·基础研究·

# 蝰蛇(缅甸亚种)毒凝血因子X激活物FVe-1的分离纯化与理化活性的研究

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**摘 要**:【目的】从蝰蛇(缅甸亚种)毒分离纯化一种凝血活性因子X激活物FVe-1,并研究其理化性质、序列测定以及凝血活性。【方法】应用阳离子交换层析、分子凝胶过滤层析分离纯化蛇毒,采用MALDI质谱测定法测定分子质量,等电聚焦电泳测定等电点,Edman降解法测定蛋白N末端氨基酸序列,发色底物法和SDS-PAGE等方法测定FVe-1的酶学特征和凝血活性。【结果】从蝰蛇(缅甸亚种)毒分离得到的FVe-1为单体蛋白,相对分子质量为13 808,等电点4.6, 0.5 mg FVe-1的凝血活性与1.5625 u的凝血酶或与54.93 ng RVVX的凝血活性相当;可激活FX,但对凝血酶和纤维蛋白原无作用;酶活性的适宜pH为6.5~7.5, 适宜温度为25~60 ℃; 为钙依赖性, 凝血活性可被EDTA和DTT抑制;FVe-1的N端序列为NH2-N-L-Y-Q-F-G-E-M-I-N;具有呈剂量依赖性的促血浆凝固作用。【结论】从蝰蛇(缅甸亚种)毒纯化出一种FVe-1是一个凝血因子X的激活物,可激活凝血因子X,但对凝血酶和纤维蛋白原的作用微弱。

**关键词**:蛇毒;蝰蛇;凝血因子X激活物
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## Purification, Biochemical Properties, and Activities of a Novel Factor X Activator (FVe-1) from *Daboia Russelli Siamensis* (Myanmar) Venom

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**Abstract**:【Objective】To purify and characterize a novel factor X activator, FVe-1 from *Daboia russelli siamensis* (Myanmar) venom. 【Methods】 FVe-1 was purified by ion-exchange chromatography and gel filtration. The hemostatic activity of FVe-1 was determined based on chromogenic substrates. The fibrinogen-clotting activity of FVe-1 was also determined. Thermal stability, pH stability, enzyme activity, and inhibition of FVe-1 were determined by its remaining procoagulant activity. N-treminal sequence was determined by the method of automated Edman degradation.【Results】 FVe-1 was achieved by chromatography with a molecular weight of 13,808 and an isoelectric point of 4.6. The hemostatic activity of 0.5 mg FVe-1 was equal to that of 1.5625 u thrombin or that of 54.93 ng RVVX. FVe-1 primarily activated FX, but did not affect on prothrombin and fibrinogen. The suitable pH and temperature range of FVe-1 was 6.5-7.5 and 25–60 ℃, respectively. The activity of FVe-1 was enhanced by $Ca^{2+}$ and inhibited by EDTA and DTT. The N-terminal sequence of FVe-1 was NH2-N-L-Y-Q-F-G-E-M-I-N.【Conclusion】FVe-1 is a factor X-activating enzyme, which could activate FX to FXa, but have minimal effect on prothrombin and fibrinogen.

**Key words**: factor X activator; snake venom; *Daboia russelli siamensis*
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Snake venoms contain toxins that interfere in the mechanism of haemostasis and thrombosis. Some of them affect platelet aggregation and/or blood coagulation. The ability of procoagulant venom proteins that can activate the coagulation cascade at specific steps enables their use in diagnostic determination of coagulation factors. Factor X (FX) is one of the key components in the blood coagulation cascade, which can be activated to form activated Factor X (FXa) by FIXa, in the presence of $Ca^{2+}$, phospholipid, and Factor VIIIa (FVIIIa); or by Factor VIIa (FVIIa), in the presence of $Ca^{2+}$ and tissue factor. The activation results from the cleavage site of the $Arg^{52}$- $Ile^{53}$ bond in the heavy chain of human FX and release of a 52-residue activation peptide. And the snake venom activators of FX are non-physiological enzymes, which can convert FX into FXa, in the presence of $Ca^{2+}$ at the same cleavage site of $Arg^{52}$-$Ile^{53}$[1]. The venoms of *Daboia russelli siamensis* have been reported to contain strong FX-activating components. Because of their biological activities, some of these venom proteins are useful for basic studies of hemostasis, and for pharmacological and clinical applications. Although we have known that the venom of *Daboia russelli siamensis* also contains some hemostatic fractions, studies about the hemostatic effect and procoagulant mechanism of *Daboia russelli siamensis* venom are minimal. In the present investigation, We purified the FX-activating fraction, FVe-1 from *Daboia russelli siamensis* (Myanmar) venom, determined the physical and chemical prope-rties, and investigated the mechanism of hemostasia of FVe-1.

