Purification and Characterization of Human Coagulation Factor V*

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We have purified human coagulation Factor V, and gel filtration on Ultrogel.

Human Factor V is a single polypeptide chain before and after disulfide bond reduction with an apparent $M_r = 335,000$ as determined by electrophoresis on 5% acrylamide sodium dodecyl sulfate gels. Human Factor V is a glycoprotein containing 15% by weight carbohydrate and there is a high content of sialic acid (88 residues/mol) compared to the other sugars.

When human Factor V is treated with thrombin, coagulation activity increases 25- to 30-fold to a specific activity of 1.7 to 2.0 units/$\mu$g. Thrombin activation is accompanied by the cleavage of three bonds in the Factor V molecule. We have detected activation intermediates with apparent $M_r = 285,000$ and $248,000$, and final products with apparent $M_r = 150,000, 121,000$, and a doublet at 95,000-91,000 as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The final products of thrombin activation of human Factor V and bovine Factor V are similar, yet the intermediates observed are different. This suggests that cleavages are made at similar locations in bovine and human Factor V, but that they occur in a different sequence.

When human Factor V is treated with the Factor V activator from Russell's viper venom, it is split into two components with apparent $M_r = 303,000$ and 95,000-91,000 and is fully activated. The increase in coagulation activity observed upon treatment of human Factor V with thrombin or the Factor V activator from Russell's viper venom seems to correlate with the generation of the doublet $M_r = 95,000-91,000$ component.

Factor V is a blood coagulation factor necessary for normal hemostasis. Patients who lack Factor V and those who acquire Factor V inhibitors have moderate to severe bleeding problems.

**EXPERIMENTAL PROCEDURES**

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The abbreviations used are: SDS, sodium dodecyl sulfate; V-CP, Factor V-activating enzyme from Russell's viper venom; PEG 6000, polyethylene glycol 6000; PAS reagent, periodic acid-Schiff reagent. The "Experimental Procedures" are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9560 Rockville Pike, Bethesda, Maryland 20014. Request Document No. 80M-1054, cite author(s), and include a check or money order for $1.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
Purification of Human Factor V—As was true for bovine Factor V (4), the keys to the successful purification of human Factor V were: 1) rapid purification steps performed without interruption; 2) inclusion of calcium in buffers to stabilize Factor V; and 3) the use of benzamidine and diisopropylfluorophosphate to prevent proteolysis of Factor V. We have purified human Factor V approximately 6000-fold (see Table 1) with a 21% yield based on coagulation assay. Ten milligrams of homogenous Factor V were obtained from 4 liters of fresh frozen citrated plasma. The isolation of Factor V was complete within 12 h after the plasma was collected. The unique step in our Factor V isolation is based on the finding that human Factor V is adsorbed to barium citrate in the presence but not in the absence of 11% polyethylene glycol 6000. Thus, we achieved a 33% purification of Factor V after this step.

After adding sodium chloride to a final concentration of 0.1 M, the Factor V eluted from barium citrate was applied to a DEAE-Sepharose column (Fig. 1). Fractions from the trailing edge of the activity peak were found to contain degraded Factor V, so these fractions were omitted from the elution pattern. This step resulted in a 16-fold purification with a 40% recovery of activity. Bolhuis et al. (14) obtained poor recoveries of Factor V activity on Sephadex G-200, but found that acrylamide-agarose-based resins (Ultrogel) allowed good recoveries of activity. We used Ultrogel AcA 34 and found that Factor V eluted in the void volume of the column. The high concentration of benzamidine made it impossible to determine protein concentration by absorbance measurements. To determine the protein elution profile for this step, we equilibrated an AcA 34 column with buffer containing 1 mM benzamidine and applied a Factor V sample eluted from DEAE-Sepharose corresponding to about one-third the amount shown in Table 1 (Fig. 2). Although chromatography in 1 mM benzamidine increased the amount of degraded factor when compared to SDS-gel electrophoresis, it did illustrate the separation of Factor V from contaminants. The AcA 34 pool from a column run with 10 mM benzamidine was concentrated to a 2-ml volume and stored at −70°C. Purified Factor V stored in this manner was stable for at least 3 months as judged by total activity and SDS-gel electrophoresis. Loss of Factor V activity due to adsorption is a problem at concentrations less than 200 μg/ml. This can be prevented by the inclusion of 5 mg/ml of bovine serum albumin in samples.

