Kinetics and mechanisms of surface-dependent coagulation factor XII activation

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\textbf{Keywords} 
Blood coagulation, factor XII, contact activation, intrinsic pathway, dextran sulfate, mathematical modeling

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\textbf{Abbreviations} 
fXII, factor XII; fXIIa, factor XIIa, an active form of FXII; DS, dextran sulfate
Abstract

Surface-induced activation of factor XII is critical part of the intrinsic pathway of blood coagulation. The mechanism of this process remains unclear: in particular, it is not known whether the initial amounts of factor XIIa, an active form of factor XII, are produced purely by factor XII contacting a surface or if traces of factor XIIa pre-exist. Furthermore, it is not known whether factor XII first has to bind to a surface before it can interact with the surface-bound factor XIIa in a two-dimensional process to become activated ("bound-substrate model") or if surface-bound factor XIIa activates a fluid-delivered form of factor XII ("free-substrate model"). To investigate these possibilities, we used mathematical modeling to implement various hypotheses. Time courses of factor XII production were generated under different initial conditions and matched with experimental data. We established that only the "bound-substrate model" fits with the majority of experimental data, whereas the "free-substrate model" does not. We also addressed the question of spontaneous activation and found that measurable differences between the models with and without spontaneous activation appear only under limiting conditions (deficit or excess of surface). As there are insufficient data regarding the system’s behavior upon such variations of surface concentration in the literature, we designed new experiments to answer this question.
1. Introduction

Activation of factor XII (fXII) upon contact with a foreign surface is the initial event in the onset of contact pathway of blood coagulation (Vogler and Siedlecki 2009). Previously, the physiological role of contact activation in hemostasis was thought to be insignificant, as individuals with an fXII deficiency do not suffer from abnormal bleedings (Sperling, Fischer et al. 2009, Caen and Wu 2010), and initially such deficiency was detected only by in vitro lab tests (Sperling, Fischer et al., Caen and Wu, Abaeva, Canault et al.). However, emerging evidence has linked the intrinsic pathway and thrombotic events: fXII-knockout mice are protected from thrombotic events (Caen and Wu 2010), while their hemostatic capacity remains normal (Renne, Pozgajova et al. 2005, Renne, Schmaier et al. 2012). In addition, the intrinsic pathway is a subject of critical importance regarding hemocompatibility and thrombus formation on biomaterials because artificial surface can induce blood clotting upon contact (Sperling, Fischer et al.). It has been shown that activation is not surface-specific and occurs on different types of surfaces. Therefore, the issue of improving hemocompatibility is not a purely surface-engineering problem (Vogler and Siedlecki) but is subject to kinetic studies regarding the balance of coagulation reactions. Successful approaches require an understanding of the contact activation process, and mathematical modeling is a useful tool for such investigations because of immense complexity of the molecular mechanism of the studied phenomenon.

The conversion of fXII to the active enzyme fXIIa triggers the series of reactions, which ultimately leads to the formation of a fibrin clot. In addition to triggering fXII conversion, the activating surface also supports the assembly of protein complexes involving fXIIa, coagulation factor XI (fXI), (pre)kallikrein, and high-molecular-weight kininogen to regulate the activation of fXII and FXI (Sperling, Fischer et al. 2009, Vogler and Siedlecki 2009). The mechanism of fXII interactions with surfaces through the activation process remains poorly understood, and several possible pathways are depicted in Fig. 1.

The major questions are: 1) does fXII undergo spontaneous activation upon contact with a surface, or is it completely autoactivated by trace amounts of fXIIa? 2) does fXII bind to the surface to become autoactivated by fXIIa (bound-substrate model), or does it bind from the bulk solution (free-substrate model)?

To answer these questions, we considered several mechanisms of autoactivation and examined the influence of trace fXIIa in purified fXII on the kinetics of the activation process. We tested these hypotheses using computer simulations and optimization procedures to compare the theoretical results with experimental data. Our approach disproved the free-substrate model, but the presence of spontaneous activation remains in question, although we designed experiments that shed new light on the process.
2. Method

2.1. Model Description

To investigate the process of fXII activation in detail, we simulated experiments on the fXII activation reaction in a buffer solution, without contribution of other proteins of the contact system. We chose dextran sulfate (DS) as a negatively charged soluble material, which is widely used (van der Graaf, Tans et al. 1982, Tankersley, Alving et al. 1983, Tankersley and Finlayson 1984, Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986, Silverberg and Kaplan 1988, Nigretto, Corretge et al. 1989, Samuel, Pixley et al. 1992, Loiseau, Randriamahazaka et al. 1996, Loiseau, Randriamahazaka et al. 1997, Rojkjaer and Schousboe 1997) and known for its ability to bind several fXII molecules to one chain for aggregation, a necessary step for activation (Silverberg and Kaplan 1988, Samuel, Pixley et al. 1992, Citarella, Wuillemin et al. 1997). Hence, dextran sulfate is a convenient tool for modelling studies.

