Activation of Factor XII by Dextran Sulfate: The Basis for an Assay of Factor XII

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A system was developed for studying the activation of factor XII (Hageman factor) in the presence of dextran sulfate (DS). Salient features of the system included low ionic strength (0.08), low concentration of factor XII (approximately 1/10,000 that in normal plasma), and an excess of exogenous prekallikrein (PK). In this system, factor XII was rapidly converted to the 80,000 molecular weight (mol wt) form of factor XIIa (α-factor-XIIa). Once formed, the factor XIIa converted PK to kallikrein at a rate that was proportional to the amount of factor XII originally present in the incubation mixture. This system was used to construct a simple sensitive assay for factor XII in plasma and other biologic samples. The kallikrein produced was measured spectrophotometrically with the chromogenic substrate (H-D-Pro-Phe-Arg-p-nitroanilide (S-2302)). This assay was shown to be independent of the high molecular weight kininogen and the PK content of the sample being analyzed. The measurements obtained were consistent with fundamental enzymologic principles and, if desired, could be processed with a simple calculator program to achieve linear standard curves. When applied to the quantitation of factor XII in plasma, the assay yielded values in close agreement with those determined by coagulant assay or by radial immunodiffusion.

RECPROCAL ACTIVATION of factor XII and PK occurs when plasma contacts a negatively charged surface, such as glass or kaolin. High molecular weight kininogen, a contact-activation cofactor that circulates as a complex with PK, accelerates the activation process by binding PK to the surface, where it is cleaved by surface-bound factor XIIa. High molecular weight kininogen may also enhance contact-activation by protecting kallikrein from proteinase inhibitors.

As an initial step, functional assays for factor XII require the conversion of the zymogen to its active form, whereupon its ability to clot factor-XII-deficient plasma or to activate PK can be determined. Insoluble agents, such as kaolin or celite, are frequently employed to bring about factor XII activation. When these are used in factor XII assays that involve PK activation, it may be desirable to remove them by centrifugation to avoid interference (light-scattering) in spectrophotometric analyses for the resulting kallikrein. However, substantial amounts of α-factor-XIIa can remain bound to the insoluble agent and thus be removed during centrifugation.

The reported use of the soluble polycylic, DS, as the activating agent in an assay for PK7 prompted our investigations into its use in an assay for factor XII. Because we intended to employ this assay for monitoring the purification of factor XII from plasma, we considered it essential for the assay to be independent of other contact factors that might be present in (or absent from) the samples analyzed. The method developed fulfilled this requirement and, moreover, proved to be suitable for the measurement of factor XII in unfractionated plasma, in plasma containing Polybrene, or in serum obtained by clotting plasma with thrombin or by recalcification in glass.

MATERIALS AND METHODS

Chemicals

DEAE-Sephadex A-50, CM-Sephadex C-50, and Sephadex G-150 were obtained from Pharmacia Fine Chemicals, Inc., Piscataway, NJ. The chromogenic substrate for kallikrein, H-D-Pro-Phe-Arg-p-nitroanilide (S-2302), was purchased from Kabi Diagnostica, Stockholm, Sweden. Dextran sulfate sodium salt (DS), mol wt 500,000, was obtained from Sigma Chemical Co., St. Louis, MO. Human thrombin, lot H-1, was obtained from Dr. D. L. Aronson. Human albumin, 5%, was obtained from Cutter Laboratories, Inc., Berkeley, CA; it contained no detectable PK-activator activity and <1 ng kallikrein/ml. Hexadimethrine bromide (Polybrene, Aldrich Chemical Co., Inc., Milwaukee, WI), soybean trypsin inhibitor (STI, Worthington Biochemical Corp., Freehold, NJ), and polyoxyethylene (20) sorbitol monolaurate (Tween 20; Fisher Scientific Co., Fairlawn, NJ) were obtained from the sources indicated. Other chemicals used were of reagent quality.

