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Kinetics of Activation and Autoactivation of Human Factor XII†

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ABSTRACT: The kinetics of the enzymic reactions that participate in the contact activation system of human plasma were examined. These reactions are potentiated by dextran sulfate, a negatively charged solute that mimics many of the effects of glass or kaolin on this system. The reactions of reciprocal activation, consisting of activation of factor XII by kallikrein and of prekallikrein by activated factor XII, follow Michaelis-Menten kinetics; values of k_{cat} and K_m for each of these reactions were determined in the presence of dextran sulfate and in its absence. In the presence of dextran sulfate, the catalytic efficiency for factor XII activation was increased 11 000-fold, and that for prekallikrein was increased 70-fold. Autoactivation of factor XII in the presence of dextran sulfate also follows Michaelis-Menten kinetics with $k_{cat} = 0.033\ s^{-1}$ and $K_m = 7.5\ \mu M$. This finding supports the concept that autoactivation is an enzymic process, initiated by traces of activated factor XII which are invariably present in factor XII preparations. At prekallikrein and factor XII levels equal to those in plasma, reciprocal activation is ~2000-fold more rapid than autoactivation. Thus, reciprocal activation is the predominant mode of factor XII activation in normal plasma.

The contact activation system of human plasma consists principally of the three glycoproteins factor XII (Hageman factor), prekallikrein (Fletcher factor), and high molecular weight (M_r)[1] kininogen (Fitzgerald factor) [for a review, see Griffin & Cochrane (1979)]. The first two are zymogens which are converted to the serine proteinases factor XIIa[2] and kallikrein by limited proteolysis. High molecular weight kininogen is a nonenzymic cofactor which circulates as a complex with prekallikrein (Mandle et al., 1976) and with factor XI (Thompson et al., 1977). It may function by enhancing the binding of both prekallikrein and factor XI to negatively charged surfaces (Wiggins et al., 1977) whereupon their proteolytic activation by factor XIIa is facilitated. Activation of factor XI results in initiation of the intrinsic coagulation pathway, whereas the activation of prekallikrein impinges on a number of systems in plasma. Substrates of kallikrein include kininogen, plasminogen (Colman, 1969), prorenin (Derkx et al., 1979), and factor XII (Cochrane et al., 1973). The ability of kallikrein to activate factor XII, coupled with the activation of prekallikrein by factor XIIa (i.e., reciprocal activation), thus affords a positive-feedback amplification system which may be extremely efficient in effecting activation once initial "triggering" levels of either enzyme are achieved.

The mechanism for generating this triggering enzymic activity is controversial. Griffin & Cochrane (1979) have suggested that a low level of enzymic activity may be inherent in zymogenic prekallikrein and/or factor XII. Others have proposed that adsorption of factor XII to a negatively charged surface produces a conformational change that results in generation of enzymic activity in single-chain factor XII (Ratnoff & Saito, 1979) or that such adsorption produces a specific, nonenzymic cleavage to form factor XIIa (Ratnoff & Saito, 1982). It has also been suggested that initiation involves substrate-induced catalysis by single-chain factor XII (Heimark et al., 1980).

The observation that factor XIIa can cleave surface-bound factor XII to form additional factor XIIa (i.e., autoactivation) led Miller et al. (1980) and Silverberg et al. (1980a) to propose that this mechanism may be an important facet of contact activation, particularly in prekallikrein-deficient plasma. Although autoactivation does not explain the origin of the initial triggering level of factor XIIa, it nonetheless provides an alternate mechanism for factor XIIa formation once such triggering is achieved.

In the present work, we have addressed the question of the relative importance of autoactivation, as opposed to reciprocal activation, by studying the kinetics of the individual reactions involved in each mode of activation. These studies have employed dextran sulfate as a soluble, negatively charged

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[1] Abbreviations: M_r, molecular weight; S-2302, H-D-prolyl-L-phenylalanyl-L-arginyl-*p*-nitroanilide; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

[2] In this work, α-factor XIIa is the M_r 80 000 form of activated factor XII, and β-factor XIIa is the M_r 28 000 form (also known as factor XII_f or Hageman factor fragment); factor XIIa (without prefix) is used to refer to either α- or β-factor XIIa, or both.

"surface" for activation. Other investigators have also used this agent in studies of the contact system (Kluft, 1978; van der Graaf et al., 1982) and have demonstrated that it induces contact activation in a manner similar to the more commonly used negatively charged surfaces such as glass or kaolin. A knowledge of the kinetic parameters for each of the reactions involved in contact activation allows the construction of kinetic models for quantitatively comparing the triggering events of this complex system.

Materials and Methods

All chemicals obtained from commercial sources were the best grade available. H-D-Pro-Phe-Arg-*p*-nitroanilide (S-2302) was obtained from Kabi Diagnostica, Stockholm, Sweden. *N*-Benzoyl-Pro-Phe-Arg-*p*-nitroanilide was purchased from Boehringer Mannheim, Indianapolis, IN. Dextran sulfate sodium salt, M_r 500 000, and soybean trypsin inhibitor were obtained from Sigma Chemical Co., St. Louis, MO. Hexadimethrine bromide (Polybrene) was purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. Human albumin, 5%, was the product of Cutter Laboratories, Inc., Berkeley, CA.

