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ORIGINAL PAPER

Purification and characterization of a fibrinolytic enzyme from *Streptomyces* sp. XZNUM 00004

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Abstract A fibrinolytic enzyme (SFE1) from *Streptomyces* sp. XZNUM 00004 was purified to electrophoretic homogeneity with the methods including ammonium sulfate precipitation, polyacrylamide gel, DEAE-Sepharose Fast Flow anion exchange and gel-filtration chromatography. The molecular weight of SFE1 was estimated to be 20 kDa by SDS-PAGE, fibrin zymography, and gel filtration chromatography. The isoelectric point was 4.9. K_m and V_{max} values were 0.96 mg/ml and 181.8 unit/ml, respectively. It was very stable at pH 5.0–8.0 and below 65 °C. The optimum pH for enzyme activity was 7.8. The optimum temperature was 35 °C. The fibrinolytic activity of SFE1 was enhanced by Na^+, K^+, Mn^{2+}, Mg^{2+}, Zn^{2+} and Co^{2+}. Conversely, Cu^{2+} showed strong inhibition. Furthermore, the fibrinolytic activity was strongly inhibited by PMSF, and partly inhibited by EDTA and EGTA. SFE1 rapidly hydrolyzed the $A\alpha$-chain of fibrinogen, followed by the $B\beta$-chain and finally the γ-chain. The first 15 amino acids of the N-terminal sequence were API-TLSQGHVDVVDI. Additionally, SFE1 directly digested fibrin and not by plasminogen activators in vitro. SFE1 can be further developed as a potential candidate for thrombolytic therapy.

Keywords Fibrinolytic enzyme · Purification · *Streptomyces* · Thrombolytic therapy

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Introduction

It is well known that thrombotic disease is regarded as a major cause of human death in the worldwide. And thrombolytic therapy is still the best way to achieve recanalization in these diseases nowadays (Marder 2009). Thrombolytic drugs used for clinical applications are divided into three generations (Wang et al. 2010). The first is composed of streptokinase (SK) (Banerjee et al. 2004), urokinase (UK) (Blasi and Sidenius 2010), etc. The second generation consists of tissue plasminogen activator (t-PA) (Kent et al. 2004), single-chain urokinase-type plasminogen activator (scu-PA, or pro-urokinase, pro-UK) (Blasi and Sidenius 2010), etc. The third generation are novel agents derived from the first or the second generations of thrombolytic agents by modern molecular biological techniques (mutants of pro-UK and t-PA) (Marín et al. 2009; Killer et al. 2010). Despite widespread used, these fibrinolytic agents suffer important shortcomings including bleeding complications, short half-life, high cost, the risk of allergic reactions and large therapeutic doses (Killer et al. 2010). Therefore, it is necessary to search for novel fibrinolytic agents from other sources. In the last decade, fibrinolytic enzymes have been identified from various sources including actinomycetes (Simkhada et al. 2010), bacteria (Wang et al. 2008; Agrebi et al. 2009), fungi (Kim et al. 2008), marine polychaete worms (Deng et al. 2010), and so on.

Actinomycetes are a special group of microorganisms, which have been demonstrated to be excellent producers of bioactive and structurally novel metabolites (Bérdy 2005; He et al. 2010; Kavitha et al. 2010). Therefore, we decided to investigate a thrombolytic agent from actinomycetes. During a screening programme on actinomycetes from rhizosphere soil of Chinese *Polygonatum sibiricum* Red.,

Streptomyces sp. XZNUM 00004 producing a strong fibrinolytic enzyme was selected. In this paper, purification and characterization of a fibrinolytic enzyme, SFE1, from *Streptomyces* sp. XZNUM 00004 were investigated.

Materials and methods

Materials

Thrombin was purchased from Hangzhou Medicine Co. (China). Bovine serum albumin (BSA), azocasein, trichloroacetic acid (TCA), L-tyrosine, ethylene diamine tetraacetic acid (EDTA), phenylmethylsulfonyl fluoride (PMSF), and ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) were obtained from Sigma-Aldrich Co. (USA). Pepstatin, aprotinin, and soybean trypsin inhibitor β-mercaptoethanol were purchased from Shanghai Sangon Co. (China). DEAE-Sepharose Fast Flow and Bio-gel Polyacrylamide Gel were acquired from BIO-RAD (USA). Fibrinogen (Human) was from FIBRORAAS (Shanghai, China). The protein molecular weight markers (SM0671 and SM0431) for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Fermentas (Canada). Urokinase was acquired from Chinese Medicine Testing Institute. All other reagents were of analytical grade.

