Centrifugal Analysis for Plasma Kallikrein Activity, with Use of the Chromogenic Substrate S-2302

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The amidolytic activity of activated kallikrein in plasma can be measured by use of the chromogenic substrate, S-2302 (H-D-Pro-Phe-Arg-pNA). Plasma prekallikrein was activated to kallikrein by exposure to 50 mg/L dextran sulfate in acetone/water (35/65 by vol) at 0 °C for 15 min. The acetone slows anti-kallikrein activity and increases the kallikrein activity by 30%. The 37 °C reaction mixture contained 0.54 mmol of S-2302 substrate per liter of Tris buffer (pH 7.5 at 37 °C). We monitored the change in absorbance at 405 nm for 60 s. The specificity of the substrate for kallikrein was demonstrated by using plasma deficient in prekallikrein (Fletcher trait) diluted with pooled normal human plasma. We recommend collecting blood specimens with sodium citrate as the anticoagulant and with use of a double-syringe technique and all-plastic containers. Plasma kallikrein activity with Chromozym-PK (Bz-Pro-Phe-Arg-pNA) as substrate (y-axis) compared with S-2302 as substrate (x-axis) gave the relation: y = 0.28x + 0.82 (r = 0.94). Day-to-day analytical variation was 2.4% for a pooled plasma with a mean value of 85.9 μkat/L. The mean (and 2 SD) for 50 healthy adults was 86.4 (32.4) μkat/L.

Additional Keyphrases: blood coagulation - prekallikrein - enzyme activity - reference interval - kinetic enzyme assay - variation, sources of

Measurement of kallikrein (EC 3.4.21.8) in plasma is of great physiological importance because of the pivotal role kallikrein plays in several functions: an early step in the coagulation cascade, the maintenance of blood pressure, and the influence on complement consumption and the fibrinolytic system.

In earlier evaluation of the kinetic activity of kallikrein in plasma, amino acid esters such as α-benzoyl-L-arginine ethyl ester and α-tosyl-L-arginine methyl ester were used as the substrates. Various methods for measuring esterolysis of these substrates were used, such as measurement of the liberated alcohol (1, 2), differential ultraviolet absorption of acids and esters, and the radioactive-methanol method (3). Because digestion of these substrates is also catalyzed by trypsin (EC 3.4.21.4), soybean trypsin inhibitor was used to eliminate interference by endogenous trypsin in the assay mixture. This technique gave some informative results, but the use of radioactive isotopes may not be desirable, and esterolytic substrates lack specificity for the amidolytic enzyme kallikrein.

There currently are three non-radioactive amidolytic substrates available commercially for use in the kinetic analysis for plasma kallikrein (PK). Two are chromogenic, S-2302 and Chromozym-PK. The third is a dipeptide fluorogenic sub-

strate with the fluorescent compound 7-amino-4-methylcoumarin linked to an arginine group. Amundsen et al. (4) first described a method for assaying plasma kallikrein with use of the chromogenic substrate Chromozym-PK, and later a modification of this method was reported by Stormorken et al. (5). A very extensive evaluation of PK activity as measured with the same substrate was reported by Kluft et al. (6, 7). Their approach was to activate plasma prekallikrein (PPK) with dextran sulfate at 0 °C and then measure with a double-beam spectrophotometer the rate of p-nitroaniline release at 37 °C. Friberger et al. (8) and Claeson et al. (9) using the substrate S-2302, presented similar methods.

PK analysis has some unique problems: activation of PPK to PK must be complete and PK activity decrease by natural inhibitors in plasma must be prevented. We address both of these problems in the present method by use of a mixture of dextran sulfate and acetone in the activation step. In addition, we chose the substrate S-2302 for its greater sensitivity and used a centrifugal analyzer to measure the rate of substrate degradation.

Materials and Methods

Apparatus

Plasma kallikrein activity was determined kinetically by, monitoring the change in absorbance at 405 nm as measured in the centrifugal analyzer (CentrififChem System 400 Analyzer; Union Carbide Corp., Tarrytown, NY 10591).