Our model (see Appendix) was derived using the standard mass action law based on the following assumptions:

1. fXII reversibly binds to a surface (see Fig. 1.2) forming a complex designated as XIX_{bound}. This process is a fast equilibrium reaction (Samuel, Pixley et al. 1992, Loiseau, Randriamahazaka et al. 1996, Loiseau, Randriamahazaka et al. 1997, Pokhilko and Ataullakhanov 1998), and because only a small amount of fXII undergoes activation at any time (Zhuo, Siedlecki et al.), the equilibrium state between free fXII and bound fXII is achieved at any given time during the entire process of activation.

2. The active form (see Fig. 1.3) is released after binding with a surface fXIIa, which is able to cleave its own zymogen fXII and other active molecules fXIIa, i.e., it has coagulant activity.

3. fXIIa cleaves other molecules of fXIIa to produce minor active forms (see Fig. 1.4). It is shown that, most likely, α-fXIIa is a primary activation product and a precursor of all minor forms including β-fXIIa which has procoagulant activity but does not cleave other molecules of fXII (Dunn and Kaplan 1982, Dunn, Silverberg et al. 1982, Golas, Parhi et al. 2010, Golas, Yeh et al. 2011, Golas, Yeh et al. 2013). There is clear evidence that upon activation in a buffer solution, minor forms are released (Dunn and Kaplan, Dunn, Silverberg et al.). Recent studies have revealed that amidolytic activity is not necessary linked to procoagulant activity and, most likely, an unknown number of active enzymes releases during activation in a buffer solution (Golas, Yeh et al.). Chromogenic substrates are sensitive to amidolytic activity, and therefore the experimental curves reflect the release of all types of active forms. To compare the results with experiments, we considered the total amount of amidolytic fragments that were generated as follows:

\[ [XII_{amidolytic}] = [XIIa] + [XIIf] \]  

As \( XII_{\mu} \) we designated all minor forms which do not have procoagulant activity.

4. After degradation, part of the fXIIa molecule remains bound to the surface and prevents other molecules of fXII from binding with DS sites occupied by it.

5. We suggested that newly generated fXIIa accumulates near the surface and is immediately reused in subsequent events, preventing other proteins from binding to the surface. In this case, the sites of DS, previously occupied by an active form, are no longer able to bind new molecules of fXII. Thus, we assigned all active forms as fXIIa and did not include a term for surface-bound form and fluid form of fXIIa and, therefore, did not use the binding equation for fXIIa in the model. It is consistent with evidences that the affinity of fXIIa to the surface is significantly greater compared to its precursor (Shore, Day et al. 1987), and the dissociation constant is low (Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986).
1986). Even though fXIIa can be washed from DS (Zhuo, Siedlecki et al.), indicating that the binding is reversible, it is possible that bound fXII does not dissociate from the surface after activation (Rojkjaer and Schousboe 1997). It is to say, that following the terms used by previous studies (Vogler, Graper et al. 1995, Zhuo, Siedlecki et al. 2006, Zhuo, Siedlecki et al. 2007), we used “binding” and “adsorption” to denote the allocation of proteins in close proximity with the surface (Vogler and Siedlecki) and did not make any assumptions with regards to nature of the binding or the adsorption.

6. Albumin was included in our model because plasma proteins compete with contact factors for binding sites on surfaces (Vogler, Graper et al. 1995, Zhuo, Siedlecki et al. 2006, Zhuo, Siedlecki et al. 2007).

However, initial assumptions turned out to be insufficient to design a model because we still had two open questions with respect to activation on the surface:

1. Does binding with the surface alone cause formation of an active form? In other words, is spontaneous activation possible? Depending on answer, we worked with two hypotheses – with and without spontaneous activation.

2. What is the role of surface binding in the autoactivation process? We considered several options depending on the bound or unbound state of fXII before cleavage by fXIIa (see Fig. 1.3). It is hypothesized that binding with a surface causes a conformational change that makes fXII more susceptible to cleavage (Griffin and Cochrane 1976, Griffin 1978, Citarella, te Velthuis et al. 2000). Thus, the primary hypothesis was the “bound-substrate mechanism”, i.e., fXII binds to a surface before it may interact with the surface-bound fXIIa via two-dimensional diffusion and becomes activated. However, we also included in our model the possibility of fXII activation from bulk solution, referred to as the “free-substrate mechanism” (surface-bound fXIIa activates a fluid-delivered form of fXII). If fXII is activated, regardless of its state (bound or unbound), the “mixed” mechanism takes place.