Purification and Characterization of Plasma Proteins. Prekallikrein was isolated from human plasma as previously described (Alving et al., 1983). It exhibited two bands with apparent M_rs of 84000 and 82 000, in a ratio of 7:3, upon $NaDodSO_4$–polyacrylamide gel electrophoresis in 6% gels (Weber & Osborn, 1969). Approximately 0.1% of the material was in an active form, as judged by its amidolytic activity toward S-2302. α-Factor XIIa (M_r 80000) and β-factor XIIa (M_r 28 000) were prepared by published methods (Tankersley et al., 1982, 1983). The purities of these enzymes were judged to be 85% and >95%, respectively, by $NaDodSO_4$–polyacrylamide gel electrophoresis. Factor XII, prepared by the method of Griffin & Cochrane (1976), was subjected to a final chromatographic step on Sephadex G-150 (Pharmacia Fine Chemicals, Piscataway, NJ) to remove Polybrene and other inhibitors included in the previous steps. The final product, M_r 80 000, was ~90% homogeneous upon $NaDodSO_4$–polyacrylamide gel electrophoresis and contained 0.08% of activated factor XII as determined by hydrolysis of S-2302. Freedom from contamination by prekallikrein or kallikrein was established by incubating the preparation (final concentration 400 μg/mL) with β-factor XIIa (0.1 μg/mL) for 25 min at 23 °C to convert any prekallikrein to kallikrein and examining the resulting mixture for amidolytic activity toward *N*-benzoyl-Pro-Phe-Arg-*p*-nitroanilide. At equal mass concentrations of enzyme, kallikrein hydrolyzes this substrate ~260 times more rapidly than α-factor XIIa and ~124 times more rapidly than β-factor XIIa (Silverberg et al., 1980a). The observed activity was accounted for by the added β-factor XIIa and the endogenous factor XIIa activity of the preparation and was not inhibited by soybean trypsin inhibitor, which inhibits kallikrein. On the basis of these experiments, we concluded that contamination with prekallikrein or kallikrein was $<1 \times 10^{-4}$% of the total protein.[3]

Kallikrein was produced by activating prekallikrein with β-factor XIIa. Prekallikrein (30 μg) and β-factor XIIa (0.1 μg) were incubated at 37 °C in 0.1 mL of 0.05 M Tris-HCl and 0.05 M NaCl, pH 8.0, buffer containing 1 mg of human albumin/mL. Full activation was achieved in ~15 min as determined by hydrolysis of 0.5 mM S-2302 (specific activity 149 μmol·min^{-1}·mg^{-1}), and the activity remained constant thereafter. The kallikrein was stored at −70 °C.

Protein concentrations were determined spectrophotometrically by employing previously determined absorption coefficients (a_{280}) of 1.2 for prekallikrein, 1.6 for β-factor XIIa, and 1.7 for factor XII and α-factor XIIa. Molar concentrations were computed by using molecular weights of 84 000 for prekallikrein and kallikrein, 80 000 for factor XII and α-factor XIIa, and 28 000 for β-factor XIIa. No corrections were made for the purities of the enzymes and zymogens.

Kinetic Studies. Activation kinetics were determined at 37 °C in 0.05 M Tris-HCl, 0.05 M NaCl, and 1 mg of human albumin/mL, pH 8.0 (Tris–albumin buffer). The inclusion of albumin served to decrease absorptive losses of enzymes (Scott et al., 1981) and the inhibition of kallikrein by dextran sulfate (Tankersley et al., 1983). The substrate S-2302, 0.5 mM in Tris–albumin buffer, was used to quantitate both kallikrein and factor XIIa by an end-point method. The sample to be assayed (5 or 10 μL) was added to 200 μL of this substrate, and the mixture was incubated at 37 °C for a suitable time (1–180 min); then 200 μL of 10% acetic acid was added to stop the reaction, and the A_{405} was determined. A molar absorption coefficient (ϵ_{405}) of 9700 for *p*-nitroaniline was used to calculate the amount of substrate hydrolyzed. For assays of factor XIIa, Polybrene (5 μg/mL) and soybean trypsin inhibitor (50 μg/mL) were included in the S-2302 substrate solution; these agents had no effect on the hydrolysis of S-2302 by factor XIIa. Polybrene prevented continued factor XII activation, and soybean trypsin inhibitor completely inhibited kallikrein when it was present. Preliminary experiments confirmed that, under these conditions, the hydrolysis of S-2302 by either kallikrein or factor XIIa was linear with time up to at least 25% of substrate hydrolyzed (i.e., up to a measured ΔA_{405} of 0.6). The activity of α-factor XIIa toward S-2302 was unaffected by the presence of dextran sulfate, and a specific activity of 14.4 μmol·min^{-1}·mg^{-1} was used to calculate the concentration of α-factor XIIa. In contrast, under the conditions employed, dextran sulfate produced a 24% inhibition of the S-2302 hydrolysis by kallikrein. Thus, kallikrein concentrations were computed on the basis of specific activities of 113 or 149 μmol·min^{-1}·mg^{-1}, depending upon whether dextran sulfate was present or absent, respectively.

