



A MATHEMATICAL MODEL FOR THE SPATIO-TEMPORAL DYNAMICS OF INTRINSIC PATHWAY OF BLOOD COAGULATION. II. RESULTS

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Abstract This paper continues our study (see Part I) where we modeled the spatio-temporal dynamics of the intrinsic pathway of blood coagulation. Here, we analyzed this model and showed that it describes the threshold behavior of coagulation. When activation is subthreshold (which produces not more than 0.07 nM factor XIa at saturating free calcium concentrations of 2 mM or higher), the concentration of generated thrombin remains below 0.01 nM. At the abovethreshold activation corresponding to factor XIa exceeding 0.07 nM, the concentration of thrombin explosively increases and then abruptly decreases. The peak concentration of thrombin reaches hundreds nM. With respect to free calcium concentration, the system also behaves in a threshold manner. For activation corresponding to 0.3 nM factor XIa, the threshold concentration of free calcium where the outburst of explosive thrombin generation occur is equal to 0.21 mM. The model simulations are in a good agreement with the experimentally recorded kinetics of thrombin generation at different concentrations of free calcium (1). Analysis of the spatial dynamics of coagulation showed that if activation exceeded the threshold level at a certain point, the concentration wave of thrombin arises and propagates at a high speed from the activation zone. The parameters of this wave depends mainly on the efficiency of the feedback loops. The feedback loops through the backbone factors of the intrinsic pathway (autoactivation of factor X or activation of factor XI by thrombin) has a potential for the unlimited propagation of the thrombin wave. With increasing activity of activated protein C (the effect equivalent to that of thrombomodulin), oscillating regimes arise in the model. The first thrombin wave is followed by several secondary running waves. The amplitudes of secondary waves increases to the periphery of the clot consolidating its surface layer. *Copyright © 1996 Elsevier Science Ltd*

Key words: blood coagulation, thrombin, intrinsic pathway, mathematical model, spatial dynamics.

Assumptions and equations of the mathematical model describing the spatio-temporal behavior of concentrations of the activated factors forming after contact triggering the coagulation system were formulated in the first part of this study (see Part I. The model description). The objectives of our modeling are: (i) to analyze quantitatively the kinetics of the intrinsic pathway of coagulation, with special regard for the calcium and activator thresholds, and (ii) to simulate the dynamics of spatial growth of the clot.

RESULTS

Figure 1 shows thrombin evolution in time for varying levels of contact activation for saturating concentrations of Ca^{2+} (2mM or higher). First, we consider a case of zero initial concentrations of all activated factors under assumption that the stationary concentration of factor XIa is a measure of the extent of activation. The quantitative evaluation of the extent of activation in experiments is difficult. Therefore, below we refer to the stationary concentration of factor XIa (in place of the corresponding level of contact activation) to specify contact activation. At low contact activations, thrombin grows with time until reaches a stationary level that is lower than 0.01 nM (Fig. 1A). Stationary concentrations of other activated coagulation factors are also extremely low. These low concentrations of the activated factors are insufficient to produce a fibrin clot.

At a certain level of activation, the situation dramatically changes. Thrombin exponentially increases and then steeply falls. Its kinetics is typically pulse-shaped (Fig. 1B). The same kinetics is characteristically displayed by the factors of the coagulation cascade within the feedback loops. We refer to this level of activation as to the activation threshold and call the corresponding concentrations of activated factors threshold concentrations. The maximum threshold concentration of thrombin is of the order of hundreds nM (see Fig. 1B). A further increase in the activation level only slightly increases the amplitude of the thrombin peak and does not change qualitatively

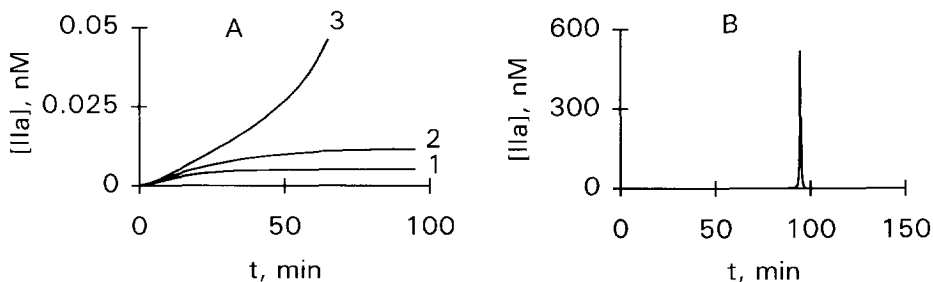


FIG. 1.

