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REVIEW

Blood flow and mass transfer regulation of coagulation

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ABSTRACT

Blood flow regulates coagulation and fibrin formation by controlling the transport, or mass transfer, of zymogens, co-factors, enzymes, and inhibitors to, from, and within a growing thrombus. The rate of mass transfer of these solutes relative to their consumption or production by coagulation reactions determines, in part, the rate of thrombin generation, fibrin deposition, and thrombi growth. Experimental studies on the influence of blood flow on specific coagulation reactions are reviewed here, along with a theoretical framework that predicts how flow influences surface-bound coagulation binding and enzymatic reactions. These flow-mediated transport mechanisms are also used to interpret the role of binding site densities and injury size on initiating coagulation and fibrin deposition. The importance of transport of coagulation proteins within the interstitial spaces of thrombi is shown to influence thrombi architecture, growth, and arrest.

1. Background

Coagulation is a tightly controlled biochemical network of reactions that leads to the generation of thrombin. This network includes zymogens, enzymes, co-factors, and inhibitors, which quickly generate thrombin at the site of injury and efficiently eliminate it in the peripheral area. Most models of coagulation, such as the cascade or waterfall models [1] and cell-based models [2], focus on the biochemical mechanisms that include a series of binding and enzymatic reactions occurring on the subendothelium and the surface of cells and microparticles. These models are useful for describing coagulation in closed systems such as clot time assays and prothrombin time (PT), that take place under static conditions in test tubes or well-plates. They may also be appropriate models for describing clot formation in vivo under conditions of stasis, for example in venous thrombosis. However, blood flow imposes additional biophysical constraints on coagulation and platelet function during intravascular thrombus formation [3].

The recognition of the relationship between blood flow and coagulation dates back at least to the 19th century with the description of red clots and white clots [4]. Red clots are associated with venous thromboembolism and originate in areas of reduced blood flow or stasis as first described by Virchow. White clots are associated with high blood flow in arterial thrombosis as described by Bizzozero in his observations in the first in vitro flow chamber [5]. The red thrombi–white thrombi nomenclature is a somewhat oversimplified picture because even arterial, white thrombi contain a significant amount of fibrin [6]. Nevertheless, these initial observations point to blood flow as a key regulator of coagulation, fibrin deposition, and thrombus architecture. Baumgartner and colleagues made the first quantitative measurements of this biophysical regulatory mechanism using in vitro flow chambers [7–9]. An inverse relationship between blood flow and fibrin deposition was observed in whole blood perfusion over the subendothelium of rabbit arteries. Since these initial studies, the influence of flow on individual coagulation reactions, thrombin generation, fibrin formation, and platelet function has been greatly aided by in vitro models, especially more recent models that take advantage of micropatterning and microfluidic technology to define fluid dynamics with spatially controlled presentation of procoagulant surfaces [10]. These technologies provide the tools for determining how geometry, injury size, and more complex flows affect coagulation in environments that mimic in vivo injuries.

The theoretical foundations for how flow influences biochemical reactions on surfaces were developed in support of the burgeoning biotechnology industry in the 1960s and 1970s. Specifically, enzymes immobilized on the wall of tubes serve as bioreactors for food and pharmaceutical processing. In their seminal work, Laidler and colleagues derived modifications to expressions of Michaelis–Menten and other enzyme kinetic models to incorporate the influence of flow on product generation [11,12]. They accounted for the transport of substrates to enzymes immobilized on a surface by convection (flow) and diffusion, which together are referred to hereafter as mass transfer. Nemerson and colleagues applied these mass transfer concepts to coagulation reactions [13].

This review focuses on the flow-based mechanisms that regulate coagulation reactions, building from a general theory of biochemical reactions on surfaces in order to organize and interpret in vitro and in vivo observations in purified, plasma, and whole blood experiments. The

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flow-based mechanisms that regulate platelet adhesion and aggregation have been reviewed elsewhere [3,14]. The review is organized as follows: (1) a brief background presented in this section, (2) the theoretical basis of how mass transfer regulates binding and enzyme reactions on surfaces, (3) the application of these theories in purified systems looking at one or a few coagulation or anticoagulation reactions, (4) the effect of injury size and geometry on the extrinsic pathway, (5) the regulation of fibrin polymerization and morphology by flow, (6) interstitial transport as a regulator of thrombus growth, and (7) a summary.

2. Mass transfer regulation of biochemical surface reactions

This section includes the governing equations that describe the relative rates of mass transfer and chemical reactions in order to give the reader the theoretical basis for how the experiments described in Sections 3–6 are interpreted. The intent here is to provide all of these equations in one place, saving the interested reader the time and effort of tracking them down across various textbooks and manuscripts. Another motivation is to provide a common language for these biophysical mechanisms that is necessary for ongoing discussions in this field. The definition of all variables and their units can be found in Appendix A.

