfamily of SERine Protease INhibitors (SERPINS). In 1980 it was revealed by computer analyses that three completely different proteins, antithrombin III (ATIII), α1-proteinase inhibitor and ovalbumin should belong to the same superfamily, now known as the serpin family. ATIII mainly inhibits thrombin, and coagulation factors IXa and Xa. α1-Antitrypsin, also known as α1-proteinase inhibitor, is considered as the prototype of the serpins, and mainly inhibits neutrophil elastase. Ovalbumin is one of four major egg white storage proteins, of which the function is still unknown. Many more proteins have been shown to belong to this superfamily since 1980, as has been summarised recently by Gettins. The structure of some of these proteins has been elucidated successfully with X-ray crystallography. Based on the known structures, the unknown structure of other serpins can be more or less predicted by homology modelling.
**Chapter 1**

**Potentiation**

The inhibitory activity of several serpins can be enhanced by heparin and other glycosaminoglycans (GAGs), which is known as potentiation. C1-Inh is a relatively weak inhibitor compared to other serpins, and can also be enhanced by GAG\(^{14,15}\). The potentiation mechanism of C1-Inh is unknown, in contrast to that of several other serpins. Understanding this mechanism may facilitate design of mutants with enhanced inhibitory activity.

**Aims of this Thesis**

This thesis describes investigations on the mechanism of potentiation of C1-Inh. This serves two main goals: 1) to investigate the structural basis of the potentiation of C1-Inh by glycosaminoglycans, and 2) to eventually facilitate application of (potentiated) C1-Inh in inflammatory diseases.

Chapter 2 is a general introduction about serpin structure and function and introduces a model for the three-dimensional structure of C1-Inh. This three-dimensional model is the basis for hypotheses investigated in later chapters. Chapter 3 describes the application of C1-Inh and glycosaminoglycans in a rat model, to analyse the efficacy of this non-covalent complex in vivo. To study the mechanism at the protein level, several mutants of C1-Inh have been produced in a recombinant expression system in the methylotrophic yeast *P. pastoris*, which is introduced in chapter 4. Chapter 5 is devoted to the inhibitory activity of 5 C1-Inh mutants with a reactive site loop elongation of 1 or 2 amino acids. A unique deletion of 55 amino acids in the non-conserved N-terminal domain was discovered in a hereditary angioedema patient. The effect of this deletion on the phenotype and the role of the unique N-terminal domain are discussed in chapter 6.

Chapter 7 deals with several other characteristics of the potentiation of C1-Inh by heparin, as well as the role of potential heparin binding residues.

**References**