Reagents

The chromogenic substrate S-2302 (H-D-Pro-Phe-Arg-pNA-2HCl, M_r = 611.6; courtesy of KABI Group Inc., Greenwich, CT 06870) was dissolved in de-ionized water to make a 6.0 mmol/L stock solution, which was stable for at least two months at 2 to 8 °C. The 0.6 mmol/L working substrate was prepared by diluting the stock solution 10-fold with tris(hydroxymethyl)methylamine (Tris) buffer (50 mmol/L, pH 7.8 at 24 °C) containing 12 mmol of NaCl per liter. Chromozym-PK (Bz-Pro-Phe-Arg-pNA-HCl, M_r = 679.3; Fentapharm AG, Basle, Switzerland) working substrate was dissolved in Tris buffer to a concentration of 0.6 mmol/L. p-Nitroanilin, 50 μmol/L aqueous standard, was a gift from Dr. Lars Mellstam (AB KABI Peptide Research, Möln达尔, Sweden). Dextran sulfate (M_w = 500 000; Pharmacia Ltd., Upsala, Sweden) was prepared by dissolving 10 mg in 30 mL of de-ionized water, then adding 70 mL of acetone. The solution was stable for at least three months at 2 to 8 °C. Fletcher (prekallikrein)-deficient plasma was kindly supplied by Dr. Charles Abildgaard (University of California, Davis Medical Center). Hageman (Factor XII)-deficient plasma was purchased from Dade Diagnostics, Inc., Miami, FL 33152. Normal human plasma was collected and pooled according to the procedure outlined below, from volunteer laboratory personnel. Plasma from a minimum of 10 healthy adults was pooled and 200-μL aliquots were stored at −50 °C in plastic containers. Acetone and all other reagents were of AR grade.

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Blood was collected by venipuncture, with 0.1 mol/L sodium citrate as anticoagulant (one part sodium citrate to nine parts blood), with use of a double-syringe collection technique. In this technique two syringes are used. The needle is inserted into the vein and 2 to 4 mL of blood is drawn. The syringe is removed, leaving the needle in place. The second syringe is attached to the needle and the specimen is collected. The purpose of the first syringe is to remove "endogenous" tissue factors that may contaminate the specimen during venipuncture. The contents of second syringe are presumed to be free of such contamination. All syringes and tubes are plastic. We centrifuged the specimens within 1 h of collection (at 15–24 °C, 1500 × g, 10 min), removed the plasma with a plastic pipette, and stored it in plastic containers. The specimens either are maintained at room temperature and assayed within 3 h or stored at −50 °C for future analysis.

The effects of spontaneous cold activation of PPK were investigated by collecting specimens and storing plasma aliquots from two subjects at 4 °C. The aliquots were assayed for PK activity with and without activation with dextran sulfate and 35 mL/dL acetate.

**Procedure for Activation and Analysis of Plasma Kallikrein**

Frozen specimens are thawed at room temperature until clear. Aliquots of Tris buffer (50 mmol/L) and dextran sulfate solutions are chilled in an ice bath before use. After 13 × 100 mm glass tubes are placed in the ice bath, 50 μL of test plasma is added to each tube. Plasma prekallikrein is activated to PK by adding 50 μL of the dextran sulfate solution, mixing, and incubating for 15 min at 0 °C. Then 1300 μL of cold buffer is added and, without delay, 40 μL of this mixture is pipetted into the sample well of the CentrifiChem transfer disc, which contains 350 μL of S-2302 working substrate solution in the adjacent reagent well.

The CentrifiChem Analyzer is programmed as follows: initial reading time = 3 s, reading interval = 1 min, temperature = 37 °C, filter = 405 nm, and blank set to "auto." Plasma kallikrein activity is measured as the change per minute in absorbance at 405 nm (ΔA/min). The results are calculated by multiplying ΔA/min times a factor derived from the molar absorptivity of p-nitroaniline (ε_pNA = 9.20 × 10³ L·mol⁻¹·cm⁻¹) and expressed in μkat/L (1 μkat = 60 U). We assume a hematocrit of 40 mL/dL. Given our conditions, the resulting plasma volume in the final assay mixture would be 1.19 μL. Then

