Proteinase Inhibitors

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Human α_2-Macroglobulin—Another Variation on the Venus Flytrap

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Proteases are necessarily ubiquitous protein enzymes that catalyze the cleavage of other proteins and thereby regulate the vital processes of an individual's life cycle. Proteases play important regulatory roles in digestion, growth, development, aging, and death of all cells and organisms.[1] Proteases regulate physiological processes by controlling the synthesis and turnover of proteins.

As one can see from early textbooks, proteases and their enzymatic mechanisms have long been investigated in all fields of biochemistry. Who does not remember the catalytic triad of serine proteases from undergraduate biochemistry courses? A remarkable feature of the hydrolytic cleavage reaction of serine proteases is the formation of the acyl–enzyme intermediate after the first reaction step where one of the two cleavage products is temporarily covalently linked to the enzyme. As a result of a hydrolysis in the second step the other cleavage product is released, returning the enzyme to its initial state.

From a medical and therapeutic point of view, proteases are promising target molecules because of their important regulatory functions. Several endogenous and exogenous factors may lead to an inappropriate production of specific proteases contributing to an abnormal physiology and diseases, such as cancer and cardiovascular and inflammatory diseases.[2] This shows that the finely tuned regulation of protein biosynthesis and protein degradation is vitally important and must be controlled with highest precision. Important key players of the protease regulation are protease inhibitors.

Mammalian proteases are divided into two groups. Exopeptidases degrade a protein by cutting the polypeptide chain residue by residue from the N or C terminus. The second group, proteinases, are endopeptidases that cleave peptide bonds within central regions of a polypeptide.[3] The proteinases consist of four classes based on their specific active center, namely the serine, aspartic, cysteine, and metalloproteinases. Usually these proteinases act first in the degradation of proteins.

Proteinase inhibitors are categorized as either class-specific or nonspecific.[3] Class-specific inhibitors are able to block only one of the four above-mentioned proteinase classes and are therefore subdivided in four subfamilies.[3] These inhibitors are relatively small in size and display higher target specificities than the nonspecific proteinase inhibitors. The high target specificity limits their activity spectrum but does not diminish their importance in the regulation of physiological processes in the body and thus their therapeutic relevance.

Bovine pancreatic trypsin inhibitor (BPTI) was the first class-specific serine proteinase inhibitor to be studied at atomic resolution.[4] In 1970 the X-ray crystal structure of this enzyme inhibitor was solved by Huber and colleagues.[4] BPTI, also known as aprotinin or Trasylol, is a small polypeptide comprising 58 amino acid residues that inhibits a variety of serine proteinasesincluding trypsin. Four years later in collaboration with others, Huber and Bode successfully solved the crystal structure of the complex between BPTI and trypsin.[5] BPTI interlocks target proteinases, resulting in a dead end for the enzyme–inhibitor complex (Figure 1). Trasylol was used for many years to reduce bleeding during cardiac surgery.[6] However, since its use is associated with increased morbidity and mortality in adults, its application has been restricted since 2008.[7]

Nonspecific proteinase inhibitors are able to block proteinases from all four proteinase classes and are composed of the α-macroglobulins only.[3] The α-macroglobulins are high-molecular-weight glycoproteins and comprise up to 10% of total serum proteins.[3,8] Owing to broad spectrum of activity α-macroglobulins are involved in the regulation of many physiological processes, such as coagulation and inflammatory processes. Their major function is the inhibition of excess endogenous and exogenous proteinases.

The most important α-macroglobulin is α_2-macroglobulin (α_2M), which has been known for more than six decades. It has been highly preserved throughout evolution[3] and it is the proteinase inhibitor for which the most biochemical information is available. Human α_2M was isolated first by Cohn and

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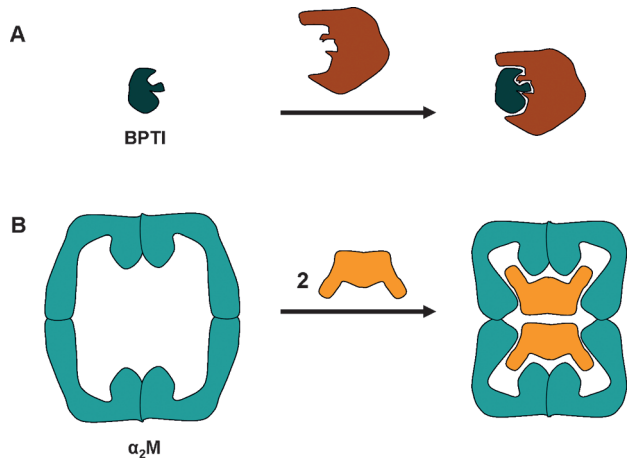