Kinetics of thrombin generation for subthreshold (curves 1 and 2) and above-threshold (curve 3) levels of activation (corresponding to 0.05, 0.06, and 0.07 nM factor XIa for curves 1, 2, and 3, respectively). Numerical simulations were performed with the parameter values specified in Table I (see Part I). The threshold value of factor XIa appeared to be 0.07 nM. The entire kinetics of thrombin generation for above-threshold activation is shown in FIG. 1B.

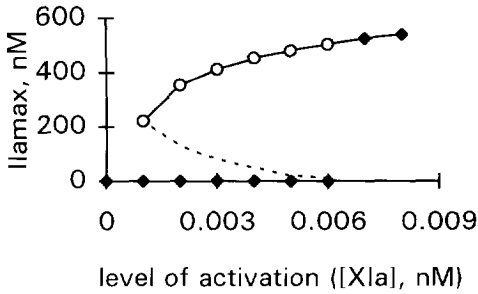


FIG. 2.

Peak thrombin concentration as a function of the activation level. Rhombi correspond to zero initial concentrations of thrombin. Circles correspond to small initial concentrations of thrombin significantly decreasing the threshold level of activation.

the behavior of the system. A 40-fold increase in the activation increases the amplitude of the thrombin peak by only 30%. Actually, only the onset time of the thrombin peak decreases with increasing activation. The amplitude of the thrombin peak is so great that a dense fibrin clot is formed at any level of activation exceeding the threshold.

If initial concentrations of activated factors differ from zero, coagulation can occur at lower levels of activation. Assume that thrombin is nonzero in the moment of activation. Figure 2 shows the maximum thrombin concentration for various levels of activation. Rhombi and circles correspond to zero and low-level initial concentrations of thrombin, respectively. Nonzero initial concentrations of thrombin significantly decrease the activation threshold. Note that below we give values of the threshold concentrations that correspond to zero initial conditions.

Kinetics of thrombin generation as a function of free calcium concentration.

To estimate the unknown kinetic constants, we analyzed a series of kinetic curves of hydrolysis of BOC-Ala-Pro-Arg-AMC, a specific fluorogenic substrate of thrombin (Fig. 3). These curves were recorded in contact-activated plasma at different calcium concentrations and reflect the kinetics of thrombin generation (1). To compare the model and experiments, we added to the model the equation describing hydrolysis of the substrate S by thrombin with $k_{cat} = 7.8 \cdot 10^3 \text{ min}^{-1}$ (2).

Comparison of the model simulations with experimental data showed that, at initial stages of coagulation (when AMC is in the range of 30-40 μM), the thrombin generation kinetics does not depend on the rates of reactions catalyzed by APC. This allows us to

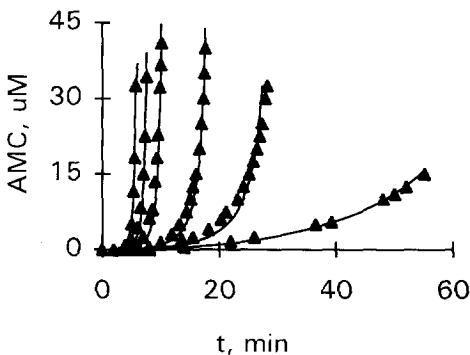


FIG. 3.

Theoretical (solid lines) and experimental (triangles) curves of thrombin generation for various Ca concentrations. Experimental curves were taken from (2). Calcium concentrations varied from 0.24 mM (the lowest curve) to 1.44 mM (the steepest curve) (2).