Plasmas

Plasmas deficient in factor XII, factor XI, PK, or high molecular weight kininogen were purchased from George King Biomedical, Inc., Overland Park, KS. Additional plasma samples deficient in high molecular weight kininogen were provided by Dr. John Pisano, National Institutes of Health, Bethesda, MD. Individual plasma samples were prepared from blood drawn from healthy volunteers after obtaining informed consent; blood drawn into plastic syringes was immediately mixed with 3.8% sodium citrate dihydrate. After centrifugation, a portion of each plasma sample was treated with Polybrene (2% in H2O) to provide a final concentration of 100 μg Polybrene/ml. The plasmas were stored in small aliquots at −70°C. A reference normal human plasma (NHP) pool was prepared from blood drawn from 4 healthy volunteers. Freshly
frozen, platelet-poor plasma for protein purification was provided by the NIH blood bank.

**Protein Purification**

The isolation of factor XII from human plasma was carried out essentially as described by Griffin and Cochrane,9 with the addition of a final chromatographic step on Sephadex G-150. The final product, at a concentration of 1 mg/ml, was stored at -70°C. When fully activated by incubation with DS (5 µg/ml) and kallikrein (0.1 µg/ml) for 15 min at 30°C, it hydrolyzed 0.5 mM S-2302 (in 0.05 M Tris-HCl, 0.05 M NaCl, 100 µg STI/ml, pH 8.0) at a rate of 11.9 µmole/min/µg. When tested without previous activation, its activity was <0.01 µmole/min/µg; thus, <0.1% of the factor XII was in an active form.

α-Factor-XIIa was prepared by dialyzing plasma against 0.05 M Tris-HCl, 0.02 M NaCl, pH 8.0, and chromatographing on DEAE-Sephadex A-50 equilibrated with the same buffer. The column was washed with this same buffer, then eluted with a linear NaCl gradient (0.02-0.4 M) in Tris-HCl, pH 8.0. Factor XII was eluted at 0.12-0.16 M NaCl. It was diluted with H2O to an ionic strength of 0.09, adjusted to pH 5.8, and applied to a column of CM-Sephadex C-50 equilibrated with 0.05 M sodium citrate, pH 5.8. The column was washed with the same buffer containing 0.08 M NaCl, then eluted with a linear NaCl gradient (0.08-0.5 M). Factor XII was activated by this chromatographic procedure; α-factor-XIIa was eluted between 0.15 and 0.23 M NaCl. These fractions were pooled, concentrated by ultrafiltration, and chromatographed on Sephadex G-150 in 0.05 M sodium citrate, 0.2 M NaCl, pH 5.0. Approximately 90% of the S-2302 activity applied was associated with a protein peak eluted at a position corresponding to a mol wt of 91,000. It hydrolyzed 0.5 mM S-2302 (pH 8.0, 30°C) at a rate of 11.2 µmole/min/µg; and this activity was not inhibited by STI. No increase in S-2302 activity occurred upon incubation with kallikrein and DS. The α-factor-XIIa preparation (0.44 mg/ml) was stored at -70°C.

β-Factor-XIIa was prepared as previously described.9 It hydrolyzed S-2302 (pH 8.0, 30°C) at a rate of 38.9 µmole/min/µg. This difference (approximately threefold) from the hydrolytic activity of α-factor-XIIa or activated factor XII is consistent with the difference in their molecular weight, as determined below.

Unless otherwise indicated, the PK used in this work was highly purified from human plasma by methods described previously.10 The preparation had a protein concentration of 1.58 mg/ml. After activation, it hydrolyzed 0.5 mM S-2302 (pH 8.0, 30°C) at a rate of 173 µmole/min/µg; it had no detectable activity toward this substrate without prior activation.

Crude PK, prepared by the following procedure, was also found suitable for use in the factor XII assay. Because it interferes with the DS activation of factor XII, Polybrene should not be added to the plasma or to the buffers used for PK preparation; therefore it is essential that only plastic or thoroughly siliconized glass equipment be employed for the steps prior to DEAE-Sephadex chromatography. Fresh citrated human plasma (20 ml) was dialyzed for 3 hr at 23°C against 0.025 M Tris-HCl, pH 8.0, then for 3 hr against 0.05 M Tris-HCl, pH 8.0. The dialyzed plasma was applied to a 2.5 x 12 cm column of DEAE-Sephadex A-50 equilibrated and eluted at 23°C with 0.05 M Tris-HCl, pH 8.0; 10 ml fractions were collected. Fractions were tested for suitability in the following manner: 45-µl portions were incubated with 5 µl of DS (50 µg/ml), and with 5 µl of DS plus 5 µl of NHP (1:100 dilution), for 30 min at 37°C. The incubates were then assayed for S-2302 activity. Fractions 4–7 produced a ΔA500/5 min of >0.2 when incubated with DS plus NHP, and <0.01 when incubated with DS alone. These fractions were pooled and concentrated to a volume of 13.5 ml to afford a PK concentration sufficient for the PK-DS assay.

