Arch Pharm Res Vol 25, No 4, 505-510, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

An Improved, Reliable and Practical Kinetic Assay for the Detection of Prekallikrein Activator in Blood Products

In Soo Shin, Yun Bo Shim, Choong Man Hong, Hyun Chul Koh[1], Seok Ho Lee, and Seung Hwa Hong

Blood Product Division. Korea Food & Drug Administration, Seoul 122-704, Korea and [1]College of Medicine, Hanyang University, Seoul, 133-791, Korea

(Received May 3, 2002)

An improved kinetic assay for prekallikrein activator (PKA), a potential vasodilator, has been developed to be used as an indicator for quality control during production of human albumin preparations. It consists of two reaction stages. In the first stage, PKA and prekallikrein are incubated at 37°C for 45 min to allow the transformation into kallikrein. Kallikrein, a serine protease, catalyzes the splitting of p-nitroaniline (pNA) from its substrate H-*D*-Pro-Phe-Arg-pNA (S-2302). The rate at which pNA is released was measured spectrophotometrically at 405 nm. Prekallikrein, a substrate of PKA was purified by DEAE ion-exchange chromatography and the major potential variations in the assay were optimized; pH 8.0 and 150 mM sodium chloride were chosen to give a proper ionic strength. Reaction times in the range of 10 to 360 min provided linear dose-response curves. The concentration of prekallikrein was adjusted to fall between 1:1 and 1:3 dilutions to generate a linear standard calibration curve. Under the optimized conditions, reproducibility was checked. In a precision test, the coefficient of variation (CV) stayed within ±4% and the dose-response curve showed a good correlation (r^2=0.999). An accuracy test with an international standard of PKA afforded a mean recovery of 97.5%.

Key words: Prekallikrein Activator, Hypotension, Kinetic Assay

INTRODUCTION

The factor XII (Hageman factor) is the zymogen of a serine protease; the active form of factor XII (factor XIIa) which initiates the sequence of enzymatic reactions leading to blood coagulation (Allen *et al.*, 2002; Kaplan, 1978; Revak *et al.*, 1974; Siv *et al.*, 1999). It is also the first component of the plasma kinin forming system. In plasma, factor XIIa, the prekallikrein activator (PKA), converts prekallikrein (PK) to kallikrein that in turn liberates the vasoactive peptide bradykinin from the high molecular weight kininogen. Kininogen circulates in blood as a complex with PK (Allen *et al.*, 2002; Domenico *et al.*, 1997 Judith *et al.*, 1997; Mandle *et al.*, 1976).

Human factor XIIa consists of two polypeptide chains joined by some disulfide bridges. It has an atomic mass of ca. 80,000 daltons, similar to that of the single chain factor XII molecule (Revak *et al.*; 1977; 1974). In addition, other active forms of factor XII activate PK readily, but have a diminished capacity in activating factor XI and, thereby, initiating the intrinsic coagulation pathway (Kaplan and Austen, 1970; Bagdasarian *et al.*, 1973; Radcliffe *et al.*, 1976). As one of these active forms, PKA has been characterized as a 28,000-dalton fragment resulting from a proteolytic cleavage of FXII by kallikrein, trypsin and factor XIa (Griffin and Austen, 1978; Kaplan, 1971). The occurrence of hypotension in patients receiving the therapeutic dose of plasma with high levels of PKA has been only sporadically reported (Alving *et al.*, 1980, 1982; Heinonen *et al.*; 1981). Although there was another report that PKA is not related to the hypotensive effect during or after the intravenous infusion of albumin solution (Turner *et al.*, 1987), PKA is considered as one of the important factors determining the safety of plasma fractionated products, such as human albumin and human immunoglobulin preparations. The specifications and guidelines regarding PKA were set by European Pharmacopiea (EP) in 1998, and the national authorities of the European Union regulate the level of PKA present in some human plasma products (EP, 1997). WHO has also issued a guideline on PKA and recommends that

Correspondence to: Seung Hwa Hong, Blood Products Division. Korea Food & Drug Administration, Seoul 122-704, Korea
Tel: 82-2-380-1711 Fax: 82-2-383-8322
E-mail: shhon8@kfda.go.kr

tests are performed in some human plasma products including human albumin preparation (WHO Technical Report Series, 1994).

