An Analysis of the Robustness and Fragility of the Coagulation System

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Abstract

The coagulation system (CS) is a complex, inter-connected biological system with major physiological and pathological roles. Adaptive mechanisms such as ubiquitous feedback and feedforward loops create non-linear relationships among its individual components and render the study of this biology at a molecular and cellular level nearly impossible. Computational modeling aims to overcome limitations of current analytical methods through *in silico* simulation of these complex interplays. We present herein an Agent Based Modeling and Simulation (ABMS) approach for simulating these complex interactions. Our ABMS approach utilizes a subset of 48 rules to define the interactions among 24 enzymes and factors of the CS. These rules simulate the interaction of each "agent", such as substrates, enzymes, and cofactors, on a two-dimensional grid of ~3,000 cells and ~500,000 agents. Our ABMS method demonstrates the robustness of the physiologic CS system over large ranges of tissue factor (TF) concentrations. The system also demonstrates fragility as complete coagulation occurs at sufficiently high concentrations of TF. Removal of individual coagulation inhibitors from the physiologic system results in system fragility at relatively lower TF concentrations. The complete removal of coagulation inhibitors leads to a system that is incapable of controlling coagulation at all TF concentrations. The synergistic effects of the inhibitory pathways create an intricate regulatory mechanism that allows sufficient clot formation while preventing system wide activation of the CS; a robust system emerges.

Introduction

Coagulation System

In the event of an injury to the endothelium, the coagulation system balances the need for localized clot formation against prevention of systemic activation. This finely tuned system is composed of an assortment of

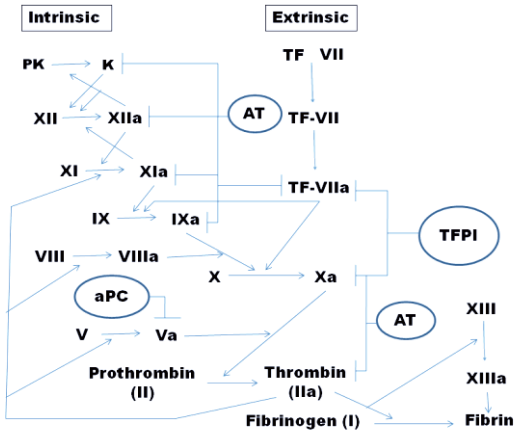

Figure 1: Schematic diagram of the coagulation cascade.

molecular and cellular "agents" (e.g. substrates, enzymes, cofactors, inhibitors, platelets, and endothelial cells) all interacting to generate a stable clot in order to rapidly obtain hemostasis[1]. The classical view of coagulation is a series of zymogen to enzyme conversions that generates a proteolytic enzyme, thrombin, which catalyzes the deposition of fibrin. In this model, activation of factor VII

(extrinsic, Fig. 1) or factor XII (intrinsic, Fig. 1) results in the formation of the multi-molecular tenase and prothrombinase complexes. These complexes eventually generate thrombin. Thrombin then cleaves fibrinogen to form fibrin monomers, which polymerize to form a three-dimensional clot. *In vivo*, clotting is initiated through the extrinsic system; deficiencies of PK, HMWK, or Factor XII are not associated with bleeding diatheses.

The CS may be viewed as a complex adaptive system, in which individual components are linked through multiple feedback and feedforward loops. These loops introduce non-linear relationships among the components. Thus, the classically portrayed coagulation pathway (Figure 1) is in actuality a static diagram that cannot adequately describe this dynamic evolutionary network.

The CS demonstrates its robustness by successfully forming clots at the site of endothelial injuries, while maintaining blood fluidity. Over the course of a lifetime, humans suffer many injuries that challenge the system yet do not result in death from either bleeding or systemic coagulation. The CS maintains this delicate balance through the negative feedback provided by the inhibitory pathways.

Clot formation is regulated *in vivo* through the antithrombin III-heparin complex (AT-H), activated protein C (aPC), and tissue factor pathway inhibitor (TFPI) (Fig. 1). These regulating systems limit excessive formation of cross-linked fibrin under hemostatic conditions. The anticoagulation systems are regulated independently, but combine to synergistically control thrombin generation.