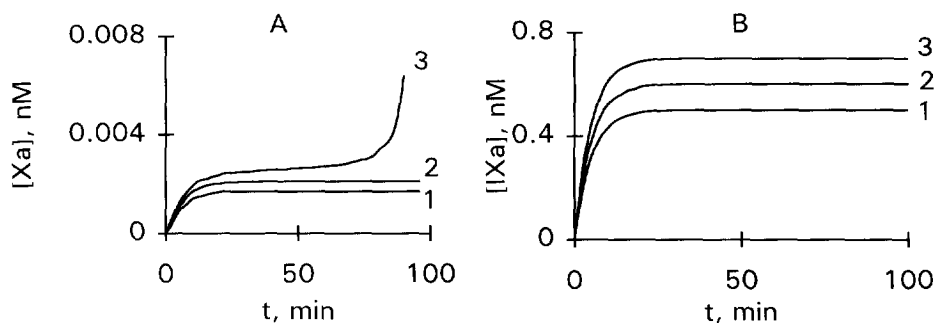


FIG. 4.

Generation kinetics of factors Xa (A) and IXa (B) for (1 and 2) subthreshold and (3) above-threshold activations that correspond to factor XIa concentrations of 0.05, 0.06, and 0.07 nM, respectively (see also caption to FIG. 1).

assume that: (i) factor VIIIa inactivation by APC has a k_m significantly exceeding the concentration of factor VIIIa, and (ii) reactions of inactivation of factors VIIIa and Va by APC do not differ in their k_{cat} / k_m values.

The optimal agreement of the model with experimental curves is achieved for k_5 and k_8 values of 0.17 min^{-1} and 0.00001 min^{-1} , respectively. The corresponding theoretical curves along with experimental data are shown in Fig. 3. Only the feedback through factor V performs well. The feedback through factor VIII exerts a weak effect on the model kinetics.

Threshold behavior of the system.

Analysis of the model showed that the system behaves in a threshold manner with respect to changes in both activation and Ca ion concentration. Figures 1A, 4A, and 4B demonstrate the kinetics of factors IIa, IXa, and Xa for various levels of activation at free calcium concentration of 2 mM (the saturating concentration). The curves in the series were generated for the levels of activation that corresponded to concentrations of factor XIa differing by 0.001 nM. It is easily seen that the threshold activation corresponds to factor XI concentration of 0.007 nM. At this concentration, a dramatic increase in the rates of production of factors IIa and Xa occurs. Factor IXa (which is not under the feedback control in the cascade) behaves distinctly. There is no qualitative change in factor IXa concentration with increasing activation. It gradually increases reaching the stationary level within the first 30 min. This result is compatible with the experimental findings on activation kinetics of factor IXa (3).

Figure 4A shows that, at above-threshold activations, simulated factor Xa begins to increase later than thrombin. This delay is accounted for by the less efficient feedback control through factor VIII (vs. factor V).

Analysis of the model showed that the dynamics of the system similarly depends on the activation level and free calcium concentration. For any fixed value of free calcium concentration, there is the corresponding value of threshold activation. The activation threshold decreases as free calcium concentration increases. And vice versa: for any

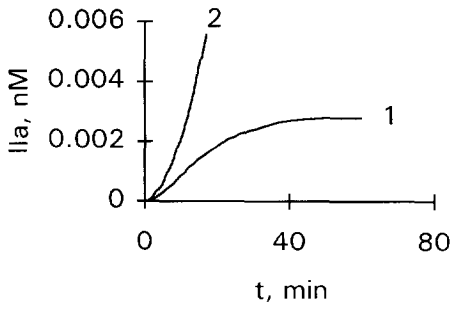


FIG. 5.

Initial kinetics of thrombin generation at the activation corresponding to 0.3 nM factor XIa for (1) subthreshold and (2) above threshold concentrations of free calcium (0.2 and 0.21 mM, respectively).

fixed level of activation, there is the corresponding threshold free calcium concentration. Figure 5 shows the thrombin kinetics for two concentrations of free calcium (0.2 and 0.21 mM) at a constant level of activation ($[XIa] = 0.3$ nM). It is clear that for this level of activation, the threshold concentration of free calcium is between 0.2 and 0.21 mM, in a good agreement with the experimental data (1).