Strain and culture conditions

Strain XZNUM 00004 was isolated from rhizosphere soil of *P. sibiricum* Red., a Chinese traditional medicinal plant. It was identified as a strain related to *Streptomyces* based on morphological and 16S rDNA sequences in our previous study (Ju et al. 2010) (Accession no. GU211008 in GenBank). A loopful of spores were scraped from the plate and inoculated into seed medium containing 2 % beef extract, 0.1 % yeast extract, 0.05 % peptone, 0.05 % glucose and 0.05 %NaCl. It was cultivated in shaking incubator (180 r/min) at 28 °C. After 24 h cultivation, a 5 % (v/v) inoculum was transferred into fermentation medium (2 % soluble starch, 0.1 % KNO_3, 0.05 % NaCl, 0.05 % K_2HPO_4, 0.05 % $MgSO_4$, 0.001 % $FeSO_4$, pH 7.2) and fermented at 28 °C at 180 r/min for 5 days. Culture supernatant of the strain was obtained via centrifugation (1,753×*g*, 10 min).

Enzyme purification

Solid $(NH_4)_2SO_4$ was slowly added to the supernatant to 80 % saturation. The mixture was stored at 4 °C overnight. The precipitate was collected by centrifugation (7,012×*g*, 10 min, 4 °C) and resuspended in 10 mM Tris–HCl buffer (pH 8.0). After removal of insoluble materials by centrifugation (7,012×*g*, 5 min, 4 °C), the suspension was applied to a 2.6 cm × 30 cm Polyacrylamide Gel column (Bio-gel P60) equilibrated with 10 mM Tris–HCl buffer (pH 8.0) at a flow rate of 1 ml/min. The active fractions were pooled and further purified using DEAE-Sepharose Fast Flow anion exchange column (2.0 cm × 6 cm) equilibrated with 10 mM Tris–HCl buffer (pH 8.0). The bound proteins were eluted with a linear gradient of 0–1 mol/l NaCl in the same buffer at a flow rate of 2 ml/min. The active fractions were pooled, lyophilized and further purified by a 1.5 cm × 75 cm Polyacrylamide Gel column (Bio-gel P30) equilibrated with 10 mM Tris–HCl buffer (pH 8.0) at a flow rate of 0.5 ml/min. The fractions with strong fibrinolytic activity were pooled, lyophilized and used as the purified enzyme preparation.

Protein concentration

Protein concentration was determined by the method of Bradford (Bradford 1976) using bovine serum albumin as the standard protein, measuring the absorbance at 595 nm.

Assay of enzyme activity on fibrin plate

Fibrinolytic activity was measured according to the fibrin plate method with some modifications (Astrup and Mullertz 1952). Briefly, 5 ml fibrinogen solution [5 mg/ml fibrinogen (Human) in 0.9 % NaCl, standing for 5 min at 45 °C] was mixed with 5 ml agar solution [0.5 % agar in 0.9 % NaCl, standing for 30 min at 45 °C]. The mixture was poured into the plate (9 cm diameter) with 50 µl thrombin solution [200 IU/ml thrombin in 0.9 % NaCl]. The plate was left for 30 min at room temperature to form a fibrin clot layer. 10 µl sample solution was placed on the plate. The plate was incubated at 37 °C for 18 h. The fibrinolytic activity was quantified by measuring the diameter of the clear zone according to the standard curve using urokinase (Zheng et al. 2000). The activity was expressed in the unit of clear zone area per µg of the protein (mm^2/µg) (Mander et al. 2011).

Native PAGE

Native PAGE was performed at room temperature with the use of a 5 % stacking gel and a 12 % separating gel in a BIO-RAD Mini-Cell electrophoresis system. Electrophoresis was run at constant voltage of 90 V in 37 mM Tris–glycine buffer (pH 8.9) until the tracking dye reached the bottom of the gel. The gel was stained with 0.25 % Coomassie blue R-250 for 20 min; and destined with 10 % methanol, 10 % acetic acid until the background was clear.

Determination of molecular weight and fibrin zymography

The molecular weight of SFE1 was determined by the method of SDS-PAGE with a 5 % stacking gel and a 12 % separating gel. Protein molecular weight markers were used as reference proteins. Gel was stained with 1 % (w/v) Coomassie Brilliant Blue R-250 and destained with destaining solution (ethanol: acetic acid: distilled water = 7:1:12, v/v/v). The molecular mass of SFE1 was also analyzed by gel filtration chromatography using a 1.0 cm × 90 cm column (Sephadex G-100) at a flow rate of 0.4 ml/min. Molecular weight markers for gel filtration chromatography included β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), carbonic anhydrase (29 kDa), and cytochrome *c* (12 kDa).

