Human Coagulation Factor V Purification and Thrombin-catalyzed Activation

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ABSTRACT Factor V was isolated from human plasma by barium citrate adsorption, polyethylene glycol fractionation, DEAE-Sepharose CL-6B chromatography, ammonium sulfate fractionation, and gel chromatography on Ultrogel 22. Degradation of Factor V during purification was largely prevented by ample use of inhibitors of proteolytic enzyme. The purified Factor V was a stable, single-chain molecule with an apparent molecular weight of 330,000. Activation of human Factor V by thrombin resulted in a 10- to 15-fold increase in activity. The activation pattern as monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis was compared with that of bovine Factor V. Differences in the patterns of thrombin activation were noticed between the two species, whereas the final products were similar. The products of human Factor V activation are two closely spaced doublets, one with an apparent molecular weight of ~110,000, and the other, ~72,000. An antibody was raised against the purified protein. Crossed immunoelectrophoresis showed that the antibody recognized Factor V both before and after activation with thrombin.

INTRODUCTION

Factor V is a high molecular weight plasma protein. It is an essential, nonenzymatic, component of the prothrombinase complex, which also comprises phospholipid, calcium ions, and the serine protease Factor X_a (1). Factor V also occurs in platelets and is released when platelets are stimulated by thrombin, for example (2, 3). When released from the platelets it binds to the platelet surface, forming part of the binding site for Factor X_a (4–8).

Bovine Factor V is more stable than its human counterpart, for which reason most of our knowledge derives from studies using bovine material (9–14). However, only recently was bovine Factor V purified to homogeneity, by Nesheim et al. (12) and by Esmon (13). These investigators stressed the importance of using quick separation techniques and of the addition of abundant protease inhibitors throughout the purification procedure. The Factor V obtained was a single-chain protein with a molecular weight of 330,000 (12). Well-defined proteolytic cleavages with concomitant increase in Factor V activity occurred on incubation with a catalytic amount of thrombin (13, 14).

Human Factor V is reportedly extremely labile and only a few attempts have been made to isolate it (9). In 1975, Rosenberg et al. (15) described a four-step procedure including isoelectric precipitation, hydroxylapatite batch adsorption and elution, polyethylene glycol fractionation, and DEAE-cellulose chromatography. The isolated protein gave a single band on isofocusing gel electrophoresis but was not characterized by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. When incubated with thrombin, the Factor V activity increased only twoto threefold, suggesting that the final product was partially degraded. More recently, Bolhuis et al. (16) described a procedure including cryoprecipitation, polyethylene glycol fractionation, gel filtration on Ultrogel 44 (LKB Produkter AB, Bromma, Sweden), and adsorption of contaminating haptoglobin to immobilized hemoglobin. It was, however, not unambiguously demonstrated that the isolated protein was indeed Factor V, e.g., that the protein was cleaved by thrombin quantitatively. In our laboratory the procedure described has not yielded a pure Factor V preparation.

This paper reports a simple method for isolating a stable, apparently undegraded form of human Factor V. The molecular weight of the isolated protein was identical with that of bovine Factor V. The activation of human Factor V by thrombin resulted in a 10- to 15-fold increase in Factor V activity. The activation products were characterized by SDS-polyacrylamide gel electrophoresis. An antibody against human Factor V was also raised.

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METHODS

DEAE-Sepharose CL-6B was obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Ultrogel 22 was from LKB Produkter AB, Bromma, Sweden, and agarose from Marine Colloids Inc., Springfield, N. J. Bovine serum albumin, soybean trypsin inhibitor, phenylmethanesulfonyl fluoride (PMSF)[1] and diisopropylphosphofluoridate (DFP) were from Sigma Chemical Co., St. Louis, Mo. Ortho brain thromboplastin was from Ortho Diagnostics, Inc., Raritan, N. J. Polyethylene glycol (PEG), with the mean molecular weight of 6,000, (PEG-6,000) was from Kebo-Grave AB, Malmö, Sweden.

Bovine as well as human thrombin was prepared essentially according to Lundblad et al. (17). They had fibrinogen clotting activities of 2,500 and 2,300 National Institutes of Health U/mg, respectively, when tested according to Fenton and Fasco (18).