\[
\text{Activity, } \text{U/L} = \frac{\Delta A}{\text{min}} \left( \frac{\text{reaction vol}}{\text{sample vol}} \right) \left( \frac{10^6 \text{ μmol/mol}}{\text{ε-light path in cm}} \right)
\]

\[
= \frac{\Delta A}{\text{min}} \left( \frac{390 \text{ μL}}{1.19 \text{ μL}} \right) \left( \frac{10^6}{9.20 \times 10^3} \right) \left( \frac{\text{μmol}}{L} \right)
\]

\[
= \frac{\Delta A}{\text{min}} \left( \frac{35623 \text{ μmol/L}}{L} \right)
\]

or

\[
\text{Activity, } \text{μkat/L} = \text{activity, } \text{U/L} \left( \frac{1 \text{ min}}{60 \text{ s}} \right)
\]

\[
= \frac{\Delta A}{\text{min}} \left( \frac{35623 \text{ μmol/L}}{60 \text{ s}} \right)
\]

\[
= \frac{\Delta A}{\text{min}} \left( \frac{594 \text{ μmol}}{L} \right)
\]

**Plasma Kallikrein Activity with Various Acetone Concentrations in the Activation Mixture**

The optimum acetone concentration in the PPK activation mixture for PK stability was determined by constructing a gradient of acetone concentrations (0–40 mL/dL), each containing 50 mg of dextran sulfate per liter, as the activation solutions. Plasma pool was activated in duplicate for 15 min with each solution in an equilibrium ratio at 0 °C, then diluted with Tris buffer. The activated plasma pools were brought to 23 °C for various timed intervals to allow response of viable PK inhibitors. Then samples under each test condition were assayed, to determine the optimum acetone concentration to use for PK stability with time.

**Reference Values**

Plasma specimens from 50 ostensibly healthy adults were assayed to determine the reference interval. These assays were done during several days on 17 men (age 19–48 years) and 33
women (age 20–60 years). Of the women in the study, three were taking oral contraceptives and three were pregnant (first or early second trimester).

In a longitudinal study, five women (24, 25, 27, 28, and 31 years of age) had specimens drawn periodically during 28 days, and their respective dates of menstruation were noted. All the specimens collected from a particular subject were frozen and thawed and then assayed in one batch, to eliminate day-to-day analytical variation. One subject (number 5) reported taking oral contraceptives during the course of this study.

Results

Assay Conditions

We adjusted the Tris buffer, as suggested by Friberger et al. (7) and Claeson et al. (8), to pH 7.8 at room temperature. Figure 1 presents the relationship of initial activity (3–63 s) to substrate concentration at pH 7.5 in the assay mixture. The $K_m$ determined from the Lineweaver–Burk plot is $2.2 \times 10^{-4}$ mol/L. The absorbance readings at 405 nm at pH 7.5 and substrate concentration of 0.54 mmol/L are presented at four timed values, i.e., 3, 63, 123, and 183 s for each of five dilutions of plasma specimens (Figure 2). With higher activities there was a tendency for absorbances to deviate from the initial linear slope based on the values at 3 and 63 s. The relationship of initial activity (3–63 s) to volume of plasma was linear (Figure 3).

Table 1. Activation Conditions: Effects of Temperature and Acetone (35 mL/dL)

<table>
<thead>
<tr>
<th>Temp. °C</th>
<th>Acetone present</th>
<th>Mean PK acty. μkat/L</th>
<th>Range* μkat/L</th>
<th>Percent activity as compared to method of choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Yes</td>
<td>89.7</td>
<td>88.5–90.3</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>No</td>
<td>73.6</td>
<td>72.5–74.8</td>
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<td>12.6</td>
<td>12.3–12.8</td>
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<tr>
<td>23</td>
<td>No</td>
<td>12.3</td>
<td>11.6–13.1</td>
<td>14</td>
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</tbody>
</table>

* Results based on four replicate determinations.

Fig. 3. Linearity of method for plasma kallikrein activity as assessed from dilutions of plasma specimens.