PKA is measured using a radioactive-chemical esterolytic assay (Kaplan *et al.*, 1970). The kallikrein, liberated from a highly purified plasma PK by the action of PKA in samples or standards, is estimated by its esterolytic action on tritiated tosyl (p-toluenesulfonyl) arginine methyl ester (TAME) or other chromogenic substrates like S-2302 (Siv *et al.*, 1999). In general, the activity of PKA in plasma products has been measured indirectly by two stages of *in vitro* reaction. In the first stage, the samples containing PKA are incubated with PK that will be transformed into kallikrein. In the second stage, the amount of kallikrein is measured by its action on the synthetic substrate, S-2302 (H-D-Pro-Phe-Arg-ρNA), indirectly (Alving *et al.*, 1980; Heinonen *et al.*, 1982; Siv *et al.*, 1999; Snape *et al.*, 1979; Yasda *et al.*, 1981).

The understanding of the kinetics involved in the enzymatic reaction of PK to kallikrein has allowed us to establish sensitive analytical methods for the quantification of low levels of PKA in therapeutic plasma fractions. Accordingly, this study presents the results of the optimized, reproducible kinetic assay we have developed to measure the PKA activity. We present data on the background amidase activity, day-to-day assays, the coefficient of variation for measuring precision and accuracy and the recovery ratio from preparations of different levels of PKA.

MATERIALS AND METHODS

Materials

The chromogenic substrate S-2302 (H-D-Pro-Phe-Arg-ρNA 2HCl, mw 611.6) was purchased from Chromogenix, Italy. A 6mM stock solution was made by dissolving 25 mg of S-2302 in 6.8 ml of distilled water and was kept at 4°C. The working solution was diluted from this stock solution by making a 0.6 mM solution using 0.05 M Tris buffer containing from 0 to 0.15 M NaCl with various pH levels from 7.3 to 8.0 at 25°C. Several pH values from 7.3 to 8.5, and concentrations of NaCl from 0 to 0.2 M, in 0.05 M Tris buffer were studied in order to determine the optimal condition for the assay.

Preparation of prekallikrein

Prekallikrein (PK) was purified by a modified method described by Snape *et al* (1979). PK source, fresh human plasma, was partially purified from Korea Red Cross. The platelet deficient human plasma was dialyzed with 0.05 M Tris-HCl buffer (pH 8.0) containing 0.02 M NaCl and 0.05 M polybrene (buffer A). It was transferred to a 26 × 40 cm column of DEAE cellulose equilibrated with buffer A and PK was eluted with the same buffer at a flow rate of 0.5 ml per minute. The peak fraction was carefully pooled with A_{280} (UV/VIS array spectrophotometer DU 7500, Beckman, USA) exceeding 3 containing PK to give about the same volume as the dialyzed plasma. All procedures were carried out at room temperature.

Assay for PKA

In the first stage, 25 μl of various concentrations of the PKA-containing samples or standard dilution in 0.05 M Tris buffer (pH 8.0) including 0.15 M NaCl (dilution buffer) was added to 100 ml of PK substrate. Then the mixture was incubated at 37°C for 45 min. Before this addition the NaCl concentration of the PK substrate was adjusted to 150 mM, then diluted 1:2 or 1:3 using the dilution buffer. In the second stage, 1 ml of 0.6 mM S-2302 substrate solution was warmed to 37°C and added to the PKA-PK mixture. In order to measure the kinetics, the change of optical density at 405 nm was monitored after a short lag time (a few seconds) from 2 to 10 minutes. The measurements were carried out using a programmed, 6-channel, UV/VIS array spectrophotometer (DU 7500, Beckman, USA).