# 1 Materials and Methods

## 1.1 Snake venom

The lyophilized venom of *Daboia russelli siamensis* (Myanmar) was purchased from Guangzhou Medical College (Guangzhou, China) and stored in a desiccator.

## 1.2 Reagents

CM-Sephadex C-50 and Superdex™ 75 were purchased from Pharmacia (Uppasala, Sweden). Human fibrinogen and thrombin were supplied from Sigma(St Louis, MO, USA). FX, FXa, prothrombin, FXa chromogenic substances and prothrombin chromogenic substances were from Hyphen BioMed. RVV-X was purchased from Enzyme Research Laboratoties (Global Coag, USA). All other reagents were of analytical grade and obtained from commercial sources (Guangzhou, China).

## 1.3 Purification of FVe-1

*Daboia russelli siamensis* (Myanmar) venom (1.0 g) was dissolved in 0.5 mol/L ammonium acetate (pH 5.8) and applied to a CM-Sephadex C-50 column (2.6 cm×80 cm). The fractions were eluted with 0.1 mol/L ammonium acetate buffer (pH 5.8) containing a linear NaCl gradient (0.5-1 mol/L) at a flow rate of 4 tubes/h. The peak containing highest hemostatic activity was chromatographed on a Superdex™ column (2.0 cm×100 cm) equilibrated with 0.02 mol/L sodium phosphate buffer (pH 7.4). Elution was performed with the same buffer. The fraction with hemostatic activity was again applied on a Superdex™ column. All steps were carried out at room temperature and monitored at 280 nm. All fractions in each step were tested for hemostatic activity as further described.

## 1.4 Melecular weight and isoelectric point

SDS-PAGE was performed according to the method of Laemmli[2]. The molecular weight standards were MBP-G-galactosidase(175 000), MBP-paramyosin (83 000), glutamic dehydrogenase (62 000), aldolase (47 500), triosephosphate isomerase (32 500), G-lactoglobulin A (25 000), lysozyme (16500), and aprotinin(6500). Isoelectrofocusing_PAGE was carried out with a pH gradient of 4.65 –9.6 generated by ampholine; Amersham Biosciences, Uppsala, Sweden), as described by Zhang et al[3].

## 1.5 Mass spectrometric analysis

MALDI-MS anaylysis was assayed on a Autoflex MALDI-TOF-TOF-MS (matrix-assisted laser desorption/ionization-time of flight mass spectr-ometer, Bruker) equipped with nitrogen laser wave-length of 337 nm and the matrix was CCA as previously described[4].

Protein mass spectra were obtained in the positive ion mode at an acceleration voltage of 20 kV, extraction voltage of 23 kV.

### 1.6 N-terminal sequence determination

According to the method of Kennedy[5], 16 μg of FV e-1 sample was applied on a 12.5% Tricine- SDS-PAGE and electrotransferred onto a polyvinylidene difluoride (PVDF) membrane. After staining with Coomassie brilliant blue, the protein band of interest was cut out. N-terminal amino acid sequencing of FV e-1 was determined by automated Edman degradation using a AB1491A protein sequencer (PE) and compared with other protein sequences at the National Center for Biotechnology Information (NCBI) database using the BLAST service.

