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Review

Maggot Kinase and Natural Thrombolytic Proteins

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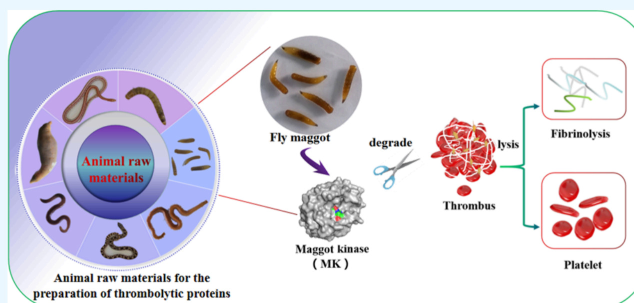

ABSTRACT: Thrombolytic enzymes constitute a class of proteases with antithrombotic functions. Derived from natural products and abundant in nature, certain thrombolytic enzymes, such as urokinase, earthworm kinase, and streptokinase, have been widely used in the clinical treatment of vascular embolic diseases. Fly maggots, characterized by their easy growth and low cost, are a traditional Chinese medicine recorded in the Compendium of Materia Medica. These maggots can also be used as raw material for the extraction and preparation of thrombolytic enzymes (maggot kinase). In this review, we assembled global research reports on natural thrombolytic enzymes through a literature search and reviewed the functions and structures of natural thrombolytic enzymes to provide a reference for natural thrombophilic drug screening and development.

1. INTRODUCTION

Vascular embolic disease poses a serious threat to human health. The dislodgement of a vein thrombus can cause pulmonary embolism, and thrombus formation in coronary or cerebral arteries can induce myocardial or cerebral infarctions, respectively.[1] Currently, the thrombolytic drugs used in domestic clinics primarily include antiplatelet, thrombolytic, and anticoagulant drugs,[2] such as streptokinase (SK), urokinase (UK), earthworm kinase, and pit-viper thrombolytic enzymes.[3] These enzymes, which are common macromolecular thrombolytic enzymes found in nature (Figure 1), are widely used in thrombotic disease treatment.

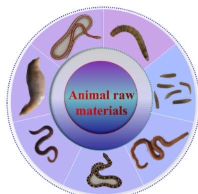

Figure 1. Natural raw materials for preparing thrombolytic enzymes.

Natural thrombolytic enzymes have important applications in the treatment of cardiovascular diseases in humans. In the physiological state, following the activation of coagulation factors in blood, produced thrombin converts fibrinogen to fibrin, which adheres to the intima of blood vessels. This fibrin on the intima is broken down by the fibrinolytic system of the body. The coagulation and fibrinolytic systems oppose each other, resulting in a dynamic equilibrium state for maintenance of normal blood circulation.[4] Coagulation factors, including fibrinogen and fibrin, play important roles in the thrombosis process, and the factors and enzymes involved in the coagulation process are important targets for the action of thrombolytic drugs. As protein biomolecules, the structure of thrombolytic enzymes affects their functions. In this review, the activities and structures of natural thrombolytic enzymes from different sources are examined to provide a reference for the development of thrombolytic enzymes.

1.1. Maggot Kinase. Since ancient times, fly maggots have been known to possess medicinal properties. The Compendium of Materia Medica details that fly maggots can be used for the treatment of "chancre, infantile paralysis, hot diarrhea, and vomiting." Maggot kinase (MK) is a newly reported thrombolytic compound isolated from fly maggots,[5] by the Chinese researcher, Liu Can. It comprises 226 amino acid residues, has thrombolytic activity in the pH range of 2−10, and begins to undergo inactivation at temperatures higher than 50 °C. The active site of MK comprises a triad of His41, Asp86, and Ser180, and it is inhibited by phenylmethylsulfonyl fluoride and aprotinin (a peptidyl-inhibiting enzyme), characteristics typical of the serine protease family. Fibrin is the primary component of a thrombus, and MK can directly dissolve thrombi by hydrolyzing fibrin (Figure 2). Fibrinogen is also involved in thrombus formation. Fibrinogen is activated

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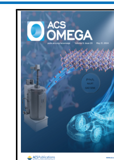