Therefore, we built our model using 6 hypotheses – 3 options of an autoactivation mechanisms times two modes of spontaneous activation (exists/does not exist).

2.2 Simulation

Equations given in the Appendix were solved using Copasi® software (collaborative project of the Virginia Bioinformatics Institute, the University of Heidelberg, and the University of Manchester).

With Copasi® we calculated the system’s time evolution under various conditions by solving differential equations corresponding to the model. To solve differential equations, Copasi® uses LSODA a method that can be applied to both stiff and non-stiff problems. For parameter estimation we performed optimization procedures to calculate unknown kinetic constants from experimental datasets. Using Copasi, we utilized datasets from one or several experimental works and used the program to fit specific kinetic constants. Parameter values were estimated using methods of optimization. These methods minimize differences between empirical datasets and numerical computations. We preferably used the simulated annealing optimization algorithm, a stochastic, time-consuming algorithm yet one of the most robust global optimization algorithms. We also used evolutionary programming, evolutionary strategy with stochastic ranking and particle swarm.

Some processes described in the modeling section are hypothetical, and therefore, a majority of the constants that had not been measured prior to optimization were required. We listed all the constants at the Table 1, where we specify whether each was derived from available sources or estimated by numerical studies. Full mathematical expressions can be found in the Appendix. First, we performed optimization procedures using initial conditions from the previously works mentioned above (Tankersley and Finlayson 1984, Shore, Day et al. 1987) to obtain set of kinetics constants that fits with experimental results from those
works. We considered mechanisms of autoactivation separately to figure out which mechanism that is able to describe previous experiments.
3. Results

3.1. Activation kinetics of FXII. Calculations with our model gave us kinetic curves of fXII activation. Typical time courses for one of version of the model are shown in Fig. 2. The concentrations of fXII, both in a free and bound forms, decreased to the zero, indicating that there was a full conversion into the active form (see Fig. 2B, C). The concentration of fXIIa reached a maximum and then decreased due to fragmentation processes (see Fig. 2A). The total amidolytic activity, which can be measured in experiments using a chromogenic substrate, increased up to the initial concentration of the zymogen (see Fig. 2F). The concentration of surface in this simulation was sufficient to bind all available fXII (see Fig. 2D). Therefore, the concentration of free surface remained constant after a slight decrease.

3.2. “Free-substrate” mechanism. First, we attempted to find the set of kinetic constants to provide quantitative fitting for all experiments with different initial values of fXII using this hypothesis. We failed to do this because the results of the numerical computation did not agree with the experimental data. To illustrate the inconsistency, we derived constants for the conflicting experiments separately. Fig. 3 demonstrates that the free-substrate mechanism does not describe dependence on the free factor concentration: the constants adjusted to describe experiments with 0.2 \( \mu M \cdot min^{-1} \) of fXII do not work for the experiment with 2 \( \mu M \cdot min^{-1} \), and vice versa. We considered the formation of complex fXII-fXIIa via Michaelis-Menten kinetics, but fitting was not improved (data not shown).

To analyze this further, we examined the system’s behavior by fixing all constants and varying the value of \( k_{free} \). The system was found to be extremely sensitive to the variation (data not shown). Moreover, for all initial concentrations of fXII, the obtained value of \( k_{fragm} \) was negligibly small (\( 10^{-9} – 10^{-13} \mu M \cdot min^{-1} \)), which contradicts the generation of ‘minor fragments’, discussed above. Taken together, these data do not support the “free substrate” mechanism.

3.3 “Bound-substrate” mechanism. The same optimization procedures were performed for the “bound-substrate hypothesis”, and the required set of constants was successfully found (see Table 1). Using this set, we obtained theoretical curves for the various initial amounts of fXII and overlaid the curves with dots corresponding to the experimental data (Fig. 4). Panels A-F show that the numerical computations fit the experimental data for various initial amounts of fXII. Hence, using the “bound-substrate” hypothesis, we can compute time courses that fit with the empirical datasets measured by at least two independent research groups. We also evaluated the combined option (“mixed hypothesis”), assuming that fXIIa can be activated both from the surface-bound form and fluid form. However, the obtained set of constants was identical to the “bound-substrate hypothesis” if the initial value of fXIIa was set to zero. When we considered the presence of active fragments with coagulant activity at time zero, we obtained the set of constants as follows:

\[
\begin{align*}
    k_{bound} &= 0.015 \text{ min}^{-1}, \\
    k_{free} &= 0.009 \mu M^{-1} \text{ min}^{-1}, \\
    k_{fragm} &= 0.014 \text{ min}^{-1}.
\end{align*}
\]

However, because the set does not provide a better fit with experimental data (data not shown), we decided to decline this hypothesis as unreasonably overcomplicated. Thus, our first step resulted in choosing the bound-substrate mechanism as the primary pathway of substrate delivery, and free-substrate mechanism does not contribute greatly even if it exists (as it can be concluded from negligibly small value of \( k_{free} \)).