Factor V assay

Factor V-deficient plasma was prepared as described (19). Factor V activity was determined by the method of Kappeler (20). The assay was performed in a Fibrometer Coagulation Timer (BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, Md.). The sample (0.1 ml) was incubated with 0.1 ml Factor V-deficient plasma and 0.1 ml brain thromboplastin at 37°C. After exactly 30 s, 0.1 ml 25 mM calcium chloride was added and the clotting time measured. Samples were diluted in 0.036 M sodium acetate, 0.036 M sodium barbitone, 0.14 M NaCl pH 7.4, containing 1% bovine serum albumin. Pooled human citrated plasma, stored in aliquots at −70°C, was used as a source of Factor V in the preparation of standard curves. 1 U of Factor V was defined as the activity found in 1 ml of the pooled plasma.

Antisera

Antisera against the purified Factor V were raised in rabbits. Approximately 100 μg of protein was diluted to a final volume of 1 ml in 0.9% NaCl and emulsified in Freund's complete adjuvant. Rabbits were immunized by subcutaneous injections, and booster doses with the same amount of antigen were given every 3rd wk until the response was satisfactory. Monospecific antisera against human albumin and prothrombin were available at the laboratory.

Electrophoretic and immunochemical methods

Agarose gel electrophoresis was run at pH 8.6 in 0.075 M barbital buffer containing 2 mM calcium lactate (21). Crossed immunoelectrophoresis and electroimmunoassay were performed as described (22, 23). SDS-polyacrylamide disc gel electrophoresis was done in gels containing 5% acrylamide (24).

Amino acid analysis

The amino acid composition of human Factor V was determined in acid hydrolysates (24 and 72 h in 6 M HCl at 110°C *in vacuo*) with standard procedures using a single-column program on a Kontron amino acid analyzer with a Durrum resin (DC4A, Durrum Chemical Corp., Palo Alto, Calif.). Half-cystine was determined as cysteic acid after performic acid oxidation (25).

Purification of Factor V

All manipulations of the samples were performed on an ice bath; chromatographies and centrifugations were run at 4°C.

Blood collection. Human plasma was obtained from the local blood bank. Approximately 400 ml blood was collected in 63 ml CPD-adenine (Travenol Laboratories, Inc., Deerfield, Ill.). After separation of blood cells by centrifugation at 5,000 *g* for 10 min the plasma was kept at 4°C for at most 1 h before the purification of Factor V was started. Freshly frozen plasma (−70°C) was occasionally used as starting material. The following protease inhibitors were added to the plasma: benzamidine hydrochloride (1 mM), soybean trypsin inhibitor (50 mg/liter), DFP (0.5 mM), and PMSF (0.5 mM).

Barium citrate adsorption. 80 ml of 1 M $BaCl_2$ was added dropwise per liter of plasma. After the mixture had been stirred for 1 h, the barium citrate was removed by centrifugation at 6,000 *g* for 10 min.

PEG-6,000 fractionation. To the supernate from the previous step solid PEG-6,000 was added (80 g/liter). After stirring for 1 h the precipitate was removed by centrifugation at 6,000 *g* for 10 min. 40 g of solid PEG-6,000 was then added per liter of supernate. The solution was stirred for 1 h, after which the precipitate was collected by centrifugation at 6,000 *g* for 10 min and the supernate was rejected.

DEAE-Sepharose chromatography. The PEG-precipitated material was dissolved in 200 ml ice-cold 50 mM Tris-HCl, 0.1 M NH_4Cl pH 7.5 containing benzamidine hydrochloride (1 mM), DFP (1 mM), PMSF (1 mM), and 10 mg soybean trypsin inhibitor. The dissolved pellet was deposited on a column (5 × 20 cm) of DEAE-Sepharose CL-6B (Fig. 1) Factor V

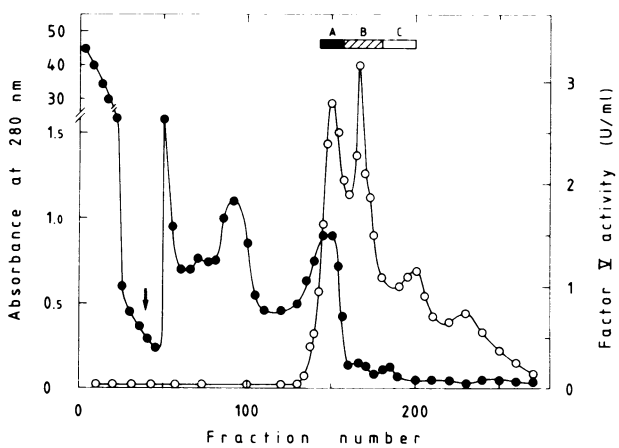

FIGURE 1 DEAE-Sepharose CL-6B chromatography of the 8–12% PEG-6,000 fraction. The column (5 × 20 cm) was equilibrated in 50 mM Tris-HCl, 0.1 M NH_4Cl pH 7.5, and 1 mM benzamidine hydrochloride. After application of the sample, the column was washed with the equilibration buffer at a rate of 150 ml/h for 5–6 h (only the last part of the breakthrough protein peak is shown). Factor V was then eluted with a linear gradient of NH_4Cl (0.1–0.35, 1.15 liter per vessel) in the equilibration buffer containing 10 mM calcium chloride. The flow rate was 150 ml/h and 10-ml fractions were collected. ●, absorbance at 280 nm; ○, Factor V activity. The arrow indicates where the gradient was started. Fractions were pooled as indicated by the horizontal bars.

