

Biochemical Reaction Networks and Blood Clotting

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Abstract

Following an injury to the walls of a blood vessel, platelet aggregation and blood coagulation are induced. Through a series of enzymatic and biochemical interactions, a blood clot is formed. Using ordinary differential equations with mass action and Michaelis-Menton kinetics to describe these interactions and computer programs like Fortran and Matlab, we are able to model blood clot formation and maintenance, both spatially-dependent biochemical reaction networks.

Biology Background

Various chemical components are involved in blood clot formation. Enzymes, like VIIa, IXa, and Xa, work in conjunction with cofactors like TF (tissue factor), VIIIa, and Va. The processes that the enzymes and cofactors facilitate are also affected by inhibitors such as APC (activated protein C), which inhibits VIIIa and Va, TFPI (tissue factor pathway inhibitor), which inhibits the actions of TF and a VIIa:Xa complex, and ATIII (antithrombin III), which inhibits thrombin, IXa, and Xa.

As shown in the Figure 1, there are various interactions that take place between enzymes, cofactors, and inhibitors. Some of these processes can occur in solution, while others can only take place in certain locations on the subendothelium or on an activated platelet. Thrombin is the most crucial component of the blood clotting system, as its presence both renders platelets active and breaks fibrinogen into fibrin, the mesh base of a blood clot. Thrombin also acts as an enzyme processor in solution, along with Xa. The tenase complex of VIIIa:IXa and the prothrombinase Va:Xa complex act only when bound to an activated platelet. One other complex, TF:VIIa, acts only when attached to the subendothelium. Enzymatic processes in this system are outlined in Table 1.

Mathematical Model

Certain aforementioned enzyme-factor complexes act as processors only when they are bound to specific blood vessel locations. Complications arise when we take into account the actual density of binding sites in a given location. Binding site density, along with enzyme concentration, shear rate, platelet count, and material-inherent surface reactivity, add complexity and realism to the traditional mass-action and Michaelis-Menton mathematical models.

Each mathematical model is constructed with its own assumptions. The model initially utilized in this project assumed that all solution materials are well-mixed, that protein flow rate is proportional to protein concentration differences, that platelet transport is different from enzyme transport due to platelet thickness, that unactivated platelets are free to flow out while activated platelets “stack” and create a difference of rate constants due to volume, and that binding sites can harbor competition, sharing, or specificity.

Modeling Experiments

The unaltered model used in this project was originally created in Fortran with 63 ordinary differential equations. Using this mathematical model, it is possible to find differences in the system’s reactions to changes. When parameter values, generally concentrations and rate constants, and shear rate, the gradient of velocity is a flowing material (in inverse seconds), are standardized, it is possible to compare the differences between a system in which platelet deposit blocks TF:VIIa activity and in which it does not (Figures 2, 3)

Experiments such as these are useful for determining the actual effect of variable concentrations of clotting components on “normal” systems. However, there is the other aspect of the “abnormal” system. In diseases like hemophilia, the system may be lacking a chemical or physical component crucial to the blood clotting process (Figure 4). Another thing that can be altered is the shear rate. In Figure 5, thrombin concentration as dependent on TF density on the subendothelium is shown at different shear rates.

Model Modifications

The model discussed is based off of a great deal of biological background and uses some of the most powerful tools of mathematical modeling. However, this model designed by Kuharsky and Fogelson is by no means entirely accurate. In order to make it more representative of the actual biological process, other components can be added. Years later, Fogelson and Tania updated the other model. They added more complex APC inhibitor interaction and alternative TFPI interaction which assumed a finite rate of inhibitor unbinding and a differing Xa/TF:VIIa post-activation action. Also, a second shell was added to create the endothelial zone, an area right above the reaction zone where molecules are more susceptible to being carried downstream. Interaction between the reaction and endothelial zones is also included in the newer model.

This model showed various differences in results. Starting with the same parameter values, enzyme concentrations appeared to change (Figure 6). It is also possible to see the result of the additional zone in Figure 7.