Background amidase activity

The background amidase activity, which is present in the plasma products towards S-2302, was measured and subtracted from the total kallikrein activity for compensation. The assay was performed in exactly the same way as the PKA assay except for the replacement of PK substrate by dilution buffer.

PKA standard curves

The variable ΔA per minute was plotted against the PKA standard (the first international standard of PKA, 85 IU per ml, NIBSC, UK) at concentrations of 4, 9, 13, 17, 26 and 35 I.U. per ml to make a standard curve. The curve was obtained at each assay using freshly prepared standard solutions.

RESULTS

Preparation of prekallikrein

In order to prepare highly concentrated prekallikrein (PK), the kallikrein activity of the carefully pooled fractions was determined. In brief, 25 μl of pooled fraction was mixed with 1 ml of S-2302 (working solution), warmed in advance to 37°C, and the mixture was checked to measure the rate of absorbance change at 37°C for at least 2 minutes. As a result, the rate of absorbance change at

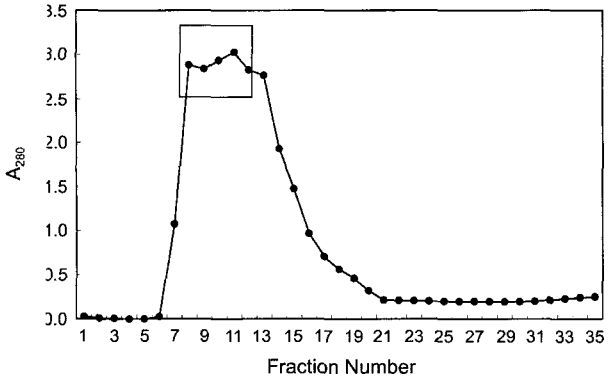

Fig. 1. Typical pattern of PK fraction in A_{280}. The fraction inside the rectangle was pooled and assayed for kallikrein activity (blank) with S-2302.

405 nm was calculated as ΔA per minute. To minimize any bias from PK substrates, the pooled fractions whose ΔA was not more than 0.001 per minute were stored at -70°C. A typical chromatogram is presented in Fig. 1.

Effect of pH and ionic strength

The pH dependence of the activation was studied over a pH range from 7.3 to 8.5 at three different NaCl concentrations (0.02, 0.05 and 0.15 M). These experiments were carried out with a single batch of PK. At all three ionic concentrations, maximal activity was seen at a pH of 8.0 (Fig. 2). The ionic strength of the medium appears to have a strong effect on the activation of PK and/or S-2302 hydrolysis. Plasma fractionated products (blood products) are in different buffers and must often be analyzed under undiluted conditions. If the ionic strength of the sample is higher than that of the standard, the PKA assay result often gives a false result. The activations of PKA were determined in 0.05 M Tris buffer (pH 8.0) containing

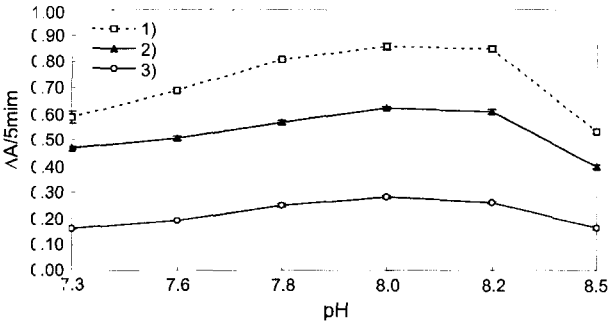

Fig. 2. Rate of S-2302 hydrolysis (ΔA/5 min) as a function of pH in 0.05 M Tris buffer containing: 1) 20 mM, 2) 50 mM and 3) 150 mM, NaCl. Assay: 25 μl of 17 I.U./ml PKA and 100 μl of PK were incubated for 45 min at 37°C. After 1000 μl of 0.6 mM S-2302 was added, the absorbance was measured for 5 min at 37°C. Dilutions were made in the appropriate buffers.