[1] *Abbreviations used in this paper*: DFP, diisopropylphosphofluoridate; PEG, polyethylene glycol; PEG-6,000, polyethylene glycol (6,000 mol wt); PMSF, phenylmethanesulfonyl fluoride.

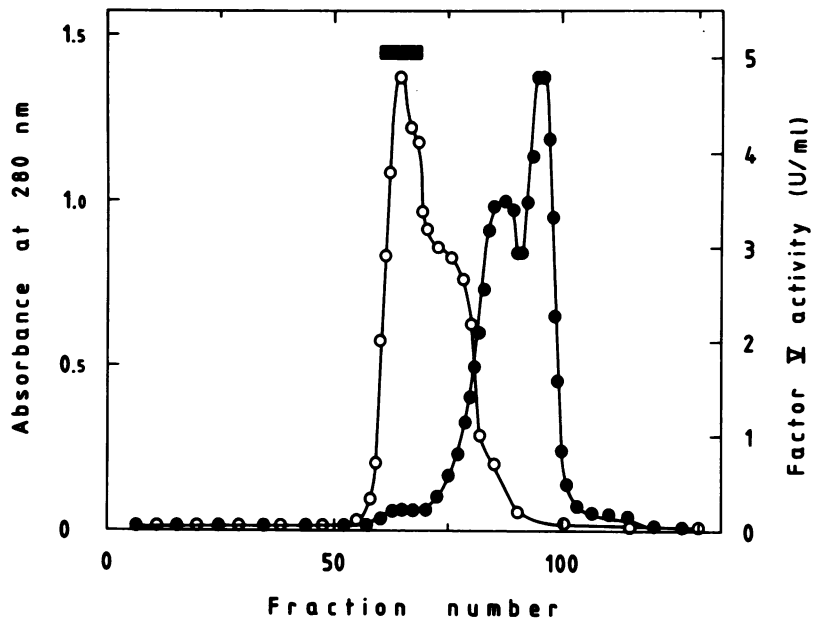

FIGURE 2 Gel chromatography on Ultrogel 22. The ammonium sulfate-precipitated DEAE-Sepharose pools A to C were separately applied to a column (1.6 × 91, 1.6 × 84, and 1.6 × 84 cm, respectively) with Ultrogel 22 in 50 mM Tris-HCl (pH 7.5), 0.1 M NH_4Cl, 10 mM calcium chloride, and 1 mM benzamidine hydrochloride. Fractions of 1.43 ml were collected at a flow rate of 5.7 ml/h. The figure shows the chromatography of pool A (Ultrogel A). ●, absorbance at 280 nm; ○, Factor V activity. Fractions were pooled as indicated by the horizontal bar.

activity was measured, pooled fractions were kept on an ice bath, and Factor V was precipitated by solid ammonium sulfate (60% saturation).

Gel filtration on Ultrogel 22. The precipitates from the previous step were collected by centrifugation at 10,000 *g* for 15 min and dissolved in a minimal volume of 50 mM Tris-HCl, 0.1 M NH_4Cl, 10 mM calcium chloride pH 7.5 containing 1 mM DFP, 1 mM PMSF, and 1 mM benzamidine hydrochloride. The samples were applied to a column of Ultrogel 22 (Fig. 2). The fractions containing Factor V activity were pooled as indicated in the figure and dialyzed overnight on an ice bath against 25 mM Tris-HCl, 0.05 M NH_4Cl, 5 mM calcium chloride pH 7.5 containing 0.5 mM benzamidine hydrochloride, and 50% glycerol. The protein solutions concentrated by the dialysis by a factor of three to four were then stored at −20°C.

Bovine Factor V was prepared essentially with the method described above.[2] The major difference between the two species was the limit of precipitation by PEG-6,000, which was 5–10% for bovine Factor V. Furthermore, the yield was higher (25–30%). The isolated bovine Factor V was undegraded and homogeneous on SDS–5% polyacrylamide gel electrophoresis.

