COAGULATION, ANTICOAGULATION AND THROMBOSIS: MOLECULAR MECHANISMS

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Introduction

The last twenty years has seen the solving of the major molecular mechanisms that control coagulation and anticoagulation. It has long been realised that these mechanisms can be modulated to meet the special requirements of tissues such as the microcirculation, which require principally anticoagulant activity, and the arterial circulation, which gives a high priority to coagulation and the prevention of haemorrhage. We can now see in structural detail how this modulation takes place, with the heparans and thrombodulin that line the microvasculature, switching antithrombin to an active inhibitory form, and converting thrombin from a coagulant protease to one that activates the anticoagulant protein C pathway. This modulation occurs through a series of often subtle changes in conformation which together give an extraordinary complexity of interactions. The advantage of such complexity is in the ability to finally balance the coagulation processes. But the disadvantage is the vulnerability of these intricate mechanisms to mutations and acquired changes. The consequence of these aberrations is thrombosis.

How antithrombin works

Antithrombin is a member of the serpin family of protease inhibitors. The serpins have evolved an extraordinary mechanism, comparable to that of a mousetrap, that allows complete inhibition of their target proteases. Antithrombin circulates in a metastable state with its reactive centre, like the bait of the mousetrap, exposed exteriorly and containing a sequence that acts as a specific substrate or bait for coagulation proteases and, in particular, for factor Xa and thrombin. As shown in figure 1A, cleavage of the reactive centre by the protease unleashes the spring formed by the reactive centre loop, with the loop moving on its proximal hinge to enter the main sheet of the molecule as its central strand. In doing so, the protease is displaced to the other end of the molecule with an accompanying destruction of some 40% of the structure of the protease. This gives the total and effectively irreversible inhibition required to halt the proteolytic cascades that otherwise lead to thrombosis.

How heparin modulates anticoagulation

Antithrombin exists in the circulation in a relatively inactive form with the key arginine at its reactive centre being obscured by an internal orientation. It is only when antithrombin binds to the heparans of the microcirculation that the reactive loop changes conformation to fully expose the arginine and hence give the active inhibitory form (fig. 2A). The exposure of the reactive loop and the activation of antithrombin is a consequence of the binding of a precisely defined pentasaccharide fragment present in both heparans and in therapeutic heparin preparations. The pentasaccharide, which is highly negatively charged, binds to a patch of positively charged arginines and lysines on the side of molecule as indicated in fig. 2B. This binding has two consequences. As well as activating antithrombin as an inhibitor of factor Xa, it also provides the attachment site for longer heparin molecules that can link to an exosite on thrombin and hence bridge and catalyze the thrombin-antithrombin complex (fig. 2B).

Hence it can be seen how the fractionated heparins can have selective functions. The low molecular weight/high affinity heparins will contain a relatively frequent presence of the critical pentasaccharide and hence will be particularly effective inhibitors of factor Xa. Whereas the higher molecular weight heparins will be less selective for factor Xa, but because they are long enough to bridge the complex they will be effective activators of the inhibition of thrombin.

Therapeutic heparin

Heparin does not naturally exist as such in the circulation. Therapeutic heparin is a highly sulphated and heterogeneous glycosaminoglycan derived from the intestine of pigs. It was previously used in unfractionated form with an average of 45 saccharides per chain. But only 1 in 3 of these chains contained the specific pentasaccharide. More recently heparin has been fragmented to yield low molecular weight/high affinity forms that range from 6 to 30 saccharide units. Although these are less efficient in bridging the thrombin antithrombin complex, they mostly contain the pentasaccharide se-
quence and hence give more effective inhibition of factor Xa.

Within the last two or three years, synthetic heparins have become available for clinical use, with the natural pentasaccharide sequence giving an effective Xa inhibitor suitable for subcutaneous administration. This pentasaccharide (fondaparinux) has 100% bioavailability and a half-life of 17 hours. In another form, the modification of the natural sequence of the pentasaccharide by the addition of an extra sulphate gives exceptionally strong binding to antithrombin and results, therapeutically, in a long-acting Xa inhibitor. Further synthetic heparins are likely to become available including longer chain forms that will inhibit thrombin as well as Xa. The special advantage of the synthetic heparins is their extraordinary specificity, with the expectation that they are unlikely to form the secondary interactions that result in platelet interactions with the risk of thrombocytopenia.

