Design of Inhibitors for Proteolytic Cascades

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General considerations for proteolytic cascades

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Abstract

Proteases are involved in the regulation of a wide variety of essential physiological processes, often by participating in a highly orchestrated sequence of events termed a ‘proteolytic cascade’. Four major proteolytic cascades with disease relevance are candidates for therapeutic intervention, namely caspase-mediated apoptosis, blood coagulation, the matrix metalloproteinase cascade and the complement cascade. Understanding the various steps involved in the functioning of a cascade is key to deciding possible points of intervention for the design of potential drug molecules. This brief review illustrates some of the common features of proteolytic cascades using the blood coagulation pathway as an example.

Introduction

Approximately 500 human genes encode proteases [1]. These are divided into mechanistic classes (e.g. serine proteases), clans (e.g. SA) and families (e.g. S1A) (see MEROPS database http://merops.sanger.ac.uk/). Certain protease families participate in some highly orchestrated proteolytic cascades to assume essential physiological functions, such as digestion, innate immunity and coagulation [3]. Within a proteolytic cascade, some proteases act as initiators, others are involved in amplification loops, while some ensure propagation (illustrated for clarity in Figure 1). In particular, four proteolytic cascades have been investigated for their potential as targets for therapeutic intervention (reviewed elsewhere in this colloquium). The caspase cascade recruits intracellular cysteine proteases taking part in the well-regulated processes governing programmed cell death. An extracellular cascade that includes the family of matrix metalloproteinases turns over the extracellular matrix component (e.g. collagens, elastin and fibrin) in events such as embryogenesis, wound healing and metastasis. The complement cascade oversees the recognition and disposal of invading micro-organisms or altered host cells, while the coagulation cascade oversees blood clotting. For clarity, the three phases of a proteolytic cascade are exemplified here using the coagulation cascade, arguably the best characterized proteolytic cascade.

The three phases of the proteolytic cascade

Under quiescent conditions, the proteases involved in cascades are present as latent inactive zymogens (Figure 1). In the initiation phase, the cascade is triggered by an external stimulus, such as injury, stress or infection. Subsequently, an initiating protease or ‘initiator’ will self-activate by autocatalysis (e.g. coagulation factor VII converts itself into factor VIIa). During the propagation phase, the initiator converts a downstream propagator into its active form by limited proteolysis (e.g. factor VIIa activates factor X into factor Xa). In the execution phase, the propagator will in turn activate an executor, as well as activating its initiator via a positive-feedback mechanism (e.g. factor Xa activates prothrombin as well as factor VII).

The cascade results in an overall rapid amplification of proteolytic activity. An advantage of an amplification system is that only a small amount of an activated, initiating protease is required to trigger a major response [4]. For example, the initiating protease in the coagulation pathway, factor VII, is present at low concentrations (10 nM), whereas prothrombin is present at relatively higher concentrations (1.4 µM). Each activation step is regulated primarily by the complementary specificity between the activating protease and the zymogen form of the activated protease.
Inhibition of the proteolytic cascade

Unlike phosphorylation/dephosphorylation cascades, which are reversible, proteolytic cascades are irreversible, and therefore must be tightly regulated to prevent serious deleterious effects of uncontrolled proteolytic activity. Disease states arising from uncontrolled activation of a cascade include disseminated intravascular coagulation in sepsis and tumour metastasis. A number of natural protein inhibitors regulate proteolytic cascades, for example serpins, TIMPs (tissue inhibitors of metalloproteinases) and complement inhibitors.

A key regulator of the coagulation pathway is antithrombin-III, which requires heparin for it to act as an efficient anticoagulant. Heparin increases its rate of inactivation of coagulation factors (factors VIIa and Xa and thrombin) by several orders of magnitude [5]. This highlights the importance of aiming to achieve high association rate constants with synthetic inhibitors of proteolytic cascades [6,7].

An important question to be considered when designing inhibitors for proteolytic cascades is: which phase in the cascade should be intercepted with inhibitors? In principle, maximal inhibition is more readily achieved by blocking the initiation phase [4]. This will limit the drive on the executioner proteases. For example, a selective thrombin inhibitor would not prevent the drive from activated factor Xa to cleave prothrombin. Nevertheless, for the past decade the pharmaceutical industry has developed more synthetic inhibitors for the executioner thrombin than for the initiator factor VIIa. An underlying reason for this apparent anomaly could be that the design of small-molecule inhibitors of a ‘specialist’ enzyme such as factor VIIa is more difficult than for proteases such as thrombin which have a broader specificity. For example, factor VIIa requires an extended surface of interaction with its respective ligands tissue factor, factor X and, in the ternary inhibitory complex, tissue factor pathway inhibitor [8]. These complex interactions induce a conformational change in the active site of factor VIIa to enhance its proteolytic activity. Consequently, in vitro it is more difficult to develop facile assays with peptide substrates and to discover inhibitors for tissue factor/factor VIIa than it is for thrombin. Nevertheless, a number of pharmaceutical companies are now focusing attention on inhibitors of factor VIIa.

However, both of these approaches are marred by the perennial issue of selectivity, since there can be a high degree of similarity between members of a proteolytic cascade. If an inhibitor has similar $K_i$ values for factor VIIa and thrombin, then the relative peak plasma concentrations (respectively 1000:1) will, by the law of mass action, result in the majority of the inhibitor partitioning to thrombin [9]. An alternative approach is to opt for the design of inhibitors that act on two or more members of the cascade. This approach can potentially achieve overall improved potency, and is exemplified by the development of dual factor VIIa/Xa or factor Xa/thrombin inhibitors [10]. Similarly, Vertex has produced broad-spectrum non-peptidic inhibitors in the caspase area [11]. In practice there are no hard and fast rules, and the merits of each approach have to be evaluated within the context of both the proteolytic cascade as well as the overall desired therapeutic effect.

Finally, with regard to the therapeutic utility of inhibitors of proteolytic cascades, factors such as the tissue distribution of the proteases must be considered, e.g. the protection of thrombin from macromolecular inhibitors when it is bound in the mesh of the fibrin clot. Also, additional activities of the target proteases must be considered, e.g. related negative-feedback pathways such as the anticoagulant pathway of protein C activation by thrombin. Overall, such complexities require testing in sophisticated preclinical pharmacological models of the disease state in order to validate a cascade target.

Conclusions

Proteolytic cascades have powerful physiological roles; however, their dysregulation can lead to serious pathological conditions. Inhibitors of proteolytic cascades thus represent attractive targets for the design of therapeutic agents. There are as yet no panacea solutions as to which stage of the cascade should be inhibited, and new facets of proteolytic cascades are still being discovered. In recent years, the advancement in screening technologies has allowed the pharmaceutical industry to target proteolytic cascades using either holistic or systematic approaches. From these differing standpoints, the future will see the further discovery of both mixed and selective inhibitors, which in turn will allow a better understanding and therapeutic evaluation of proteases involved in cascades, such as caspases, matrix metalloproteinases, complement and coagulation factors.

References

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