Fibrin zymography has been shown to be a sensitive quantitative technique. The gel used for fibrin zymography contained a protease substrate, fibrin. Samples were unheated in SDS-loading buffer lacking reducing agent. Following electrophoresis, SDS was removed in the gel by exchange in Triton X-100. This allowed the fibrinolytic enzyme in the sample to renature and activate. By overnight incubation in an appropriate buffer, the activity was revealed by an absence of protein staining in the region where fibrin had been digested. Specifically, Fibrin zymography was performed out according to Kim et al. (1998) with slight modifications. Fibrinogen (2.0 mg/ml) and thrombin (1 U/ml) were mixed with 12 % polyacrylamide gel solution. Following electrophoresis of the purified enzyme on the fibrin gel, the gel was soaked in 2.5 % Triton X-100 containing Tris–HCl (50 mM) buffer (pH 7.8) and distillation water for 30 min at room temperature, respectively. Subsequently, the gel was incubated in 30 mM Tris–HCl buffer (pH 7.8) containing 200 mM NaCl at 35 °C for 16 h. The gel was stained with Coomassie blue for 1 h and then destained.

Determination of isoelectric point

Isoelectric focusing analysis of SFE1 was carried out using disc electrophoresis in 7.5 % polyacrylamide gel containing 3 % ampholyte with pH range 3.0-10.0. The cylindrical gels with and without 1 μg SFE1 were run using 5 % (v/v) H_3PO_4 as anolyte and 2 % (w/v) NaOH as catholyte. Focusing was carried out at a constant voltage of 100 V until the current was close to zero. The gel containing sample was stained with 1 % (w/v) Coomassie Brilliant Blue R-250 and destained with destaining solution (ethanol: acetic acid: distilled water = 7:1:12, v/v/v). The blank gel was cut into segments of 5 mm length from anolyte to catholyte. The pH was determined after the gel segments eluted in 1 ml bidistilled water for 24 h. The standard curve was made according to pH and the gel length. The isoelectric point (p*I*) of SFE1 was estimated by the length of the protein band to anolyte according to the standard curve (Li 1998).

Effect of pH on fibrinolytic enzyme activity and stability

The optimal pH for SFE1 was determined through measuring the enzyme activity within a pH range of 4.0–10.0 at 37 °C for 18 h. The pH stability of SFE1 was estimated by measuring the remaining fibrinolytic activity after incubating the enzyme for 1 h at 37 °C with different buffers. The following buffer systems were used: 100 mM sodium acetate buffer (pH 4.0–5.0), 100 mM phosphate buffer (pH 6.0–8.0), and 100 mM glycine-NaOH buffer (pH 9.0–10.0).The enzyme activity was measured according to the fibrin plate method. Maximum activity was represented 100 % and other sample activities were expressed as a relative percentage to the maximum.

Effect of temperature on fibrinolytic enzyme activity and stability

The optimal temperature of SFE1 was determined by measuring the enzyme activity at different temperatures (25–85 °C) for 18 h. The thermal stability of SFE1 was evaluated by measuring the remaining fibrinolytic activity after incubating the enzyme in various temperatures (25–85 °C) for 1 h at pH 7.8. The fibrinolytic activity was assessed by the fibrin plate method.

Effect of metal ions and protease inhibitors on fibrinolytic activity

The effect of seven protease inhibitors on the activity of SFE1 was studied using pepstatin (0.1 mg/ml), aprotinin (0.1 mg/ml), soybean trypsin inhibitor (0.1 mg/ml), PMSF (1 mM), EGTA (1 mM), EDTA (1 mM) and β-mercaptoethanol (1 mM). SFE1 was incubated with these inhibitors at corresponding concentration for 30 min at 37 °C and the residual activity was measured by the fibrin plate method. The activity of SFE1 measured in the absence of inhibitors was taken as 100 %.

The effect of various metal ions on the activity of SFE1 was investigated using NaCl, KCl, $CaCl_2$, $MnCl_2$, $MgCl_2$, $ZnCl_2$, $CuCl_2$, $CoCl_2$ and $FeSO_4$. SFE1 was incubated with different metal ion saline solutions (5 mM) for 2 h at 37 °C. The residual activity was measured by the fibrin plate method. The activity of SFE1 assayed in the absence of metal ions was taken as 100 %.