**PK-DS Assay for Factor XII**

The PK-DS substrate solution used for the assay of factor XII was prepared in the following manner. To 18 ml of 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0 buffer was added 100 µl of PK, 2 ml of 5% human albumin, and 110 µl of DS (1 mg/ml in H2O). Aliquots (45 µl) of this solution were then carefully pipetted into the bottom of conical polypropylene tubes of 1.5 ml capacity (Eppendorf). The solution was kept on ice during the pipetting procedure (~2 hr) and a positive displacement pipetter (Scientific Manufacturing Industries, Emeryville, CA) was used. The tubes were promptly frozen at -70°C. Alternatively, the crude PK preparation (13.5 ml, see above) was cooled on ice and treated with 1.6 ml of 5% albumin, 0.8 ml of 0.05 M Tris-HCl, 1.0 M NaCl, pH 8.0, and 88 µl of DS (1 mg/ml). This solution was then aliquotted as described above.

Plasma samples were diluted 1:1,000 with 0.05 M Tris-HCl, 0.05 M NaCl, 1 mg albumin/ml, pH 8.0, in polypropylene tubes. Other materials to be assayed (column fractions, purified factor XII, etc.) were similarly diluted such that the approximate factor XII content was 0.01%-0.2% of that of NHP (i.e., 2-50 µg/ml). Standards were prepared by diluting NHP 1/500 in the same buffer; this solution represented a factor XII content of 200% NHP in this assay, and it was further diluted to provide 3-8 different levels of factor XII (see Fig. 4). Diluted samples were stable for at least 4 hr at room temperature, and could be frozen and thawed several times without affecting the measured factor XII content. A total of 20 samples (including standards) could be conveniently assayed in one run, with additions being performed at 15-sec intervals. The tubes of PK-DS substrate, prepared as described above, were warmed to 37°C on a hot-block for 15–20 min, then 5 µl of diluted sample was admixed. After 10 min, 500 µl of 0.2 mM S-2302, 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0, prewarmed to 37°C, was added, and the mixture was further incubated for exactly 5 min; then 100 µl of 20% acetic acid was added to stop the reaction. The absorbance at 405 nm was determined against a blank consisting of 500 µl of S-2302 solution and 150 µl of 20% acetic acid; results were reported as ΔA500/5 min. The ΔA500/5 min values observed for the standards were used to construct a standard curve, as described in Results. A programmable calculator (Texas Instruments TI-59) was used to generate the least-squares standard curve and to interpolate the factor XII levels in samples from the measured ΔA500/5 min. The program listing, documentation, and user instructions will be supplied by the authors upon request.

**Other Methods**

Protein solutions were concentrated by positive-pressure ultrafiltration in Amicon stirred cells equipped with PM-10 membranes. Protein concentrations were determined by Lowry assay with bovine albumin as standard, or by spectrophotometry (A500), employing previously determined absorption coefficients. Radial immunodiffusion of factor XII was performed by the procedure of Mancini et al.,12 goat antiserum to human factor XII was provided by Dr. John Griffin, Scripps Clinic, La Jolla, CA. It was adsorbed with 1/10 volume of factor-XII-deficient plasma and used at a final dilution of 1:90 in 1% agarose. Plasma samples were assayed undiluted, and standards consisted of purified factor XII diluted in factor-XII-deficient plasma to provide concentrations of 0, 8, 16, 32, and 64 µg/ml. Factor XII coagulant activity was determined by the activated partial thromboplastin time with factor-XII-deficient plasma as substrate.13 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-
PAGE) was carried out on 6% gels, as described by Weber and Osborn. Figure 1 shows SDS-PAGE gels of the purified proteins used in this work. Unreduced factor XII and α-factor-XIIa both demonstrated a major band corresponding to a mol wt of 80,000. Reduction of α-factor-XIIa resulted in the appearance of bands corresponding to mol wts of 53,000 and 28,000, whereas no change was noted upon reduction of factor XII. β-factor-XIIa appeared as a single band (mol wt 28,000), whether reduced or unreduced. Unreduced samples of PK and of kallikrein both demonstrated two closely spaced bands of mol wts 82,000 and 84,000, with the latter predominating (~70%). Upon reduction, PK was unchanged, whereas kallikrein revealed major bands at mol wts of 50,000, 34,000, and 32,000 and two minor bands at 29,000 and 21,000. (The latter bands arise from further proteolysis of the 50,000 mol wt heavy chain.) These SDS-PAGE patterns for PK and kallikrein are consistent with the results reported by others.