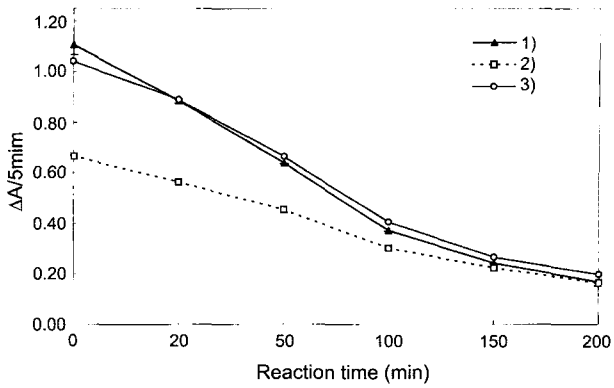

Fig. 3. PKA activity as a function of NaCl concentration in 0.05 M Tris buffer pH 8.0. Assay: 25 μl of PKA in, 1) the Tris buffers containing NaCl concentrations shown at the abscissa, 2) 40 mM sodium caprylate in 100 mM NaCl and 3) 300 mM glycine at pH 6.8. Incubated with 100 μl of PK (diluted 1:2 in the appropriate buffers) at 37°C for 45 min. After 1000 μl of 0.6 mM S-2302 was added, and ΔA was followed for 5 min at 37°C.

various concentrations of NaCl (0 to 0.2 M). PKA activities were measured for three samples, each containing 17 I.U./ml of PKA diluted in different buffer (Fig. 3). The highest PKA-catalyzed reaction rate of PK was observed under 0.05 M Tris buffer (pH 8.0) without added NaCl, but the results between samples (1, 3 and 2) showed some differences (CV=20.7%). At high NaCl concentrations, the deviation between samples was gradually reduced and the variation coefficient of 0.15 M NaCl was much less (CV=7.1%). This assay method should be available for the detection of PKA in various blood products, including human albumin preparation, because it produces the consistent results under various conditions including additives and pH levels of products. Consequently, 0.15 M NaCl was chosen as the optimal concentration of choice for this assay.

PK-PKA Reaction Time

The activation rates of PK to kallikrein at various concentrations of PKA (4~35 IU per ml) were studied. The hydrolysis of PK was measured continuously for 5~365 min at various concentrations of PKA. The plot of PKA concentration rate against time was a straight line (r^2=0.97), the slope of which was proportional to the rate of kallikrein generation (Fig. 4). Although little variation in result was observed in the initial 10 min reaction period, quite reproducible results were obtained after 45 min. These results imply that insignificant technical or temporal differences in the initial stage of the reaction appear to exert a strong effect on the final results. Therefore, the reaction time was set at 45 min for the best results.

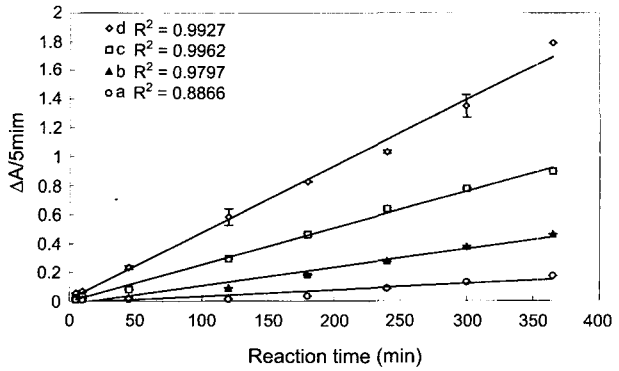

Fig. 4. The rate of activation of PK to K at different concentrations of PKA (a: 4, b: 9, c: 18, and d: 35 I.U./ml) was studied. PK substrate was incubated with PKA at 37°C for 5 to 365 min. Assay: 25 μl of PKA in 0.05 M Tris buffers containing 0.150 M NaCl at pH 8.0 (dilution buffer) was incubated with 100 ml of PK (diluted 1:2 with the dilution buffer) at 37°C for the appropriate reaction time. After 1000 μl of 0.6 mM S-2302 was added, ΔA was followed for 5 min at 37°C.