Assay of fibrinogenolytic activity

Fibrinogenolytic activity was detected via a modified fibrinogenolytic assay (Cha et al. 2010). 180 µl fibrinogen (2.0 % human fibrinogen in 50 mM Tris–HCl buffer, pH 7.8) was mixed with 20 µl purified enzyme (0.2 mg/ml) and incubated at 37 °C. 20 µl mixture solution was withdrawn at various intervals (5 min, 15 min, 30 min, 1 h, 2 h, 3 h and 4 h) and boiled for 5 min to terminate the reaction. The samples were analyzed by SDS-PAGE.

Determination of kinetic constants

The kinetic constants, K_m and V_{max} of SFE1 were calculated by the method of Lineweaver–Burk double-reciprocal plot (Lineweaver and Burk 1934) with azocasein as a substrate. Purified enzyme (10 µl) was mixed with 240 µl of azocasein solution (0.1–4.0 mg/ml azocasein in 50 mM Tris–HCl buffer pH7.8). After incubation at 37 °C for 10 min, the reaction was terminated by adding 500 µl 10 % (w/v) trichloroacetic acid, followed by standing in ice water for 10 min and centrifuged (10,000 r/min, 10 min, 4 °C). The absorbance of the supernatant was measured at 340 nm. One unit (IU) of enzyme activity was expressed as the amount of the enzyme causing an increase in absorbance of 0.001 per minute at the assay condition.

Analysis of N-terminal amino acid sequence

SFE1 was applied to SDS-PAGE, transferred to a polyvinylidene difluoride (PVDF) membrane and stained with Coomassie Blue. The stained band was used directly for analysis of N-terminal amino acid sequence by a Procise 491 amino acid sequencer (Applied Biosystems, USA) at College of Life Sciences, Peking University.

Evaluation of the anticoagulant effect of SFE1 in vitro

The anticoagulant effect of SFE1 in vitro was estimated by the method of Lu et al. (2010) with minor modifications. In brief, 1 ml fresh whole blood was added to 1 ml 200 IU/ml of SFE1 solution (0.9 % NaCl, pH 7.4) in a glass test tube. The mixture was incubated at 37 °C for 3 h. The fresh whole blood was obtained from healthy male rat. As positive and negative controls, 200 IU/ml urokinase (0.9 % NaCl, pH 7.4) and normal saline were used instead of SEF1, respectively.

Analysis of thrombolysis mechanism of SFE1 in vitro

The fresh whole blood was placed to form blood clot at 4 °C. 1 g blood clots were heated at 85 °C for 30 min to deactivate the plasminogen. 1 ml 200 IU/ml of SFE1 solution (0.9 % NaCl, pH 7.4) was added to heated and unheated blood clots in a glass test tube, respectively. The mixtures were incubated at 37 °C. Thrombolysis mechanism was analyzed according to the dissolution rates of heated and unheated blood clots.

Results

Enzyme purification

SFE1 fibrinolytic enzyme was purified by the four-step procedure described in the section 'Enzyme purification'. After the final purification step, the enzyme showed a single band on SDS-PAGE (Lane 1 of Fig. 1a) and nature PAGE (Fig. 1b), which indicated that the enzyme was a monomeric protein. 1.1 mg fibrinolytic enzyme was obtained from 3 liters culture supernatant. It was purified 8.8-fold with a recovery of 2.2 % and a specific activity of 2750.6 IU/mg of protein (Table 1).

Molecular weight and isoelectric point

The molecular weight of SFE1 was estimated to be 20 kDa by SDS-PAGE (Lane 1 of Fig. 1a) and fibrin zymography (Lane 2 of Fig. 1a), corresponding with that determined by gel filtration. The isoelectric focusing electrophoresis of SFE1 was shown in Fig. 2b. The p*I* of SFE1 was measured about 4.9 according to the standard curve of pH-Gel length (Fig. 2).

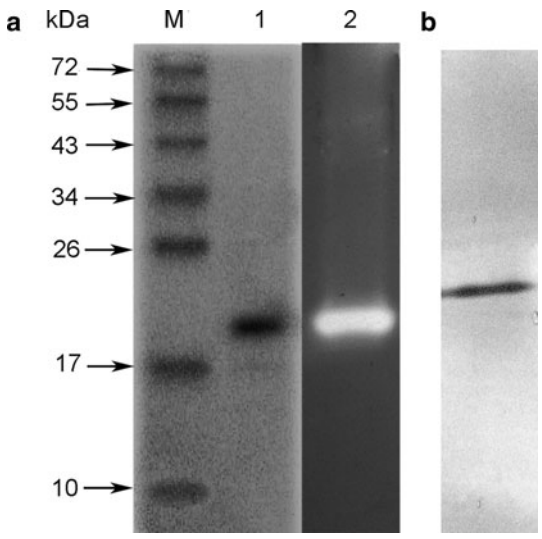

Fig. 1 SDS-PAGE and nature PAGE of SFE1. **a** *Lane M* molecular mass markers; *Lane 1* purified enzyme; *Lane 2* fibrin zymography of purified enzyme; **b** nature PAGE of SFE1