The kinetics of thrombin generation as a function of the rate of Protein C activation.

We studied how the kinetics of thrombin generation depends on the rate constant for protein C activation by thrombin at free calcium concentrations of 2 mM or higher ($f(Ca)$, see Part I). The peak amplitude of thrombin decreases with increasing k_{apc} (Figs. 6A and 6B). For $k_{apc} > 0.06$ min⁻¹, the model gives several peaks of thrombin generation with decreasing amplitudes. Damped oscillations of all activated factors involved in the operation of the feedback loops occur in model system in this case. The amplitude of the first thrombin peak as well as the interpeak intervals decrease with increasing k_{apc} . Figure 6B demonstrates the thrombin kinetics for $k_{apc} = 0.1$ min⁻¹. Analysis of the simplified models showed that the appearance of several thrombin peaks is accounted for by transition of the system into the oscillating regime. The

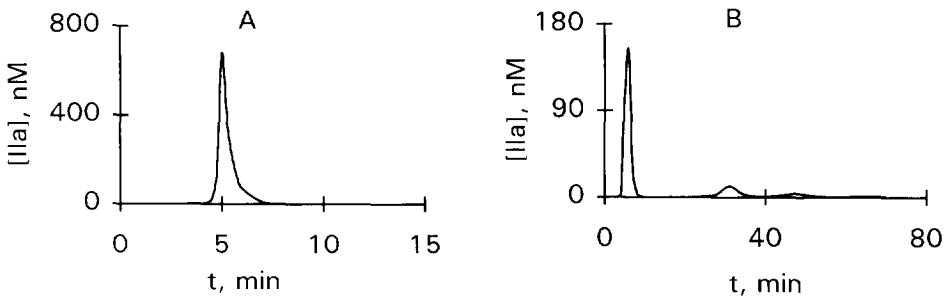
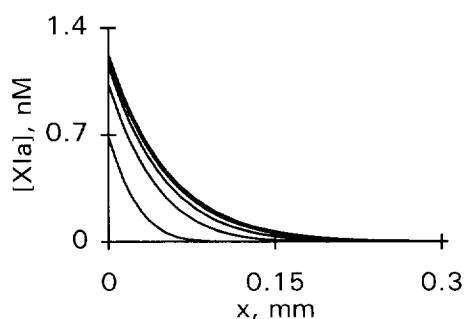


FIG. 6.

Kinetics of thrombin generation simulated for (A) normal and (B) higher than normal constants for protein C activation by thrombin ($k_{apc} = 0.0014$ min⁻¹ and $k_{apc} = 0.1$ min⁻¹, respectively).

FIG. 7.



Spatial profiles of factor XIa concentration from $t = 1$ min (lower curve) at 3-min intervals.

decrease in the amplitude of the next thrombin peaks is caused by prothrombin depletion. If we assume that no precursor is depleted, equiamplitude peaks are repeated infinitely long in the range of k_{apc} values under study.

Spatial dynamics of the clot growth.

Figure 7 shows the concentration of factor XIa as a function of the distance from the boundary where activation is applied. This boundary simulates the edge of the injury region. With increasing distance from the activating boundary, the concentration of diffusing factor XIa decreases. The first profile depicted in Fig. 7 is the concentration profile of factor XIa established 1 min after the activation was started. Other profiles are successively shown at a 3-min interval. The inactivation of factor XIa occurring at any point of the space leads to the situation that the diffusion profile of factor XIa does not change any more 10 min after its diffusion from the boundary began. The shape of the stationary profile of factor XIa is determined by the rate constant for its inactivation by plasma inhibitors (h_{11}) and by the diffusion coefficient (D).