Activation of Factor V by thrombin

Factor V (0.1–0.3 mg/ml) in 50% glycerol, 25 mM Tris-HCl, 50 mM NH_4Cl pH 7.5, 0.5 mM benzamidine hydrochloride, 5 mM calcium chloride was kept on an ice bath. Bovine or human thrombin (stock solution of 3,000 and 650 U/ml, respectively) was then added to a final concentration of 2.5 U/ml. Samples of the incubate (100–200 μl) were removed at various intervals and added to an equal volume of 50 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol. No further proteolysis of Factor V, as judged by SDS-polyacrylamide gel electrophoresis, could be detected in this buffer. Aliquots were also drawn, diluted 1,000–100,000 times in the Factor V assay buffer and immediately assayed for Factor V activity. After 20 min incubation on an ice bath, the reaction temperature was raised to 37°C to accelerate the activation process, and after another 10–15 min additional thrombin was added (2.5 U/ml).

RESULTS

Comments on the purification of Factor V. Because human Factor V activity in plasma is known to be very labile, probably because of its sensitivity to proteolytic enzymes, the isolation was performed at 0°–4°C without interruption. Several protease inhibitors were added throughout the purification. The isolation procedure was designed to avoid lengthy dialysis and concentration steps, as summarized in Table I.

Citrated human plasma, kept at 37°C, rapidly lost its Factor V activity, and after 6 h it had ~30% of that originally found, while bovine plasma kept under identical conditions still had ~60–70%. When freshly frozen plasma was used as starting material without added DFP, the final product contained not only undegraded Factor V, but also several lower molecular weight forms representing partially degraded Factor V. The barium citrate adsorption of plasma was included to remove the vitamin K-dependent proteins and reduce the risk of formation of small amounts of

[2] B. Dahlbäck and J. Stenflo, unpublished results.

TABLE I
Purification of Factor V

Fraction	Volume	Total protein	Total Factor V activity	Specific activity	Purification	Recovery
	ml	*Absorbance units at 280 nm*	*U*	*U/ml/A_{280}*	*X-fold*	%
Plasma	1,770	101,000	3,800	0.038	1	100
Barium citrate supernate	1,800	100,000	3,000	0.03	0.8	79
8–12% PEG-6,000 precipitate	200	12,720	1,800	0.14	3.7	47
DEAE-Sepharose CL-6B						35
Pool A	148	115	444	3.9	102	
Pool B	250	39	650	16.7	439	
Pool C	208	27	250	9.3	244	
Ultrogel 22						6
A	15	1.05	54	51	1,350	
B	11.5	1.04	104	100	2,630	
C	11.5	0.51	58	114	3,000	

thrombin or other active coagulation factors during subsequent steps. When this step was excluded, the limits of precipitation of Factor V in the following PEG-6,000 fractionation were higher (~12–18% instead of 8–12%) and electroimmunoassay showed a considerable amount of prothrombin (30–50% of that in plasma) in the dissolved precipitate. The PEG-6,000 precipitation gave a fourto fivefold purification and rapidly reduced both the volume and the total amount of protein. The dissolved precipitate proved suitable for direct application to the DEAE-Sepharose CL-6B column (Fig. 1). This step was concluded ~16–20 h after the blood had been collected and produced a 30-fold (pool A) to 120-fold (pool B) purification with a recovery of ~60%. Factor V was bound very firmly to the column and was eluted late in the gradient. Factor V precipitated by 60% ammonium sulfate at this stage of purity could be stored at 4°C for weeks without any significant loss of activity. Pools A to C from the DEAE-Sepharose were separately chromatographed on a column with Ultrogel 22 (abbreviated Ultrogel A to C, see Table I). The chromatogram obtained when DEAE-Sepharose pool A was applied to the column with Ultrogel 22 is shown in Fig. 2. DEAE-Sepharose pools B and C gave chromatograms similar to that of pool A. After dialysis against glycerol/ buffer and 4 mo of storage at −20°C no loss of Factor V activity or degradation was noticed, as judged by SDS-polyacrylamide gel electrophoresis. The specific activities in the best Factor V preparations were 50–100 U/ml per absorbance unit at 280 nm, and on incubation with thrombin they were as high as 500–1,500 U/ml per absorbance unit at 280 nm.