Future Work

The project thus far has consisted of learning the workings of the enzymatic biological system involved in blood clotting by reading journal articles, becoming familiar with previous models, and converting the Fortran code to Matlab for ease of modification and further work. The goal is to, after obtaining a working Matlab model, add other interactions such as that of enzyme XI with soluble tissue factors, account more consistently for the effects of blood flow, adjust enzyme/cofactor/inhibitor levels, and explore the complex intervention of immune response. These additions, especially the extra enzyme, are subjects of controversy in the clotting community and have never before been included in coagulation models.

Figures and Tables

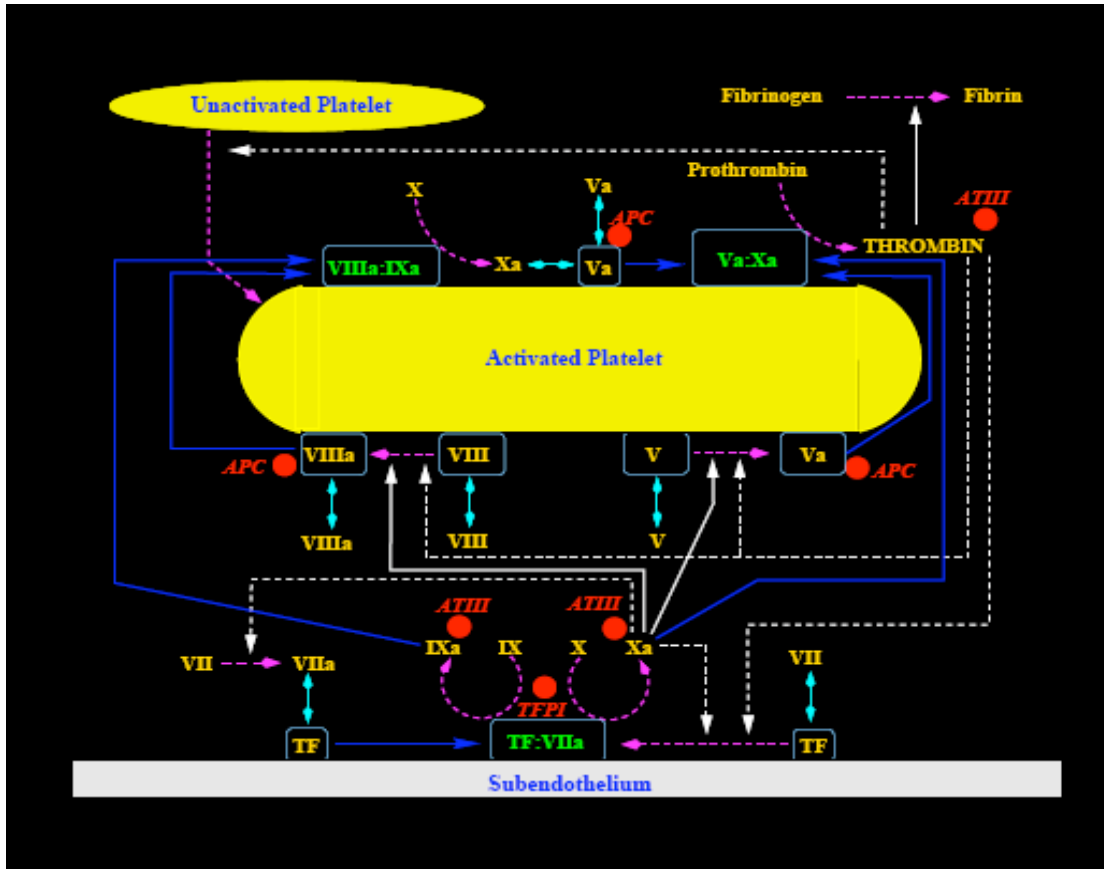


Figure 1- Enzymatic Interactions

| Location | Input | Processor | Product |
|---------------|-------------|----------------------|----------|
| Act. Platelet | X | Tenase VIIIa:IXa | Xa |
| Act. Platelet | Prothrombin | Prothrombinase Va:Xa | Thrombin |
| Solution | V | Thrombin | Va |
| Solution | VII | Thrombin | VIIa |
| Solution | VIII | Thrombin | VIIIa |
| Solution | V | Xa | Va |
| Solution | VII | Xa | VIIa |
| Solution | VIII | Xa | VIIIa |
| Subendo. | IX | TF:VIIa | IXa |
| Subendo. | X | TF:VIIa | Xa |