3.4. The origin of the initial amount of fXIIa. The next step was to determine whether the system has the trace of an enzyme at the initial time due to contamination of purified fXII used in activation studies or the system producing a sufficient amount of fXIIa via spontaneous activation.

For our model, eliminating spontaneous activation corresponded to assigning a value of zero to the autoactivation constant. According to previous experimental work, we detected...
non-zero amidolytic activity in the initial system, and, as FXIIa and minor forms have amidolytic activity, so with Eq.1 we had:

\[ [\text{XII}_{\text{amidolytic}}]_{t=0} = [\text{XII}_a]_{t=0} + [\text{XII}_f]_{t=0} \] (2)

As part of fitting procedures we considered three possibilities:

1) activation is triggered by contaminating amounts of fXIIa (0.1 – 0.7%, measured by amidolytic activity (Tankersley and Finlayson 1984, Shore, Day et al. 1987)), so

\[ [\text{XII}_{\text{amidolytic}}]_{t=0} = [\text{XII}_a]_{t=0} + [\text{XII}_f]_{t=0} = 0 \] (3)

We found that \( k_{\text{bound}} = 0.015 \text{min}^{-1}, k_{\text{fragm}} = 0.013 \text{min}^{-1} \) and obtained kinetic curves (see Fig.4, solid lines)

2) activation is triggered by spontaneous activation upon binding with a surface, so;

\[ [\text{XII}_{\text{amidolytic}}]_{t=0} = [\text{XII}_f]_{t=0} + [\text{XII}_a]_{t=0} = 0 \] (4)

We found that \( k_{\text{bound}} = 0.012 \text{min}^{-1}, k_{\text{fragm}} = 0.008 \text{min}^{-1}, k_{\text{spont}} = 0.0005 \text{min}^{-1} \) and obtained kinetic curves (see Fig.4, dotted lines)

3) activation is triggered by both mechanisms independently

In that case we also eliminate initial concentration of minor forms, and the fitting procedures resulted in \( k_{\text{spont}} = 0 \), indicating that if there was an initial presence of forms with coagulant activity, spontaneous activation is unnecessary and difficult to observe.

To establish the difference in models with or without spontaneous activation, we examined the system’s behavior while varying the concentration, i.e., the dependence of the total amount of fXIIa generated upon the surface concentration. We obtained a distinctive bell-shaped curve “level of activation versus dextran sulfate surface concentration” (see Fig. 5, insets).

Hence, there are three modes:

1) surface-limiting condition (deficiency of DS): plateau was reached due to exhaustion of the surface area available for binding of fXII (see Fig. 6A);
2) activation-to-completion: all available fXII is converted to minor forms, but the activation is faster if there is no spontaneous activation (see Fig. 6B);
3) protein-limiting condition (excess of DS): the rate of activation is so slow that the s-shape is lost and the curve become a line (see Fig. 6C).

For numerical estimation, we also used experimental data (Rojkjaer and Schousboe 1997), and illustrated it in Fig. 5. We found a qualitative difference in the system’s behavior: if spontaneous activation occurs, the process activation goes to completion for a much narrower range of surface concentrations (see Fig. 5, inset A) but its yield is larger upon excessive surface concentrations (see Fig. 5, inset B). Furthermore, the fXII activation rate at large surface concentrations tends to be non-zero, while it is naturally tends to be zero without spontaneous activation due to "surface dilution" (best seen at low fXII concentration, e.g. Fig. 5 inset B).

Regarding the influence of albumin, we showed that albumin does not significantly affect the time-course as the affinity of albumin for DS is significantly smaller compared to fXII (37).
4. Discussion and conclusions

The aim of our study was to gain insight into the mechanism of fXII activation on DS by comparing hypotheses with the available experimental data using mathematical modelling (for short summary, please refer to Table 2).

We found that the “bound-substrate mechanism” is the likely mechanism of activation, which utilizes “the idea of aggregation” (Samuel, Pixley et al. 1992) where binding with a surface is a prerequisite for activation. The limitations of the model and of the existing experimental studies do not allow any reliable conclusions with regard to the specifics of the two-dimensional interactions of fXIIa and fXIIa, freedom of surface movement for these factors, or effective surface diffusion range. However, it seems likely that it is surface density and not volume concentration of fXII that determined the rate of activation. Specially designed experiments where the former is varied and the latter is kept constant (or vice versa) can be the ultimate test of this conclusion.