If the activating signal (that is, the factor XIa influx from the left boundary of the segment under consideration) remains subthreshold, thrombin and other activated factors have the diffusion concentration profiles similar to that of factor XIa. If the activation is above threshold, a thrombin wave starts from the boundary after a certain delay. This wave has a steep front (Fig. 8) significantly differing in shape from the diffusion profile of factor XIa (Fig. 7). Following the thrombin wave, the wave of fibrin propagates forming the fibrin clot.

For the parameter values specified in Table 2 (see Part I), the time delay after which the thrombin wave starts is 5 min. The speed of the thrombin wave changes during its propagation. With increasing the distance from the injury site, the wave speed and amplitude are slowed down until thrombin disappear almost completely. The fibrin wave runs after the thrombin wave and form the fibrin clot fixing the area of the presence of thrombin.

Some time after the first thrombin wave started, several successive thrombin waves are born within the fibrin clot. However, the maximum amplitude of these secondary waves is tens times smaller than that of the first thrombin wave, possibly because of depletion of precursors of the activated factors within the growing clot. The small amplitude of the secondary thrombin waves makes their contribution to the overall size of the fibrin clot insignificant. In contrast to the first thrombin wave, secondary waves have the amplitude that initially, while the wave propagates at the maximum speed, increases and reaches the maximum value at the edge of the formed fibrin clot. Therefore,

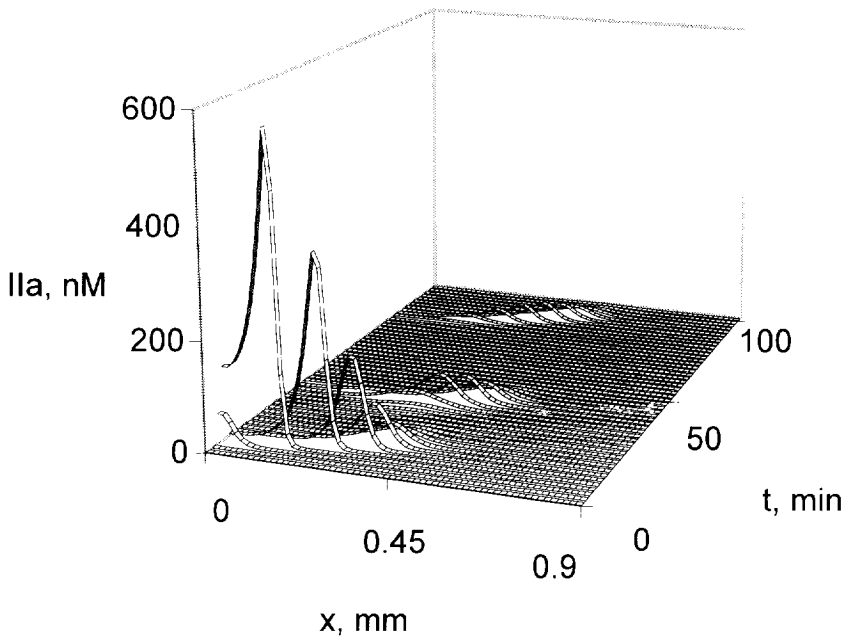


FIG. 8.

Spatio-temporal dynamics of thrombin propagation from the site of activation. Values along the time axis increase in the direction going away from the reader. Thrombin profiles are presented at 2-min intervals from the beginning of activation of the system. Figure illustrates the appearance of the secondary thrombin waves 30 and 80 min after triggering of the system. Values of the parameters used in simulations are specified in Table II (except for k_{apc} taken as 0.01 min^{-1}).

additional fibrin production is also maximum at the edge of the clot increasing its thickness predominantly at the edge. In the parameter ranges considered, the fibrin concentration at the edge of the clot may exceed 1.5-2 times that in the center.

Figure 8 shows the spatio-temporal dynamics of thrombin propagation from the site of activation (left boundary). It illustrates the appearance and spreading of the second and third waves of thrombin at time 31 - 45 min and third 78 - 100 min, respectively.