### 1.7 Measurement of hemostatic activities

Four different groups were established at various concentration intervals. Each concentration was also tested 6 times. The FV e-1 groups were given of the doses of 6.00, 3.00, 1.50, 0.75, and 0.38 mg/mL. Thrombin was used as a positive control with the dose of 250.00, 125.00, 62.50, 31.25, 15.63, 7.81, and 3.91 U/mL. RVV-X was used as another positive control with the dose of 1200.00, 600.00, 300.00, 150.00, 75.00, 37.50, and 18.75 ng/mL. Normal saline was used as a negative control. The hemostatic activities of thrombin and FV e-1 were measured according to the method of the coagulation time determined by Williams[6]. The citrated plasma (100 μL) was incubated with 100 μL of 0.01 mol/L Tris-HC1 buffer (pH 7.3) containing 0.15 mol/L NaCl at 37 ℃ for 3 min. 100 μL of 0.05 mol/L $CaCl_2$ plus thrombin (100 μL) or FV e-1 (100 μL) were added to the pre-incubation mixture respectively.The clotting time of the plasma was then recorded.

### 1.8 Effect of FV e-1 on human blood factor X

The activation of purified human FX was followed by using the FXa differential chromogenic substance[7]. Purified human blood factor X (100 U) was incubated at 37 ℃ in 50 mmol/L Tris-HCl (pH 7.4) containing 0.1 mol/L NaCl, 0.01 mol/L $CaCl_2$, and 12 μg of FV e-1 in a total reaction volume of 500 μL. Aliquots (50 μL) were taken at 5, 10, 15, 20, 25, 30, 35, 40, and 45 min intervals, and 50 μL of FXa differential chromogenic substance solution [dissolved in 50 mmol/L Tris-HCl (pH 7.4) containing 0.1 mol/L NaCl (final concentration of 0.2 mmol/L)] was added. The absorbance was recorded at 405 nm. FXa was used as a positive control, and normal saline was used as a negative control. In addition, at 3, 7, 15, 30, 45, 60, 90, and 120 min intervals, aliquots (20 μL) were removed from FV e-1 group and analyzed by SDS-PAGE.

### 1.9 Effect of FV e-1 on human prothrombin

Using the method of Hofmann[8], the activation of the purified prothrombin was followed by using the thrombin differential chromogenic substance. Purified human prothrombin (6.67 μg/μL) was incubated at 37 ℃ in 50 mmol/L, Tris-HCl (pH 7.4) containing 0.1 mol/L NaCl and 10 mmol/L $CaCl_2$ with FV e-1 (12 μg). Aliquots (50 μL) were removed at various times. 450 μL of thrombin differential chromogenic substance solution [5 mg/mL, dissolved in 50 mmol/L Tris-HCl (pH 7.4) containing 0.1 mol/L NaCl] was added into the aliquots. The absorbance was recorded at 405 nm. Thrombin was used as a positive control, and normal saline was used as a negative control.

### 1.10 Effect of FV e-1 on fibrinogen

FV e-1 (20 μL, concentrations were 6.00, 3.00, 1.50, 0.75, and 0.38 mg/mL, respectively) and 200 μL of human fibrinogen solution (3 mg/mL) in 50 mmol/L Tris-HCl buffer (pH 7.4, containing 0.1 mol/L NaCl and 10 mmol/L $CaCl_2$) were mixed and incubated at 37 ℃. The clotting time was recorded. Thrombin was used as a positive control, and normal saline was used as a negative control.

### 1.11 Effect of heat on venom stability

FV e-1 (20 μL, 1 mg/mL) was preincubated respectively at 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, and 85 ℃ for 15 min. The samples were cooled on ice. The activation of purified human FX was followed by using the FXa differential chromogenic substance as described above. The samples untreated by FV e-1 was used as control. These values were expressed as percentages relative

to control[9]. Each temperature was tested 3 times.

### 1.12 Effect of pH on venom stability

FⅤe-1 (1 μL, 1 mg/mL) was preincubated at 37 ℃ for 30 min in different buffers (pH 5.5–12), and the activation of purified human factor Ⅹ was determined as described above. The samples untreated by FⅤe-1 was used as control. These values were expressed as percentages relative to control[9]. Each pH was also tested 3 times.