Analysis of Human Factor V by SDS-Polyacrylamide Gel Electrophoresis—Human Factor V is a single-chain poly peptide with an apparent Mr = 355,000 as determined by electrophoresis on 3% acrylamide-SDS gels before and after disulfide bond reduction (see Fig. 4, zero time point gel). In addition to staining with Coomassie blue, Factor V stains with PAS reagent, indicating that it is a glycoprotein (data not shown). The apparent molecular weight for human Factor V derived from SDS-gel electrophoresis is an estimate since Factor V is a glycoprotein (20). However, this molecular weight is similar to the values reported for bovine Factor V. Nesheim et al. (4) found a Mr = 330,000 in sedimentation equilibrium experiments, and Esmen (5), who used SDS-polyacrylamide gel electrophoresis, reported a value of 290,000.

Amino Acid and Carbohydrate Composition—The amino acid and carbohydrate composition of our human Factor V preparation is compared with the composition for bovine Factor V reported by Nesheim et al. in Table II. The compositions are similar except that human Factor V has a higher half-cystine and phenylalanine content and lower proline content compared to bovine Factor V.
Amino acid benzamidine. The units on the vertical axis are: Absorbance at separation of Factor V from contaminant proteins, the column (2
mined on samples diluted 10- to 100-fold from which the corresponding dilution of buffer was subtracted.

In order to illustrate the column was eluted with buffer containing
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Carbohydrate
Factor V. We find that human Factor V has an unusual carbohydrate composition. The presence of mannose and N-acytlygalactosamine indicates that both N- and O-linked oligosaccharides are present (29). Most remarkable is the large amount of sialic acid present. This was seen in two different Factor V preparations. Nesheim et al. (4) reported 65 mol of N-acetylgalactosamine/330,000 g of bovine Factor V, while we found only 20 mol of N-acetylgalactosamine/335,000 g of human Factor V. It is possible that part of this difference is due to incomplete hydrolysis of N-acetylgalactosamine on our part since our hydrolysis conditions were milder than those employed by Nesheim et al. (4).

Extinction Coefficient—We determined the concentration of Factor V using the synthetic boundary method of Babul and Stellwagen (26). We assumed a refractive increment of 4.1 fringes/mg of glycoprotein/ml. The results obtained when amino acid and carbohydrate composition data were used to calculate the Factor V concentration were in good agreement with the concentration measured in the ultracentrifuge. The extinction coefficient, E280, was calculated to be 8.9.

Activation of Factor V by thrombin—When purified human Factor V was incubated with catalytic amounts of thrombin, there was a 25- to 30-fold increase in activity as measured by coagulation assay. In several experiments the specific activity of thrombin-activated Factor V ranged from 1.7 to 2.0 units/µg. Concomitant with the increase in clotting activity was the disappearance of the M1 = 335,000 component and the appearance of several new lower molecular weight components on SDS gels. In order to study the mechanism of activation of Factor V by thrombin, we slowed the reaction by lowering the temperature to 4°C. When human Factor V is incubated with 2 units of human thrombin/ml, there is a gradual increase in clotting activity (Fig. 3). After a 15-min incubation with thrombin at 4°C Factor V, activity had increased 5.5-fold. At 15 min the reaction temperature was raised to 37°C and the activation process was rapidly completed. In this experiment Factor V activity increased 29-fold; the fully activated Factor V had a specific activity of 1.9 units/µg.