The regulatory systems are limited by the physiologic concentrations of the components. Once the regulatory systems are overwhelmed, systemic coagulopathies (e.g. disseminated intravascular coagulopathy, traumatic induced coagulopathy, and coagulopathy associated with cardiac arrest) ensue; the diseases that favor coagulation result from the fragility of the CS. The system fails to localize clot formation when the initiating concentration of TF is too great for the inhibitory pathways to suppress system wide activation. Once this threshold is exceeded, the activation of the CS results in a disease state.

Computational Modeling

Computational systems biology is an emerging field that provides tools to analyze and understand complex adaptive systems such as the CS[5,6]. A major advantage of this approach is its rapid, real time analysis of multiple biological systems; each may function as a highly coordinated independent network interacting with other networks in the group at one or more branch points. These independent networks can be thought of as small molecular machines, which work co-operatively to form a large, multi-component molecular machine producing one or more physiological responses. Understanding the mechanism and co-operative nature of these networks as well as predicting the physiological response to appropriate pharmaceutical agents is an extremely difficult and intricate task. Advanced systems biology techniques, e.g., computational technology, may achieve this goal with consequent major applications in understanding patho-physiological conditions and their treatment.

Table I: Entity Table

Entity	Description
IX	Factor IX (Christmas factor) Activates X. - Forms tenase complex
VIII	Factor VIII. Co-factor of IX – Forms tenase complex
VIIIa	Activated factor VIII
VIIIa1	Factor VIIIa spontaneously dissociates into inactive VIIIa1 + VIIIa2
VIIIa2	Factor VIIIa spontaneously dissociates into inactive VIIIa1 + VIIIa2
IXa-VIIIa	tenase complex - activates X
IXa-VIIIa-X	IXa-VIIIa-X complex
VII	Factor VII. Activates IX and X.
VIIa	Activated factor VII
II	Factor II (prothrombin). Activates F, V, VII, XIII
IIa	Activated factor II (thrombin)
X	Factor X. Activates II. Co-factor of V – forms prothrombinase complex
V	Factor V. Co-factor of X – forms prothrombinase complex
Va-Xa	Prothrombinase complex – activates II
TF	Tissue Factor. Activates X in combination with VIIa
TF-VIIa	TF-VIIa complex
AT	Antithrombin III – inhibits TF-VIIa, IIa, IXa, XIa, XIIa and Xa
AT-Xa	AT-Xa complex
AT-IXa	AT-IXa complex
AT-IIa	AT-IIa complex
AT-TF-VIIa	AT-TF-VIIa complex
TFPI	Tissue Factor Pathway Inhibitor - inhibits VIIa-TF, Xa
TFPI-Xa	TFPI-Xa complex
TF-VIIa-Xa-TFPI	TF-VIIa-Xa-TFPI complex

To date, most computational models of the coagulation system have focused on using ordinary or partial differential equations (ODEs and PDEs)[7-9]. Differential equations describe the change in the states of the variables of the system over time and are derived from known or hypothesized kinetics. ODE models can readily simulate coagulation *in vitro* as it is a relatively homogenous system[10]; however, such models face significant limitations when modeling *in vivo* hemostasis due to complicating factors such as non-stationarity, spatial heterogeneity and the effects of blood flow. Therefore, derivation of differential equations suitable for *in vivo* modeling becomes problematic.

In order to address the shortcomings associated with ODE models, we present the application of a computational systems biology approach using agent-based

modeling and simulation (ABMS) to understand the CS and determine the role of coagulation inhibitors in preventing complete activation of thrombin. ABMS provides a powerful alternative to differential equations[11-13]. ABMS is a relatively new modeling paradigm derived from cellular automata (CA)[14,15]. ABMS has mobile autonomous entities (agents) that can move through space. Each agent is allowed to assume a finite number of states, determined by a pre-defined set of rules. Every agent is individually updated at the end of each operating period according to the pre-set rules. The rules are a function of the current state of the agent and the state of its neighbors.