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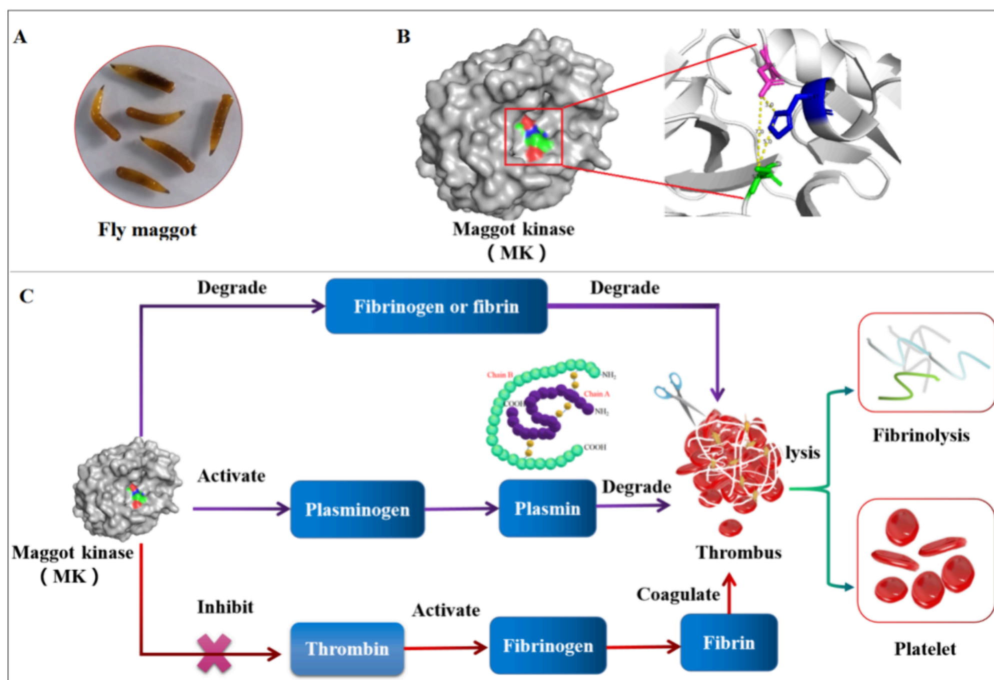

Figure 2. Thrombolytic mechanism of maggot kinase. (A) Fly maggots as a raw material for the preparation of maggot kinase. (B) Structure of maggot kinase. (C) The pathway of thrombolysis by maggot kinase.

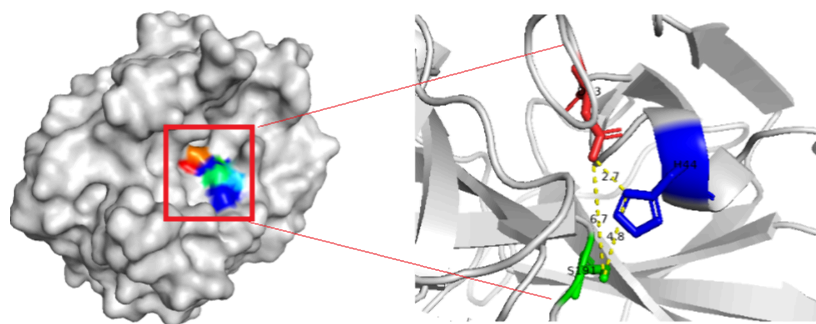

Figure 3. Structure of earthworm kinase (lumbrokinase).

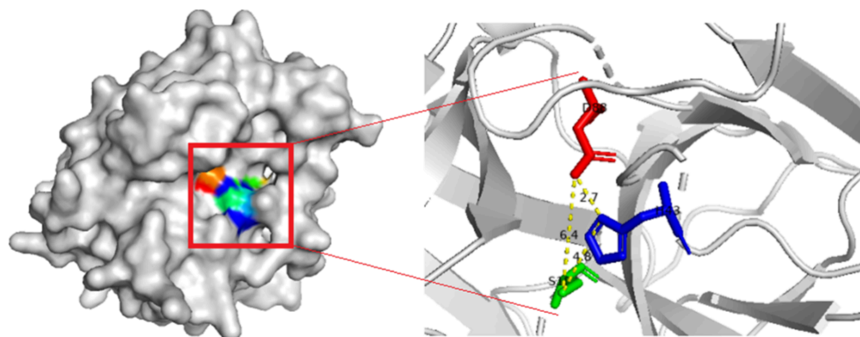

Figure 4. Structure of the snake venom thrombolysis enzyme (fibrolase).

by thrombin and can agglutinate with platelets to form thrombi. MK can decompose fibrinogen via fibrinogen α-, β-, and γ-chain degradation; however, it degrades the fibrinogen α-chain more efficiently than it hydrolyses the β- and γ-chains. MK can inhibit thrombus formation by lowering fibrin concentration and reducing the thrombus formation matrix. By degrading thrombin and activating plasminogen, MK can exert antithrombosis effects via multiple target points.