Factor V activated under the same conditions was analyzed by SDS-polyacrylamide gel electrophoresis. In the experiment shown in Fig. 4, samples were reduced with 2-mercaptoethanol and the gels were stained with Coomassie blue. Identical gel

Both human and bovine Factor V are glycoproteins containing 13% and 12% carbohydrate, respectively. Detailed carbohydrate composition data is not available for bovine Factor V. We find that human Factor V has an unusual

| Component       | Human g | Bovine g |
|-----------------|---------|---------|
| Aspartic acid   | 259     | 299     |
| Threonine       | 156     | 121     |
| Serine          | 266     | 204     |
| Glutamic acid   | 317     | 293     |
| Proline         | 134     | 199     |
| Glycine         | 149     | 146     |
| Alanine         | 140     | 131     |
| Half-cystine    | 47      | 24      |
| Valine          | 117     | 108     |
| Methionine      | 45      | 43      |
| Isoleucine      | 150     | 124     |
| Leucine         | 336     | 240     |
| Tyrosine        | 150     | 94      |
| Phenylalanine   | 132     | 88      |
| Histidine       | 73      | 71      |
| Lysine          | 157     | 148     |
| Arginine        | 134     | 110     |
| Tryptophan      | 30      | 21      |
| Fucose          | 8       | ND      |
| Mannose         | 31      | ND      |
| Galactose       | 38      | ND      |
| N-Acetylgalactosamine | 15 | ND |
| N-Acetylgalactosamine | 20 | 65 |
| Sialic acid     | 86      | ND      |
| Protein (%)     | 86      | 88      |
| Carbohydrate (%)| 13      | 12      |

* This study. Composition expressed as residues/335,000 g of glycoprotein (residues/290,000 g of amino acids).
* Data taken from Nesheim et al. (4). Composition expressed as residues/329,000 g of glycoprotein (residues/290,000 g of amino acids).
* Determined as cystic acid according to Hirs (21).
* Determined by the method of Edelhoch (22).
* Determined by the method of Liu and Chang (34).
* ND, Not determined.
* Determined from amino acid analysis.
* Determined by the method of Warren (25).

FIG. 1 (left). Chromatography on DEAE-Sepharose. The units on the vertical axis are: A, absorbance at 280 nm; B, Factor V activity; C, NaCl concentration. See "Methods" for details. A280 was determined on samples diluted 10- to 100-fold from which the A280 of a corresponding dilution of buffer was subtracted.

FIG. 2 (center). Gel filtration on Ultrogel AcA 34 in 1 mM benzamidine. The units on the vertical axis are: A, Absorbance at 280 nm; B, Factor V activity. During the purification of Factor V this column was eluted with buffer containing 10 mM benzamidine. This concentration of benzamidine makes it impossible to estimate protein concentration by absorbance measurements. In order to illustrate the separation of Factor V from contaminant proteins, the column (2 x

95 cm) was equilibrated at 4°C with 0.15 M NaCl/20 mM Tris, pH 7.4/5 mM CaCl2 containing 1 mM benzamidine. This experiment. A280 was determined on samples diluted 10-fold from which the A280 of a 10-fold dilution of buffer was subtracted.

FIG. 3 (right). Activation of Factor V by thrombin. Factor V (200 µg/ml) in a buffer containing 0.15 M NaCl/20 mM Tris, pH 7.4/5 mM CaCl2/2 mM benzamidine was equilibrated at 4°C. Thrombin (2 units/ml) was added and at the indicated times samples were taken, diluted 20,000- to 40,000-fold, and immediately assayed. At 15 min, the activation mixture was equilibrated at 37°C to accelerate the activation process to completion.