The prothrombin time (PT) is commonly utilized in assessing the coagulation status of patients. Excess TF initiates the coagulation cascade and thereby tests the fidelity of the extrinsic pathway as a part of the whole cascade. As these tests are performed on human plasma, except for the bolus of the initiator, all factors of the clotting cascade are typically present at their normal plasma levels (Table III).

Few therapeutic modalities address the system wide activation of the CS in disease states such as DIC, TIC, and coagulopathy associated with cardiac arrest. These pathophysiologic events are virtually impossible to study *in vivo*. Our model attempts to provide a virtual laboratory by which these disease states may be simulated. The conditions under which the CS maintains its robustness and demonstrates its fragility are described below.

Coagulation Model

The model described in this paper is designed to simulate clot formation in the *in vitro* environment. Therefore, a limited subset of substrates, reactions, and products from the intrinsic, extrinsic, common, AT-H, and TFPI pathways have been included (Table I & II). In order to create realistic simulations, physiologic concentrations of factors were used in the *in silico* experiments (Table III). The rates associated with the reactions were taken from the literature and were assumed to be performed in saturating phospholipid and calcium conditions. The simulations were designed to test experimental conditions that examine both the robustness and fragility of the CS.

Materials and Methods

Coagulation Model

The ABMS in this paper uses a two dimensional particle system whereby particles move freely and interact on a discrete spatial grid. In this specific model, we define the particles of the system as the reactants, enzymes, and products in the entity table (Table I). The spatial grid is set as a two dimensional grid where the agent's location is identified by its x and y coordinates. Each coordinate pair (x, y) delineates a unique location. The number of grid locations used in these simulations is 3,000. The number of agents in the simulations is on the order of 10^6. Each time step of the simulation represents 0.01 seconds.

Table II: Rule Table

#	Reaction	Pathway
1	VII + TF → VII-TF[7,19-21]	Extrinsic
2	VII + TF ← VII-TF[7,19-21]	Extrinsic
3	VIIa + TF → VIIa-TF[7,19-21]	Extrinsic
4	VIIa + TF← VIIa-TF[7,19-21]	Extrinsic
5	VIIa-TF + VII → VIIa-TF + VIIa[7,8,22]	Extrinsic
6	Xa + VII → Xa + VIIa[7,8,22]	Extrinsic
7	IIa + VII → IIa + VIIa[7,8,22]	Extrinsic
8	VIIa-TF + X → VIIa-TF-X[7,21,23]	Extrinsic
9	VIIa-TF + X ← VIIa-TF-X[7,21,23]	Extrinsic
10	VIIa-TF-X → VIIa-TF + Xa[7,24,25]	Extrinsic
11	VIIa-TF + Xa → VIIa-TF-Xa[7,25,26]	Extrinsic
12	VIIa-TF + Xa ← VIIa-TF-Xa[7]	Extrinsic
13	VIIa-TF + IX → VIIa-TF-IX[7,27,28]	Extrinsic
14	VIIa-TF + IX ← VIIa-TF-IX[7,27,28]	Extrinsic
15	VIIa-TF-IX → VIIa-TF + IXa[7,27,28]	Extrinsic
23	VIIIa + IXa → VIIIa-IXa[7,28]	Intrinsic
25	VIIIa + IXa ← VIIIa-IXa[7,28]	Intrinsic
26	VIIIa-IXa + X → VIIIa-IXa-X[7,28,29]	Intrinsic
27	VIIIa-IXa + X ← VIIIa-IXa-X[7,28,29]	Intrinsic
28	VIIIa-IXa-X → VIIIa-IXa-Xa[7,28,29]	Intrinsic
29	VIIIa → VIIIa1 + VIIIa2[7,30]	Intrinsic
30	VIIIa ← VIIIa1 + VIIIa2[7,30,31]	Intrinsic
31	VIIIa-IXa → VIIIa1 + VIIIa2 + IXa[7,32]	Intrinsic
32	Xa + II → Xa + IIa[7,8,33,34]	Common
33	IIa + VIII → IIa + VIIIa[7,8,23]	Common
34	IIa + V → IIa + Va[7,8,28,35,36]	Common
35	Xa + Va → Xa-Va[7,28]	Common
36	Xa + Va ← Xa-Va[7,28]	Common
37	Xa-Va + II → Xa-Va-II[7,28]	Common
38	Xa-Va + II ← Xa-Va-II[7,28]	Common
39	Xa-Va-II → Xa-Va + IIa[7,28]	Common
40	Xa + TFPI → Xa-TFPI[7,23,28]	TFPI
41	Xa + TFPI← Xa-TFPI[7,23,28]	TFPI
42	TF-VIIa-Xa + TFPI → TF-VIIa-Xa-TFPI[7,23,37]	TFPI
43	TF-VIIa-Xa + TFPI ← TF-VIIa-Xa-TFPI[7,23,37]	TFPI
44	TF-VIIa + Xa-TFPI → TF-VIIa-Xa-TFPI[7,23,37]	TFPI
45	AT + Xa → AT-Xa[7,8,38]	AT
46	AT + TF-VIIa → AT-TF-VIIa[7,8,33]	AT
47	AT + IXa → AT-IXa[7,8,38,39]	AT
48	AT + IIa → AT-IIa[7,8,40]	AT

