



Cascading into the Thrombin-Thrombomodulin Complex Comprehending a Substitution in Thrombomodulin

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Abstract

According to the Centers for Disease Control every year more than 60,000 Americans die of excessive blood clots or thrombophilia, and many more suffer from this disorder. Clotting disorders pose unique problems for women because of their impact on reproductive health and pregnancy. Thrombin, a protease that cleaves fibrinogen and activates platelets, plays a key role in the generation of blood clots. Several mechanisms prevent excessive thrombin activity. Thrombin that diffuses away from the clot binds to its receptor, thrombomodulin. Thrombomodulin-bound thrombin can no longer generate blood clots. Instead, it cleaves and activates Protein C which shuts down further thrombin generation. The substitution Gln387Pro in thrombomodulin inhibits Protein C activation and interferes with this feed-back inhibition. The Laconia SMART (Students Modeling A Research Topic) Team used 3D printing technology to model thrombin in association with thrombomodulin. Amino acid residues within the catalytic site (Ser195, His57, and Asp102) and the two substrate binding sites, exosite I (Lys36-Arg77) and exosite II (Arg 93-Lys240) of thrombin, involved in these interactions are highlighted. Understanding the relation between structure and function of the thrombin-thrombomodulin complex may lead to new therapeutics for clotting disorders.

What is Blood Clotting?

Blood clotting is a natural process within the body that prevents excessive bleeding when a blood vessel is injured. Blood clots are made of blood cells and fibrin that immediately plug the injured blood vessel and prevent blood loss (Figure 1). Blood clotting is a very complex process that involves the activation of a number of proteases in a cascade-like manner that finally results in the activation of thrombin. Thrombin is the primary protease responsible for the generation of blood clots. There are two different pathways that cause thrombin activation. Upon blood vessel injury, Tissue factor comes in contact with clotting factors present in blood initiating the *extrinsic pathway*. Blood clots can also be initiated by inflammation and may involve factor XII and other clotting factors that constitute the *intrinsic pathway*. Two of the most common blood clotting disorders are thrombophilia or a tendency for too much blood clotting, and hemophilia or not enough blood clotting. Thrombophilia is associated with Deep Vein Thrombosis (Figure 2), a condition where a blood clot is formed in a vein located deep in the body. This condition not only causes discomfort but can also be life threatening. The blood clot can break loose, travel to the lung and block a major blood vessel causing sudden death. Similarly, thrombosis of the arteries may cause heart attacks, stroke and placental problems during pregnancy. Hemophilia, on the other hand, can lead to excessive blood loss. Blood is not able to clot fast enough resulting in excessive bruising (Figure 3) and bleeding. Understanding the mechanisms that cause blood clotting abnormalities can improve our ability to treat and prevent these adverse health outcomes.

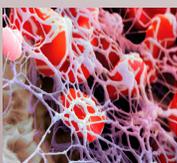


Figure 1. Blood Clot (1)

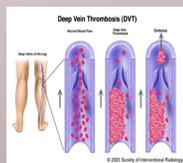


Figure 2: Deep Vein Thrombosis (2)



Figure 3: Hemophilia (3)

Anticoagulation by the Thrombin-Thrombomodulin Complex

Thrombin is the central enzyme in blood clotting cascade that activates platelets and cleaves fibrinogen to make the platelet-fibrin blood clots. Excessive thrombin generation or activity is regulated by many different mechanisms. Thrombomodulin is a receptor for thrombin that participates in feedback inhibition of thrombin generation. When thrombin is bound to thrombomodulin it cannot activate platelets or cleave fibrinogen to make blood clots. Instead it cleaves and activates protein C. Activated protein C degrades factors V and VIII reducing the production of thrombin. Given the importance of the thrombin-thrombomodulin complex in the regulation of blood coagulation, the SMART Team generated a 3D-model of the complex to examine the location of the Gln387Pro substitution in thrombomodulin. The Gln amino acid at the position 387 is shown in the relation of the exosite and the catalytic site on thrombomodulin.

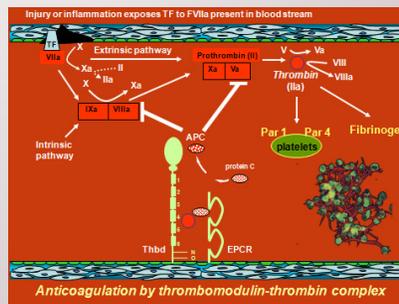


Figure 4: The Blood Clotting Cascade. The role of Thrombomodulin (Thbd) in feed back inhibition of thrombin generation is highlighted. Roman numerals represent blood clotting factors; activated state is represented by the letter "a"; EPCR, Endothelial Protein C Receptor; APC, Activated Protein C, Par, Protease Activated Receptor.

Structure of the Thrombin-Thrombomodulin Complex



Figure 5: Thrombin Model

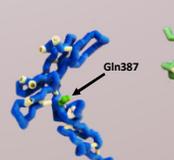


Figure 6: Thrombomodulin Model

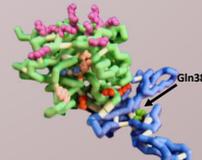


Figure 7: Thrombin-Thrombomodulin Model

Colors: Exosite I in Red; Exosite II in Violet; Catalytic Site in Light Pink; Thrombomodulin in Blue; Gln387 in green.