Activation of Prekallikrein: (*a*) *α-Factor XIIa without Dextran Sulfate.* Prekallikrein (five different concentrations ranging from 0.147 to 1.176 μM) in Tris–albumin buffer was incubated at 37 °C with α-factor XIIa (0.275 nM final concentration). At six different times (1–25 min) during the incubation, aliquots of the mixture were assayed for kallikrein as described above. The concentration of kallikrein produced was a linear function of time, and the rate of activation was computed for each initial prekallikrein concentration from the slope of the least-squares line fitted to these data points.

(*b*) *α-Factor XIIa plus Dextran Sulfate.* Six different concentrations of prekallikrein (0.049–1.176 μM), in Tris–albumin buffer containing 5 μg of dextran sulfate/mL, were employed. α-Factor XIIa (0.138 nM final concentration) was added, and the mixture was assayed for kallikrein at six to eight time points ranging from 0.5 to 5 min. Kallikrein formation was linear with time during the first 3 min; these points were used to generate least-squares regression lines from which the rates of activation were computed.

[3] When factor XII was allowed to autoactivate with dextran sulfate, the activity was not inhibited by soybean trypsin inhibitor but was completely inhibited by the factor XIIa inhibitor from corn (Hojima et al., 1980), which does not inhibit kallikrein. When prekallikrein (0.004% by weight of the factor XII) was added to the factor XII and the mixture subsequently allowed to activate, activity not inhibited by corn inhibitor was easily demonstrable, accounted for all of the prekallikrein added, and was completely inhibited by soybean inhibitor.

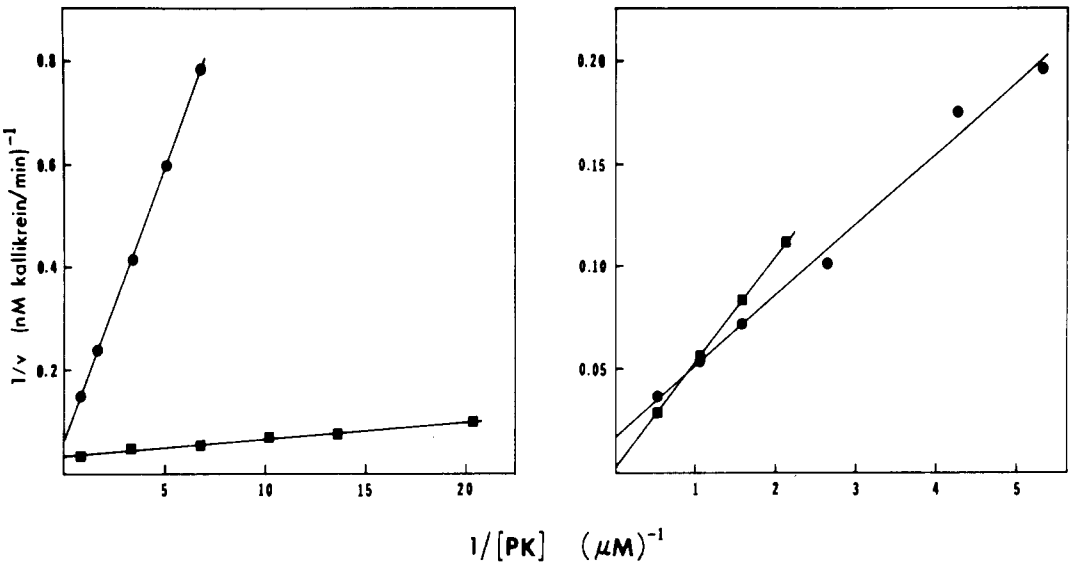


FIGURE 1: Lineweaver–Burk plots for the activation of prekallikrein by α-factor XIIa (left panel) and β-factor XIIa (right panel) in the presence (■) or absence (●) of dextran sulfate (5 μg/mL). The activation reactions were carried out at 37 °C in 0.05 M Tris-HCl, 0.05 M NaCl, and 1 mg of albumin/mL, pH 8.0. The concentration of β-factor XIIa was 0.287 nM; that of α-factor XIIa was 0.275 nM [(●) no dextran sulfate] or 0.138 nM [(■) plus dextran sulfate]. The kinetic constants derived from these plots are presented in Table I.

(*c*) *β-Factor XIIa without Dextran Sulfate.* These studies employed six concentrations (0.188–1.88 μM) of prekallikrein. The β-factor XIIa concentration was 0.287 nM; incubation mixtures were assayed for kallikrein at 1-min intervals during the first 5 min.

(*d*) *β-Factor XIIa plus Dextran Sulfate.* Prekallikrein (0.47, 0.63, 0.94, and 1.88 μM) in Tris–albumin buffer containing 5 μg of dextran sulfate/mL was activated with 0.287 nM β-factor XIIa. Kallikrein was assayed at six time points during the first 10 min of incubation.

Activation of Factor XII: (*a*) *Autoactivation with Dextran Sulfate.* Factor XII (six different concentrations from 1.15 to 4.59 μM) was incubated in Tris–albumin buffer containing 25 μg of dextran sulfate/mL. At frequent intervals (1.5–4 min) during the course of the incubation, aliquots were assayed for factor XIIa by means of S-2302 containing Polybrene and soybean trypsin inhibitor as described above. These assays were performed 8–14 times during the first 35–60 min of each run. Factor XIIa generation with time was sigmoidal (see Figure 2); these data were analyzed as described under Results.