Grid locations in this model are designated as either empty or occupied by one or more substrates, enzymes, or reaction products. The agents are allowed to move freely about the grid. The movement, joining, and breaking are governed by probability rules. The movement parameter determines the extent of each agent's motion (0 implies every cell is stationary). The joining parameter determines the extent of interaction between adjacent agents. The breaking parameter defines the extent of disruption of agents that have joined. This model sets the probability of joining and breaking based on experimentally determined kinetic constants. The movement parameter is set at 1.

The agents are allowed to interact with all their neighbors, but meaningful interactions are limited to those in the rule table (Table II). The neighborhood of each agent in this model is defined as all agents located in the same grid location. After each time step, the agents move in a random manner to an adjacent grid location.

ABMS modeling requires the assignment of the probability of conversion associated with each chemical reaction as defined in the rule table (Table II). Reductionist *in vitro* experimental techniques have allowed a detailed understanding of the individual chemical reactions involved in the process of coagulation. The information obtained by studying the individual reactions is used as the basis for the rules governing the updating of the ABMS at each time step. We assigned a probability of conversion value related to the kinetics of the reactions. The initial configuration is random; each substrate and enzyme is assigned a predefined number of agents based on their desired initial concentration (Table III).

Table III: Baseline plasma concentration of coagulation factors.

Agent	Initial Concentration (μM)	# of Agents
TF	Varies	Varies
II	1.4	280,000
V	0.02	4,000
VII	0.01	2,000
VIIa	0.0001	20
VIII	0.0003	60
IX	0.09	18,000
X	0.17	34,000
AT	3.4	68,000
TFPI	0.0025	500

In vivo, prothrombinase and tenase complexes are each formed through a combination of three factors. The prothrombinase complex is formed by a combination of prothrombin, factor Xa and factor Va; the intrinsic tenase complex is formed when factors VIIIa and IXa combine with factor X. These three-body complexes are not directly simulated in ABMS, as *in vivo*, they must arise through sequential combination of two molecules. Thus, we utilized a sequential two-body collision approach to generate each complex.

The ABMS is designed to represent the *in vitro* environment. In this case, the spatial grid is in the shape of a rectangle allowing the particles to interact and bounce off the edge of the grid. There are no platelets, RBCs, or WBCs in the system as *in vitro* coagulation tests are run on acellular plasma. The PT experiments were terminated when 99% of the initial fibrinogen was converted to fibrin monomers. When running PT experiments, an additional end condition of 138 seconds was defined. This time equates to an INR > 10 which is a commonly reported value in clinical laboratories.

Computation

Netlogo v4.1, a software package designed to run ABMS, was utilized to perform the simulations[16]. The user determines the subset of reactants, the subset of reactions, the subset of coagulation factors, rate constants, initial factor concentrations, and termination conditions for each simulation. The concentration of every coagulation factor was output every 100 time steps (1 virtual second). The output of each simulation was stored in a comma separated file. All simulations were carried out on an Intel based desktop personal computer running Microsoft Windows XP. Up to six simulations were run in parallel. Each simulation ran up to 72 hours depending on the initial and stop conditions. Simulations were terminated after 13,800 iterations(138 virtual seconds) if the terminating conditions were not met.