Table 1- Enzymatic Processes

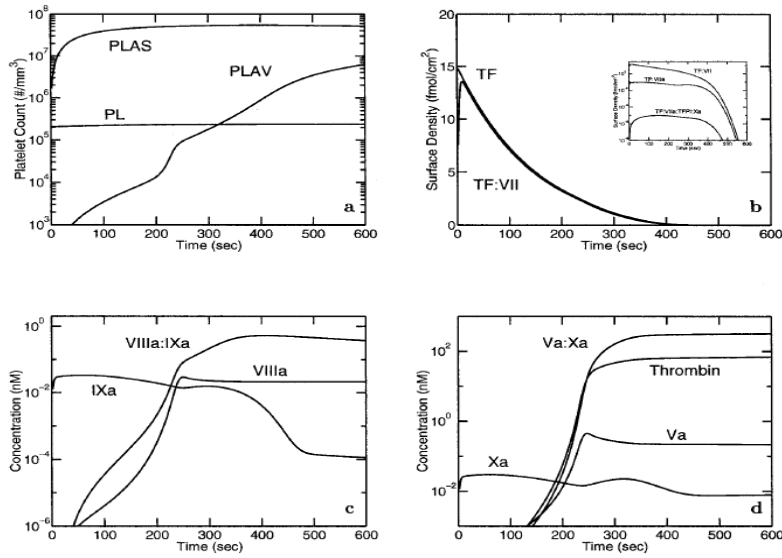


Figure 2- Platelet Deposit Blocks TF:VIIa Activity

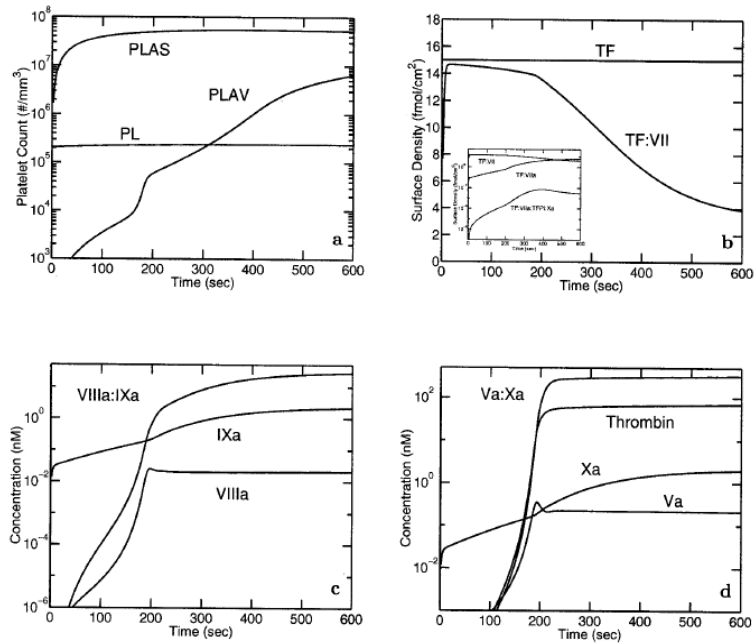


Figure 3- Platelet Deposit Does Not Block TF:VIIa Activity

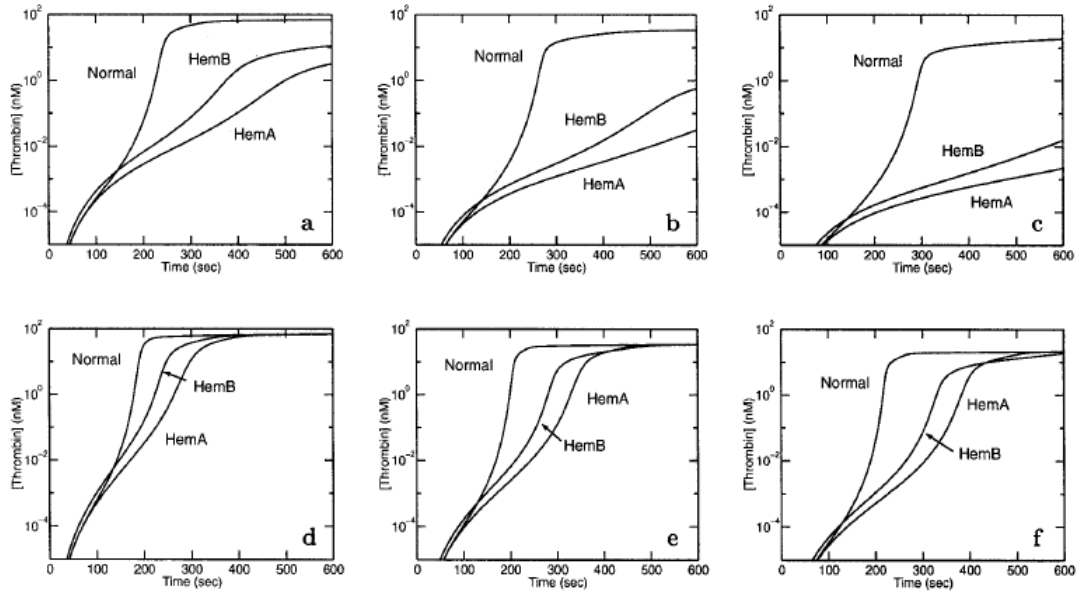


Figure 4- Hemophilia Comparison

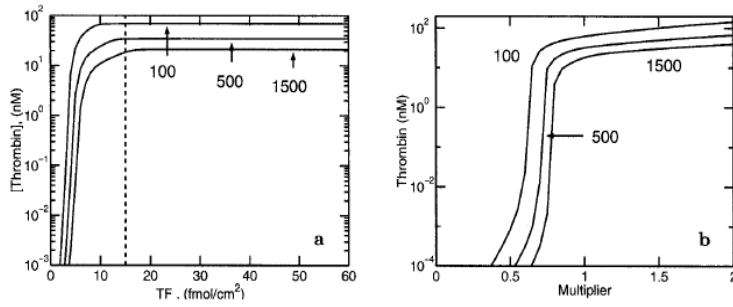


Figure 5- Shear Rate Comparison