(*b*) *Kallikrein without Dextran Sulfate.* Factor XII (0.31, 1.25, 2.50, and 5.61 μM) in Tris–albumin buffer was treated with kallikrein (0.36 μM) and incubated at 37 °C. The mixture was assayed for factor XIIa at five times (2.5–30 min) during the incubation; a linear increase in factor XIIa concentration with time was observed.

(*c*) *Kallikrein plus Dextran Sulfate.* Seven different concentrations of factor XII (0.162–3.44 μM), in Tris–albumin buffer containing 25 μg of dextran sulfate/mL, were incubated with kallikrein (0.413 nM). Factor XIIa activity was determined at 10-s intervals during the first 60 s. The plots of factor XIIa formed with time were somewhat concave (see Figure 4), particularly at the higher concentrations of factor XII, because of the contribution of autoactivation to the total rate of factor XIIa production. The analyses of these data are described under Results.

Results

The S-2302 activity of kallikrein is inhibited by dextran sulfate, and the extent of inhibition is markedly decreased in the presence of albumin (Tankersley et al., 1983). Studies conducted in the course of the present work demonstrated that, when kallikrein was assayed as described under Materials and Methods (i.e., kallikrein in buffer containing 1 mg of albumin/mL), dextran sulfate (5 μg/mL) produced a 24% inhibition of its S-2302 activity. This percentage inhibition was independent of the kallikrein concentration. Moreover, the same degree of inhibition was observed when prekallikrein was activated in the presence of dextran sulfate and the resulting kallikrein then assayed. These findings allowed us to compute the concentration of kallikrein on the basis of the activity toward S-2302 by making appropriate corrections for inhibition by dextran sulfate when it was present. The S-2302 activity exhibited by factor XIIa (either α- or β-factor XIIa) was not inhibited by dextran sulfate when assayed in the presence of 1 mg of albumin/mL.

Table I: Kinetic Constants for the Activation of Prekallikrein by α- and β-Factor XIIa in the Presence or Absence of Dextran Sulfate[a]

activator	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)
α-factor XIIa	1.03 (0.95-1.13)	1.80 (1.65-2.00)	0.57 (0.56-0.58)
α-factor XIIa + dextran sulfate	3.6 (3.3-4.0)	0.091 (0.077-0.112)	39.1 (37.2-41.0)
β-factor XIIa	3.5 (2.2-9.4)	2.1 (1.2-7.2)	1.67 (1.51-1.83)
β-factor XIIa + dextran sulfate	40 (25-130)	37 (22-171)	1.12 (1.11-1.13)

[a] Kinetics were studied under the conditions described in the legend to Figure 1. Values in parentheses are 95% confidence ranges.

Activation of Prekallikrein. Lineweaver–Burk plots for the activation of prekallikrein by α- and β-factor XIIa in the presence and absence of dextran sulfate are shown in Figure 1. The kinetics constants, k_{cat} and K_m, obtained from these plots are presented in Table I. Dextran sulfate produced a substantial decrease in the K_m for prekallikrein activation by α-factor XIIa, as well as an increase in the k_{cat} for this reaction. Thus, the activation is accelerated by dextran sulfate at all concentrations of prekallikrein. In contrast, dextran sulfate increased both k_{cat} and K_m for prekallikrein activation by β-factor XIIa. Therefore, dextran sulfate may either potentiate

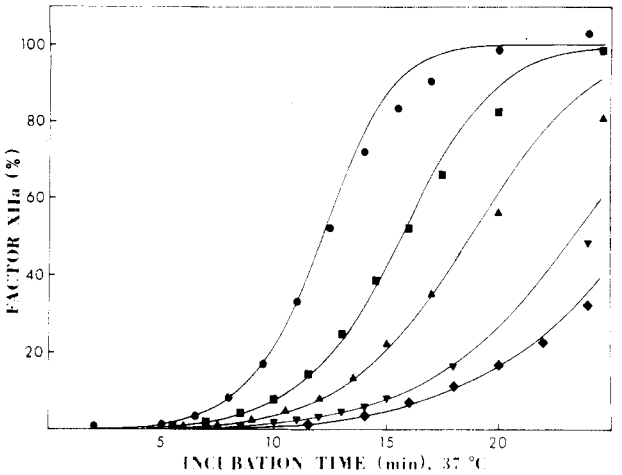


FIGURE 2: Autoactivation of factor XII by dextran sulfate. Mixtures containing factor XII, dextran sulfate (25 μg/mL), and albumin (1 mg/mL) in 0.05 M Tris-HCl and 0.05 M NaCl, pH 8.0, were incubated at 37 °C for the times indicated. Aliquots (5 μL) of the reaction mixtures were assayed for factor XIIa as described under Materials and Methods. The initial concentrations of factor XII were 3.44 (●), 2.29 (■), 1.72 (▲), 1.38 (▼), and 1.15 (♦) μM. The solid lines are the autoactivation progress curves computed after assuming K_m = 7.5 μM and k_{cat} = 0.033 s^{-1}.

or inhibit this activation, depending upon the prekallikrein concentration.

Autoactivation of Factor XII. Incubation of Factor XII with dextran sulfate resulted in the generation of Factor XIIa. Autoactivation progress curves are shown in Figure 2. The early phase of the activation follows an exponential curve, as expected for an autocatalytic process. These experiments employed no added enzyme other than that contained in the factor XII preparation, which was estimated to be about 0.08% activated and to contain $<1 \times 10^{-4}$% kallikrein.