Characterization of Factor V by SDS-polyacrylamide gel electrophoresis. The Factor V obtained after chromatography on Ultrogel 22 of DEAE-Sepharose pool A was nearly homogeneous (Fig. 3). Analysis on 10% gels showed no additional protein bands. The molecular weight of human Factor V was estimated relative to that of bovine Factor V, which is reported to be 330,000 (12). Before and after reduction of disulfide bridges, mixed human and bovine Factor V appeared as a single band on 5% polyacrylamide gel electrophoresis, with SDS indicating also that the molecular weight of human Factor V was 330,000. Factor V obtained after chromatography on Ultrogel 22 of DEAE-Sepharose pools B and C contained several electrophoretic components with lower molecular weight (components B and C, Fig. 3). Like single-chain Factor V, these bands disappeared completely upon activation with a catalytic amount of thrombin,

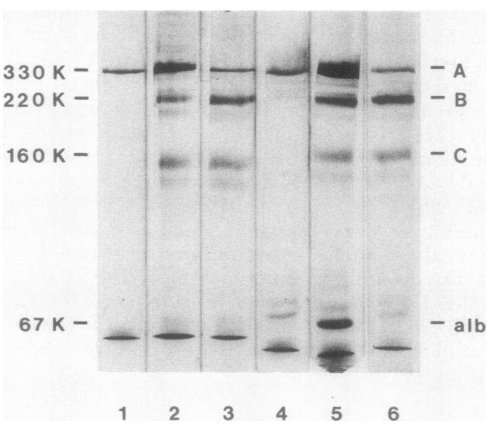

FIGURE 3 SDS–5% polyacrylamide gel electrophoresis of human Factor V obtained from Ultrogel A to C. Samples 1–3 were unreduced and 4–6 reduced. The relative molecular weights of the components are indicated at the left. Samples 1 and 4, from Ultrogel A; samples 2 and 5, Factor V from Ultrogel B; samples 3 and 6, Factor V from Ultrogel C. Approximately 15 μg protein was loaded on gels 1, 3, 4, and 6 and 25–30 μg protein on gels 2 and 5. The tracking dye is seen as a band at the bottom of each gel.

indicating that they represented partially degraded Factor V. In Ultrogel B and C a small amount of Factor V activity was eluted earlier than the main Factor V peak. On SDS-polyacrylamide gels, this material gave a pattern identical with that of the protein of the mean peak in the same column, and probably represented an aggregated form of Factor V.

Occasionally the Factor V preparation contained a contaminant, which was rendered on reduced gels only (component alb in Fig. 3), and had an apparent molecular weight of 66,000 after reduction of disulfide bridges. On agarose gel electrophoresis it migrated like albumin, to which it is also immunochemically related. It was probably albumin molecules linked together by disulfide bridges. In most preparations this polymeric albumin was not visible, and in no other preparation was it as obvious as in Factor V from Ultrogel B, shown in Fig. 3.

Amino acid composition. The amino acid composition of human Factor V is shown in Table II, together with the composition reported for bovine Factor V. The composition of our bovine Factor V preparation (not shown) was in good agreement with that reported by Nesheim et al. (12). The carbohydrate content of human Factor V was assumed to be identical to that of bovine Factor V (12), which gives a molecular weight of the apoprotein of 279,000. The major difference in amino acid composition between the two species was the higher half-cystine content in human Factor V. This difference was found in two preparations.

TABLE II
Amino Acid Composition of Human Factor V

Amino acid*	Human	Bovine‡
Aspartic acid	252	299
Threonine§	161	121
Serine§	245	204
Glutamic acid	305	293
Proline	144	199
Half-cystine	83	24
Glycine	161	146
Alanine	142	131
Valine‖	135	108
Methionine	61	43
Isoleucine‖	102	124
Leucine	252	240
Tyrosine	81	94
Phenylalanine	91	88
Lysine	139	148
Histidine	62	71
Arginine	108	110
Total	2,524	2,443

* Composition expressed as residues per molecule assuming a molecular weight of the apoprotein of 279,000.
‡ Data from Nesheim et al. (12). The composition of bovine Factor V is shown for comparison.
§ Extrapolated to zero-time hydrolysis.
‖ 72-h hydrolysis value.