Fig. 4. Effects of various acetone concentrations (mL/dL) on activity of plasma kallikrein remaining after intervals at 23 °C.

Activation of Prekallikrein to Kallikrein

We recommend activating PPK at 0 °C with dextran sulfate and acetone for 15 min. Dextran sulfate was used to initiate the activation process. Acetone and low temperatures (0 °C) were used to prevent PK inhibition (Table 1). Figure 4 demonstrates that 35 mL of acetone per deciliter of activation mixture is the optimum concentration for stabilizing the PK activity, because with lower concentrations the activity decays faster. Activation of PPK with and without acetone in the assay mixture, based on five split-sample trials, is compared in Figure 5. Figure 6 demonstrates that PK activity was about 30% greater when acetone was used. The optimum time for maximum PPK activation with acetone was 15 min (see Figure 5).

We examined the possibility of PPK activation during specimen collection (Table 2). Specimens were collected from four subjects under conditions simulating those that may occur in routine collections. As noted in Table 2 for either the single- or double-syringe technique, for storage up to 60 min or 180 min, at 23 °C or at 4 °C, the activity when glass was used (Conditions I–P) varied from 68 to 79% of that for the proposed method (Condition A). When plastic was used for these

Fig. 5. Mean activity and range of activities of plasma kallikrein after various intervals of incubation with dextran sulfate and either no acetone or 35 mL of acetone per deciliter.
Table 2. Effects of Blood-Collection Conditions and Short-Term Storage Conditions on Plasma Kallikrein Activity

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Mean</th>
</tr>
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<tbody>
<tr>
<td>A D, P, 23, 60</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
</tr>
<tr>
<td>B D, P, 23, 180</td>
<td>109</td>
<td>107</td>
<td>107</td>
<td>102</td>
<td>106</td>
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<tr>
<td>C S, P, 23, 60</td>
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<td>98</td>
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<td>93</td>
<td>99</td>
</tr>
<tr>
<td>D S, P, 23, 180</td>
<td>111</td>
<td>107</td>
<td>99</td>
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<td>110</td>
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</tr>
<tr>
<td>G S, P, 4, 60</td>
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<td>104</td>
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<td>I D, G, 23, 60</td>
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<td>J D, G, 23, 180</td>
<td>78</td>
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<td>73</td>
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<tr>
<td>K S, G, 23, 60</td>
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<td>77</td>
<td>59</td>
<td>75</td>
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<td>L S, G, 23, 180</td>
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<td>77</td>
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<td>68</td>
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<tr>
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<td>85</td>
<td>79</td>
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<td>77</td>
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<td>N D, G, 4, 180</td>
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<td>75</td>
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<td>75</td>
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<tr>
<td>P S, G, 4, 180</td>
<td>69</td>
<td>78</td>
<td>71</td>
<td>53</td>
<td>68</td>
</tr>
</tbody>
</table>

* D, double-syringe technique; S, single-syringe technique; P, plastic containers; G, glass containers; 23, 23 °C; 4, 4 °C; 60, 60 min; 180, 180 min. <sup>b</sup> PK value (μkat/L) for Condition A: Subject 1, 79.7; Subject 2, 53.5; Subject 3, 85.6; Subject 4, 72.0.

same variations (Conditions B–H), the activity varied from 99 to 114% of the proposed method. In the case of plastic containers, the value at 180-min storage often seemed somewhat greater than at 60-min storage. In addition, storage at 4 °C for the plastic containers seemed to result in a somewhat higher value than at 23 °C storage. However, we did note spontaneous cold (4 °C) activation of PPK on longer storage in specimens from two of the subjects. Table 3 presents the plasma kallikrein activity with and without dextran sulfate-acetone activation at 1.5, 24, and 36 h of storage at 4 °C for each of two subjects. The PPK was activated to PK in the cold before the 24th hour for Subject 1 and between 24 and 36 h for Subject 2, possibly demonstrating spontaneous activation at 4 °C.