Autoactivation of factor XII may be depicted as

$$\text{XIIa} + \text{XII} \underset{}{\overset{K_s \simeq K_m}{\rightleftharpoons}} \text{XIIa–XII} \xrightarrow{k_{cat}} \text{XIIa} + \text{XIIa}$$

The rate of formation of product, XIIa, is equal to k_{cat} times the product of the enzyme concentration and the degree of saturation of the enzyme by substrate, $\bar{y}$:

$$\text{rate} = \frac{d[\text{XIIa}]}{dt} = k_{cat}[\text{XIIa}]\bar{y} \qquad (1a)$$

where

$$\bar{y} = \frac{[\text{XII}]}{[\text{XII}] + K_m} \qquad (1b)$$

In the early phase of the reaction, $\bar{y}$ is essentially constant, but the concentration of enzyme, which in this case is also the product, is increasing rapidly. The differential form of the rate equation may be rearranged and integrated to give

$$\ln [\text{XIIa}]_t = \ln [\text{XIIa}]_0 + k_{cat}\bar{y}t \qquad (2)$$

Thus, a plot of ln $[\text{XIIa}]_t$ vs. t should produce a straight line with a slope of $k_{cat}\bar{y}$ and an intercept of ln $[\text{XIIa}]_0$. The data shown in Figure 2 were plotted in this manner (Figure 3). $[\text{XIIa}]_0$, obtained from the y intercept of these plots, ranged from 0.06 to 0.11% of the factor XII concentration, in good agreement with the directly measured value. From the slopes of the initial, linear portion of these plots, values for $k_{cat}\bar{y}$ were obtained at each concentration of factor XII. The kinetic constants k_{cat} and K_m were then computed from the intercepts of a double-reciprocal plot of these data (Figure 3, inset). These constants were employed in a computer program to

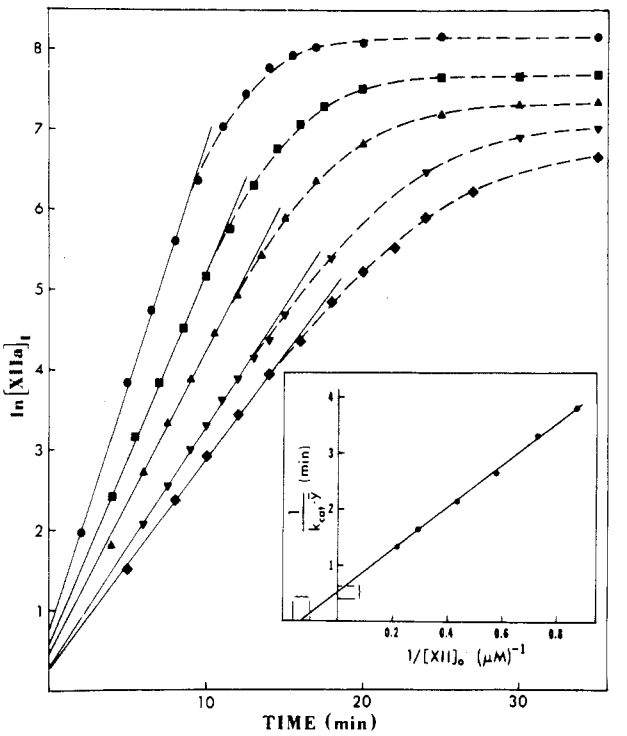


FIGURE 3: Semilogarithmic transform of the data from Figure 2. The ordinate is the natural logarithm of the factor XIIa concentration (in nanomolar) measured at time t. The slopes of the initial, linear portion of these plots provide values for $k_{cat}\bar{y}$ at each concentration of factor XII. (●) [XII] = 3.44 μM, $k_{cat}\bar{y}$ = 0.611 min^{-1}; (■) [XII] = 2.29 μM, $k_{cat}\bar{y}$ = 0.465 min^{-1}; (▲) [XII] = 1.72 μM, $k_{cat}\bar{y}$ = 0.378 min^{-1}; (▼) [XII] = 1.38 μM, $k_{cat}\bar{y}$ = 0.302 min^{-1}; (♦) [XII] = 1.15 μM, $k_{cat}\bar{y}$ = 0.262 min^{-1}; (not shown) [XII] = 4.59 μM, $k_{cat}\bar{y}$ = 0.744 min^{-1}. Inset: Double-reciprocal plot constructed with these values of [XII] and $k_{cat}\bar{y}$; 95% confidence limits of the intercepts are denoted by braces; k_{cat} = 0.033 s^{-1} (0.028–0.040 s^{-1}), K_m = 7.5 μM (6.1–9.7 μM).

generate theoretical progress curves for autoactivation. This program made use of the relationship[4]

$$\frac{d[\text{XIIa}]}{dt} = \frac{k_{cat}}{2}\left\{[\text{XII}] + [\text{XIIa}] + K_m - \sqrt{([\text{XII}] + [\text{XIIa}] + K_m)^2 - 4[\text{XII}][\text{XIIa}]}\right\} \qquad (3)$$

to evaluate the instantaneous rate of autoactivation, from which the changes in [XII] and [XIIa] could be computed over small intervals of time (0.2 min). Reiteration of this procedure produced the curves shown in Figure 2. The agreement of the experimental data with these theoretical curves is quite good in the early phase of the autoactivation reaction.