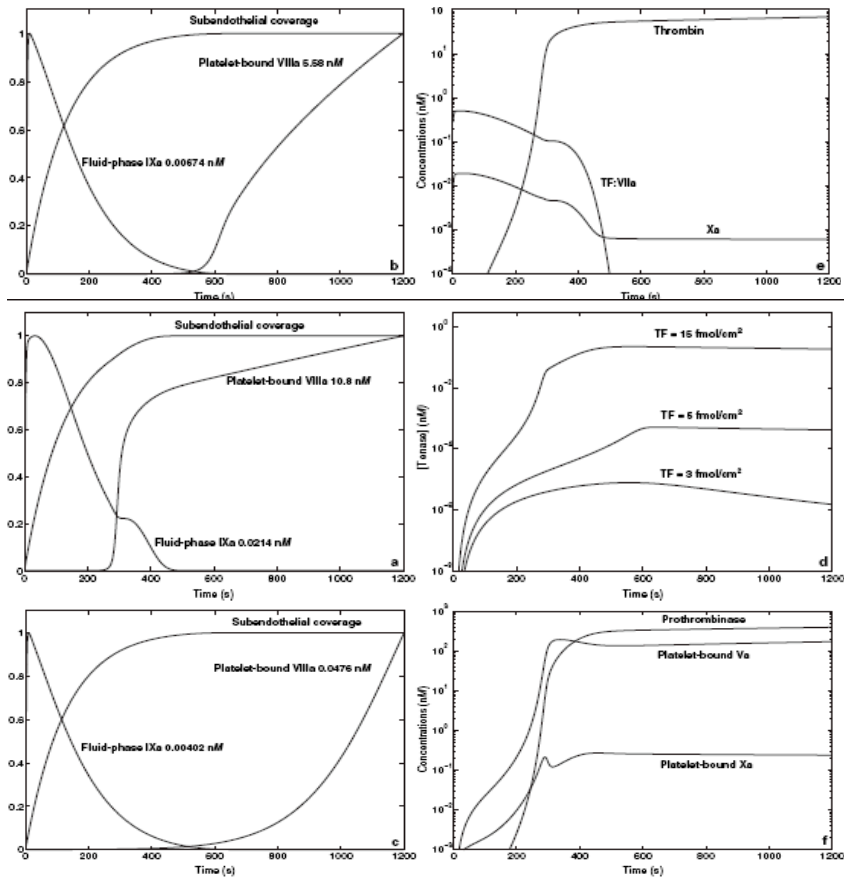


Figure 6- Enzyme Concentrations

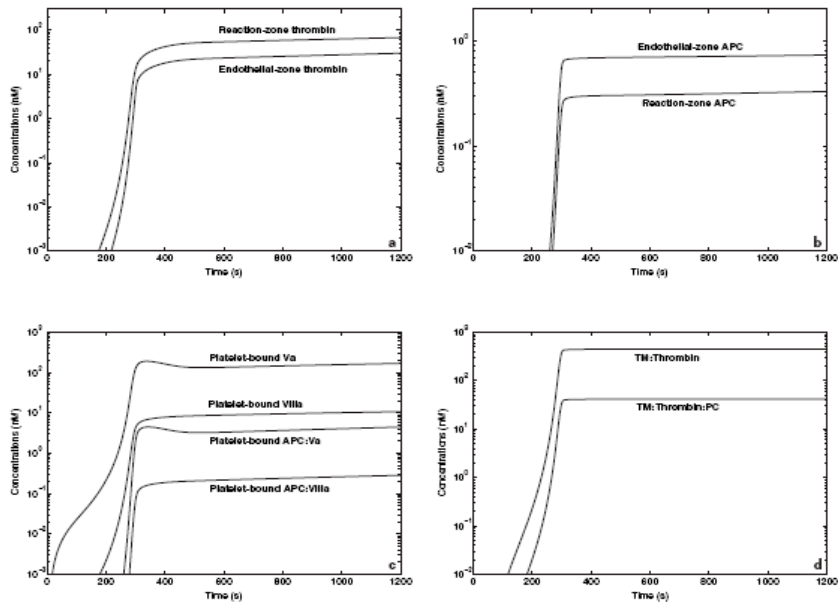


Figure 7- Zone Comparison

References

Fogelson, Aaron L, and Nesy Tania. "Coagulation under Flow: The Influence of Flow-Mediated Transport on the Initiation and Inhibition of Coagulation." Pathophysiology of Haemostasis and Thrombosis 34 (2005): 91-108.

Goldbeter, Albert, and Daniel E Koshland. "An amplified sensitivity arising from covalent modification in biological systems." Proc. Natl. Acad. Sci. USA 78.11 (Nov. 1981): 6840-6844.

Kuharsky, Andrew L, and Aaron L Fogelson. "Surface-Mediated Control of Blood Coagulation: The Role of Binding Site Densities and Platelet Deposition." Biophysical Journal 80 (Mar. 2001): 1050-1074.