The next question regarded the mechanism that triggers contact activation, i.e., the origin of the initial amount of fXIIa, which subsequently is able to support its own activation. Is the presence of a surface sufficient for activation? If so, spontaneous activation would be induced by binding with a surface. If there is no spontaneous activation, the trigger is a conversion of fXII to fXIIa by traces of fXIIa that are inevitably present in the preparation of fXII (Vogler and Siedlecki 2009). It was shown that purified fXI failed to induce clotting in fXII-depleted plasma (Zhuo, Siedlecki et al. 2007). Therefore, such traces are either vanishingly small or contain minor fragments without coagulant activity. Previous models have reported the system’s insensitivity to the level of active fXII at time zero (Gregory and Basmadjian 1994). The existence of active forms that do not cleave fXII was shown by adding $\alpha$-fXIIa to the activation solution. It was observed that even significant amounts of this form did not influence the time course of activation (Tans and Griffin 1983, Tans, Rosing et al. 1983, Silverberg and Kaplan 1988). This result can be explained by inability of $\beta$-fXIIa to bind to surfaces because the surface-binding site is localized the heavy chain, and $\beta$-fXIIa consists of a light chain and sometimes of the small fragment of a heavy chain (so-called doublet (Dunn and Kaplan 1982, Dunn, Silverberg et al. 1982)). The relative proportion of enzymes with different activities is thought to depend on activator surface properties (Zhuo, Siedlecki et al. 2006, Zhuo, Siedlecki et al. 2007, Vogler and Siedlecki 2009, Golas, Parhi et al. 2010, Golas, Yeh et al. 2011, Golas, Yeh et al. 2013). Therefore, our results may not be applicable to experiments on other types of surfaces than DS.

In our study, we assumed that $\alpha$-fXIII is the precursor of any other minor fragments, which is in line with empirical studies. Nevertheless, we considered the possibility of the generation of minor fragments with amidolytic activity directly from the fXII cleaved by fXIIa, but such a model did not produce curves that corresponded with experiments (data not shown).

To find the difference in the behavior of the system with or without spontaneous activation, we calculated kinetic curves for different surface concentrations, because it has been shown that a variation in surface concentration significantly affects the initiation of the contact pathway. An increase in the surface available for binding causes a decrease in the total amount of fXII converted to active form (Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986, Nigretto, Corretge et al. 1989, Loiseau, Randriamahazaka et al. 1996, Citarella, Wuillemin et al. 1997, Rojkjaer and Schousboe 1997) due to a diminished density of fXII on the surface (Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986). In addition, for surface concentrations less than optimum, the yield of activation drops dramatically (Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986, Nigretto, Corretge et al. 1989, Loiseau, Randriamahazaka et al. 1996, Citarella, Wuillemin et al. 1997, Rojkjaer and Schousboe 1997)
and correlates with a decrease in the total number of \( \text{fXII} \) molecules bound to the surface (Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986). The total yield depends on the protein-surface ratio, and the maximum yield is achieved before the surface becomes saturated by protein (Griep, Fujikawa et al.) and the reaction becomes surface-limited (Loiseau, Randriamahazaka et al.). Hence, it is possible that excess of surface causes an increase in the distance between surface-bound molecules, lowering the possibility of interaction between them if only \( \text{fXII} \) bound to the surface could be activated, which is consistent with our “bound-substrate model”. Our calculation also was in agreement with the experimental evidence that complete hydrolysis of \( \text{fXIIa} \) cannot be achieved in a buffer solution in a surface-limited condition (high \( \text{fXII} \)-surface ratio) (Golas, Yeh et al.). This finding is consistent with the hypothesis that tight localization of \( \text{fXII} \) on the surface increases the efficiency of autoactivation. (Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986, Nigretto, Corretge et al. 1989, Loiseau, Randriamahazaka et al. 1996, Citarella, Wuillemin et al. 1997, Rojkjaer and Schousboe 1997).

Thus, we proved that the role of the surface as the essential substrate, though the ability of the surface to induce spontaneous activation remains unknown.

The distinctive feature in our model was the explicit account taken of the role of the surface concentration and molecular interactions with \( \text{fXII} \). Previously, activation in the presence of DS was thought to be a purely enzymatic Michaelis-Menten-like or first-order reaction (Tankersley, Alving et al. 1983, Tankersley and Finlayson 1984, Shore, Day et al. 1987), though some studies have interpreted the process as a set of interrelated physical and enzymatic reactions (Zhuo, Siedlecki et al.) and even suggested a stochastic mechanical point of view (Golas, Yeh et al.). Regarding the initiation of a surface-catalyzed contact pathway, the schemes that did not explicitly include the surface (or include only extreme cases (Loiseau, Randriamahazaka et al.)) failed to adequately describe the system’s behavior and functioned only for fitting the data of the particular group. Moreover, no difference has been made between the different catalytic forms of \( \text{fXIIa} \). The mechanism of fragmentation also remains unknown, but we show that \( \alpha-\text{fXIIa} \) is most likely a primary activation product (Shore, Day et al.) and a precursor of all minor forms (Dunn and Kaplan 1982, Dunn, Silverberg et al. 1982, Golas, Parhi et al. 2010, Golas, Yeh et al. 2011, Golas, Yeh et al. 2013).