Table 1 Summary of SFE1 purification

Step	Total protein (mg)	Total activity (IU)	Specific activity (IU/mg)	Yield (%)	Purification factor
Culture supernatant	429.0	134,768.0	314.1	100.0	1.0
80 % $(NH_4)_2SO_4$	81.4	43,144.3	530.0	32.0	1.7
Bio-gel P60	11.7	13,198.8	1,132.9	9.8	3.6
DEAE-Sepharose FF	4.7	6,176.1	1,311.3	4.6	4.2
Bio-gel P30	1.1	3,025.6	2,750.6	2.2	8.8

Effect of pH and temperature on fibrinolytic enzyme activity and stability

To determine whether pH had effect on the activity and stability, SFE1 was treated in different pH from 4.0 to 10.0. As indicated in Fig. 3, pH had a great influence on activity and stability of SFE1. The optimum pH was 7.8, but in the range of pH 5.0–8.0, SFE1 was also relatively stable. To further investigate the role of temperature in controlling the activity and stability, SFE1 was put in different temperature from 25 to 85 °C at pH 7.8. As displayed by the results, it was active below 65 °C, and the maximum activity was observed at 35 °C (Fig. 4).

Effect of metal ions and protease inhibitors on the fibrinolytic enzyme activity

To test whether metal ions and protease inhibitors could participate in the regulation of fibrinolytic enzyme activity, nine metal cations (Na^+, K^+, Mn^{2+}, Mg^{2+}, Zn^{2+}, Co^{2+} and Cu^{2+}) and seven protease inhibitors (pepstatin, aprotinin, soybean trypsin inhibitor, PMSF, EGTA, EDTA and β-mercaptoethanol) were introduced into our study. As shown in Table 2, Na^+, K^+, Mn^{2+}, Mg^{2+}, $Zn^{2+}$and Co^{2+} enhanced the activity, while Cu^{2+} showed strong inhibition. In addition, the fibrinolytic activity of SFE1 was strongly inhibited by a typical serine protease inhibitor PMSF (1 mM), but the other serine protease inhibitors mentioned above (aprotinin, soybean trypsin inhibitor) had no obvious effects on the enzyme activity. The fibrinolytic activity of SFE1 was also decreased by EDTA (1 mM) and EGTA (1 mM) (Table 2).

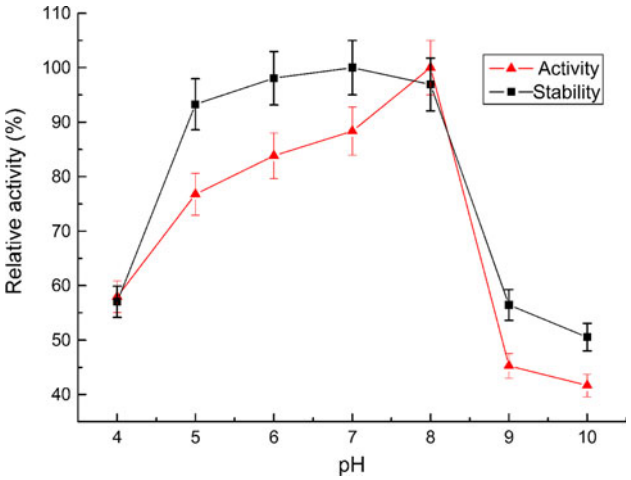

Fig. 3 Effect of pH on the activity and stability of SFE1. The optimal pH for SFE1 was determined by measuring the fibrinolytic activity within a pH range of 4.0–10.0 at 37 °C for 18 h. The pH stability of SFE1 was estimated by measuring the remaining fibrinolytic activity after incubating the enzyme for 1 h at 37 °C with different buffers: 100 mM sodium acetate buffer (pH 4.0–5.0), 100 mM phosphate buffer (pH 6.0–8.0), and 100 mM glycine-NaOH buffer (pH 9.0–10.0)