Simulations

Unless otherwise stated, modeling of the system was performed under conditions that simulated literature derived mean physiologic concentrations of each soluble factor in normal humans (Table 3). The simulations were designed to determine the effects of varying the initiating TF concentration on clot formation.

Results

Four sets of simulations were performed to demonstrate the robustness of the CS (Table IV). The experiments terminated when all the fibrinogen was converted to fibrin due to systemwide coagulation or the elapse of 138s. The initial conditions of the system were varied 1) alteration of the initial TF concentration and 2) removal of coagulation inhibitors from the physiologic system.

Systemic response to varying the coagulation parameters are as follows: 1) Experiment 1: Normal physiological conditions for the coagiulation system. These conditions include both the AT and TFPI inhibitors. The system is able to prevent systemic clot formation at TF concentrations less than 500pM. 2) Experiment 2: Effects of lack of AT. The lack of AT leads to the system wide activation of the coagulation system at TF concentrations greater than 1 pM. 3) Experiment 3: Effects of lack of TFPI. The lack of TFPI leads to systemic clot formation for TF concentrations greater than 50pM. The total lack of inhibitors (AT and TFPI) results in systemwide activation over all TF concentrations (Table IV).

Table IV: Prothrombin times as a function of initiating TF concentration. The Normal column represents the physiologic system including all inhibitors. The AT column is the system that lacks AT. The TFPI column lacks TFPI. The NI column lacks both AT and TFPI.

TF (pM)	Normal (s)	AT (s)	TFPI (s)	NI (s)
1	138	138	138	118
10	138	76.4	138	97.8
25	138	26.6	138	63.1
50	138	34.7	138	21.7
100	138	24.0	48.8	19.5
500	33.9	14.3	28.9	13.0
1000	30.8	13.0	23.9	11.3
10000	27.5	12.4	21.0	10.8
100000	27.4	12.2	21.3	10.5

Figure 2 is a graphical representation of the PT plotted against the log of the initiating TF concentration. The plot of the normal physiologic system has a sharp break that is a result of the fragility of the system. Once a threshold of initiating TF concentration is reached, the system is no longer able to control clot formation. In contrast, the system that lacks inhibitors is incapable of preventing system wide clot formation and demonstrates a typical dose response relationship of an enzyme catalyzed system.

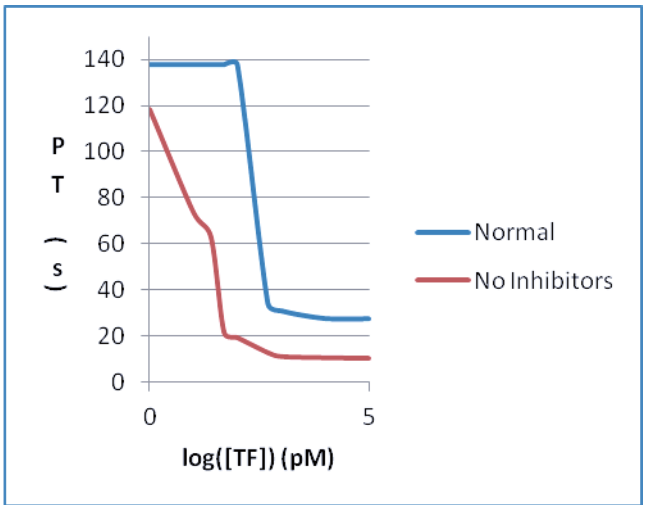

Figure 2: Plot of the PT vs. the log of the initiating TF concentration. The blue line represents the normal physiologic system that includes all inhibitors. The red line represents the system that lacks both AT and TFPI. The normal physiologic system has a sharp threshold that represents the failure to prevent system wide activation, hence fragility.