Autoactivation of factor XII in the absence of dextran sulfate was almost unmeasurably slow; incubation of factor XII (3.44 μM) for 6 h at 37 °C, pH 8.0, resulted in <1% activation. From this finding, we concluded that dextran sulfate accelerates autoactivation of factor XII at least 100-fold.

[4] The rate of an enzyme-catalyzed reaction is equal to k_{cat}[ES], where [ES], the concentration of enzyme–substrate complex, is obtained from the equilibrium relationship $K_m \simeq K_s = ([E]_{tot} - [ES])([S]_{tot} - [ES])/[ES]$. In the usual treatment, $[E]_{tot}$, and therefore [ES], is $\ll [S]_{tot}$, and thus $[ES] \simeq [E]_{tot}[S]_{tot}/(K_m + [S]_{tot})$ and rate $\simeq k_{cat}[E]_{tot}[S]_{tot}/(K_m + [S]_{tot})$ (cf. eq 1). When $[E]_{tot}$ is *not* $\ll [S]_{tot}$ (as is the case in the later stages of an autocatalytic reaction), then [ES] may not be negligible in comparison to $[S]_{tot}$. Solving the quadratic equilibrium equation for [ES] and multiplying by k_{cat} yield the relationship shown as eq 3.

Table II: Kinetic Constants for Factor XII Activation[a]

activator	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)
autoactivation with dextran sulfate	0.033 (0.028-0.040)	7.5 (6.1-9.7)	0.0044 (0.0042-0.0046)
kallikrein alone	0.010 (0.005-0.16)	11 (5-430)	0.00096 (0.00091-0.00101)
kallikrein with dextran sulfate	5.7 (5.1-6.5)	0.51 (0.43-0.63)	11.2 (10.8-11.6)

[a] At 37 °C, pH 8.0; see text for details. Values in parentheses are 95% confidence ranges.

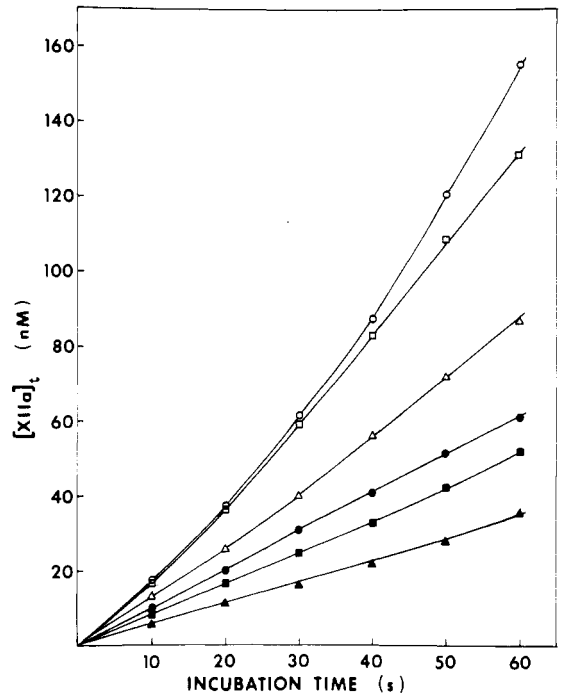


FIGURE 4: Progress curves for activation of factor XII by kallikrein in the presence of dextran sulfate. Factor XII was incubated at 37 °C with kallikrein (0.413 nM), dextran sulfate (25 μg/mL), and albumin (1 mg/mL) in 0.05 M Tris-HCl and 0.05 M NaCl, pH 8.0, for the times indicated. Aliquots (10 μL) of the reaction mixture were assayed for factor XIIa as indicated under Materials and Methods. The initial concentrations of factor XII were 3.44 (○), 1.72 (□), 0.75 (△), 0.40 (●), 0.26 (■), and 0.162 (▲) μM.

Activation of Factor XII by Kallikrein. The activation of factor XII by kallikrein in the absence of dextran sulfate was extremely slow even at the high molar ratios of kallikrein/factor XII (ranging from 0.064 to 1.16) used in this study. A Lineweaver–Burk plot for this reaction (four concentrations of factor XII) produced a least-squares line which intersected the axes very close to the origin (not shown). There was thus a large degree of uncertainty in the values for k_{cat} (0.010 s^{-1}) and K_m (11 μM) obtained from this plot, as manifested by the large range in the 95% confidence limits for these constants (Table II). The catalytic efficiency (k_{cat}/K_m), however, could be determined accurately from the slope of this plot.

