α-Thrombin

The physiological process that prevents significant blood loss from damaged tissues, blood vessels or organs following vascular injury is referred to as hemostasis. Thrombus or blood clot formation occurs when the hemostatic response becomes activated in an uninjured or slightly injured vessel. α-Thrombin is the final enzyme involved in the blood clotting cascade and is well known for its role in blood hemostasis, inflammation, and wound healing.

α-Thrombin is synthesized in the liver and secreted into the blood in an inactive form (prothrombin), a vitamin K-dependent zymogen that is activated to yield thrombin at sites of vascular injury following upstream activation of the coagulation cascade. In the coagulation cascade, thrombin catalytically cleaves the bonds of fibrinogen into networks of fibrin to form the solid fibrin gel of a hemostatic plug or a pathologic thrombus. These networks then trap lots of erythrocytes, forming a scab which serves to protect the organism from excessive blood loss upon injury and to maintain blood fluidity within the vascular system. In addition, thrombin amplifies the clotting process by activating Factors V, VIII, XI and XIII. Conversely, thrombin also aids the anticoagulant process by activation of protein C.

Thrombin is a serine protease which works by cleaving two sites within the fibrinogen molecule after two arginine residue sites. Other examples of serine proteases are trypsin and chymotrypsin, enzymes involved in digestion. Thrombin consists of two polypeptide chains: a light chain (A chain) which consists of one alpha helix and a Heavy chain (B chain) which consists of 2 beta barrels and several alpha helices. The A chain is composed of 36 residues and is non-essential for catalysis. The B chain is composed of 259 amino acids and is derived from the carboxyl terminal sequence of prothrombin. The active site is located in between the 2 beta barrels and contains three amino acids critical for catalysis: Ser195, Asp102, and His57.

When leeches draw blood, the blood does not clot until after the leech releases its grip. This is due to a protein that the leech secretes called hirudin. Hirudin is an anticoagulant protein and stops the function of the thrombin. Thrombin contains three key surface sites critical for regulatory function. The first subsite is called the anion-binding exosite-1 (ABE-1), which serves as a binding site for natural substrate fibrinogen, hirudin, and heparin cofactor II. The ABE-1 is made up of residues Arg67 through Glu80. The second site of importance called the anion-binding exosite-2 (ABE-2), binding site of heparin cofactor II and antithrombin. The last site of importance is the protein’s active site, also the binding site of direct inhibitors.
The blood clotting function of thrombin can be inhibited in three distinct ways: at the Active Site, Exosite I, or Exosite II. Thrombin inhibitors come in two forms, non-polymeric and polymeric. In the non-polymeric form the inhibitor binds to the active site and morphs the active site, making it difficult for thrombin to pull and cleave fibrinogen. In the polymeric form, the inhibitor binds to either Exosite 1 or Exosite 2 and blocks thrombin differently. When Exosite 1 is plugged, fibrinogen can’t bind there, inhibiting thrombin. When heparin cofactor II is bound to exosite 2 and anti-thrombin is present, thrombin is inhibited at both Exosite 2 and the active site, morphing thrombin drastically. Polymeric inhibitors bind to exosites instead of active sites. There are three main polymeric inhibitors: Hirudin, Herapin cofactor II, and Antithrombin.

Venous and arterial thrombosis (blood clots) are one of the most common causes of death. Thrombin inhibitors stop the function of thrombin and prevent the formation of blood clots. Blood clots can travel through the body and cause several medical conditions ultimately leading to a heart attack, hence generating the need for Thrombin inhibition through rational drug design.

References