Although our model is also based on several assumptions, we avoided including hypothetical mechanisms such as so called “autoinhibition” (reactions in which \( \text{fXIIa} \) itself inhibits the conversion of \( \text{fXII} \) to \( \text{fXIIa} \) (Zhuo, Siedlecki et al.)). Our model successfully described the system’s behavior and its dependence on different variables and fit with the large range of experimental data (Tankersley, Alving et al. 1983, Tankersley and Finlayson 1984, Shore, Day et al. 1987, Loiseau, Randriamahazaka et al. 1996, Rojkjaer and Schousboe 1997, Jesty, Rodriguez et al. 2005).
Author Contributions

VAT planned research, developed the model, carried out all simulations, analyzed the data, and wrote the paper. ANS contributed to model design and implementation, analyzed the data, and edited the paper. MAP conceived the study, planned research, analyzed the data, and edited the paper.

Acknowledgements

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References


Table 1. List of kinetic constants and parameters

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Reaction</th>
<th>Value/hypothesis</th>
<th>Bound-substrate</th>
<th>Free-substrate</th>
<th>Mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{spont}$</td>
<td>Spontaneous activation</td>
<td>$0.5^a$</td>
<td>$0.3-0.9^a$</td>
<td>$0.5^a$</td>
<td></td>
</tr>
<tr>
<td>(min$^{-1}$)</td>
<td>$XII_{bound} \xrightarrow{k_{spont}} XIIa$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{bound}$</td>
<td>Bound-substrate autoactivation</td>
<td>$12^a$</td>
<td>$15^a$</td>
<td>$12^a$</td>
<td></td>
</tr>
<tr>
<td>(min$^{-1}$)</td>
<td>$XII_{bound} + XIIa \xrightarrow{k_{bound}} XIIa + XIIa$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{free}$</td>
<td>Free-substrate autoactivation</td>
<td>$0.3-1.5^a$</td>
<td>$0.3-9.9^a$</td>
<td>$9^a$</td>
<td></td>
</tr>
<tr>
<td>(nM$^{-1}$ min$^{-1}$)</td>
<td>$XII_{free} + XIIa \xrightarrow{k_{free}} XIIa + XIIa$</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>$k_{fragm}$</td>
<td>Fragmentation</td>
<td>$0.5^a$</td>
<td>$13^a$</td>
<td>$8^a$</td>
<td></td>
</tr>
<tr>
<td>(min$^{-1}$)</td>
<td>$XIIa + XIIa \xrightarrow{k_{fragm}} XIIa + XIIf$</td>
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<td></td>
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<tr>
<td>$N^b$</td>
<td>Binding</td>
<td>$81^a$</td>
<td>$184^a$</td>
<td>$176^a$</td>
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<td></td>
<td>$XII + N \cdot Surface \xrightarrow{k_{12}} XII_{bound}$</td>
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<tr>
<td>$k_{12}$</td>
<td>(nM)</td>
<td>$170nM$ (Samuel, Pixley et al. 1992)</td>
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<tr>
<td>$k_A$</td>
<td>$A + Surface \xrightarrow{k_A} A_{bound}$</td>
<td></td>
<td>$307\mu M$ (Anderot, Nilsson et al. 2009)</td>
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<td>(µM)</td>
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</table>

$^a$ Values obtained by optimization procedures (for the free-substrate hypothesis, the range is given)