Table II

Comparison of amino acid and carbohydrate composition of human and bovine Factor V

| Component       | Human g | Bovine g |
|-----------------|---------|---------|
| Aspartic acid   | 259     | 299     |
| Threonine       | 156     | 121     |
| Serine          | 266     | 204     |
| Glutamic acid   | 317     | 293     |
| Proline         | 134     | 199     |
| Glycine         | 149     | 146     |
| Alanine         | 140     | 131     |
| Half-cystine    | 47      | 24      |
| Valine          | 117     | 108     |
| Methionine      | 45      | 43      |
| Isoleucine      | 150     | 124     |
| Leucine         | 336     | 240     |
| Tyrosine        | 150     | 94      |
| Phenylalanine   | 132     | 88      |
| Histidine       | 73      | 71      |
| Lysine          | 157     | 148     |
| Arginine        | 134     | 110     |
| Tryptophan      | 30      | 21      |
| Fucose          | 8       | ND      |
| Mannose         | 31      | ND      |
| Galactose       | 38      | ND      |
| N-Acetylgalactosamine | 15 | ND |
| N-Acetylgalactosamine | 20 | 65 |
| Sialic acid     | 86      | ND      |
| Protein (%)     | 86      | 88      |
| Carbohydrate (%)| 13      | 12      |

* This study. Composition expressed as residues/335,000 g of glycoprotein (residues/290,000 g of amino acids).
* Data taken from Nesheim et al. (4). Composition expressed as residues/329,000 g of glycoprotein (residues/290,000 g of amino acids).
* Determined as cystic acid according to Hirs (21).
* Determined by the method of Edelhoch (22).
* Determined by the method of Liu and Chang (34).
* ND, Not determined.
* Determined from amino acid analysis.
* Determined by the method of Warren (25).
patterns were obtained when 2-mercaptoethanol was omitted. During the 4°C incubation the component with an apparent $M_r = 335,000$ disappears, and three new components appear with apparent $M_r = 295,000$, 248,000, and 121,000. The first two are intermediates in the activation process in that they are not present in fully activated Factor V. The $M_r = 121,000$ band is a final product of the activation process. After 1 min of incubation with thrombin at 4°C, only the $M_r = 295,000$ and $M_r = 121,000$ bands have become visible. At 3 min, the $M_r = 248,000$ band appears. When the temperature is shifted to 37°C, the $M_r = 295,000$ band, and later, the $M_r = 248,000$ band disappear. Coincident with the disappearance of the $M_r = 295,000$ and $M_r = 248,000$ bands is the appearance of a doublet component, with apparent $M_r = 95,000$ and 91,000. The production of the $M_r = 95,000-91,000$ doublet seems to coincide with the appearance of most of the Factor V activity. In some activation experiments the $M_r = 121,000$ component also appears as a doublet. All of the bands which stained with Coomassie blue also stained with PAS reagent. In addition another band appeared in gels that were stained with PAS reagent that was not visible in Coomassie blue-stained gels. This band appeared to be a final product of Factor V activation. It had an apparent $M_r = 190,000$ (actually assumed to be 150,000, see below) on 5% acrylamide-SDS gels. These results indicate that all of the fragments which result from thrombin activation of Factor V contain carbohydrate.