In contrast, factor XII activation by kallikrein in the presence of dextran sulfate was rapid. Plots of factor XIIa generated with time are shown in Figure 4. The nonlinearity of these plots indicated that autoactivation was contributing to the total rate of factor XIIa formation. It is, of course, impossible to study "pure" kallikrein activation of factor XII in the presence of dextran sulfate, because the factor XIIa formed begins immediately to catalyze autoactivation. In the early stage of the reaction, however, when the factor XIIa concentration is low, the contribution of autoactivation to the total rate of factor XIIa production is minimal. We used the early time points (i.e., 10–30 s; Figure 4) to approximate the rates of factor XIIa generation due to kallikrein; a double-reciprocal plot of these rates vs. factor XII concentration gave values of 7.2 s^{-1} and 0.78 μM for k_{cat} and K_m, respectively (not shown). These approximate values, together with the previously determined kinetic constants for autoactivation (see above), were then used in a reiterative computer program that calculated, for each time point in Figure 4, the concentration of factor XIIa produced by autoactivation alone. Each point could then be corrected for autoactivation to obtain the concentration of factor XIIa formed by kallikrein. The extent of this correction ranged from <1% at early time points and low factor XII levels to as much as 24% for the 60-s point at the highest factor XII concentration. After such a correction, essentially linear plots of factor XIIa formed vs. time were obtained, and the slopes of these plots provided the rates of kallikrein-catalyzed factor XII activation at each concentration of factor XII. A Lineweaver–Burk plot of these corrected kinetic data is shown in Figure 5. Values of k_{cat} (5.7 s^{-1}) and K_m (0.51 μM) were not appreciably different from those obtained from the uncorrected rates, although the 95% confidence limits for these constants were substantially improved.

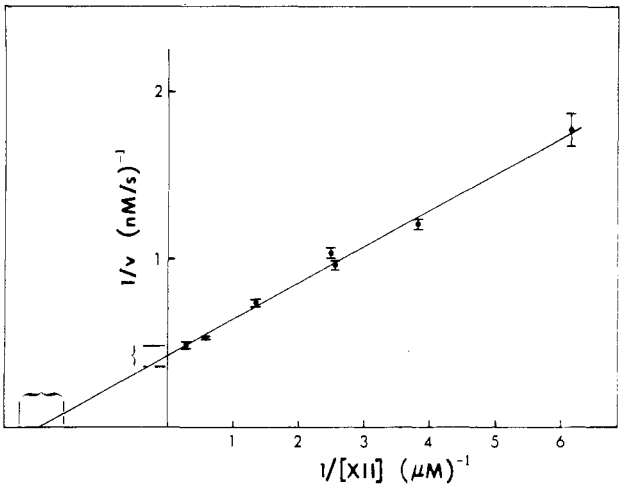


FIGURE 5: Lineweaver–Burk plot for the activation of factor XII by kallikrein in the presence of dextran sulfate. The rates of factor XIIa formation presented in Figure 4 were corrected for autoactivation as described under Results. The 95% confidence limits for these rates are denoted by vertical bars, and the corresponding limits for the intercepts are indicated by braces; k_{cat} = 5.7 s^{-1} (5.1–6.5 s^{-1}), K_m = 0.51 μM (0.43–0.63 μM).

Kinetic constants for factor XII activation are summarized in Table II. The most striking feature is the pronounced enhancement afforded by dextran sulfate in the activation by kallikrein. The kinetic constants for factor XII autoactivation are rather unimpressive in comparison to those for activation by kallikrein.

Discussion

Factor XII and α-factor XIIa, but not β-factor XIIa, are strongly adsorbed to negatively charged surfaces (Revak et al., 1978). The adsorption appears to be electrostatic, brought about by a positively charged region that is present in the heavy chain of α-factor XIIa but absent from β-factor XIIa. Prekallikrein is also adsorbed, albeit weakly, to negatively charged substances; adsorption is enhanced (~7-fold) by high molecular weight kininogen (Wiggins et al., 1977). Such adsorption of prekallikrein may produce a partial unfolding of the molecule, resulting in an increased susceptibility to pro-

teolytic activation. This may account for the increase in k_{cat} produced by dextran sulfate for activation by α- or β-factor XIIa. The pI of prekallikrein is 8.7 (Kaplan et al., 1972), and thus, it is positively charged at pH 8.0. Adsorption to dextran sulfate, producing a complex with net negative charge, may result in electrostatic repulsion of β-factor XIIa (pI = 4.2; Venneröd & Laake, 1974) and a consequent increase in the K_m for this reaction. In contrast, the K_m for activation by α-factor XIIa is decreased in the presence of dextran sulfate, presumably because of the strong affinity of α-factor XIIa for negatively charged substances. At a prekallikrein concentration of 0.3 μM (a concentration approximately equal to that in plasma), the overall effect of dextran sulfate is to decrease (by 26%) the rate of prekallikrein activation by β-factor XIIa, whereas activation by α-factor XIIa is accelerated 20-fold. These observations provide a method for distinguishing these two forms of factor XIIa based upon their relative ability to activate prekallikrein in the presence and absence of dextran sulfate.

Our studies on the concentration dependence of factor XII autoactivation demonstrate that the reaction follows Michaelis–Menten kinetics. This finding supports the conclusion that autoactivation is an enzymic process, as proposed by Silverberg et al. (1980b). Mechanisms invoking either nonenzymic cleavage (Ratnoff & Saito, 1982) or a conformational change producing an active center without bond breaking (Ratnoff & Saito, 1979) to account for the activating influence of negatively charged surfaces would not be expected to demonstrate Michaelis–Menten kinetics. Progress curves for autoactivation (Figure 2) are characterized by an exponential increase in factor XIIa with time, a finding inconsistent with activation being the result of contamination by kallikrein. Such curves might be misinterpreted as indicating a "lag phase", i.e., a stage in which enzymic catalysis was absent. However, when these data were plotted according to eq 2, a linear relationship obtained throughout the initial phase of the experiment (Figure 3). This behavior indicates a continuously increasing concentration of enzyme at any time >0, making it obvious that no lag phase exists. Although the catalytic efficiency of autoactivation is low, the exponential nature of autocatalysis and the high concentration of enzyme produced by this process results in an impressive maximal rate of factor XIIa formation.