Figure 9 shows the corresponding fibrin profiles at equal time intervals. In the first phase, the clot grows at a very high rate reaching 50% of the final size within first 4 min of its growth. The first thrombin wave provides 86% of the fibrin clot. The thickening of the edge of the clot is clearly seen (Fig. 9). Figure 10 shows the movement of the fibrin front as the time dependence of the displacement of the point corresponding to a fibrin concentration of 600 nM.

Using the model, we analyzed how the system behaves at varying activations. The maximum amplitude of the thrombin wave appeared to be almost independent of the level of the abovethreshold activation and exceeds the maximum achievable at

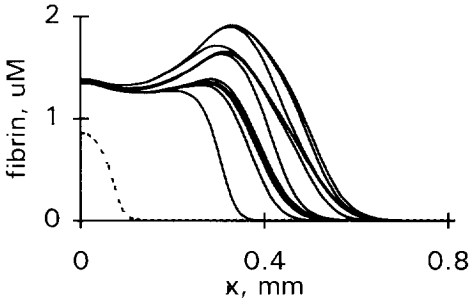


FIG. 9.

Gradual increase in the fibrin density at the edge of the clot resulting from the appearance of three successive thrombin waves. The corresponding thrombin profiles are shown in FIG. 8. Fibrin profiles are shown at 5-min intervals. The dashed line corresponds to the time point of 6 min.

subthreshold activations by five orders of magnitude. The size of the clot also slightly depends on the level of activation (Fig. 11).

Effect of activation of factor XI by thrombin on the spatial dynamics of the system.

It is of particular interest to understand what might be the effect of the feedback activation of factors of the coagulation cascade whose activated forms are proteinases (i.e., factors distinct from factors V and VIII which have no enzymatic activities). Several such reactions are described in the literature. They include activation of factor XI by thrombin (4) and autoactivation of factor X (5). These reactions form positive feedback loops whose effects on the spatial dynamics of coagulation is essentially different (as we show below) from those exerted by feedback activation of factors V and VIII.

In this paper, we also examined the role of factor XI activation by thrombin in thrombin generation and spreading. Earlier, the rate and Michaelis constants for activation of factor XI by thrombin in the purified system were determined to be $7.8 \cdot 10^{-3} \text{ min}^{-1}$ and 50 nM, respectively (4). However, in the presence of fibrinogen (6) or in plasma (7), no evidence that this reaction occurs was obtained. It might well be that the competition for thrombin between its numerous substrates decreases the effective/apparent rate constant for activation of factor XI. We can evaluate the rate (V) of factor XI activation by thrombin in the presence of fibrinogen. If $K_{m1} = 2 \text{ uM}$, $I = 10 \text{ uM}$, and $XI = 50 \text{ nM}$, we obtain $V = k_{11} \cdot XI / (XI + K_{m11\text{eff}})$, where $K_{m11\text{eff}} = 300 \text{ nM}$. Therefore, $k_{11\text{eff}} = 1.1 \cdot 10^{-3} \text{ min}^{-1}$, seven times smaller than in the absence of fibrinogen.

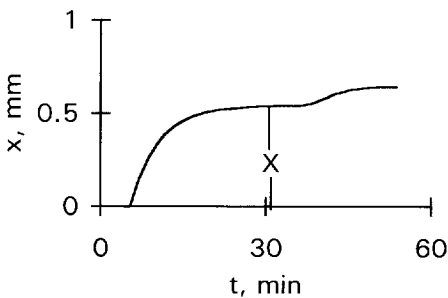


FIG. 10.

Displacement of the clot edge in time. X is the clot size after the first thrombin wave passed (fixed level of fibrin is 170 nM). The curve corresponds to the numerical computations shown in FIG. 9.

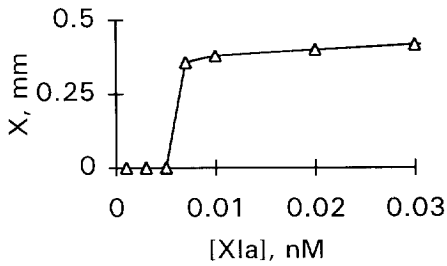


FIG. 11.