**RESULTS**

*Inhibition of Kallikrein by DS*

The development of a factor XII assay that depends on the DS-induced activation of factor XII and subsequent factor-XIIa-catalyzed activation of PK requires the accurate quantitation of the kallikrein produced. As shown in Fig. 2, DS significantly inhibited the hydrolysis of S-2302 by kallikrein. The inhibition appeared to be similar to that observed when kallikrein is exposed to surfaces, in that the process was irreversible and had both an immediate and a time-dependent phase. Furthermore, the loss of kallikrein activity produced by DS was considerably less when...
crude kallikrein samples (containing primarily IgG) were used, or when whole human plasma was activated with DS.

Albumin, Tween 20, and gelatin were found to be effective in preventing both the surface-mediated loss of kallikrein and the inhibition by DS. In addition to preventing the inhibition of kallikrein by DS, however, gelatin interfered with the DS-induced activation of factor XII, whereas albumin and Tween 20 did not. We chose an albumin level of 5 mg/ml for use in further studies. At this level, DS (5 μg/ml) produced <10% inhibition of kallikrein (3 μg/ml) in 10 min.

Activation of PK

The rate of PK activation by NHP, factor XII, and activated forms of factor XII was examined both in the presence of DS and in its absence. In the presence of DS (5 μg/ml) and albumin, rates of PK activation by NHP (1:1,000), factor XII (20 ng/ml), and α-factor-XIIa (20 ng/ml) were approximately the same, and were considerably greater than that produced by an equal (mass) concentration of β-factor-XIIa (Fig. 3). The brief lag phase (~0.5 min) observed in the PK activation by NHP or by factor XII was absent when α- or β-factor-XIIa was the activating species.

When DS was omitted from the PK-albumin mixture, no activation occurred with NHP or with factor XII. The omission of DS resulted in a twofold increase in the rate of activation by β-factor-XIIa; that is, DS inhibited this activation by 50%. In contrast, DS greatly accelerated the activation of PK by α-factor-XIIa (~35-fold); hence, in the absence of DS, the activation rate was only 3% of that observed in its presence.

The NHP used in these experiments contained factor XII, as determined immunochemically, at a concentration of 25 μg/ml (see below). Thus, the observation (Fig. 3) that a 1:1,000 dilution of NHP activates PK-DS (after the lag period) slightly faster than does 20 ng α-factor-XIIa/ml suggests that all, or nearly all, of the factor XII in the diluted plasma is converted to α-factor-XIIa during the first 0.5–1 min of incubation. Once formed, α-factor-XIIa catalyzes the conversion of PK to kallikrein, apparently in a first-order reaction (see below). Thus, if the transition of factor XII to α-factor-XIIa is complete, the rate of PK activation should be proportional to the concentration of factor XII originally present, and measurement of the kallikrein generated in this system can be used as the basis of an assay for factor XII.

PK-DS Assay for Factor XII

A typical standard curve is shown in Fig. 4. Although a direct plot of kallikrein generated (expressed as ΔA405/5 min) versus factor XII content produces a usable standard curve (Fig. 4, broken line), it is convenient to transform these data so that a linear function is obtained. Two factors contributing to the observed curvature are the depletion of PK during the first stage of the assay, resulting in a progressively lower rate of kallikrein formation as the reaction proceeds, and the depletion of S-2302 during the second stage of the assay (i.e., during the measurement of the kallikrein formed in the first stage), resulting in a decreased rate of S-2302 hydrolysis with time.