### 1.13 Effect of $Ca^{2+}$ on FⅤe-1 activation

FⅤe-1 (1 μL, 1 mg/mL) was preincubated at 37 ℃ for 30 min in Tris-Hcl buffer (0.05 μmol/L, pH 7.4) containing various concentrations of $Ca^{2+}$ (0–15 mmol/L), and the activation of purified human factor Ⅹ was determined as described above. The samples by untreated FVe-1 was used as control. These values were expressed as percentages relative to control[9].

### 1.14 Effect of inhibitors on FⅤe-1 activation

FⅤe-1 (1 μg) was incubated with several inhibitors: EDTA (5 mmol/L), PMSF (1 mmol/L), DTT (5 mmol/L), and aprotinin (0.05 mmol/L) at 37 ℃ for 30 min, respectively. The activation of purified human factor X was determined as described above. FⅤe-1 untreated was used as control. These values were expressed as percentages relative to control[9].

### 1.15 Statistical analysis

Data are presented as mean ±SEM. Statistical significance was determined by Student's *t*-test. Value with a *P*-value<0.05 was considered to be statistically significant.

## 2 Results

### 2.1 Purification of FⅤe-1

The purification of FⅤe-1 from *Daboia Russelli Siamensis* (Myanmar) Venom was achieved by using 3 steps (Fig.1). Ion exchange chromat-ography of crude venom using a CM-Sephadex C-50 column yielded 11 fractions (Fig.1A). The fifth fraction (FⅤ) had the highest hemostatic activity. Its hemostatic activity, expressed by the thrombin coagulation time, was (400.5 ± 0.5) s/32 μg ($n$=6). Further chromatography of FⅤ using a column of Superdex™ 75 column resulted in 5 fractions: Fa, Fb, Fc, Fd and Fe (Fig.1B). The hemostatic activity of Fe was (321 ± 0.5) s/32 μg ($n$=6), which was higher than other fractions. The collection of Fe was then applied to another Superdex™ 75 column resulted in one peak. The single peak was named FⅤe-1 (Fig.1C). The hemostatic activity of FⅤe-1 was (286.5 ± 0.6) s/32 μg ($n$=6). From 1 g of crude venom, 8 mg of FⅤe-1 was obtained (Table 1). The hemostatic activity of 0.5 mg FⅤe-1 was equal to that of 1.5625 u thrombin or that of 54.93 ng RVVX.

### 2.2 Melecular weight and isoelectric point

SDS-PAGE analysis gave a calculated molecular weight of 13 ku (Fig.2), and MALDI-TOF- MS analysis gave the molecular weight of 13 808 (Fig.3). Isoelctric focusing study indicated that FVe-1 was an acid protein with a pI of 4.6(Fig.4).

### 2.3 N-terminal sequence determination

The N-terminal sequence of FⅤe-1 were determined as NH2-N-L-Y-Q-F-G-E-M-I-N. FⅤe-1 had a difference sequence, when compared with the N-terminal sequences of other previously isolated factor X activators from *Daboia Russelli Siamensis* such as RVV-Ⅹ and FⅠa, using the data at the National Center for Biotechnology Information (NCBI) database.

### 2.4 Measurement of hemostatic activities

The clotting time of normal rabbit plasma was (624 ± 0.63) s ($n$=6). It was shown the clotting time of FⅤe-1 increased with the decreased dosages (Table 2) as well as that of RVV-Ⅹ and thrombin

**Table 1 Purification of a factor X activator from Myanmar *Daboia russelli siamensis* venom** (mean±SEM, $n$=6)

| Column | Protein/mg | Yield/% | Procoagulant activity (s/32 μg) |
|---|---|---|---|
| CM-Sephadex C-50 | 100 | 10.0 | 400.5 ± 0.5[1)] |
| Superdex™ 75 gel filtration | 30 | 3 | 321.0 ± 0.5[1)] |
| Superdex™ 75 gel filtration | 8 | 0.8 | 286.5 ± 0.6[1)] |