The clot size observed after the first thrombin wave passed as a function of the activation level (see also FIG. 10).

To account for the contribution of the activation of factor XI by thrombin, we added the corresponding equation to the set of equations [1]-[8] (see Part I):

$$\frac{dXIa}{dt} = k_{11} \cdot IIa + v_{tok} - h_{11} \cdot XIa$$

Here, k_{11} and h_{11} are the rate constants for factor XI activation by thrombin and inactivation by plasma inhibitors, respectively; v_{tok} is a parameter characterizing the level of contact activation ($v_{tok} = h_{11} \cdot [XIa]_{st}$). The values of $[XIa]_{st}$ were taken from experiments described in (1). The values of all kinetic constants are given in Table I.

The equation describing factor XIa kinetics contains the term $k_{11} \cdot IIa$. We varied k_{11} from 0 to 10^{-2} min^{-1} (4) without any significant effect on the activation kinetics of other factors and on fibrin formation in the homogeneous case. However, the spatial dynamics of thrombin (and fibrin) formation changes dramatically. For $k_{11} > 10^{-6} \text{ min}^{-1}$, thrombin spreads in the space as a wave that, after a short transient, has the constant amplitude and speed and never stops until reaches the natural boundaries of the medium. The speed of this wave depends on the value of k_{11} .

Figure 12 shows the coordinate of thrombin wave front versus time for various values of k_{11} . A possibility for factor XI to be activated by thrombin (even with the rate constant k_{11} as low as 10^{-5} min^{-1} that precludes its experimental measuring) dramatically changes the pattern of the clot growth. The growth proceeds continuously, and the clot become infinit in size.

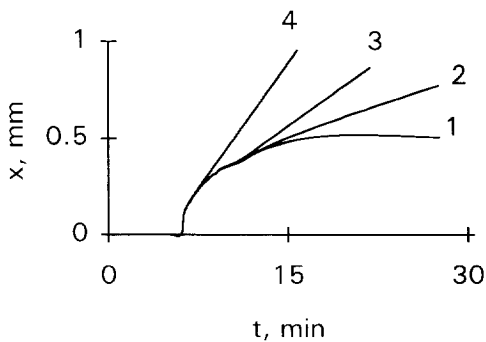


FIG. 12.

Dynamics of the clot growth for various rate constants for activation of factor XI by thrombin. Curves 1, 2, 3, and 4 are simulated for k_{11} 0, 10^{-5} , 10^{-4} , and $7.8 \cdot 10^{-3} \text{ min}^{-1}$, respectively). Other parameter values are those as specified in TABLE II.

DISCUSSION

Our aim (among others) was to describe quantitatively the kinetics of thrombin generation observed experimentally at different free calcium concentrations. Therefore, we treated the appropriate model variables as explicit functions of the free calcium concentration. The rates of activation of factors X and II in purified systems plotted against free calcium concentration were reported to be sigmoid curves (8,9). We used these dependencies in the model to bring it in accord with the experimental kinetics of thrombin generation at different free calcium concentrations described in (1). However, when constructing the model, we ignored the other known but less pronounced calcium dependencies (such as those of the rates of inactivation of factors Xa and IIa by AT). We also studied the involvement of protein C in the control of coagulation. Recent growing interest in protein C is accounted for by its great physiological importance in the blood coagulation system. Much of recent studies were accentuated on the dynamics of protein C activation in disseminated intravascular coagulation. However, information on the kinetic constants of APC functioning is as yet incomplete. Therefore, in our model analysis of protein C effects, we broadly varied the constants for protein C activation by thrombin.

Preliminary analysis of the homogeneous (point) model showed that, if PC activation is taken into account, the model acquire a dynamic instability in a broad range of the model parameters. Is this instability functionally necessary? We can not answer this question on the basis of experimental data accumulated to date. However, examination of the spatial dynamics of the clot growth shows that this instability results in the appearance of several secondary waves of polymerization causing the consolidation of the edge of the clot. The secondary thrombin waves may be of physiological significance because it seems beneficial for the clot to have the consolidated edge. The equation for protein C activation in our model is written in the simplest form, without regard for the cofactor effects of protein S or factor Va (10,11). In our future study, we will try to understand whether these effects are significant for the spatial dynamics of the clot growth.