Activation of Factor V by thrombin. The conclusion that the products of the purification procedure were indeed Factor V was based on the high specific activity of the protein and on the finding that activation with both human and bovine thrombin resulted in complete conversion of the protein to lower molecular weight products. Catalytic amounts of thrombin were used, and the reaction mixtures were kept on an ice bath to facilitate identification of early activation intermediates. After 10 min the temperature was increased to 37°C, and after another 10 min an additional aliquot of thrombin was added. The Factor V activity was followed and the reaction products were examined with SDS-polyacrylamide gel electrophoresis (Fig. 4). The pattern obtained was compared with that of thrombin-catalyzed activation of bovine Factor V, and the nomenclature of Nesheim and Mann (14) was used. Activation of human Factor V by human and by bovine thrombin gave identical patterns on SDS-polyacrylamide gels. The high molecular weight form of Factor V (component A) was quickly cleaved even at the low initial temperature, and electrophoretic components A_1 and D (and to some extent, also component B) were formed with a concomitant fivefold increase in Factor V activity. Component D, which on reduced gels appeared as a closely spaced doublet, was stationary throughout the incubation and appeared to be an end product. After increasing the temperature to 37°C there was an additional twoto threefold increase in activity. Component A_1 (and B) disappeared at the same time as component E and F appeared. The gel pattern was the same when the reaction temperature was 37°C throughout the experiment, although early intermediates were less easily seen.

Thrombin activation of Factor V obtained from the more heterogeneous Ultrogel B fraction was also followed to determine whether the electrophoretic components B and C, which were not easily observed during activation of intact Factor V, were indeed related to Factor V. Both bands B and C disappeared during the activation, and it therefore appears that these components were partially degraded Factor V. During the activation of intact Factor V, electrophoretic component B was seen as a faint band, whereas component C, in our system, could not be demonstrated as an intermediate. After completed activation the patterns were identical whether homogeneous high molecular weight Factor V or partially degraded Factor V was used. The Factor V activities found are also shown in Fig. 4. The increase in Factor V activity during thrombin activation was the same whether Factor V from Ultrogel A or B was used, whereas the more degraded Factor V from Ultrogel C increased only two- to threefold in activity.

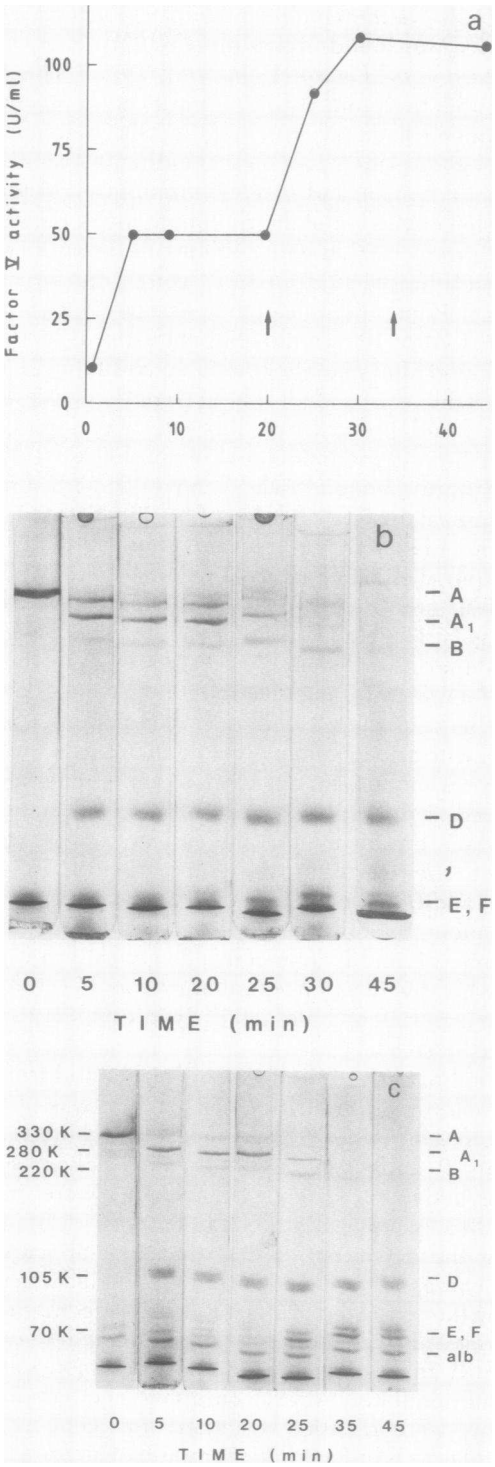

FIGURE 4 Activation of intact human Factor V by thrombin. Human Factor V from Ultrogel A (10 U/ml) was incubated with bovine thrombin (2.5 U/ml). The reaction mixture was kept on an ice bath; after 20 min (first arrow) it was equilibrated at 37°C, and after 34 min (second arrow) an additional aliquot of thrombin (2.5 U/ml) was added. At the times indicated aliquots were removed and subjected to SDS–5% polyacrylamide gel electrophoresis. (a) Factor V activity, (b) gels with unreduced samples, and (c) reduced samples are shown. Components and relative molecular weights are indicated. Approximately 15 μg protein was loaded on each gel. The tracking dye is seen as a band at the bottom of each gel.