Loading dose of venom was 1.0 g. 1)$P$ < 0.001

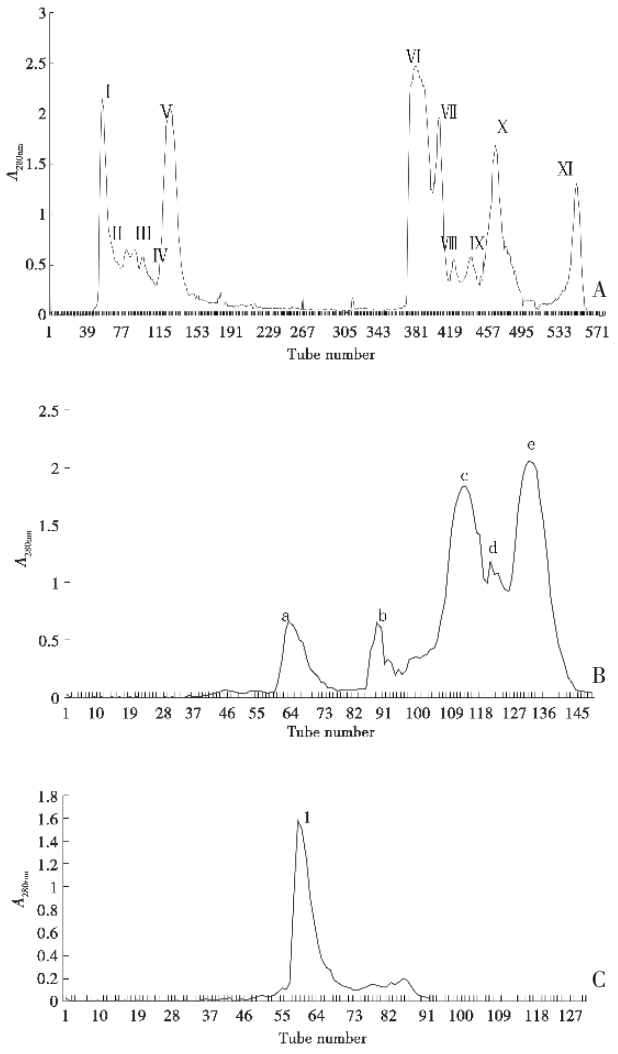


**Fig.1　Purification of purification of a factor X activator from Myanmar Daboia russelli siamensis venom**

A: CM-Sephadex C-50 ion exchange chromatography of Myanmar *Daboia russelli siamensis* venom. B: Superdex™ 75 gel filtration of FⅤ. C: Superdex™ 75 gel filtration of FⅤe.

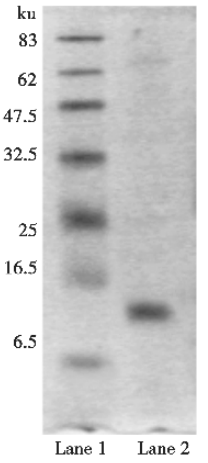


**Fig.2　Tricine-SDS-PAGE of FⅤe-1**

Lane 1: Molecular mass markers; Lane 2: FⅤe-1. The protein was stained with 0.25% Coomassie brilliant blue R-250 solution.

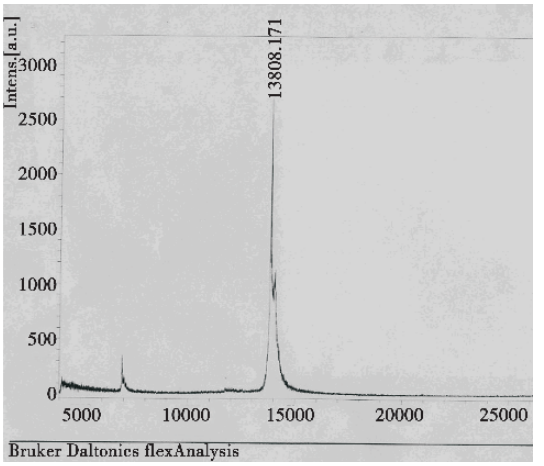


**Fig.3　Determination the molecular weight of FⅤe-1 by MALDI-MS**

did.