Analyzing the spatial growth of the clot, we assumed that major parameters of clot formation are those that determine the production of thrombin and fibrin monomers. Despite the known complexity of the fibrin-monomer polymerization and stabilization of the clot structure (12), we ignored these processes in the model.

The model predicts that the mechanism underlying the thrombin spreading is clearly distinct from simple diffusion. Due to the exponential generation kinetics, the wave front of thrombin is steep and propagates at a high speed. At every point of the space where thrombin reached by diffusion, rapid exponential production of thrombin from prothrombin begins and increases the steepness of the thrombin wave front. Its steepness, in turn, determines the speed of propagation of the thrombin wave. The model clot stops to grow when it reaches the point where the stationary concentration of factor XIa becomes subthreshold in the presence of thrombin.

The model behaves as described only if there is no activation of factor XI by thrombin or analogous reactions. The role of these reactions remains as yet uncertain because these reactions were found only in the model system of purified coagulation factors (4). The constants for these reactions were reported to be very low (4). We showed that the reactions similar to cleavage of factor XI by thrombin that form the feedback loops can significantly change the spatial dynamics of coagulation. If thrombin itself generates

factor XIa at each point of the space in the above-threshold quantities, then the process of thrombin propagation becomes self-sustaining. These quantities of factor XIa are very low. In addition, the feedback loop from thrombin to factor XI involves a relatively great number of steps of the reaction cascade. Therefore, even at the extremely low rate constant for the activation of factor XI by thrombin, a self-sustaining wave of coagulation arises in the system and propagates until it reaches the natural boundaries of the medium.

We also showed that such a role of the reactions like the activation of factor XI by thrombin could not be revealed within the frames of models for homogeneous kinetics of the process. We suppose that only experiments on the spatial dynamics of the clot growth can clarify whether these reactions actually occur.

The occurrence of such reactions leads to infinite growth of the clot. Indeed, clots are always localized. Two explanations can be offered for this discrepancy: (i) reactions of such types do not occur naturally, even at very low rates, and (ii) there exist mechanisms that stop the growth of the clot. These mechanisms are able to stop the clot growth despite the existence of the feedback activation of the main factors of the coagulation cascade (distinct from those with cofactor activities). At present, it is difficult to choose between the explanations. The second explanation seems to be plausible because all the known proteinases display more or less weak nonspecific activity (13). The coagulation cascade is formed by proteinases whose nonspecific activities can easily provide the type of feedbacks under discussion. These feedbacks are dangerous for the organism. Therefore, it is conceivable that there also exist special mechanisms confining the growing clot in size even in the presence of such undesirable activities. The model does not take into account many processes important for coagulation. However, our analysis showed that not all reactions inhibiting the coagulation can stop the continuous growth of the clot. Classical inhibitors of coagulation (such as antithrombin III) that persist in the blood are unable to prevent the infinite growth of the clot. Increasing their concentration, we can increase the threshold for triggering the coagulation to an arbitrarily high value. However, if the coagulation was triggered, it propagates infinitely over the whole space.

Earlier, we proposed a hypothesis (14) that reactions promoting the continuous growth of the clot do not exclude the possibility that the clot can be localized. Due to these reactions, the clot is growing faster. However, simultaneously with the exponential generation of thrombin in plasma, autocatalytic production of the enzyme that stops the thrombin formation is triggered. Autocatalysis in the production of such an enzyme may be provided by the involvement of the cofactor which is formed in the course of coagulation. The most suitable candidate for the role of this enzyme is protein C because its cofactor protein S is also activated by thrombin. More detailed analysis of the hypothesis of the autowave propagation of the anticoagulant that terminates the clot growth is now under consideration.

The results obtained indicate that it is important to study experimentally the spatial effects in the blood coagulation system. The most uncertain processes are those that terminate the clot growth.

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