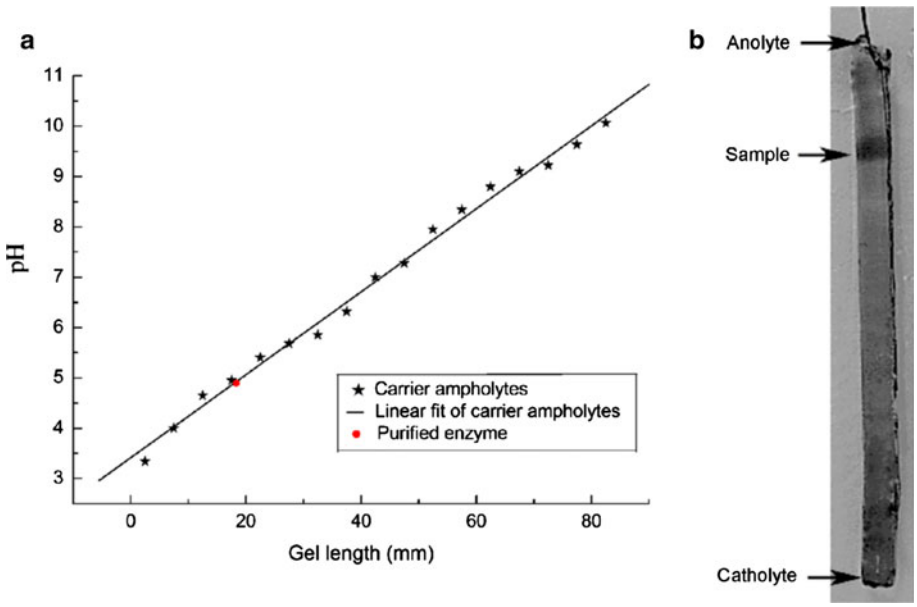

Fig. 2 Isoelectric focusing analysis of SFE1. **a** The standard curve of pH-Gel length; **b** disc electrophoresis was carried out in 7.5 % polyacrylamide gel containing ampholyte with pH range 3.0–10.0. The gel was stained for proteins with 0.25 % Coomassie brilliant blue R-250 solution

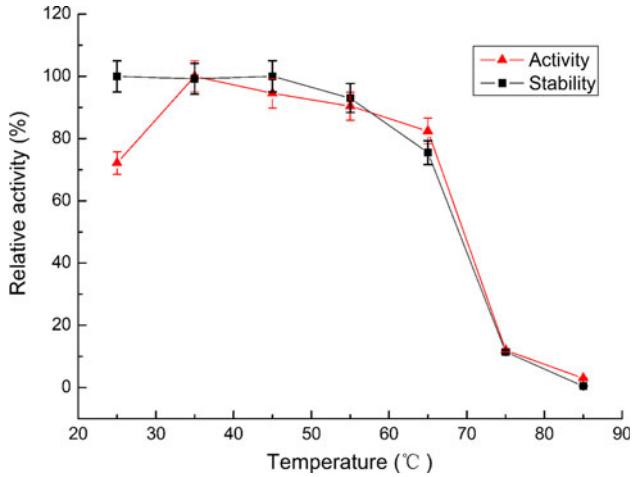

Fig. 4 Effect of temperature on the activity and stability of SFE1. The optimal temperature of SFE1 was determined by measuring the fibrinolytic activity at different temperatures for 18 h. The thermal stability of SFE1 was evaluated by measuring the remaining fibrinolytic activity after incubating the enzyme in various temperatures for 1 h at pH 7.8

Table 2 Effect of metal ions and protease inhibitors

Metal ion or inhibitor	Concentration	Residual activity %
None		100.0 ± 0.5
NaCl	5 mM	124.2 ± 2.6
KCl	5 mM	112.3 ± 2.2
$CaCl_2$	5 mM	101.3 ± 2.3
$MnCl_2$	5 mM	134.6 ± 2.9
$MgCl_2$	5 mM	122.6 ± 2.8
$ZnCl_2$	5 mM	111.5 ± 1.2
$CuCl_2$	5 mM	39.3 ± 2.5
$CoCl_2$	5 mM	131.3 ± 2.9
$FeSO_4$	5 mM	100.7 ± 1.1
Pepstatin	0.1 mg/ml	97.5 ± 0.8
Aprotinin	0.1 mg/ml	100.9 ± 1.8
Soybean trypsin inhibitor	0.1 mg/ml	99.9 ± 0.3
PMSF	1 mM	16.4 ± 2.0
EGTA	1 mM	59.9 ± 0.8
EDTA	1 mM	62.6 ± 1.0
β-mercaptoethanol	1 mM	97.8 ± 1.6

Results are presented as means ± SD ($n = 3$)

Hydrolysis of fibrinogen by the purified fibrinolytic enzyme

To explore the fibrinogenolytic activity of SFE1, SDS-PAGE was performed. Interestingly, the hydrolysis rate was significantly different with the chains of fibrinogen. The $A\alpha$-chain was completely degraded within 5 min, and the $B\beta$-chain was completely degraded within 60 min. The γ-chain was mostly hydrolyzed in 2 h (Fig. 5).