Thrombin-catalyzed activation of bovine Factor V was also studied (Fig. 5) to facilitate comparison of the products with those of human Factor V. In the experiment presented in Fig. 5 the reaction temperature was 37°C. No difference in the pattern of thrombin activation could be detected if the reaction mixture initially was kept on an ice bath. Our activation pattern of bovine Factor V seemed identical to those on record (13, 14), and the nomenclature of Nesheim and Mann (14) was adopted. There were distinct differences between the two species in the activation pattern, although the final products were similar. As previously mentioned, the dominating intermediate in human Factor V activation was component A_1, whereas in the activation of bovine Factor V, components B and C were predominant. These components closely resembled the additional electrophoretic components B and C in partially degraded human Factor V. The cause of this difference between the activation of human Factor V in the pure system and of the proteolysis that had occurred during the purification is not clear, but it may be due to cleavage of human Factor V by some protease other than thrombin during purification.

The molecular weight of single-chain bovine Factor V has been determined by Nesheim et al. (14) to 330,000 by sedimentation equilibrium analyses. When the logarithms of this value and the reported molecular weights of the bovine Factor V activation products (14) were plotted against the relative mobilities (on reduced SDS-5% polyacrylamide gels) of the corresponding protein bands, a straight line was obtained. This plot was used as molecular weight standard curve in the determination of the apparent molecular weights of the human components (Table III).

Following activation of human Factor V by thrombin, aliquots were removed and stored at 22°, 4°, and at −20°C to determine the stability of the factor V_a activity. No differences in stability between the aliquots stored at the three temperatures could be noticed. After 24, 48, and 72 h of storage ~70, 30, and 7%, respectively, of the original Factor V_a activity was retained. A parallel aliquot, 20 mM in EDTA and stored at 4°C, retained 30% of its activity after 3 h, 7% after 24 h, and 1% after 48 h, indicating that human Factor V_a, like bovine Factor V_a (13), requires calcium ions to retain its biological activity.

Immunochemical studies. Crossed immunoelec-

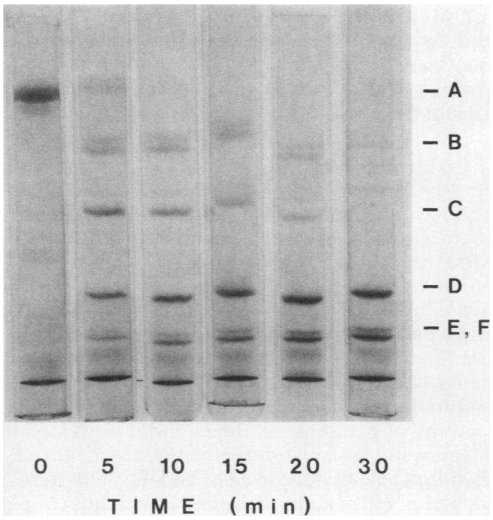

FIGURE 5 SDS–5% polyacrylamide gel analysis of the activation of bovine Factor V by thrombin. Bovine Factor V (40 U/ml) was incubated with bovine thrombin (2.5 U/ml) at 37°C. After 17 min an additional aliquot of thrombin (2.5 U/ml) was added. At various intervals samples were drawn, reduced, and subjected to SDS–5% polyacrylamide gel electrophoresis. Approximately 20 μg protein was loaded on each gel. The tracking dye is seen as a sharp band at the bottom of each gel.

trophoresis of Factor V was run both before and after activation by thrombin (Fig. 6). Before activation, Factor V migrated to a position just behind the α_1-antitrypsin band, whereas after activation it migrated more anodally. The surface under the immunoprecipitate was larger after activation by thrombin, a finding suggesting that the activated protein had lost antigenic determinants. When plasma was analyzed by electroimmunoassay with the antiserum against Factor V, a rocket-like precipitate could clearly be seen, whereas it was faint and barely visible when serum was used.

TABLE III
Molecular Weight of Human Factor V and Its Activation Products

Component	Bovine Factor V		Human Factor V
	Nesheim and Mann	Esmon	
		mol wt	
A	330,000	280,000	330,000
A_1			280,000
B	205,000	210,000	220,000
C	150,000	Not reported	160,000
D	94,000	115,000	110,000
E	74,000	73,000	74,000
F	71,000		71,000

The apparent molecular weights of human Factor V and its activation products were determined by electrophoresis on 5% SDS-polyacrylamide gels after reduction of disulfide bridges. The bovine Factor V activation products with the molecular weights given by Nesheim and Mann (14) were used to construct the calibration curve. The data of Esmon (13) are also included for comparison.