The concept of a rate-limiting step is a familiar one in describing the kinetics of complex biochemical networks. There are approximately one hundred reactions in coagulation, but there are a few that are particularly slow relative to the rest and limit how fast thrombin is generated. These slowest steps are referred to as rate-limiting. For example, the dissociation of factor Xa (FXa) from a lipid surface before reassociation with FVa on another lipid surface can be the rate-limiting step in thrombin generation [15]. In a closed system, one in which no mass enters or leaves and where procoagulant stimuli are mixed homogenously in solution, we need only consider the intrinsic kinetics of each reaction in the fluid phase. Coagulation assays like PT, activated partial thromboplastin time (APTT), or calibrated automated thrombogram (CAT) are closed systems. Coagulation in an open system, one in which mass can enter or leave, such as in the formation of an intravascular clot, is fundamentally different than closed systems in two ways. First, most reactions occur on the vessel wall rather than in the fluid phase. Even though many coagulation assays in closed systems include phospholipids or platelets as surfaces to promote the assembly of enzyme complexes, these surfaces are suspended homogenously in the fluid phase. Second, these reactions happen in the presence of flowing blood. These features require that models of coagulation reactions under flow consider not only the intrinsic kinetics of each reaction, but also the transport of products and reactants to and from the surface. Under certain conditions that we outline below, transport of reactants to the surface can be rate-limiting.

Coagulation reactions take place on a variety of surfaces including the subendothelium, endothelial cells, tissue factor (TF) exposing cells or microparticles, and the phosphatidylserine-rich lipid surface of highly activated platelets. There are two general types of surface reactions that are important in coagulation; binding reactions and enzymatic reactions.

Binding reactions are typically modeled with first-order Langmuir kinetics:

$$\frac{\partial C_s}{\partial t} = k_{on} C_S (C_{s0} - C_s) - k_{off} C_s, \tag{1}$$

where C_s is the substrate volume concentration in the bulk; c_s is the surface concentration of the bound substrate; c_{so} is the total number of binding sites per unit area; and k_{on} and k_{off} are the association and dissociation rate constants. Note that we use upper case C (e.g., mol/m^3) for volume concentration and lower case c (e.g., mol/m^2) for surface concentration. Eq. (1) states in the language of differential equations that the change in the bound concentration of the substrate with time (left-hand side) is equal to the difference between substrate binding and unbinding events (right-hand side). An example is the binding of FVII/FVIIa to its co-factor TF to form the TF:FVIIa (extrinsic tenase) complex.

Enzymatic reactions are typically modeled with Michaelis–Menten kinetics:

$$\frac{\partial C_P}{\partial t} = \frac{V_{max} C_s}{K_M + C_S}, \tag{2}$$

where C_P is the product concentration; V_{max} is the maximum reaction rate at saturating substrate concentrations; and K_M is the Michaelis constant. These include the activation of zymogens to enzymes by complexes formed by binding reaction, for example the conversion of FX to FXa by TF:FVIIa. Both binding and enzymatic reactions depend on the substrate concentration, which is dictated by the relative rate of transport of substrate from the bulk to the wall relative the association rate of the substrate.

To calculate the rate of transport under flow, we need to consider both diffusion and convection (transport by flow), which are collectively called mass transfer. Mass transfer can be thought of as an additional rate process, which in combination with reaction kinetics, determines the rate of substrate consumption. Fig. 1 depicts the influence of the relative rates of diffusion, convection, and reaction on the substrate consumption by a binding reaction that follows Langmuir kinetics on the lower boundary. Flow moves from left to right between the two walls that make up the upper and lower boundaries by a pressure driven flow. The substrate is introduced on the left boundary at a normalized concentration of one. Moving from top to bottom down the columns, the flow goes from low to high. Moving from left to right across the rows, the association (k_{on}) rate becomes faster. When mass transfer is slow relative to reaction kinetics, we call a system transport-limited. In these cases, the substrate binds to the wall faster than it transports to the wall, leading to a concentration gradient between the surface and the bulk (far-right column in Fig. 1). For an enzymatic reaction that is transport-limited, the substrate concentration is lower near the wall, thus the rate of product formation is reduced. In an experiment, if the measured product generation increases with increasing flow velocity, then it is likely transport-limited. Conversely, when mass transfer is fast relative to reaction kinetics, we call the system reaction-limited. Here, the concentration of the substrate near the surface is close to the concentration in the bulk because it binds at a similar rate that it transports to the wall (far left-hand column of Fig. 1). In an experiment, if product generation is independent of flow velocity, then it is likely reaction-limited. Under reaction-limited conditions, flow can be a potent inhibitor of coagulation by diluting products in the near-wall region that may serve as substrates in subsequent reactions.

We can estimate whether a system is reaction-limited or transport-limited and reveal other important dynamics about a biochemical reaction or set of reactions by calculating a few dimensionless parameters. These dimensionless parameters come from scaling the conservation of species equation (convection–diffusion equation) by the appropriate geometric and dynamic variables. The details of this scaling procedure are beyond the scope of this review, but interested readers can find an introduction to scaling concepts in textbooks [16,17] and review articles specifically related to scaling arguments in binding and enzymatic reactions [18–20]. In lieu of a rigorous mathematical derivation based on first principles, here we present “engineering approximations” of the relative rates of different transport and reaction processes. These approximations give order-of-magnitude estimates of different rate processes.

We can determine the importance of different rate processes by comparing their fluxes. Flux is defined as the rate that a solute moves through, or is consumed/generated at, a surface and has units of moles or mass per unit time per unit area (e.g. mol s^{-1} m^{-2}). Let's consider the case of a substrate flowing through a vessel with a diameter, d,