The activation of factor XII by kallikrein is greatly accelerated by dextran sulfate; the catalytic efficiency is increased more than 10000-fold. Most of this increase appears to be the result of an increase in k_{cat}, suggesting that binding of factor XII to dextran sulfate produces a conformational change that greatly increases its susceptibility to proteolytic activation. McMillin et al. (1974) observed changes in the circular dichroism spectrum of factor XII upon binding to quartz or ellagic acid consistent with a conformational change in the molecule. Dextran sulfate also decreased the K_m for activation of factor XII by kallikrein. Although considerable uncertainty exists in the measured K_m of this reaction in the absence of dextran sulfate, the decrease was approximately the same as that observed in the activation of prekallikrein by α-factor XIIa (~20-fold).

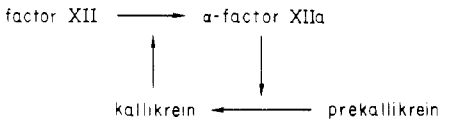
Our studies did not address the question of which molecular species of factor XIIa was formed by activation of factor XII. Dunn et al. (1982) found that the M_r 80000 form, α-factor XIIa, was the initial species produced by either autoactivation or kallikrein activation of factor XII in glass tubes, and similar results have been reported for factor XII activation induced by dextran sulfate (van der Graaf et al., 1982). Thus, the two

Table III: Comparison of Autoactivation to Reciprocal Activation in the Generation of Activated Factor XII

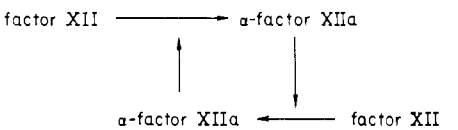
mode of activation	initial % activation[a]	time required for 50% activation[b] (s)
autoactivation	1	3080
autoactivation	0.1	4890
autoactivation	0.01	6700
reciprocal activation	1	1.6
reciprocal activation	0.1	2.5
reciprocal activation	0.01	3.5
reciprocal activation	5×10^{-13}	13.2

[a] For reciprocal activation, the percentage activation of each zymogen. 5×10^{-13}% corresponds to one molecule of kallikrein and factor XIIa per milliliter. [b] The calculations employed effective turnover numbers of 0.00127 s^{-1} for autoactivation and 2.45 s^{-1} (i.e., the average of 2.1 s^{-1} for factor XII activation and 2.8 s^{-1} for prekallikrein activation) for reciprocal activation. The time required for 50% activation is [ln (50/initial % activity)]/($k_{cat}\bar{\nu}$).

modes of factor XII activation may be depicted as indicated below. One mode is reciprocal activation:



The other mode is autoactivation:



At plasma levels (~0.3 μM) of factor XII and prekallikrein, the effective turnover numbers ($k_{cat}\bar{\nu}$) for these reactions, in the presence of dextran sulfate, are 2.1 s^{-1} for factor XII activation by kallikrein, 2.8 s^{-1} for prekallikrein activation by α-factor XIIa, and 0.00127 s^{-1} for factor XII activation by α-factor XIIa. Since the rates, as well as the initial reactant concentrations, for both of the reactions involved in reciprocal activation are very similar, the overall process is kinetically analogous to autoactivation except that the apparent turnover number is ~2000-fold greater. A doubling time (i.e., the time required for the concentration of factor XIIa to double) can be calculated for each process by dividing 0.693 by the apparent turnover number; the doubling time for autoactivation is 550 s, whereas that of the reciprocal activation scheme is only 0.28 s. The two modes of activation are compared further in Table III, in which the times required for 50% activation are calculated for various initial levels of activation. It should be emphasized that it is kinetically immaterial whether the initial activity is due to bona fide active enzyme or to low levels of enzymic activity inherent in the zymogen form. Moreover, for reciprocal activation, this initial activity may derive from either prekallikrein or factor XII, or both. The tremendous amplification provided by the reciprocal activation process is illustrated by the bottom line of data of Table III; given one molecule of active enzyme per milliliter, 50% activation will be achieved in about 13 s.

Our kinetic investigations were carried out at an ionic strength of 0.085, and we have previously reported (Tankersley et al., 1983) that the reactions of reciprocal activation are accelerated at low ionic strength. Autoactivation was also found to be enhanced at low ionic strength (data not shown); thus, these reactions may be considerably slower at physiologic ionic strength. In plasma, however, high molecular weight

kininogen greatly enhances reciprocal activation but apparently has little effect upon autoactivation (Silverberg et al., 1980b). In addition, the effect of plasma proteinase inhibitors in modulating contact activation reactions may differ, depending upon the mode of activation. It would appear, however, that reciprocal activation plays the major role in the generation of factor XIIa in normal plasma; autoactivation may provide a mechanism for contact activation in the absence of prekallikrein and high molecular weight kininogen.

Registry No. Blood coagulation factor XII, 9001-30-3; blood coagulation factor XIIa, 37203-62-6; kallikrein, 9001-01-8; prekallikrein, 9055-02-1; dextran sulfate, 9042-14-2.

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