$^b$ It has been reported that the number of XII molecules to one DS500 chain ranged from 50 to 200 (Samuel, Pixley et al. 1992, Citarella, Wuillemin et al. 1997) so we decided to include them in fitting procedures to balance the effect of pH variation, because pH is thought to affect the rate of activation by moderation of binding properties (Griep, Fujikawa et al. 1985, Griep, Fujikawa et al. 1986, Gregory and Basmadjian 1994, Citarella, Wuillemin et al. 1997). It must be noted that we did not pay attention to the difference in the optimal value of N, because there are no reliable experimental data. So we let the system choose values of N from the aforesaid range of values.
<table>
<thead>
<tr>
<th>FXII autoactivation mechanism</th>
<th>Free-substrate model</th>
<th>Bound-substrate model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic behavior of the system</td>
<td>Overall kinetics: see fig. 3 Agreement with experiments: Does not quantitatively fit with published data (Shore, Day et al.), (Tankersley, Alving et al.) Conclusion: Data used for validation do not support the &quot;free substrate&quot; mechanism</td>
<td>Overall kinetics: see fig. 4 Agreement with experiments: fits with published data with various initial concentrations of FXII ((Shore, Day et al.), (Tankersley, Alving et al.) and various surface concentrations (Rojkjaer and Schousboe 1997) Conclusion: additional experiments should be designed for wider range of protein-to-surface ratio to compare calculated behavior with empirical data and to prove/disprove that activation goes to completion in the smaller range of surface concentrations (see Fig. 5, inset A) but its yield is bigger upon excessive surface concentrations (see Fig. 5, inset B)</td>
</tr>
<tr>
<td>With spontaneous activation</td>
<td></td>
<td></td>
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<tr>
<td>Without spontaneous activation</td>
<td>Overall kinetics: see fig. 4 Agreement with experiments: fits with published data with various initial concentrations of FXII ((Shore, Day et al.), (Tankersley, Alving et al.) and various surface concentrations (Rojkjaer and Schousboe 1997) Conclusion: additional experiments should be designed for wider range of protein-to-surface ratio to compare calculated behavior with empirical data and to prove if If protein-to-surface ratio allows all fXII available convert to minor forms, the activation is faster if there is no spontaneous activation (see Fig. 6B).</td>
<td></td>
</tr>
</tbody>
</table>
Model Equations

\[
\frac{d[XIIa]}{dt} = \left( \frac{d[XIIa]}{dt} \right)_{\text{activation}} + \left( \frac{d[XIIa]}{dt} \right)_{\text{autoactivation}} + \left( \frac{d[XIIa]}{dt} \right)_{\text{surface-induced}} - \left( \frac{d[XIIa]}{dt} \right)_{\text{fragmentation}} \quad (A1)
\]

\[
\frac{d[XII]}{dt} = \left( \frac{d[XIIa]}{dt} \right)_{\text{autoactivation}} - \left( \frac{d[XIIa]}{dt} \right)_{\text{surface-induced}} - \left( \frac{d[XIIa]}{dt} \right)_{\text{activation}} \quad (A2)
\]

\[
\frac{d[XII]}{dt} = -\left( \frac{d[XIIa]}{dt} \right)_{\text{activation}} + \left( \frac{d[XIIa]}{dt} \right)_{\text{surface-induced}} + \left( \frac{d[XIIa]}{dt} \right)_{\text{autoactivation}} \quad (A3)
\]

• Surface-induced (or spontaneous) activation

\[
\frac{d[XIIa]}{dt} = k_{\text{spont}}[XII_{\text{bound}}] \quad (A4)
\]

• Autoactivation

\[
\left( \frac{d[XIIa]}{dt} \right)_{\text{autoactivation}} = k_{\text{bound}}[XII_{\text{bound}}][XIIa] + k_{\text{free}}[XII][XIIa] \quad (A5)
\]

where \(k_{\text{bound}}\) and \(k_{\text{free}}\) describe the bound-substrate and free-substrate hypotheses, respectively.

• Fragmentation

\[
\left( \frac{d[XIIa]}{dt} \right)_{\text{fragmentation}} = -k_{\text{fragm}}[XIIa][XIIa] \quad (A6)
\]

where \(k_{\text{fragm}}\) describes the release of minor fragments from factor XIIa.

• Surface

To complete the system, time evolution of surface binding sites and generation of the [fXII – surface] complex should be taken into account:

\[
\frac{d[S]}{dt} = -\frac{1}{N} \frac{d[XII]}{dt} \quad (A7)
\]

\[
[XII_{\text{bound}}] = \frac{[S][XII]}{k_{12}(1+[XII]+[A])+[S]} \quad (A8)
\]

where \(N\) is the number of factor XII molecules that can bind to a single polymer surface molecule. The equation for \([XII_{\text{bound}}]\) is derived assuming equilibrium irreversible binding, and \([A]\) is albumin, representing the competitive adsorption of blood proteins to the surface (\(k_{12}\) and \(k_A\) are binding constants of factor XII and albumin, respectively)
Figure Captures

Figure 1. Reaction scheme. (1) At the initial time point of the system, there are zymogen fXII (white) and active enzyme fXIIa (grey) localized near the surface fXII can bind with a surface and then proceed to fXIIa or with fXIIa/surface directly. (2) Spontaneous activation requires binding with a surface but not localization near other active forms. “Bound-substrate” autoactivation occurs after binding with a surface and two-dimensional interaction with fXIIa. “Free-substrate” autoactivation does not require prior independent binding with a surface and proceeds after interaction fXIIa with fXII delivered directly from a bulk solution. (3) Fragmentation is the final step and requires two fXIIa molecules to find each other on a surface.