Discussion

The components of the CS interact strongly at the molecular and cellular level; they operate as a coherently linked system that generates both pathological and physiological responses. The highly complex CS renders identification of the root cause of many known coagulation defects virtually impossible. To date the contribution of each system as part of an integrated network has not been attempted. We have developed an ABMS of the CS cascade that allows systematic evaluation of each of its components – individually and as a complex entity. The model allows a comprehensive analysis of the CS cascade that provides insight into understanding and predicting the pathophysiologic responses arising from variations in its molecular and cellular components.

Only by creating models, which account for these seemingly diverse but clearly connected processes can one hope to improve our overall understanding of the coagulation process and to create more powerful diagnostic and therapeutic options. We have employed an ABMS in our current approach due to the potential ability to quantitatively analyze individual components of each system at every point of simulation. ABMS is a dynamic modeling and simulation tool that allows the study of dynamic non-linear networked systems. ABMS represents a non-reductionist approach of studying the biologic process as a whole, while retaining the information at an individual level. The complexity of the system has stymied experimental efforts to gain a system level understanding of the coagulation cascade and its subnetwork components. ABMS may readily provide elucidation of the pathophysiology of diseases related to the coagulation system. The model may prove informative regarding individual disease processes such as genetic and acquired disorders of coagulation.

The initial results of this paper indicate that the *in vitro* coagulation system can be readily simulated using ABMS. The disease states that result from the system wide activation of the coagulation system are a result of the failure of the CS to localize clot formation when perturbed by large TF concentrations. The inhibitory systems play a large role in preventing this system wide activation.

AT appears to be the inhibitor that plays the most significant role in the prevention of system wide activation. This *in silico* observation correlates with the clinical finding that human diseases are associated with AT deficiencies, but not TFPI deficiencies. Therefore, monitoring and supplementing AT may provide tools for the clinician in the treatment and prevention of coagulopathies.

Ideally, simulation of the *in vivo* environment will follow from this initial model. Expansion of the model will require the addition of blood flow, endothelial cells, white blood cells, platelets, and the full complement of coagulation proteins. Such a model will provide insight

into complex disease processes that are impossible to obtain using laboratory techniques. Thus, computational systems biology allows the design and implementation of experiments that would be unethical and dangerous in the clinical setting; instead, creation of previously unavailable diagnostic and therapeutic strategies becomes possible.

ABMS allows a real time analysis of the coagulation system that cannot be obtained through *in vivo* experiments. Additionally, ABMS provides an opportunity to understand the complex interplay among the various subsystems. The advantages of ABMS include the ability to simulate the non-linear aspects of the coagulation system. Moreover, the model is flexible and able to account for changes such as lack of inhibitors, absence of factors, or therapeutic interventions associated with disease processes. As new mediators are discovered they are easily added to the model.

Another major advantage of ABMS is the ability to monitor each coagulation factor as 'clotting' proceeds. This implies that the effect of a large number of factors that influence coagulation (*e.g*. natural and pharmaceutical anticoagulants, natural and pharmaceutical fibrinolytic agents, and intrinsic and external inflammation mediators) can be simulated readily. These ABMS are expected to provide information regarding the overall progress of clotting as well as individual coagulation factors as a function of time. Thus, ABMS of the coagulation cascade affords the ability to simulate the effect of heparins / low molecular weight heparins, coumadins, and factor Xa / thrombin inhibitors at a systemic level for the first time.

The last and most important advantage of the model is its ability to exhibit *emergent behaviors* through which outputs produce unanticipated results. These *in silico* results may then be biologically confirmed. Such properties are particularly useful in the discovery of diagnostic and therapeutic interventions. Comprehensive modeling of the traditional coagulation cascade allows unlimited virtual experimentation on the effects of local and systemic injury on coagulation.

Computational modeling allows the creation of rapid and inexpensive virtual laboratories to generate and test hypotheses. More importantly, simulations provide clinical tools to design and test novel therapeutic strategies, while affording opportunities to predict adverse events during drug development. Ultimately, bedside simulations will allow personalized medicine to calculate replacement factor concentrations for individual hemophiliac patients, heparin doses can be titrated, etc… Future applications of the model include discovery of new mediators, understanding the proximal and distal effects of interactions between systems, discovery of new diagnostic and therapeutic options, and development of new software and algorithms for simulation.

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