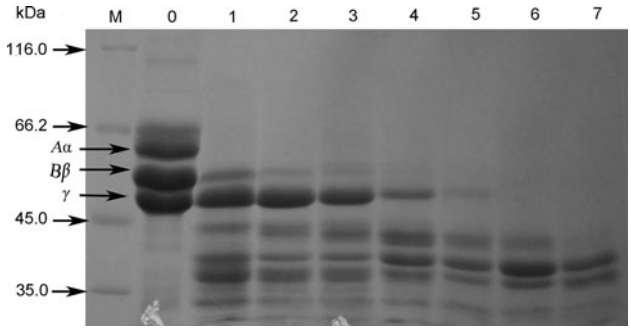

Fig. 5 Degradation of fibrinogen by SFE1. *Lane M* molecular mass markers; *Lane 0* fibrinogen control without SFE1; *Lanes 1–7* degradation products after 5, 15, 30, 60, 120, 180 and 240 min incubation at 37 °C, respectively

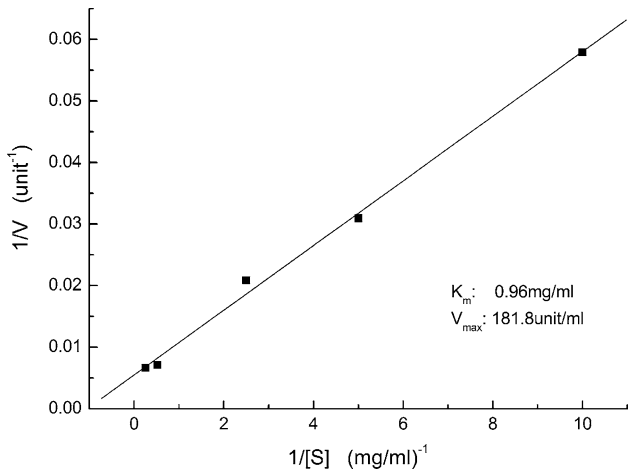

Fig. 6 Lineweaver-Burk double-reciprocal plot of SFE1

Kinetic parameters of the fibrinolytic enzyme

To evaluate the kinetic constants of SFE1 for the proteolytic reaction, the initial velocities of enzyme reactions were determined at various concentrations of the azocasein substrate. The K_m and V_{max} values of SFE1 for azocasein were 0.96 mg/ml and 181.8 unit/ml, respectively (Fig. 6).

N-terminal amino acid sequences of the fibrinolytic enzyme

The N-terminal amino acid sequence of SFE1 was analyzed via the automated Edman method after SDS-PAGE and electroplating. The N-terminal 15 amino acids sequence was APITLSQGHVDVVDI. The amino acid sequence was analyzed using standard protein–protein BLAST (blastp) in NCBI protein databases (http://www.ncbi.nlm.nih.gov/BLAST) and revealed similarity only with hypothetical protein.

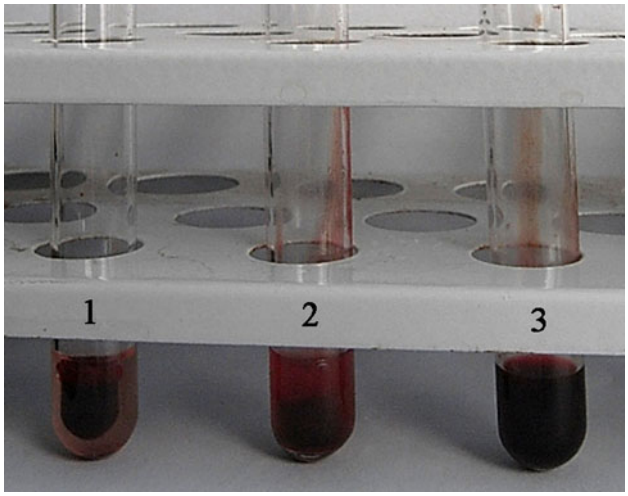

Fig. 7 Effect on the anticoagulant of SFE1 in vitro. 1. 0.9 % (w/v) NaCl (as a negative control); 2. 200 IU/ml UK (as a positive control); 3. 200 IU/ml SFE1

The anticoagulant effect of SFE1 in vitro

As shown in Fig. 7, no blood clots were observed in the test tube of SFE1 after 3 h (Fig. 7-3). In the test tube of UK, the blood clots were partly formed after 3 h (Fig. 7-2). Meanwhile, clotting had occurred in the test tube of normal saline (Fig. 7-1). The result indicated that the enzyme exhibited an efficient anticoagulant effect in vitro.