Thrombin: a coagulant and an anticoagulant

Few molecules have been better structurally studied than that of thrombin. As summarised by Huntington and Baglin, the structures show how evolution has adapted the molecule to give interactions that alter and modulate its proteolytic function. The active site of thrombin is buried in a valley formed by two flanking loops (60-Loop and gamma-Loop in fig. 3A). These loops limit access to the active site and hence determine the specificity of cleavage by thrombin. Also shown in figure 3A are the anion-binding exosites I and II. Exosite I binds fibrinogen and is responsible for the sequestration of thrombin in fibrin clots. This binding site is also competed for by thrombomodulin, which as well as blocking the binding fibrinogen switches the cleavage preference of thrombin to the anticoagulant protein C. Exosite II has a key inhibitory role in providing the site that heparin binds to in the bridging complex with antithrombin (fig. 2C). Inevitably the story is even more complicated than this. For example, exosite I is competed for not only by fibrinogen and thrombomodulin, but also by the protease activated receptors (PARs) of the platelets. In this way, thrombin specificity is affected by multiple interactions, depending on its tissue location and to what it is bound.

Thrombin inhibitors

The principal inhibitor of thrombin in the plasma is antithrombin. Therapeutically, however, there is now much interest in specific inhibitors, several of which are derived from blood sucking parasites and bats. A number of peptide inhibitors have individually evolved that bind to thrombin and block its activity as illustrated diagrammatically in figure 3B. Examples are hirudin, a 65-residue polypeptide from the medicinal leech, that reacts with both the reac-
tive site and exosite I. From this, various recombinant modified hirulogs have been developed, such as hirugen. The availability of crystallographic structures has also allowed the design of synthetic peptides, such as PPACK, that specifically block the active site. From these derivatives have been developed, including melagatran, a small inhibitor of thrombin, available in an orally available prodrug form as ximelagatran. Similarly, an arginine-based compound, argatroban, is now in use for the treatment and prevention of heparin induced thrombocytopaenia.

Conclusions: Aberrations and thrombosis

As summarised above, knowledge of the structural changes involved in the control of coagulation has opened new prospects for therapy. Clinically, the new understandings also provide insights into the aberrations that result in thromboembolic disorders. This is readily demonstrated with respect to antithrombin. As expected, mutations that directly effect the inhibitory activity of antithrombin, such as mutations at the active centre, result in familial thromboembolism. Mutations at the proximal hinge of the reactive loop, or of the shutter that triggers the conformational change (fig. 1A), slow the entry of the loop and diminish the inhibitory activity of the antithrombin. But this slowing also allows the main sheet of the molecule to open and, as a consequence, the loop of another molecule to insert to give intermolecular linkage with a variety of disadvantageous consequences. Alternatively, mutations in the distal hinge of the molecule allow the insertion of the whole intact reactive loop to give the irreversible and inactive latent form. This form preferentially binds the most active isof orm of antithrombin in the plasma, β-antithrombin, with a consequent vulnerability to severe episodic thromboembolism. Mutations at the heparin binding site cause a mild predisposition to thrombosis in the heterozygote due to the lack of activation as an inhibitor of Xa and to the failure to form the bridging complex with heparin that accompanies the inhibition of thrombin. Such mutations usually result in a low affinity for heparin but unexpectedly, an even more severe disease can result from mutations that increase the affinity for heparin. These are frequently accompanied by a diminution of inhibitory activity with the seriousness of the consequences being due to the preferential binding of the mutant antithrombin to the sites on the vasculature that are normally occupied by the highly active antithrombin. These are just some of the changes that result in disease. The reassuring development is that we can now understand in detail how each of these dysfunctions occur. The overall conclusion is that – as with all complex mechanisms – everything that can go wrong, will go wrong!

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Figure 2. Heparin activation of antithrombin. (A) The obscured reactive centre arginine is revealed when (B) the heparin pentasaccharide on the right binds to antithrombin and expels the reactive loop. (C) The complex of thrombin with antithrombin is induced and stabilised by the bridging of longer chain heparins with exosite II on thrombin.
References