The integrated form of the Michaelis-Menten equation may be employed to relate the concentration of kallikrein ([K]) produced in the first stage of the assay to the concentration of p-nitroaniline ([P]) produced in the second stage:

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[K] = \frac{K_m}{k_{cat}} \ln \left( \frac{[S]_0}{[S]_0 - [P]} + \frac{[P]}{k_{cat} \cdot t} \right)
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where \(K_m\) and \(k_{cat}\) are, respectively, the Michaelis-Menten constant and the turnover number for the hydrolysis of S-2302 by kallikrein, \([S]_0\) is the initial concentration of S-2302, and \(t\) is the (second stage) incubation time. The conversion of PK to kallikrein in the first stage of the assay approximates a first-order process, so that if the conditions and incubation time in

![Fig. 3. Activation of PK in the presence of DS. Aliquots (45 μl) of a solution containing PK (7.8 μg/ml), DS (5.5 μg/ml), and albumin (5 mg/ml) in 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0, were treated with 5 μl of NHP (1:1,000 dilution; □), factor XII (20 ng/ml; ○), α-factor-XIIa (20 ng/ml; △), or β-factor-XIIa (20 ng/ml; ▲), and incubated at 37°C for the times indicated. The amount of kallikrein produced (ΔA405/5 min) was determined by hydrolysis of 0.2 mM S-2302.](Image)
Fig. 4. Standard curve for the PK-DS assay of factor XII. Dilutions (1:500 to 1:4,000) of NHP were prepared in 0.05 M Tris-HCl, 0.05 M NaCl, 1 mg albumin/ml, pH 8.0; a 1:1,000 dilution of NHP represents a factor XII content of 100% NHP. Five microliters of each dilution was admixed with 45 µl of PK-DS (7.8 µg PK/ml, 5.5 µg DS/ml, 5 mg albumin/ml in 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0) and incubated at 37°C for 10 min, whereupon 500 µl of 0.2 mM S-2302 in 0.05 M Tris-HCl, 0.05 M NaCl, pH 8.0, was added. Incubation was continued for 5 min, then 100 µl of 20% acetic acid was added, and the A was determined (s). The concentration of p-nitroaniline ([P]) was computed from the Amax, and was used to evaluate the function shown on the right ordinate (m). Complete activation of the PK-DS produced a mm of 1.05, from which [P]max = 0.128 mM and K = 0.371 mM (see text; equation 5). The initial concentration of S-2302, and Km, the Michaelis-Menten constant for hydrolysis of S-2302 by kallikrein, have values of 0.182 mM and 0.2 mM, respectively. The regression line of the transform has a correlation coefficient of 0.9994.

Transforming the standard curve to linear form by means of equation 4 can be easily accomplished with a small programmable calculator. Two advantages accrue from such a transformation: It provides a means of reliable interpolation of unknowns with relatively few dilutions of NHP, and it allows statistical methods to be used for fitting the line to the data points.

**Assay of Plasma Samples**

This assay was used to determine the factor XII levels in individual plasma samples from normal donors as well as plasmas deficient in components of the contact-activation system (viz., PK, factor XII, factor XI, and high molecular weight kininogen). The results of these assays are shown in Table 1. Notably, Polybrene (100 µg/ml) did not affect the factor XII level as measured by this assay, nor did prior clotting of the plasma samples by treatment with thrombin (0.5 U/ml) or by recalcification in glass test tubes (1 hr, 37°C). The measured values for the deficient plasmas (except for factor-XII-deficient plasmas) fell within the normal range, indicating that PK, factor XI, or high molecular weight kininogen need not be present in the plasmas analyzed. The independence of this assay from the requirement for high molecular weight kininogen was confirmed by preparing mixtures of high molecular weight kininogen-deficient plasmas with factor-XII-deficient plasma and analyzing them by this method. The measured factor XII content of these
plasmas was not affected by the inclusion of factor-XII-deficient plasma (added to provide high molecular weight kininogen without altering the level of factor XII) in the assay mixture (Table 2).