The exosites shown in the model act as a binding site to create the thrombin-thrombomodulin complex. Exosite I (Lys36, Arg67, Lys70, Arg73, Arg75, Arg77) binds to thrombomodulin, fibrinogen and other procoagulant substrates while exosite II (Arg93, Arg97, Arg101, Arg126, Arg165, Lys169, Arg173, Arg175, Arg233, Lys235, Lys236, Lys240) strengthens these interactions and serves as the primary site for thrombin binding to heparin and glycoprotein Iba. The catalytic site (Ser195, His57, Asp102) cleaves the fibrinogen and other thrombin substrates. The Gln387 amino acid on thrombomodulin is depicted in green.

Thrombomodulin Gln387Pro Mutation in Mice

Mice carrying the Gln387Pro substitution showed a significantly reduced ability of thrombomodulin to activate Protein C but did not show acute thrombosis (4). But when the mutation was examined in the context of pregnancy it was found to be associated with placental abnormality and pregnancy loss (5). This occurred only when the presence of Gln387Pro substitution in the baby was combined with a thrombophilic polymorphism in maternal blood clotting factor V. This polymorphism, called factor V Leiden, is found in 4 to 7% of Caucasian populations. The genetic nomenclature and experiments that led to this observation are outlined in Figure 8 and the results are shown in Table 1. When female FVQ/Q ThbdPro/+ mice were crossed with ThbdPro/+ males, none of the FVQ/+ ThbdPro/Pro embryos survived pregnancies. When the cross was reversed and female ThbdPro/+ mice were crossed with FVQ/Q ThbdPro/+ males, FVQ/+ ThbdPro/Pro embryos had a normal survival rate. Thus, fetal loss was triggered when the maternal prothrombotic state coincided with fetal gene defects that reduced the activation of the protein C anticoagulant pathway with in the placenta (5). This experiment demonstrates the importance of thrombomodulin-mediated anticoagulation in the placental vascular bed. The substitution Gln387Pro may alter the structure of the thrombomodulin-thrombin complex and disrupt its function. To examine this possibility the SMART Team identified the Gln387 residue in the model of the complex. The thrombomodulin Gln387 residue is located within a beta sheet. While glutamine is polar and can interact with other polar or charged amino acids, proline is rarely involved in such interactions. Unlike glutamine, the amino acid proline has a distinctive cyclic side chain that is connected to the protein backbone twice. This gives proline a conformational rigidity that destabilizes beta sheets and alpha helices and produces a 'turn' or a 'kink' in the protein. This could disrupt thrombomodulin binding to exosite I of thrombin.



Figure 8: Pedigree Cross

Figure 8: Mothers with the Leiden mutation do not support the growth of babies who are homozygous for the thrombomodulin Pro mutation (left panel). In the reverse genetic cross (right panel) mice homozygous for the thrombomodulin Pro mutation survive normally in a different mother. The normal alleles of factor V (Fv) and thrombomodulin (Thbd) are designated by the superscript "+". The mutant alleles are designated by the superscript "Q" for factor V Leiden and "Pro" for the thrombomodulin Pro mutation, respectively.

Table 1: The results of the genetic experiment shown in Figure 8 are tabulated. Rows 1 and 2 show the number and percentages of each genetic type of embryos obtained from the genetic cross corresponding to the left and right panels, respectively, in Figure 8.

Table 1: Genotype of Embryos (6)

Stage of Analysis	Parental genotype		Genotype of live embryos			Number (%age) of aborted embryos	Number (%age) of pregnancies analyzed
	Female	Male	FVQ ⁺ Thbd ⁺	FVQ ^Q Thbd ⁺	FVQ ⁺ Thbd ^{Pro/Pro}		
15.5 dpc	FVQ ⁺ Thbd ⁺	Thbd ^{Pro/Pro}	20 (42.0%)	27 (57.0%)	0	42 (47.2%)	89 (9)
15.5 dpc	Thbd ⁺	FVQ ^Q Thbd ⁺	26 (28.5%)	38 (43.2%)	34 (3.6%)	4 (4.3%)	92 (10)

Conclusions

- Thrombomodulin-mediated Protein C activation plays an important role in the maintenance of placental function.
- The Gln387 residue of thrombomodulin lies within a beta sheet structure. The Gln387Pro substitution is predicted to disrupt the beta sheet and affect the ability of thrombomodulin to interact with thrombin or Protein C.
- Further experiments are needed to determine if Gln387Pro substitution causes a kink or turn in the protein and inhibits binding between exosite I and thrombomodulin or of the complex with Protein C.

References:

1. Source of Figure 1: "What is Hemophilia." web article <http://www.hemophiliaalliance.com/hemophilia-overview/what-is-hemophilia.aspx>
2. Source of Figure 2: Web article posted by Berges, John (2010) <http://www.wisconsin.edu/med/medschool/2010/02/20/DeepVeinThrombosis/>
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4. Waiser G, et al. "A Targeted Point Mutation in Thrombomodulin Generates Viable Mice with a Prothrombotic State." *J. Clin. Invest.* 101, 1983-91 (1998)
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6. An et al. "Heparin Rescues Factor V Leiden-associated Placental Failure Independent of Anticoagulation in a Murine High-risk Pregnancy Model." *bioRxiv* preprint doi: <https://doi.org/10.1101/2013.09.02.024007>; this version posted September 2, 2013. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.