Reliability of the Method

Normal human pooled-plasma aliquots were stored at either −20 or −50 °C and were assayed with and without acetone in the activation mixture during the 30 succeeding days. The results (Figure 7) indicate that normal human plasma stored at −50 °C and assayed with acetone produced the most consistent results. No loss of activity was observed during the month. Twenty-six aliquots of normal plasma stored at −50 °C were assayed, one per day, during 30 days. The values ranged from 81.1 to 90.0 μkat/L (mean, 85.9 μkat/L; SD, 2.1 μkat/L; CV, 2.4%).

The method was tested for PK specificity by diluting pooled plasma with various volumes of Fletcher-deficient plasma and assaying for PK. The results (Table 4) demonstrate that Fletcher-deficient plasma showed essentially no activity and that the other results compared well with the expected values.

The necessity for Factor XII activation of PPK to PK was tested by using Factor XII (Hageman trait)-deficient plasma to dilute normal human plasma, thereby comparing PK activity for a range of Factor XII values. The results (Figure 8) show the critical level for Factor XII to lie between 25 to 33% of that contained in normal human plasma. When Factor XII concentrations were decreased, the time necessary for peak activation was observed to increase (Figure 9).

In Figure 10 we compare PK activities with S-2302 and Chromozym-PK as substrates. The final concentration for both substrates were equal, i.e., the reaction volume concentration contained 0.54 mmol of each substrate per liter of Tris buffer (pH 7.5 at 37 °C). There is excellent correlation (r = 0.94), but the activity with S-2302 was about 3.5 times that with Chromozym-PK.

Reference Intervals for Healthy Adult Subjects

The distribution of our results for healthy adults is plotted in Figure 11. The three women taking oral contraceptives all
had significantly greater PK values than did the other women in the group. There was no significant difference between PK values for women not taking oral contraceptives and those for the men. The mean value for these 50 individuals is 86.4 (2 SD 32.4) µkat/L. Figure 12 shows the day-to-day variation in PK values in five healthy women during 28 days. Their individual means and day-to-day variations are presented in Table 5. Subject number 5 was taking oral contraceptives during the study.

Discussion

Major problems in setting up an assay for plasma kallikrein include (a) collecting and preserving the specimen without activating PPK to PK until it is deliberately activated by dextran sulfate; (b) activating PPK to PK as completely as possible without allowing combination with naturally occurring inhibitors; and (c) developing an automated, rapid, and reliable assay that is specific for PK.

First, to avoid prior activation of PPK to PK, we used the described double-syringe collection technique. Glass containers should not be used, because glass may cause premature activation of PPK and the subsequent combination of PK with various naturally occurring inhibitors. The specimens stored at 4 °C maintained greater activity than those at 22 °C during the first 3 h after collection. However, we used 23 °C for specimen processing before analysis since possible spontaneous cold activation of PPK may occur via Factor XII activation (6, 10, 11, 12). We have observed this phenomenon (Table 2) and therefore suggest separating plasma from cells and assaying specimens within 3 h, or storing at −50 °C for future analysis.

The second problem—activation of PPK to PK without combination with in vivo inhibitors—has been dealt with in various ways by other workers (1, 3, 5–9, 13). Current procedures for PPK activation include descriptions of the effectiveness of various PPK activators as well as methods to minimize the influence of PK inhibitors. Dextran sulfate, kaolin, ellagic acid, and acetone were compared as PPK activators by Kluft et al. (6, 7) and Friberger et al. (8). Low activation temperatures (0 °C) were shown to decrease the influence of PK inhibitors (13) and have been used by many investigators (1, 6–8). High dilution ratios of activated plasma to buffer were used to decrease the concentration of inhibitors (6–8, 9) and acetone was used to denature them (3, 6). Using a combination of these methods, we describe an activation procedure most suitable for an automated PK assay. Dextran sulfate was selected as the activator because it is water soluble and therefore problems of insoluble activators (such as kaolin) were avoided in the centrifugal analysis. Acetone was added to the activation step because it reportedly increases PK activity and stability by decreasing the effect of the inhibitors (3, 8). We show that 35 mL/dL acetone in the activation mixture is optimum for PK stability; concentrations greater than 35 mL/dL cause an increase in protein precipitation and turbidity in the activation mixture and do not appear to increase PK activity or stability. This acetone concentration in the activation mixture increased results by about 30% over the range tested as compared with no acetone. Thus, the three advantages of acetone use are: (a) greater PK activity, (b) increased PK stability at peak activation, and (c) a smaller range of variability at peak activation. Because PK inhibitors are both time- and temperature-dependent (1, 6, 14), we ac-