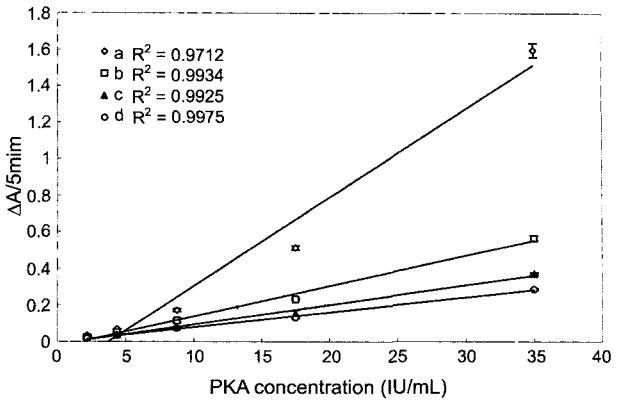

Fig. 5. The rate of activation of PK to K at different concentrations of PK dilution rate (a: no dilution, b: 1:1, c: 1:2, and d: 1:3 to dilution buffer) was studied. Assay: 25 μl of PKA in dilution buffer was incubated with 100 μl of appropriately diluted PK at 37°C for 45 min. After 1000 μl of 0.6 mM S-2302 was added, ΔA was followed for 5 min at 37°C.

PK concentration

In order to optimize the concentration of PK, the concentration of NaCl was adjusted to 0.15 M with a buffer containing Tris 0.05 M and NaCl 2 M at pH 8.0, then diluted 1:1, 1:2 and 1:3 with dilution buffer containing Tris 0.05 M and NaCl 0.15 M at pH 8.0. The linearity of standard curves was compared (Fig. 5). The value for r^2 with no dilution was somewhat reduced to 0.971, demonstrating that it is out of the range of the Beer-Lawhert Law. However, 1:1 to 1:3 diluted PK showed good linearity ($r^2 \geq 0.993$).

Reproducibility

Day-to-Day assays (Precision): We performed day-to-day assays using 4, 9, 13, 17, 26 and 35 I.U./ml of PKA for 7 days (0, 1st, 2nd, 4th, and 7th days). The correlation factor was 0.999 and the daily dose-response curves showed linearity, r^2=0.999 (Fig. 5). The data was re-arranged to confirm the precision of dose-response curves and the CV of 35~9 IU/ml of PKA fell within 4% (Table 1).

Detection limit: To calculate the detection limit, we used t-value (Dunnett's) for statistical analysis. The PKA range was adjusted to 1, 2, 4, 9, 13, 17, 26 and 35 I.U. per ml with dilution buffer. All test ranges showed the CV within 15%, and the upper range of dose showed CV within 3% and r^2 =0.999 (Table 2).

Recovery tests (Accuracy): To confirm the accuracy of the assay, recovery was measured by spiking the PKA standard to commercial human albumin 30% solution (ICN, USA). The standard solutions of PKA were adjusted to 4, 9, 13, 17, 26 and 35 I.U. per ml with the dilution buffer to fall within the range of the predetermined dose-response curves. After the PKA standard had been spiked to the albumin 1:1 to give up to 22 I.U. per ml,

Table I. Precision of the dose-response curve for PKA assay

PKA concentration (I.U./ml)	35	26	17	13	9	4
Mean ΔA/10 min	0.6823 ± 0.02759	0.5149 ± 0.02056	0.3193 ± 0.01418	0.2338 ± 0.00539	0.1593 ± 0.00688	0.0708 ± 0.00923
CV%	4.0	4.0	4.4	2.3	4.3	13.0
t-value (Dunnett's)	99.1	74.8	46.4	34.0	23.1	10.3

where, n=12 at each concentration of the PKA standard, and critical t = 2.669, when df = 55, p = 0.01, and r^2 = 0.999.