1.2. Lumbrokinase. In the 1980s, earthworm kinase (lumbrokinase), a type of serine protease, was isolated from the earthworm, *Eisenia fetida*, by the Japanese scientist, Hisashi Mihara.[6] Hisashi Mihara et al.[7,8] isolated six of its components, which have different molecular weights and isoelectric points (pI range: 3.40−4.85). Earthworm kinase (Figure 3) exerts anticoagulant and thrombolytic effects. For example, it can degrade clotting factors in blood, thereby inhibiting platelet

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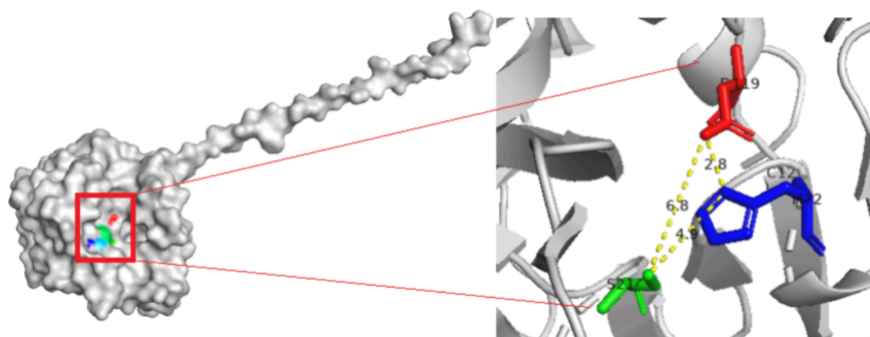

Figure 5. Structure of UFE.

aggregation.[9,10] In addition, it stimulates tissue plasminogen activator (t-PA) release from vascular endothelial cells, thereby increasing t-PA activity. Earthworm kinase can directly activate plasminogen, thereby playing a role in thrombus degradation; it can also degrade fibrin. Studies have shown that earthworm kinase has good thermal stability (stable at 55 °C for 1 h) and is active at pH 4−11. Currently, earthworm kinase is clinically used to treat patients with thrombotic diseases and this treatment associated with increased fibrinogen levels and platelet aggregation.[11,12]

1.3. Snake Venom Thrombolytic Enzymes. Snake venom is primarily a mixture of proteins, and contains enzymes and various toxins, such as proteohydrolases, phospholipases, arginine vinylase, and other hydrolytic enzymes.[13] In addition, it contains the antithrombotic component, snake venom thrombolysis enzyme (fibrolase).[14] The relative molecular weights of the thrombolytic enzymes in snake venom range from 22 to 58 kDa.[15] Thrombin-like snake venom thrombolytic enzymes can selectively degrade fibrinogen, and serine-like snake venom thrombolytic enzymes exhibit the characteristics of arginine esterases, which specifically act on the β-chain of fibrinogen.[16] In addition, they can induce direct fibrin breakdown.[17] Snake venom thrombolytic enzymes (Figure 4) isolated from the venom of the Jiangzhe pit viper can promote t-PA release and reduce the concentrations of fibrinogen activators and alpha-2-plasmin inhibitor (a2-PI), thereby enhancing the activity of the fibrinolytic system and inducing rapid thrombus dissolution.[18] Clinically, thrombolytic enzymes from snake venom are often used for the treatment of peripheral vascular diseases, such as hypercoagulable states and cerebral infarction.