**Table 4. Activity of Activated Plasma Kallikrein in Various Mixtures of Fletcher(Prekallikrein)-Deficient Plasma and Normal Human Pooled Plasma (NHPP)**

<table>
<thead>
<tr>
<th>NHPP, mL per dL of NHPP and PPK-deficient plasma mixture</th>
<th>Activity of mixture, µkat/L</th>
<th>Activity as percent of PK activity of NHPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>86.7</td>
<td>100.0</td>
</tr>
<tr>
<td>50</td>
<td>43.1</td>
<td>49.7</td>
</tr>
<tr>
<td>33</td>
<td>29.0</td>
<td>33.4</td>
</tr>
<tr>
<td>20</td>
<td>18.8</td>
<td>21.7</td>
</tr>
<tr>
<td>0</td>
<td>0.6</td>
<td>0.7</td>
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</table>

* Based on three replicate determinations.

Fig. 7. Plasma kallikrein activity of pooled plasma under various storage conditions.

Fig. 8. Percent conversion of plasma prekallikrein to plasma kallikrein, based on the measured plasma kallikrein activity as a function of Factor XII in the test plasma. A plasma deficient in Factor XII was diluted in various ratios with normal human pooled plasma to construct the range of Factor XII values.

Fig. 9. Plasma kallikrein activities compared with and without acetone (35 mL/dL) in the activation mixture at various incubation times with a plasma containing 25% of the Factor XII expected to be present in pooled normal human plasma.
tivated PPK in the cold (0 °C) to retard the effects of PK inhibitors. The results (Table 3) show the effects of temperature and acetone addition on the activation of PPK in NHPP. From the evidence, we conclude that use of dextran sulfate and 35 mL/dL acetone activation at low temperature (0 °C) results in the most suitable conditions for PPK activation and stability.

We determined the Michaelis–Menten constant ($K_m$) at pH 7.5 to be $2.2 \times 10^{-4}$ mol/L for the substrate S-2302 and for normal pooled human plasma as the enzyme source. This value is in close agreement with that reported by Friberger et al. ($K_m = 1.8 \times 10^{-4}$ mol/L), who used purified kallikrein in a similar system (8), and Claeson et al. ($K_m = 2.0 \times 10^{-4}$ mol/L) (9). The $K_m$ as determined from Figure 1, indicates the desirable S-2302 substrate concentration to be 2.2 mmol/L, assuming that 10 times the $K_m$ were to be used, while we used 0.54 mmol/L substrate or about 2.5 times the $K_m$. This was done to decrease substrate consumption, because its cost is relatively high. This concentration did not appear to affect the assay if the rate of reaction was measured within the first 60 s after the reaction is initiated (see Figure 2).

The specificity of the S-2302 substrate for PK was verified by making various dilutions of Fletcher (PPK)-deficient plasma with pooled normal human plasma (Table 2). These results indicate that no other plasma enzyme showed significant activity for the substrate.

For in vitro procedures, three elements are necessary for the activation of PPK to PK: negatively charged surface particles, kinogen of high molecular mass, and Factor XII (15, 16). Factor XII in normal plasma was diluted by adding Factor XII (Hageman)-deficient plasma. We found that the critical amount for Factor XII lies between 25% to 33% of that contained in normal plasma when 15-min activation was used (Figure 8). For this activation time and 25% Factor XII in normal plasma, only 5% to 10% of the total PK activity was observed (Figure 9). When incubation of this activation mixture was prolonged, the PK activity seen at 15 min was
Table 5. Mean and Day-to-Day Variability of Plasma Kallikrein Activity in Each of Five Healthy Women as Monitored during 28 Days