Glycoproteins have been reported to give anomalously high apparent molecular weight values when subjected to electrophoresis in SDS on polyacrylamide gels (20). Segrest and Jackson found that this anomalous behavior decreases when increasing concentrations of acrylamide are used. We examined the migration of the products of thrombin-activated Factor V on 5, 7.5, 10, and 12.5% acrylamide-SDS gels. No change in apparent molecular weight was noted for the $M_r = 121,000$ and $M_r = 95,000-91,000$ components when the acrylamide concentration was increased. However, the $M_r = 190,000$ component reached a minimum apparent $M_r = 150,000$ as the acrylamide concentration was increased. In addition, no new low molecular weight Coomassie blue- or PAS reagent-staining activation components were identified on the higher percentage of acrylamide gels. When Factor V is activated by thrombin at 22°C, the results obtained are identical with those shown in Fig. 4 except that both the $M_r = 295,000$ and $M_r = 248,000$ components are seen 1 min after thrombin is added. When Factor V from the trailing edge of the activity peak on DEAE-Sepharose was further purified on AcA 34 and analyzed by SDS-polyacrylamide gel electrophoresis, three components were seen. The first had an apparent $M_r = 335,000$ and corresponded to intact Factor V. The other components had apparent $M_r = 248,000$ and 150,000. When this preparation was treated with thrombin, all three high molecular weight components disappeared and the products and specific activity were identical with fully activated Factor V. The $M_r = 150,000$ component present here stained well with Coomassie blue, distinguishing it from the one detected during activation of intact Factor V with thrombin, which only stained with PAS reagent. The $M_r = 248,000$ component may correspond to the $M_r = 248,000$ component seen during thrombin activation of intact Factor V. The Coomassie blue-staining $M_r = 150,000$ component is not seen during activation of intact Factor V. These additional components may represent Factor V cleaved during the purification process by a protease other than thrombin.

Activation of $^{125}$I-labeled Factor V by Thrombin—In order to more firmly establish that the $M_r = 150,000$ PAS-positive component was a final product of Factor V activation, and to look for additional components that do not stain with Coomassie blue or PAS reagent, we treated $^{125}$I-labeled Factor V with thrombin. Fig. 5 shows the radiochromatogram of a 7.5% SDS-polyacrylamide gel of thrombin-activated $^{125}$I-labeled Factor V. The peak in gel slice 6 corresponds to the $M_r = 150,000$ component. The peak in slice 9 corresponds to the $M_r = 121,000$ component. The peak in slice 13 represents the $M_r = 95,000-91,000$ doublet which is not resolved in the radiochromatogram. The peaks in slices 21 and 24 were present in the $^{125}$I-labeled Factor V preparation before treatment with thrombin and probably represent highly radiolabeled contaminants. Activation time courses of $^{125}$I-labeled Factor V incubated with thrombin showed patterns identical with those shown in Fig. 4 except that the $M_r = 150,000$ component was visible as a final product. The appearance of the $M_r = 150,000$ and $M_r = 95,000-91,000$ components seemed to correspond with the disappearance of the $M_r = 248,000$ component. Analysis of $^{125}$I-labeled Factor V on SDS gels with acrylamide concentrations ranging from 5 to 12.5% disclosed no other components not previously detected.

Activation of Factor V by V-CP—V-CP is an enzyme isolated from Russell's viper venom which activates Factor V (7). When Factor V was incubated with 10 µg/ml of V-CP at 37°C, Factor V activity increased 26-fold within 10 min to a specific activity of 1.8 units/µg. Coagulation activity was not further increased by the addition of 2 units/ml of thrombin. In the experiment shown in Fig. 6, we activated Factor V with 10 µg/ml of V-CP at 37°C and examined the cleavage patterns by SDS-polyacrylamide gel electrophoresis. By 15 min the $M_r = 335,000$ component had disappeared and was replaced by two components, one with apparent $M_r = 303,000$ and the other, a doublet, with apparent $M_r = 95,000$ and 91,000. The $M_r =$
FIG. 6. Activation of Factor V by V-CP. Factor V (200 μg/ml) in a buffer containing 0.15 M NaCl/20 mm Tris/ pH 7.4/5 mm CaCl₂/2 mM benzamidine was equilibrated at 37°C. Then 10 μg/ml of V-CP were added and at the indicated times samples were taken and boiled in a buffer containing sodium dodecyl sulfate and 5% 2-mercaptoethanol for 2 min. At 15 min, the activation mixture was equilibrated at 4°C. At 20 min, 2 units/ml of thrombin were added. At 30 min, the activation mixture was again equilibrated at 37°C. Samples containing 10 μg of Factor V were electrophoresed on 5% polyacrylamide gels containing sodium dodecyl sulfate with a 4% stacking gel according to Laemmli (19). The position of the tracking dye is marked with India ink.