1.4. *Urechis unicinctus* Fibrinolytic Enzyme. In 2006, Wang Dianliang et al.[19] isolated the thrombolytic component, *Urechis unicinctus* fibrinolytic enzyme (UFE), from the annelid, *U. unicinctus*. This fibrinolytic enzyme comprises a series of isozymes, including UFE I, II, III, and IV,[20] which possess significant anticoagulant and thrombolytic activities and good biosafety.[21] The pI of monocyclic spiny grasshopper fibrinolytic isozymes ranges from 4.5 to 8.5,[22,23] and their relative molecular weights range from 10 to 45 kDa (UFE I, II, III, and IV have molecular weights of 5.1, 26.7, 20.8, and 11 kDa, respectively).[24] In vivo studies in animals have shown that UFE (Figure 5) exhibits significant thrombolytic activity, does not induce hemolytic reactions in the body, activates thrombolysinogen, and degrades fibrin (fibrinogen),[25] with no toxic side effects.

1.5. Nereis virens Thrombolytic Enzyme. Nereis kinase (NK) (Figure 6), a thrombolytic protein isolated from the nereis virens, has a molecular weight ranging from 9−40 kDa.[26] Lu Guohui et al.[27] used prokaryotes to induce NK expression and found the active protein to be a tetramer with a molecular weight of 36 kDa. Another NK (N−V proteinase, N−V P), discovered in 2007 by Zhang Yunlong et al.,[28] was isolated from a sandworm of the intertidal polychaete fauna. It is a novel thrombolytic enzyme (named N−V protease) of the serine protease family. N−V protease is a single chain protein with an isoelectric point of 4.5.[29] It hydrolyzes the α-chain of fibrinogen with high efficiency and its β- and γ-chains with lower efficiency. Its efficiency for fibrinogen chain hydrolysis is in the order, α-chain > β-chain > γ-chain. The optimum pH for thrombolytic enzymes isolated from *Perinereis aibuhitensis* Grube is 8.0, with excellent activity in 6.0−9.0 range.[30]

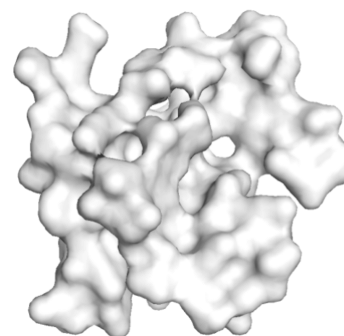

Figure 6. Structure of NK.

1.6. Yellow Mealworm Fibrinolytic Protease. The yellow mealworm, commonly known as bread worm, is an economic insect with a wide range of applications. Tenebrio fibrinolytic proteins (TFPs) (Figure 7), which exhibit significant fibrinolytic activity without observable hemolysis, were discovered by Wu Yanling et al.[31] in 2011. They have an isoelectric point of 8.34, a relative molecular mass of 56.1 kDa, an optimal pH of 7.5, and an optimal temperature range of 25−45 °C.[32] Liu Jianwei et al.[33] used mice to evaluate the effects of TFPs on thrombosis inhibition and found that they inhibited thrombus formation in mouse tails. The recombinant expression of thrombolytic enzymes in *Xanthomonas campestris* has also been studied. Han Yali et al.[34,35] used *Pichia* yeast to construct a thrombolytic protein expression model for the isolation of recombinant fibrinolytic enzymes, providing a reference for the establishment of a cell TFP production factory.

1.7. Streptokinase. Streptokinase (SK) is the first protein thrombolytic drug to be used in clinical practice (Figure 8). It is a nonenzymatic, secreted protein expressed by the prokaryotic microorganism, *Streptococcus hemolyticus*, that dissolves thrombi by activating plasminogen. In 1933, William

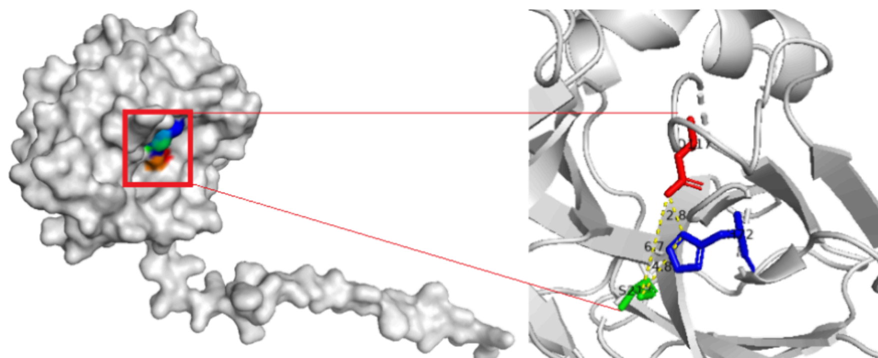

Figure 7. Structure of the TFP.