Table II. Detection limit of the dose-response curve for PKA quantification

PKA concentration (I.U./ml)	35	26	17	13	9	4	2	1
Mean ΔA/min	0.0842 ± 0.00189	0.0752 ± 0.0012	0.0358 ± 0.0009	0.0295 ± 0.0005	0.0146 ± 0.0003	0.0064 ± 0.0005	0.0032 ± 0.0004	0.0016 ± 0.0002
CV%	2.2	1.6	2.4	1.8	1.8	8.2	12.7	14.3
t-value (Dunnett's)	233.1	208.4	99.0	81.7	40.3	17.7	8.79	4.53

where, all tests were performed with quantification, critical t = 2.492, when df = 24, p = 0.01, and r^2 = 0.999

Table III. Reproducibility of PKA assay

Sample	lot 1			lot 2		
PKA (I U./ml)	21.48	10.48	6.05	21.46	10.66	5.87
CV%	1.5	3.4	3.8	0.9	2.2	2.8
Recovery (%)	94.6	92.2	106.6	94.5	93.9	103.3

The PKA standard was spiked to 22 I.U./ml of 1/2 diluted ICN 30% human albumin solution, then further diluted to 1/4 and 1/8. PKA concentration was subtracted, with both blank values and sample values, at each dilution point.

further serial dilution was performed. The average recovery was 97.5%. The CV fall was within 4% over the entire tested range (Table 3).

DISCUSSION

It has been demonstrated that PKA in an infusion solution is a causative agent of hypotension (Alving *et al.*, 1978; Domenico *et al.*, 1997; Heinonen *et al.*, 1982, 1981; Judith *et al.*, 1997; Rob *et al.*, 1982). A linear correlation between the PKA content in an infusion solution and hypotension in patients has also been demonstrated (Heinonen *et al.*, 1982, 1981). Therefore, it is very important to establish a simple, convenient and reproducible assay to detect PKA in infusion solutions. To optimize the assay conditions, the effects of pH, ion strength, PK and reaction time were studied.

Firstly, it appears that ion strength affects the generation of kallikrein and chromogenic signal more strongly than pH (Tankersley *et al.*, 1980). In 150 mM of NaCl, relatively small levels of PKA were observed upon the changes of buffers. EP also recommends 150 mM ionic strength adjusted with NaCl in the dilution buffer. Hence, we decided to employ the optimal pH of 8.0 and the ionic strength of NaCl 150 mM in the dilution buffer. Secondly, the reaction time showed good linearity with tested conditions, indicating that PK was sufficiently supplied during the tested reaction time. Because the relatively high salt concentration interferes with the production of kallikrein during the first stage of assay, it seems that the amount of PK remained abundant in the reaction tube during the tested reaction time. It has been demonstrated that human plasma products may contain several enzymes that can hydrolyze S-2302 such as kallikrein and plasmin (Alving *et al.*, 1980; Kuwahara, 1980; Lüben *et al.*, 1981; Snape *et al.*, 1979). In PKA assay it is essential to exclude the influence of these and possibly other enzymes, as well as the color or slight turbidity caused by the sample. In the present assay, this was done by using blank assay without PK substrate for each sample and standard, although the large number of blank assays showed poor linearity for ΔA per min (<0.001).