95,000-91,000 component seems to be identical with the \( M_r = 95,000-91,000 \) component obtained by activation with thrombin. When the temperature was lowered to 4°C and 2 units/ml of thrombin were added, the high molecular weight doublet rapidly disappeared and the \( M_r = 121,000 \) component appeared. Raising the temperature to 37°C did not further alter the gel pattern.

**DISCUSSION**

Purified human Factor V preparations have been reported by Rosenberg et al. (13) and Bolhuis et al. (14). Rosenberg et al. did not characterize their product by SDS-polyacrylamide gel electrophoresis. Bolhuis et al. found that their preparation was a single component with \( M_r = 300,000 \) when electrophoresed on 5% polyacrylamide gels, but these workers did not show samples reduced with 2-mercaptoethanol. Using their method we have found that the high molecular weight component seen on SDS-polyacrylamide gels without 2-mercaptoethanol disappears upon reduction and is therefore unrelated to intact Factor V. Our human Factor V appears as a single high molecular weight polypeptide chain on SDS-polyacrylamide gels in the presence and absence of 2-mercaptoethanol. Comparing the specific activities of thrombin-activated Factor V, we estimate that the previously reported human Factor V preparations were less than 10% pure. Our preparation is activated 25- to 30-fold when treated with thrombin. Previous human Factor V preparations were activated only 2- to 3-fold (13) and 8-fold (14), suggesting that they also contained Factor Vα.

Human and bovine Factor V have similar mobilities upon electrophoresis on 5% polyacrylamide-SDS gels (data not shown). Nesheim et al. (4) found the molecular weight of their purified bovine Factor V preparation to be 330,000 as determined by sedimentation equilibrium and sedimentation velocity studies. Using this value for the molecular weight of our bovine Factor V preparation, we estimate an apparent \( M_r = 335,000 \) for human Factor V. Bartlett et al. (30) reported molecular weights between 800,000 and 1,000,000 for purified bovine Factor V and partially purified human Factor V. However, the gel filtration method employed by these workers assumes that Factor V is a globular protein. Ittyerah et al. (31, 32) have proposed that bovine Factor V is composed of two subunits: an h subunit (\( M_r = 290,000 \)) which is similar to the component reported by Esmon (5) and Nesheim et al. (4), and an \( l_2 \) subunit which is a disulfide-linked dimer with \( M_r = 400,000 \). We do not find any component resembling the latter in our human Factor V preparation. Human Factor V is a glycoprotein containing 13% carbohydrate. There is an unusually large amount of sialic acid present in human Factor V. This suggests the possibility of a novel, highly sialated oligosaccharide structure(s) (29). Whether sialic acid residues have any importance in the function of Factor V remains to be determined.

When human Factor V is activated by thrombin, we observe two components that are activation intermediates and three components that are final products. In Table III these components, identified by their apparent molecular weights, are compared with those components observed during the activation of bovine Factor V by thrombin. The correspondence between bovine and human peptides seems likely but has not yet been rigorously established.

There are three bonds in the Factor V molecule cleaved by thrombin, one of which is also cleaved by V-CP. At 4°C, thrombin forms the final product with \( M_r = 121,000 \) and the intermediate of \( M_r = 295,000 \) components. At a slower rate the \( M_r = 295,000 \) intermediate disappears and the \( M_r = 248,000 \) component becomes apparent.