Purified enzyme FⅤe-1 readily cleavs a number of commercially available chromogenic substrates that have been designed for human blood FⅩ. The parameters for chromogenic substrate conversion by the venom activator were higher than by normal saline (Table 5, Fig.5). And data for SDS-PAGE analysis also showed that FⅤe-1 could cleavage FⅩ (Fig.5). These results suggested that FⅤe-1 could active FⅩ to FⅩa.

However, in the test of chromogenic substrates to prothrombin, the parameters of FVe-1 had no change compared with the normal saline group. This suggested that FVe-1 had no catalytic efficiency on prothombin.

In the test of fibrinogen-clotting activity, FⅤe-1 at dosages of up to 0.6 mg/100 μL could not coagulate human fibrinogen. This suggested that FⅤe-1 had no effect on human fibrinogen.

### 2.5　Effect of heat and PH on venom stability

The residual hemostatic activity at pH 7.4 of thermal treated FⅤe-1 was investiged after incubation for 15 min at various temperatures, The hemostatic activity of FⅤe-1 decreased markedly above 60 ℃. Heated for 15 min at 85 ℃, the activity

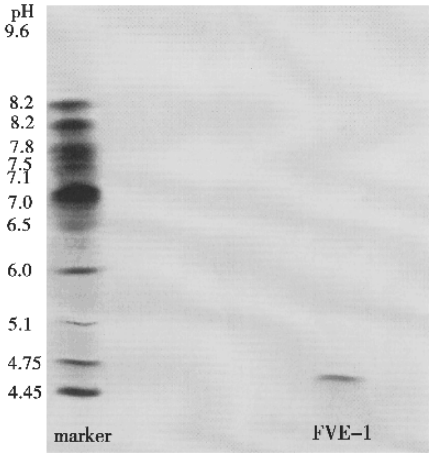


**Fig.4 Isoelectric focusing analysis of FⅤe-1**

**Table 2 Procoagulant activity of FⅤe-1 on rabbit poor-platelet plasma** (mean±SEM, $n$=6)

| Concentration/(mg/mL) | Coagulation time/s |
|---|---|
| 6.00 | 57.5 ± 0.5 |
| 3.00 | 98.0 ± 0.6 |
| 1.50 | 139.0 ± 0.6 |
| 0.75 | 178.5 ±0.5 |
| 0.38 | 287.5 ± 0 .6 |

**Table 3 Procoagulant activity of thrombin on rabbit poor-platelet plasma** (mean±SEM, $n$=6)

| Concentration/(U/mL) | Coagulation time/s |
|---|---|
| 250.00 | 6.5±0.5 |
| 125.00 | 13.0±0.6 |
| 62.50 | 17.0±0.6 |
| 31.25 | 26.5±0.5 |
| 15.63 | 64.0±0.6 |
| 7.81 | 89.5±0.5 |
| 3.91 | 146.5±0.5 |

was reduced by 50%(Fig.6). On the other hand, FVe-1 was stable over a wide pH range of 6.5 to 11.5 (Fig. 7). Furthermore the value of the activity of FVe-1 reached maximum at about pH 7. The activity was lost progressively below pH 5.5 and above pH 12.

### 2.6 Effect of metal ions on FⅤe-1 activation

The activity of FⅤe-1 was enhanced significantly by $Ca^{2+}$ and weakly by EDTA and DTT. When FⅤe-1 was incubated with casein in Tris-HCl (pH 8.5) containing 0–15 mmol/L $Ca^{2+}$, a dose-dependent increase of activity was observed. At 5 mmol/L, nearly 90% of activity of FⅤe-1 was observed (Fig.8). And when FⅤe-1 incubated with EDTA (5 mmol/L) and DTT (5 mmol/L), respectively (Table 6), the activity of FⅤe-1 decreased significantly. However the activity of FⅤe-1 did not change at the present of PMSF (1 mmol/L) and Aprotinin (0.05 mmol/L), respectively (Table