Figure 2. Typical kinetics obtained by numerical simulation of the bound-substrate autoactivation hypothesis. These curves show the time course of concentration of factors and surface during the activation process. Panel A shows generation of primary activation product, α-fXIIa ([XIIa]) Initial concentration of α-fXIIa is 0.1% of initial fXII concentration. Burst production of these forms began to decline because this form cleaved itself into a minor form (see Panel E) Panel B shows the time evolution of the precursor, fXII ([XII]). Initial amount of fXII (300nM) fully converted into active forms because there was enough surface to bind with and become activated (see Panel D) Panel C shows the concentration of surface-protein complexes ([XIIb]) replicating the time course of fXII because at any time there was an equilibrium between the free and bound forms of fXII. Panel D shows the decrease of the surface concentration ([S]) due to the irreversible conversion of fXII into active forms, which prevented subsequent binding with these sites. Panel E shows that concentration of β-fXIIa ([XIIfr]) increased and reached a plateau when all of the generated α-fXIIa cleaved itself. Panel F shows the total amidolytic activity ([XII_amidolytic]) as a sum of concentrations of α- and β-forms is described by a characteristic s-shaped curve. It reached plateau after full conversion of fXII into active forms.

Figure 3. Results of computer simulation for the free-substrate hypothesis. Yield of activation ([XII_amidolytic]/[XII]tot) vs time. Panel A: best fitting with results of activation studies for [XII]tot = 0.2μM (open triangulars) (Shore, Day et al.). Inset (Panel C): comparison of theoretical curves obtained with the same set of constants as on panel D and experimental data for [XII]tot = 0.2μM (open triangulars) (Shore, Day et al.). Panel B: comparison of theoretical curves obtained with the same set of constants as on panel A and experimental data for [XII]tot = 3.44μM (solid triangulars) (Tankersley, Alving et al.). Inset (Panel D): best fitting with results of activation studies for [XII]tot = 3.44μM (solid triangulars) (Tankersley, Alving et al.)

Figure 4. Theoretical curves for a “bound-substrate” autoactivation hypothesis. Total concentration of active form with amidolytic activity ([XII_amidolytic]) vs time. Theoretical curves were simulated using the set of constants obtained from optimization procedures. The solid line corresponds to the set without spontaneous activation, and the dotted line corresponds to the nonzero value of that constant. Panels A-F represent comparisons between theoretical curves (solid and dotted lines) and experimental data for different initial amounts of fXII (0.2 (solid square), 1.15 (solid diamond), 1.38 (solid triangular), 1.72 (solid circle), 2.29 (open circle), 3.44 (open square).
Figure 5. The dependence of the yield of activation on concentration of the surface. Empirical data (solid circles, (Rojkjaer and Schousboe 1997)) and results of computer simulation for a “bound-substrate” autoactivation hypothesis with (dotted line) and without (grey solid line) spontaneous activation. Theoretical curves were obtained via computer simulations at the same initial conditions that were used in the experiment \([XII]_{t=0} = 0.25\mu M\) and various amounts of DS, incubation time \(t = 30 \text{ min}\). Inset A: Theoretical curves obtained for \([XII]_{t=0} = 2\mu M\) \([XII]_{0} = \) and incubation time \(t = 30 \text{ min}\). The vertical line corresponds to the concentration of DS used in fitting procedures (0.05\mu M). Inset B: Theoretical curves obtained for \([XII]_{t=0} = 0.2\mu M\) and a prolonged incubation time \(t = 30 \text{ min}\). The vertical line corresponds to the concentration of DS used in fitting procedures (0.05\mu M).

Figure 6. The dependence of the activation kinetic curves on the protein – surface ratio. The curves illustrate the release of active forms during the process of activation (\([XII]_{\text{amidolytic}}\) vs time). The solid line corresponds to the “bound-substrate” autoactivation hypothesis without spontaneous activation. The dotted line corresponds to the “bound-substrate” autoactivation hypothesis with spontaneous activation assuming that all the active traces in purified fXII were in the β-form. Panel A: surface-limiting condition (deficiency of DS). Panel B: activation-to-completion. Panel C: surface-limiting condition (excess of DS).
A tree of mathematical models of factor XII activation with dextran sulfate was developed.

Simulations suggested that factor XII autoactivation is a bound-substrate reaction.

The available experiments are equally described using either spontaneous activation or trace factor XIIa.

Experiments under surface-limiting conditions could distinguish between these mechanisms.
4. Figure 1

1. Binding
2. "Bound-substrate" autoactivation
3. "Free-substrate" autoactivation
4. Spontaneous activation
5. Fragmentation
Figure 2
4. Figure 3

A

B

C

D
4. Figure 6