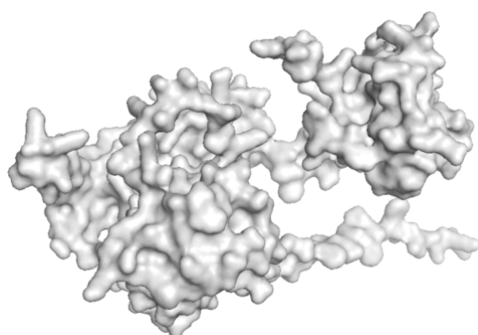

Figure 8. Structure of SK.

Tillett et al.[36] first observed that β-hemolytic *Streptococcus sp.* could secrete a thrombolysis-promoting substance. In 1945, Christensen et al.[37] found that this substance could activate plasminogen, thereby indirectly degrading thrombi. They named this substance SK. Its relative molecular weight ranges from 47 to 50.2 kDa, and its isoelectric point is 5.0.[38,39] The optimal reaction pH for SK is within the 7.3−7.6 range. SK is not an enzyme but functions as a cofactor to promote plasminogen activation.[40,41] Thus, SK indirectly promotes the activation of plasminogen, forming a complex with it at a 1:1 molecular ratio, and promotes the cleavage of the plasminogen molecule polypeptide chain. This phenomenon exposes the active centers of the enzyme and induces the conversion of plasminogen to plasmin, which plays a role in thrombolysis.[42−45] SK has Asp and His as its active centers, with no Ser residue sites; therefore, it is not a serine protease. Clinically, SK is characterized by a rapid onset of action and short half-life of 10−20 min.

1.8. Urokinase. UK, a protease with thrombolytic effects, is isolated from human urine or renal cell tissue cultures. UK has a relative molecular weight within the 33−54 kDa range, a pI between 8.4 and 9.7,[46,47] and an optimal reaction pH between 7.5 and 8.0.[48] In the human body, UK comprises large (54 kDa) and small (33 kDa)[49] molecular weight UKs, which are related to kidney stone formation. When their secretion is within normal physiological levels, stone formation is inhibited; however, when their concentrations decrease, stone formation occurs.[50]

Unlike SK, which indirectly activates plasminogen, UK can specifically cleave the peptide bond between the Arg561 and Val562 residues in plasminogen, thereby transforming plasminogen into plasmin (Figure 9). Consequently, this enzyme breaks down coagulation factors V and VIII and inhibits platelet aggregation. In addition, fibrinolytic enzymes break down fibrin clots and fibrinogen in the vasculature, effectively inhibiting thrombosis and dissolving thrombi.[49]

1.9. Natto Kinase. In 1987, Hiroyuki Sumi et al.[51] isolated a strain of *Bacillus subtilis* var. natto from fermented natto beans and found that it produced an alkaline serine protease with thrombolytic activity. This protease could decompose fibrous proteins in soybeans and was named NK (Figure 10). NK has a molecular weight of 27.7 kDa, a pI within the 8.6−8.9 range,[52] an optimal reaction pH of 7.0, and exhibits a relatively stable enzymatic activity between pH 6.0 and 9.0;[53] it has an optimal reaction temperature of 40 °C.[54] It is 1.36 times more active than UK and can induce the production of fibrinolytic enzymes through facilitating t-PA activity for thrombus and fibronectin degradation, making it a well-established natural thrombolytic enzyme in early research.[55] The gene encoding NK has a sequence length of 1473 bp,[56−58] and contains three parts and 381 amino acids. The signal

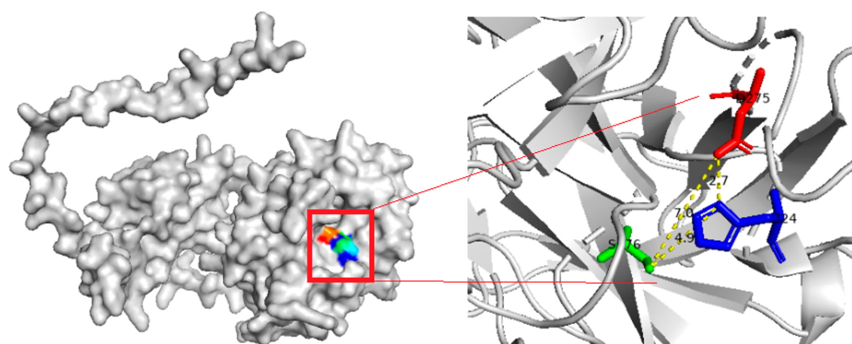

Figure 9. Structure of UK.