After warming to 37°C we observe the progressive formation of \( M_r = 150,000 \) and \( M_r = 95,000-91,000 \) components. When bovine Factor V is treated with thrombin, the molecule is split initially into two components with apparent \( M_r = 205,000 \) and 150,000 (6). Both of these components stain with Coomassie blue and are intermediates in that they are not present in fully activated bovine Factor V. The human \( M_r = 248,000 \) component seen during thrombin activation of Factor V may correspond to the \( M_r = 205,000 \) component of Nesheim and Mann (6) and the \( M_r = 220,000 \) component of Esmon (5). This assumption is supported by the fact that Esmon has shown that the bovine Factor V, \( M_r = 220,000 \) component can be cleaved by V-CP to yield the \( M_r = 140,000 \) and \( M_r = 73,000 \) components (7). We find that the disappearance of the human \( M_r = 248,000 \) component during thrombin activation of Factor V correlates with the appearance of the \( M_r = 150,000 \) component that does not stain with Coomassie blue and the \( M_r = 95,000-91,000 \) doublet. The \( M_r = 295,000 \) component which is seen as an intermediate during the activation of human Factor V does not seem to correspond to any component observed during the activation of bovine Factor V.

The components seen in partially degraded human Factor V are similar to the two intermediates seen during the activation of bovine Factor V by thrombin. The reason that different intermediates are observed when human and bovine

| Component | Human Factor V | Bovine Factor V | Bovine Factor Vα |
|-----------|----------------|----------------|-----------------|
| Factor V  | 335            | 330            | 290             |
| Activation intermediates | 295 | 205 | 220 |
| Final products | 150 | 92 | 73 |

\( ^a \) Apparent molecular weights expressed \( \times 10^3 \).

\( ^b \) This study.

\( ^c \) Nesheim and Mann (6) and Nesheim et al. (33).

\( ^d \) Esmon (5).

\( ^* \) Not found.
Factor V are activated with thrombin may be explained most simply by a different order of bond cleavages (7). It is clear that the apparent molecular weights used to designate the components in this activation scheme may not represent the true values. This can be expected because of their large size and the fact that they are heavily glycosylated. The true molecular weights of Factor V and the components of Factor V, will require the isolation and characterization of the intermediate and end products of Factor V activation. We do not know whether the molecular weights of the various human components are actually larger than the corresponding bovine products as appears from comparison of data from different laboratories.

In contrast to thrombin, V-CP produces products of apparent $M_r = 95,000-91,000$ and $M_r = 303,000$. The fact that Factor V which has been treated with V-CP and then thrombin gives a g gel pattern identical with Factor V which has been treated with thrombin alone suggests that this bond is also cleaved by thrombin. Cleavage of the V-CP-sensitive bond results in complete activation of Factor V. When Factor V is treated with thrombin as in Fig. 3, most of the Factor V activity does not appear until the $M_r = 55,000-91,000$ component is visualized. It therefore seems probable that cleavage of the Factor V molecule to produce the $M_r = 95,000-91,000$ component by either thrombin or V-CP is the necessary event required for the development of Factor V coagulation activity. The development of small amounts of Factor V activity during the 4°C incubation in Fig. 4 might be explained by the formation of small amounts of the $M_r = 55,000-91,000$ component which stain too faintly to be seen. This conclusion is different from those made by workers studying the activation of bovine Factor V by thrombin, who concluded that the appearance of activity correlated with the appearance of the bovine $M_r = 94,000$ (6) or $M_r = 110,000$ (5) component or the disappearance of the bovine $M_r = 150,000$ component (33).

After this work was completed we received a manuscript by Dahlback (35) that reports the successful purification of human Factor V by a method different from that reported here. This preparation was essentially identical with ours although it was obtained in a much lower yield. After treating human Factor V with thrombin, Dahlback also observed activation intermediates that were different from those occurring during activation of bovine Factor V in agreement with our results.

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Addendum—We have recently measured high affinity binding of human Factor V to platelets. Sodium dodecyl sulfate-gel electrophoresis of platelet pellets discloses that the $M_r = 121,000$ and $M_r = 95,000-91,000$ components bind to platelets, but that the $M_r = 150,000$ component does not. This indicates that the $M_r = 150,000$ component is not really a component of Factor V, as we imply in our paper but rather that it is an “activation peptide.”