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, years</th>
<th>No. specimens collected</th>
<th>Mean plasma kallikrein acty., μkat/L</th>
<th>Day-to-day (combined analytical and physiological) variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31</td>
<td>10</td>
<td>86.0</td>
<td>3.5, 4.1, 5.6</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>9</td>
<td>89.3</td>
<td>5.0, 6.1, 6.3</td>
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<tr>
<td>3</td>
<td>25</td>
<td>12</td>
<td>107.8</td>
<td>6.6, 6.8, 6.1</td>
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<tr>
<td>4</td>
<td>28</td>
<td>10</td>
<td>105.4</td>
<td>4.3, 4.6, 4.1</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>10</td>
<td>118.4</td>
<td>6.3, 5.3, 5.8</td>
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</tbody>
</table>

about fivefold greater at 30 min. The extended lag phase apparently resulted from the decreased quantity of Factor XII. This decreased activity would probably be interpreted as a low PPK concentration in the usual 15-min activation period. Thus, low PK activity in the clinical setting may be due to: (a) low PPK, (b) decreased Factor XII, (c) decreased high-molecular-mass kininogen, or (d) premature activation of PPK to PK before analysis. The first three conditions may be tested by mixing the test plasma with an equal volume of normal plasma. This will either restore PK activity, therefore suggesting that Factor XII or high-molecular-mass kininogen is deficient, or will not restore normal activity, indicating decreased concentrations of PPK.

Premature PPK activation, the fourth condition, is determined by testing the plasma for PK activity without dextran sulfate/acetone activation. In a study in progress, for example, we noted the reference interval for PK activity in pregnant women to be 70–130 μkat/L. We had first noticed that these data included some very low results, 10–60 μkat/L. These results were produced when too little care was given to specimen preparation. Blood specimens collected with sodium citrate were placed in a refrigerator (4 °C) for 8–16 h before being centrifuged. We speculate that the low results were the result of spontaneous cold activation of PPK to PK and the subsequent inactivation by anti-kallikreins as described by Kluft (6). We could determine which specimens were improperly processed by assaying each specimen with and without dextran sulfate/acetone (35 mL/dL) activation. Figure 13 represents data on 10 cases with low PK activity (11–59 μkat/L) after dextran sulfate/acetone activation. It was noted that "endogenous" PK activity of these same specimens without dextran sulfate/acetone treatment was high (8–30 μkat/L), suggesting prior activation of PPK. The properly processed specimens had low "endogenous" activity, usually <5 μkat/L. Once having instituted strict collection procedures without refrigeration of specimens before analysis, we no longer observed PK activity (after treatment with dextran sulfate/acetone) less than 70 μkat/L for pregnant women.

In our calculation of the plasma kallikrein activity we made at least two important assumptions. The first assumption relates to the molar absorptivity of p-nitroaniline, the second to the assumed value of the hemocrit.

The molar absorptivity for p-nitroaniline that we used was based upon the determination of the absorbance of a 50 μmol/L aqueous standard.

The hemocrit plays a direct role in the calculation of plasma kallikrein activity, because it affects the initial dilution of plasma. In our method whole blood is diluted 10-fold with sodium citrate solution. In our calculations, we assume a hemocrit of 40 mL/dL to be rather typical of the specimens that one would assay in the clinical setting. Because the citrate added to blood remains in the plasma, not penetrating the blood cells, if the hemocrit is 40 mL/dL the net effect would be to dilute the plasma six volumes of plasma with one volume of sodium citrate solution. For a hemocrit of 35 mL/dL we would have 6.5 volumes of plasma diluted with one of sodium citrate; for a hemocrit of 50 mL/dL it would be five volumes of plasma with one of citrate. To put it another way, given a hemocrit of 35 mL/dL, because we assumed a hemocrit of 40 mL/dL, we would have overestimated the kallikrein activity by 2%; given a hemocrit of 50 mL/dL, we would have underestimated the kallikrein activity by about 4%.

In summary, this procedure appears to be an excellent way to assay PK activity in many specimens, rapidly and efficiently, and with a high degree of precision and reproducibility. The adaptability of centrifugal analyzers also makes this instrument an excellent apparatus for other assays based on the use of chromogenic substrate.

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References


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