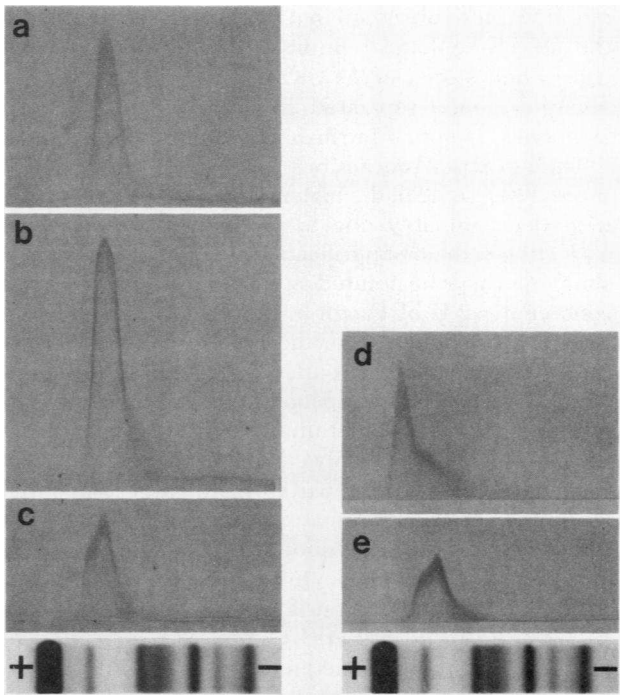

FIGURE 6 Crossed immunoelectrophoresis of human Factor V. Factor V from Ultrogel C (*a*), Factor V from Ultrogel B (*b*), Factor V from Ultrogel A (*c*), and human Factor V from Ultrogel A after (*d*) and before (*e*) activation with human thrombin (2.5 U/ml) at 37°C are shown.

DISCUSSION

The extreme lability of human Factor V in plasma, used in preparing Factor V-deficient plasma by aging, is the main reason why isolation procedures yielding an undegraded product have not been reported previously (9, 15). The lability can presumably be explained by the high susceptibility of Factor V to proteolysis during blood collection as well as during the initial purification steps. The successful purification of the somewhat more easily handled bovine Factor V (12, 13) drastically improved the prospects of successful purification of human Factor V. The isolation procedure for human Factor V described in this paper uses fresh plasma, several protease inhibitors, and a low temperature (0°–4°C). The initial steps are quick and performed without interruption. The final product is stable for >4

mo. It was also observed that the Factor V activity after the DEAE-Sepharose chromatography was stable for days when stored at 4°C. This indicates that human Factor V, once separated from proteases and their zymogens, is not an intrinsically labile protein.

Though strict precautions were taken to minimize proteolysis, some of the material obtained was partially degraded, probably due to proteolysis during blood collection or the first purification steps. In this context it should perhaps be pointed out that the starting material contained ~2 U of Factor V per milliliter, indicating that when obtained from the blood bank the plasma Factor V was already partly activated. If the local conditions allow human blood to be collected directly into anticoagulant containing protease inhibitors, the final product will probably contain a smaller amount of degraded Factor V. The partially proteolyzed material was eluted from the ionic exchange column at higher salt concentration than undegraded Factor V, as has also been reported for bovine Factor V (13).

The final yield of the purification was low. This may have been caused in part by premature activation of Factor V and rapid subsequent loss of activity. When bovine Factor V was isolated with essentially the same procedure, the yield was higher and comparable to those previously reported (12, 13). Furthermore, there were no partially degraded forms of the bovine factor.

The activation of human Factor V, followed by SDS-polyacrylamide gel electrophoresis, differed from that of bovine Factor V. Thus, component A_1 could not be demonstrated on bovine Factor V activation; and component C, although present in the partially degraded human Factor V, was apparently not an intermediate in the activation of human Factor V in our system. The final activated products were, however, similar. Human component D, which on reduced SDS-polyacrylamide gels appeared as a doublet, has a somewhat higher molecular weight (110,000) than bovine component D (94,000) (12), whereas human and bovine components E and F have virtually identical molecular weights (74,000 and 71,000, respectively).

The possibility of isolating human Factor V will advance our understanding of the role played by human Factor V in the prothrombinase complex and as part of the platelet Factor X_a receptor. Furthermore, the availability of a monospecific antibody will be useful in the development of methods to supplement the conventional clotting assay in the evaluation of various disorders affecting the coagulation system.

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