Comparison With Other Assays

The factor XII levels in 13 individual plasma samples, as determined by the coagulation assay indicated in Materials and Methods, were compared to the values obtained by the PK-DS assay (Fig. 5A). A satisfactory correlation ($r = 0.931$; slope $= 1.01$) exists between the results of these methods. Factor XII was also quantitated by radial immunodiffusion; standards prepared by diluting purified factor XII in factor-XII-deficient plasma were employed, thus allowing an absolute determination of the factor XII concentration. The reference NHP pool contained 25 μg factor XII/ml; individual plasmas ranged from 7.9 to 33.0 μg/ml (32%-132% of NHP). The correlation between the results of immunochemical determination and the PK-DS assay (Fig. 5B) was excellent ($r = 0.991$; slope $= 0.98$).

Factors Affecting the Assay

A number of variables were examined in detail to define the limitations of the procedure. These included the extent of dilution of the plasma samples, the effect of Polybrene, the ionic strength, and the purity of the PK used.

Although plasma samples were typically diluted 1:1,000 for assay, low levels of factor XII in plasma could be accurately quantitated by performing the
assay on less dilute samples. For example, mixtures of factor-XII-deficient plasma and NHP were prepared to contain 50%, 10%, 5%, and 1% NHP; when assayed at dilutions of 1:1,000, 1:200, 1:100, and 1:20, respectively, the measured factor XII levels in these mixtures were 50.6%, 9.9%, 5.1%, and 0.87%.

Polybrene, at a final concentration of 2 μg/ml in the PK-DS incubation mixture, substantially inhibited (by >90%) the activation by NHP; at a final concentration of 0.2 μg/ml, however, no inhibition was noted. Therefore, it was possible to quantitate factor XII accurately in plasma samples containing Polybrene (100 μg/ml), since the final concentration of Polybrene in the incubation mixture was only 0.01 μg/ml.

The ionic strength of the activation mixture had a profound effect on the assay (Fig. 6). Maximal activation occurred under the standard assay conditions (I = 0.08), whereas at an ionic strength of 0.15, no kallikrein was generated from PK-DS by NHP. This observation was further explored by examining the time course of PK activation by NHP in the presence of DS at different ionic strengths. The lag phase was significantly lengthened by increasing the ionic strength above 0.08.

Crude PK preparations, consisting of the “γ-globulin fraction” not adsorbed by an anion exchanger, often contain quantities of factor XII that result in a substantial rate of kallikrein formation upon addition of DS alone. They therefore give high blank values and are unsuitable for use in the present assay. The modified preparative procedure described in Materials and Methods combines low ionic strength with a high adsorbent-to-sample ratio and thus yields crude PK sufficiently free of factor XII for use in the PK-DS assay. When 13 individual normal plasmas were assayed with this crude PK and with purified PK, the correlation coefficient between assays was 0.985 (slope = 0.96; mean variation = 4.6%).

We also examined the possibility of using factor-XII-deficient plasma in place of purified (or crude) PK in this assay. Factor-XII-deficient plasma was diluted 1:3 with 0.075 M Tris-HCl, 7.5 μg DS/ml, pH 8.0, to afford approximately the same concentrations of PK and DS as those in the PK-DS substrate normally employed. Although activation of the PK in this solution did occur when it was incubated with NHP (1:10,000 final), the use of such material as a substrate for the assay of factor XII was unsatisfactory, inasmuch as the standard curve had a substantial sigmoidal curvature. When the time course of the activation by NHP was examined (cf., Fig. 3), the lag phase was observed to be significantly longer (3-5 min) than when purified PK-DS was employed, suggesting that the plasma proteinase inhibitors present in factor-XII-deficient plasma were interfering with the factor XII activation phase of the reaction.

**DISCUSSION**

When dilute plasma samples are incubated with DS and an excess of PK, factor XII is rapidly converted to α-factor-XIIa, which in turn catalyzes the conversion of PK to kallikrein. Measurement of the rate of kallikrein formation then provides a measure of the amount of α-factor-XIIa produced and thus of the amount of factor XII present in the plasma. High molecular weight kininogen is not required in this assay system, although other experiments (not described) demonstrated that activation of dilute plasma by DS in the absence of exogenous PK required both high molecular weight kininogen and PK, in accord with results reported by others.7,17