Figure 1. Proteinase inhibition by proteinase inhibitors. A) The class-specific bovine pancreatic trypsin inhibitor (BPTI, 58 amino acid residues) binds to the active site of the proteinase trypsin thereby inhibiting all proteolytic activity. B) The considerably larger nonspecific proteinase inhibitor α_2-macroglobulin (α_2M, tetramer comprising about 6000 amino acid residues) binds covalently the differing target proteinases within a bait region leading to a conformational change. The proteinase is thereby captured inside α_2M by a mechanism that is reminiscent of a Venus flytrap.

colleagues in 1946.[9] It is a huge and abundant glycoprotein containing four identical subunits with a molecular weight of 740 kDa. Each subunit is a multidomain molecule comprising 1451 amino acid residues.[10] The inhibitory activity of the homotetrameric α_2M is due to a unique "Venus flytrap" mechanism (Figure 1), by which the α_2M captures the active target proteinase irreversibly in a specific bait region.[8,11] Proteinase binding induces conformational changes of the inhibitor α_2M.[10] As a result a buried thioester group between a cysteine and a glutamine residue is exposed.[10] Subsequently, the thioester group can react with the prey proteinase thereby covalently binding the α_2M and the captured proteinase. Alternatively, small nucleophilic molecules, such as methylamine (MA), open the thioester bond, inducing allosteric conformational changes comparable to proteinase capture.[10]

The trapping of proteinase by α_2M also results in the presentation of a receptor binding domain (RBD) on the surface of the α_2M molecule.[8] Subsequently, the α_2M–proteinase complex is bound by specific cell surface receptors that do not interact with free α_2M. In the reticuloendothelial system, complexes are internalized into receptor-presenting cells by receptor-mediated endocytosis followed by proteolytic degradation within the lysosomes. These processes ensure the rapid removal of the inhibitor and its cargo from the circulation.[10,12,13]

In humans, there are no complete α_2M deficiencies known. This leads to the assumption that the absence of α_2M might be lethal. Imbalances in the activity of α_2M are known to contribute to a range of human diseases including AIDS and Alzheimer's disease.[14,15]

To gain more insight into the mechanism of α_2M, Marrero et al. have now determined the crystal structure of MA-induced human α_2M and compared it with electron microscopy images of protease–α_2M complexes.[16] The achieved crystallographic resolution of 4.3 Å sufficed only for the interpretation of electron density maps at the level of secondary-structure elements.[16] Atom types and positions could not be measured unambiguously. At this resolution, for example, the backbone of the polypeptide chain has the shape of a tube and side chains can only be guessed. The experimental details point to a crystallographer's nightmare because all crystallization efforts yielded only one single crystal suitable for X-ray diffraction analysis. The quaternary structure and crystal packing of α_2M have been carefully interpreted with various crystallographic computing techniques.

The results presented by Marrero et al. are certainly remarkable with respect to biological insights into biochemical regulation processes.[16] But we must also consider that it was only a century ago that Max von Laue designed and interpreted the first X-ray diffraction experiments on single crystals.[17] These crystal structure analyses of simple compounds like NaCl and KCl opened the door for chemists to determine molecular structures in three dimensions at atomic resolution.[18] This led to the development of detailed concepts on the stereochemistry of mechanisms, reaction pathways, and molecular interactions. If one compares the first crystal structure analyses with sizes of particles determined nowadays (e.g. the prokaryotic ribosome[19]), the molecular unit has increased by six orders of magnitude. The financial cost and technical effort have increased accordingly.

Concerning crystallization, it is still not possible to predict the optimal conditions for a particular protein or another biopolymer. Too many parameters have to be taken into account and often the most critical or sensitive parameter is not known. So, it is still a trial-and-error procedure to get crystals suitable for X-ray diffraction experiments and screening with hundreds of conditions supported by robotic pipetting and imaging devices is the technical state of the art. Even then, sometimes just a single crystal must suffice.

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