**Table 4 Procoagulant activity of RVV-X on rabbit poor- platelet plasma** (mean±SEM, $n$=6)

| Concentration/(ng/mL) | Coagulation time/s |
|---|---|
| 1200.00 | 18.6 ± 0.2 |
| 600.00 | 35.8 ± 0.4 |
| 300.00 | 53.6 ± 0.3 |
| 150.00 | 80.5 ± 0.7 |
| 75.00 | 120.7 ± 1.4 |
| 37.50 | 140.5 ± 2.7 |
| 18.75 | 190.3 ± 1.7 |

**Table 5 $A_{405nm}$ of specifical chromogenic substrate reacting with FXa activated by FⅤe-1 or NS at different times** (mean±SEM, $n$=3)

| $t$/min | FⅤe-1 | Normal saline |
|---|---|---|
| 5 | 0.281 ± 0.005[1)] | 0.066 ± 0.004 |
| 10 | 0.432 ± 0.020[1)] | 0.020 ± 0.004 |
| 15 | 0.849 ± 0.071[1)] | 0.071 ± 0.004 |
| 20 | 1.577 ± 0.028[1)] | 0.070 ± 0.004 |
| 25 | 1.836 ± 0.068[1)] | 0.067 ± 0.002 |
| 30 | 2.091 ± 0.025[1)] | 0.069 ± 0.005 |
| 35 | 2.177 ± 0.036[1)] | 0.069 ± 0.004 |
| 40 | 2.191 ± 0.032[1)] | 0.072 ± 0.003 |
| 45 | 2.176 ± 0.030[1)] | 0.071 ± 0.003 |

1) $P < 0.0001$

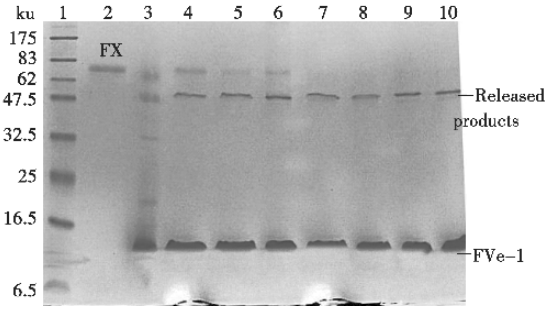


**Fig.5 Analysis of activation of FX by FⅤe-1 on SDS-PAGE at different time intervals**

Lane 1: Molecular mass markers; Lane 2: FX; Lane 3–10: FX+FⅤe-1 3, 7, 15, 30, 45, 60, 90, 120 min, respectively.

6). These results indicated that FVe-1 was not a serine-type proteinase but a metalloproteinase.

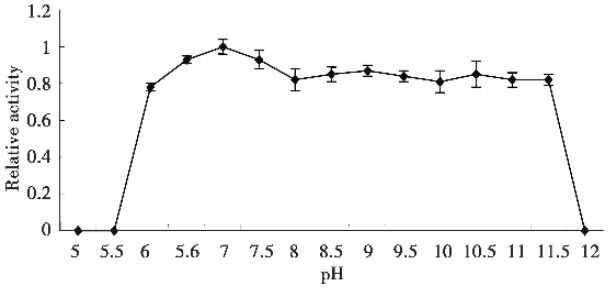


**Fig.7　The activity of FVe-1 pretreated at different pH**

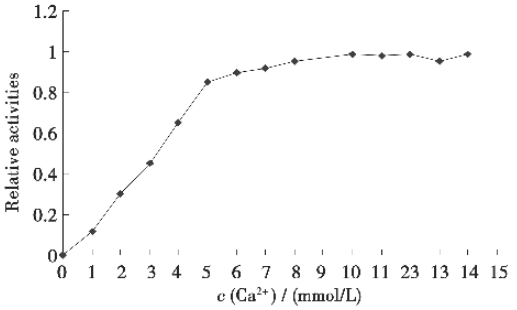


**Fig.8　The activity of FVe-1 pretreated at different concentrations of $Ca^{2+}$**

**Table 6　Effects of several inhibitors on the activity of FVe-1**

(mean ± SEM, $n$=3)