In the reproducibility test, this assay demonstrated both precision and accuracy. In day-to-day assays, the constant variation was small and the detection limit was below 1 I.U. per ml with the statistical model. However the CV was somewhat raised in 4 I.U. per ml and the mean recovery of PKA was 97.5%. With its high degree of reproducibility and linearity, we consider that the assay can be used in quality control of albumin, even though it was accepted that the albumin preparation generally has a low content of PKA (Kerry *et al.*, 1982). For validation of this test method, further research with tests of albumin preparations in related laboratories is needed. As a validation test for PKA assay, a comparison of end-point assay and an investigation of immuno-globulin preparations are also required.

REFERENCES

Allen, P., Kaplan, M. D., Kusumam, J. and Michael, S., Pathways for bradykinin formation and inflammatory disease. *J. Allerg. Clin. Immun.*, 109(2), 195-209 (2002).

Alving, B. M., Hojima, Y., Pisano, J. J., Mason, B. L., Buckingham, R. E., Mozen, M. M. and Finlayson, J. S., Hypotension associated with prekallikrein activator (Hageman-factor fragments) plasma protein fraction. *N. Engl. J. Med.*, 229, 66-70 (1978).

Alving, B. M., Tankersley, D. L., Mason, B. L., Rossi, F., Aronson, D. L. and Finlayson, J. S., Contact-activated factors: contaminants of immunoglobulin preparations with coagulant and vasoactive properties. *J. Lab. Clin. Med.*, 96, 334-346 (1980).

Bagdasarian, A., Talamo R. C., and Colman, R. W., Simple assay for prekallikrein activators. *Immunol. Commun.*, 2(1), 93-103 (1973).

Council of Europe. European Pharmacopoeia, 3rd edition; p. 95, (1997).

Domenico, R., Anna, R., and Girolamo, C., Pharmacology of the kallikrein-kinin system. *Pharmacol. Res.*, 35(6), 513-515 (1997).

Friberger, P., Gnos, M., Gustavsson, S., Laurell, L. and Claeson, G., A new specific substrate for the determination of plasmin activity. In Scully, M. F. and Kakkar, V. V. (Eds.): *Chromogenic peptide substrates: chemistry and clinical usage.* Churchill Livingstone, Edinburgh London and New York, pp. 121-127, (1978).

Griffin, J. H., Role of surface in surface-dependent activation of Hageman factor (blood coagulation factor XII). *Proc. Natl. Acad. Sci.*, 75(4), 1998-2002 (1978).

Hannele T. and Suomela. H., An optimized assay for prekallikrein activator activity in human plasma products. *Thromb. Res.*, 27(1), 35-44 (1982).

Heinonen, J., Peltola, K., Himberg, J. J. and Suomela, H., Vasodilator effect of plasma protein fraction correlates with

prekallikrein activator activity. *Ann. Thorac. Surg.*, 33(3), 244-249 (1982).

Heinonen, J., Peltola, K., Himberg, J.J., and Suomela, H., Hypotensive effect of prekallikrein activator (PKA) in plasma protein fraction (PPF). *Develop. Biol. Standard*, 48, 129-130 (1981).

Judith, C., Annie, M., Shen, Y. and Andrew, H., Kallikreins and kinins in inflammatory-like events in the reproductive tract. *Pharmacol. Res.*, 35(6), 537-540 (1997).

Kaplan, A. P. and Austen, K. F., A prealbumin activator of prekallikrein. II. Derivation of activators of prekallikrein from active Hageman factor by digestion with plasmin. *J. Exp. Med.*, 133(4), 696-712 (1971).

Kaplan, A. P., and Austen, K. F., A pre-albumin activator of prekallikrein. *J. Immunol.*, 105(4), 802-811 (1970).

Kaplan, A. P., Initiation of the intrinsic coagulation and fibrinolytic pathways of man: the role of surfaces, Hageman factor, prekallikrein, high molecular weight kininogen, and factor XI. *Prog. Hemost. Thromb.*, 4, 127-75 (1978).