Thrombolysis mechanism of SFE1 in vitro

To estimate thrombolysis mechanism of SFE1 in vitro, the rates of heated and unheated blood clots digested by SFE1 were compared. The result showed that the rate of unheated blood clots digested by SFE1 was similar to that of heated blood clots. Therefore, SFE1 directly digested fibrin and not by plasminogen activators such as streptokinase, urokinase and tissue plasminogen activator.

Discussion

Thrombolytic therapy is still the best way to achieve recanalization in thrombosis diseases nowadays. Despite some thrombolytic agents' widespread use, all of them have drawbacks (Killer et al. 2010). Therefore, it is indispensable to screen new thrombolytic agents from diverse sources. In this study, we described purification and characterization of a fibrinolytic enzyme, designated as SFE1, from *Streptomyces* sp. XZNUM 00004. The enzyme was purified to electrophoretic homogeneity by combination of chromatographic steps on DEAE-Sepharose Fast Flow anion exchange and gel-filtration chromatography. Molecular mass of the purified enzyme was estimated to be approximately 20 kDa by SDS-PAGE and gel filtration chromatography. The molecular mass of SFE1 was lower than that of the fibrinolytic enzymes from *Streptomyces* sp. CS684 (35 kDa) (Simkhada et al. 2010), a marine bacterium *Bacillus subtilis* A26 (28 kDa) (Agrebi et al. 2009), *Bacillus subtilis* LD-8547 (30 kDa) (Wang et al. 2008) and *Perenniporia fraxinea* mycelia (42 kDa) (Kim et al. 2008). With respect to the effect of pH and temperature, the optimum pH (7.8) of SFE1 was similar to FP84 (7.5) from *Streptomyces* sp. CS684, but its optimum temperature, 35 °C, was different to FP84 (45 °C) (Simkhada et al. 2010). The p*I* was about 4.9, which was similar to that of lumbrokinase (F-I-0) (4.85) (Nakajima et al. 1993)and scolonase (4.8) (You et al. 2004). The p*I* of SFE1 was higher than that of PM-1 (4.4) (Ahn et al. 2003), N–V protease (4.5) (Zhang et al. 2007), and NJF (4.4) (Deng et al. 2010); but lower than subtilisin DFE (8.0) (Peng et al. 2003) and NJP (9.2) (Wang et al. 2011).

The first fifteen N-terminal amino acid residues were Ala-Pro-Ile-Thr-Leu-Ser-Gln-Gly-His-Val-Asp-Val-Val-Asp-Ile. In NCBI Blast searches, it revealed similarity only with hypothetical protein. However, except this, it did not show any homology with other fibrinolytic enzyme reported so far. Furthermore, SFE1 was strongly inhibited by a typical serine protease inhibitor PMSF (1 mM). SFE1 was also decreased 40.1 % by EDTA (1 mM) and 37.4 % by EGTA (1 mM). Taken together, we suggest that SFE1 can be a fibrinolytic enzyme with both serine and metalloprotease activity as reported by Simkhada et al. (2010) and Wang et al. (1999).

To investigate the thrombolysis mechanism of SFE1 in vitro, the fibrinolytic activity of SFE1 on heated and unheated blood clots was examined. SFE1 formed a similar-rate for both blood clots. SFE1 also exhibited fibrinogenolytic activity by rapidly hydrolyzing the $A\alpha$-chain, then the $B\beta$-chain, and the last γ-chain. Furthermore, we also found that SFE1 showed an efficient anticoagulant effect, which was similar to PPFE-I (Lu et al. 2010). These results indicated that SFE1 was a direct-acting fibrinolytic and fibrinogenolytic agent and not by plasminogen activators such as streptokinase, urokinase and tissue plasminogen activator.

In conclusion, the fibrinolytic enzyme SFE1, obtained from *Streptomyces* sp. XZNUM 00004, exhibits a profound fibrinolytic activity. Therefore, *Streptomyces* may become a source for thrombolytic agents to treat thrombosis. Further studies on the physiological function of SFE1 are proceeding.

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