| Inhibitors | Concentration/(mmol/L) | Relative activity/% |
|---|---|---|
| EDTA | 5 | |
| | 10 | 98.1 ± 4.9 |
| L-cysteine | | |
| DTT | 5 | 20.8 ± 3.8 |
| Aprotinin | 0.05 | 99.4 ± 2.9 |
| PMSF | 1 | 99.6 ± 5.8 |

## 3　Discussions

Studies *Daboia russelli siamensis* venom have been conducted for more than seventy years. It is known that Russell's viper venom contains a number of hemostatic components that can activate FX, FV, and prothrombin[10].

In this paper, we have described the purification and characterization of the FX-activating fraction, FVe-1 from *Daboia Russelli Siamensis* (Myanmar) Venom.

With a 3-step procedure, we obtained a purified FX-activating fraction, FVe-1. Its molecular weight was 13 808 u and its pI was 4.6, showing a difference when compared with RVV-X obtained by Esnouf and Williaams, FIa obtained by Sun huanhuan, et al, and other factor χ activators from different snake venoms[7-8]. In the previous studies, there were at least two kinds of FX-activating components in *Daboia russelli siamensis* venom[11-12]. Judging from the above findings, we suggest that FVe-1 was a new FX-activating protein from *Daboia russelli siamensis* (Myanmar) venom, or a different derivation of Viper russellii. We found that FVe-1 could shorten clotting time, and the hemostatic activity of 0.5 mg FVe-1 was equal to that of 1.5625 u of thrombin or 47.58 ng of RVV-X.

The activation of the extrinsic coagulation pathway was initiated by an expression of prothrombinase. FXa could convert prothrombin to thrombin. Thrombin could directly activate platelets and cleaved fibrinogen to fibrin monomers[13]. In order to understand the relationship between FVe-1 and this process, we determined the activities of FVe-1 on prothrombin and FX in vitro. In light of our data, we found that FVe-1 could increase the activity of FX by a concentration-time relationship. However, we found that FVe-1 did not affect the activity of prothrombin directly.

Fibrinogen and fibrin also played essential roles in blood clotting. During coagulation, the soluble fibrinogen is converted to insoluble fibrin, and this process was initiated by thrombin. In this study, we found that FVe-1 had no effect on human fibrinogen in vitro. The results suggested that FVe-1 could not cleave or clot fibrinogen directly.

FVe-1 could activate factor X at the present of $Ca^{2+}$ as well as other factor X activators from Daboia, Vipera, Cerastes, Echis, Calloselasma, and Bothrops venom, When lack of $Ca^{2+}$, the hemostatic activity of FVe-1 could be completely suppressed, it was showed that $Ca^{2+}$ was necessary for the efficient activation of FVe-1. The optimal concentrations of calcium were above 5 mmol/L. Moreover, the activity

of FⅤe-1 was inhibited by EDTA and DTT, but did not affected by PMSF and Aprotinin. Factor X activators isolated from snake venoms could be classified as either metallopro-teinases or serine proteases [2]. Metalloproteinases could be activated by the dependent of $Zn^{2+}$ and/or $Ca^{2+}$, and inhibited by metal chelating agents. The evident suggested that the enzyme of FⅤe-1 was a metalloproteinase.

In conclusion, FⅤe-1 is a novel FX-activating enzyme, but has no effects on prothrombin and fibrinogen.

These activities indicate the potential therapeutic application of FⅤe-1 for hemostasis. Therefore, FⅤe-1 will probably find their application as FX-activators in blood coagulation research and in diagnostic research kits. Moreover, since the stability of natural venom is not ideal; it is more interesting to produce medical venom products by molecular cloning and expression. The N-terminus amino acid sequences of FⅤe-1 have been determined, which would be very useful in following studies.

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