Kaplan, A. P., Kay, A. B. and Austen, K. F., A prealbumin activator of prekallikrein. 3. Appearance of chemotactic activity for human neutrophils by the conversion of human prekallikrein to kallikrein. *J. Exp. Med.*, 135(1), 81-97 (1972).

Kerry, P. J., Curtis, A. D., Catherine, J., and Thomas, D. P., Standardization of prekallikrein activator (PKA): the 1st British Reference Preparation for PKA. *British J. Haematol.*, 52, 275-281 (1982).

Kuwahara, S. S., Prekallikrein activator (Hageman factor fragment) in human plasma fraction. *Transfusion*, 20, 433-439 (1980).

Lüben, G., Quast, U. and Geiger, H., Prekallikrein activator levels and side effects with human albumin preparations. *Develop. Biol. Standard*, 48, 123-127 (1981).

Lundbland, J. L., *In vitro* assay for prekallikrein activator (PKA). *Develop. Biol. Standard*, 44, 107-114 (1979).

Mandle, R. J., Colman, R. W., and Kaplan, A. P., Identification of prekallikrein and high-molecular-weight kininogen as a complex in human plasma. *Proc. Natl. Acad. Sci.*, 73(11), 4179-4183 (1976).

Marley, P. B. and Gilbo, C. M., Temperature sensitivity within the pasteurization temperature range of prekallikrein activator in stable plasma protein solution (SPPS). *Transfusion*, 21, 320-324 (1981).

McMillin, C. R., Saito, H, Ratnoff, O. D., and Walton, A. G., The secondary structure of human Hageman factor (factor XII) and its alteration by activating agents. *J. Clin. Invest.*, 54(6), 1312-1322 (1974).

Radcliffe, R. Nemerson, Y. and Emerson, Y., Mechanism of activation of bovine factor VII. Products of cleavage by factor Xa. *J. Biol. Chem.*, 251(16), 4749-802 (1976).

Revak, S. D., Cochrane, C. G., and Griffin, J. H., The binding and cleavage characteristics of human Hageman factor during contact activation. A comparison of normal plasma with plasmas deficient in factor XI, prekallikrein, or high molecular weight kininogen. *J. Clin. Invest.*, 59(6), 1167-75 (1977).

Revak, S. D., Cochrane, C. G., Johnston, A. R. and Hugli, T. E., Structural changes accompanying enzymatic activation of human Hageman factor. *J. Clin. Invest.*, 54(3), 619-27 (1974).

Rob, F., Van, R., Joa, C. B., Donald, M. S., Johan, D. and Jan. A. V. M., Bradykinin-mediated hypotension after infusion of plasma-protein fraction. *J. Lab. Clin. Med.*, 100, 288-295 (1982).

Siv, F., Nils-Ove, H., Halvard G. and Kjell, B., Contact activation factors in plasma from women on estrogen replacement therapy after ovariohysterectomy. *Thrombosis Research*, 93, 161-170 (1999).

Snape, T. J., Griffith, D., Vallet, L. and Wesley, E. D., The assay of prekallikrein activator in human blood products. *Develop.Biol. Standard*, 44, 115-120 (1979).

Tankersley, D. L., Fournel, M. A. and Schroeder, D. D., Kinetics of activation of prekallikrein by prekallikrein activator. *Biochemistry*, 19, 3121-3127 (1980).

Turner, P. J., Young, I. F., Marley, P. B., Herrington, R. W. and Schiff, P., Albumin solutions- Their production and quality control. *Develop. Biol. Standard*, 67, 119-127 (1987).

World Health Organization Technical Report Series. Control of albumin and plasma protein fraction. No. 840, Annex 2, Part B. 14. pp. 80-85 (1994).

Yasda, J., Ueno, G., Kuratsuka, K., Homma, R., Hara, S., Kazama, M. and Ito, H., Japanese minimum requirements for albumin preparations: recent amendments and current problems. *Develop. Biol. Standard.*, 48, 143-152 (1981).