Although DS inhibits the amidolytic activity of kallikrein (Fig. 2), this inhibition could be substantially decreased by including albumin in the assay system. Gelatin was even more effective than albumin in preventing the inhibition of kallikrein by DS; however, when gelatin was used in place of (or in addition to) albumin in the PK-DS assay, little or no activation of PK occurred upon treatment with dilute NHP or factor XII. These observations suggest that gelatin binds strongly to DS and thereby prevents its interaction with kallikrein (which would produce inhibition of

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**Fig. 6.** Effect of ionic strength on the activation of PK-DS by NHP. Solutions (50 μl) containing PK (7 μg/ml), DS (5 μg/ml), albumin (5 mg/ml), and NaCl (0.05–0.10 M) in 0.05 M Tris-HCl, pH 8.0, were treated with 5 μl of NHP (1:1,000) and incubated for 10 min at 37°C. The amount of kallikrein produced (ΔA280/5 min) was determined by hydrolysis of 0.2 mM S-2302.
kallikrein amidolytic activity) and with factor XII (which would result in increased susceptibility of the factor XII to proteolytic activation). In contrast, the binding of albumin to DS appears to be sufficient to prevent substantial inhibition of kallikrein while still allowing the adsorption of factor XII and its subsequent activation by kallikrein.

Under the conditions described for the PK-DS assay, DS produced a 35-fold increase in the rate of PK activation by α-factor-XIIa and a 4,000-fold increase in the rate of factor XII activation by kallikrein. The rate of α-factor-XIIa generation in a mixture of factor XII and excess PK is thus increased by more than 10³ (i.e., 35 x 4,000) in the presence of DS. Detailed kinetic studies on the potentiating effect of DS in these activation reactions will be reported separately. Briefly, we have found that in the presence of DS at low ionic strength, these reactions proceed with a velocity sufficient to ensure complete activation of factor XII in a short time (~30 sec), even though the initial concentration of “triggering” enzyme is so low as to be undetectable. Because of the reciprocal activation phenomenon, the triggering enzyme could be trace amounts of either factor XIIa or kallikrein, or even low enzymatic activity inherent in the zymogens, factor XII and PK, as has been suggested by Griffin.18 Thus, the large excess of PK employed in the PK-DS assay (typically 3,000 times the concentration of factor XII) serves not only as the substrate for α-factor-XIIa, but also as the source of factor XII activator (in the form of trace amounts of kallikrein or as inherent enzymatic activity of zymogenic PK).

When normal plasma samples were assayed for factor XII by the PK-DS method, as well as by procoagulant assay and by radial immunodiffusion, the various assays demonstrated substantial accord (Fig. 5). For example, the plasma factor XII level of Orientals (particularly Oriental females), as measured by clotting and immunologic methods, has been reported to be lower than that of whites.19 Our study included plasmas from one Chinese female and from two Chinese males; the factor XII levels in these plasmas, as determined by the PK-DS method, were 36% and 72%–73%, respectively. Additionally, new plasma samples were obtained from 8 of the 13 individuals after an interval of 6 mo and subjected to the PK-DS assay. No significant difference was noted in the factor XII levels of a given donor (correlation coefficient, 0.98; mean variation, 5.9%), suggesting that circulating factor XII levels remain reasonably constant with time.

The sensitivity of the PK-DS assay to changes in the ionic strength (Fig. 6) is striking, the activation ranging from essentially maximal at ionic strength below 0.095 to virtually zero under physiologic conditions (I ~ 0.15). Recently, Silverberg and Griffin reported kinetic studies of the activation of PK in mixtures containing PK, factor XII, DS, and IgG.20 Although our results (Fig. 3) are qualitatively similar to theirs, we observe a rate of PK activation, for a given amount of factor XII, approximately 50 times greater than they report. These investigators examined the reaction in 0.1 M sodium phosphate, pH 7.0 (I ~ 0.2), which may account for the lower rate of activation.

A similar effect has been previously described for the activation of PK by β-factor-XIIa,21 i.e., the activation rate is increased by decreasing the ionic strength. In view of the reported prorenin activator activity of kallikrein22 and the role of renin in maintaining sodium balance in plasma, the increased factor XII activation at low ionic strength may be one aspect of a general homeostatic mechanism.

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Activation of factor XII by dextran sulfate: the basis for an assay of factor XII

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