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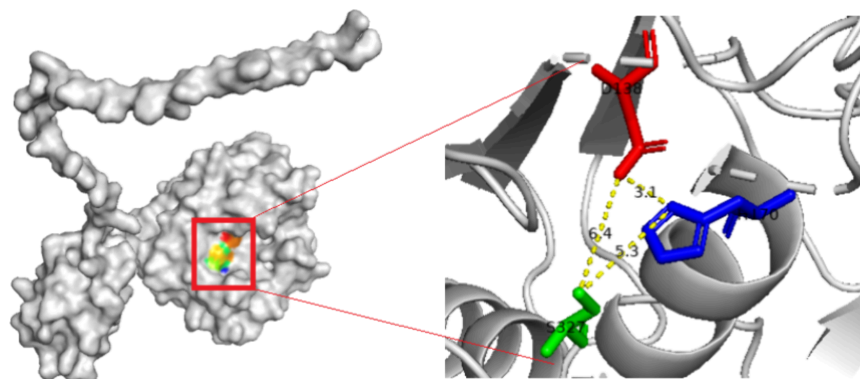

Figure 10. Structure of NK.

peptide, which mediates protein secretion, comprises the N-terminal end and the first to 29th amino acids. The leading peptide, which assists in the correct folding of proteins, comprises the 30th to 106th amino acids. The remaining 275 amino acids constitute the mature NK.[59,60]

1.10. Hirudin. In 1884, British scientist, John Haycraft, first discovered the existence of an anticoagulant substance in blood-sucking leeches. In 1904, Jacoby extracted this active ingredient, a natural polypeptide, from the pharynx of leeches and named it hirudin (Figure 11). The isoelectric point of hirudin is within the 3.5−4.0 range, and its relative molecular mass is between 7 and 9 kDa. Its optimum pH and temperature are 8.5 and 55 °C, respectively.[61] Hirudin is structurally stable and retains its high activity following gastric digestion, rendering it an orally available drug. Hirudin can directly bind to thrombin at a 1:1 ratio, forming an irreversible complex that results in thrombin inactivation, thereby blocking the conversion of fibrinogen to fibrin. It also inhibits thrombin from binding to platelets and inhibits platelet aggregation.[62]

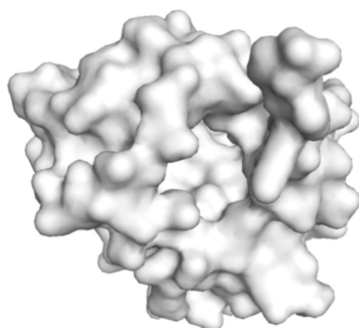

Figure 11. Structure of hirudin.

1.11. Clinical Uses of Thrombolysis Proteins. The clinical applications and side effects of natural thrombolytic proteins are shown in Table 1. Currently, lumbrokinase, urokinase, snake venom thrombolytic enzymes, hirudin, and streptokinase have been put into clinical use as thrombolytic drugs for the prevention and treatment of thromboembolic diseases. Natto kinase is mainly used as a nutraceutical to promote blood circulation. However, Maggot kinase, yellow mealworm fibrinolytic protease, nereis virens thrombolytic enzyme, and UFE are still in the preclinical research and development stage and have not yet become clinical drugs.

2. COMPARISON OF THE SPATIAL STRUCTURES OF THROMBOLYTIC ENZYMES

Protein structure determines the nature and function of proteins; thus, there may exist structural similarities between thrombolytic enzymes from different sources. The three-dimensional structure of MK was compared with those of fibrinolytic enzymes from different species, and the similarity was characterized using the root-mean-square deviation (RMSD) value. If RMSD was 0, the two structures were considered identical. If RMSD was <3, the two structures were considered similar, with lower RMSD values indicating higher similarity levels.[120] As shown in Figure 12, MK was compared with other fibrinolytic enzymes, and RMSD values were compared as follows: urokinase (UK) (0.745) < snake venom fibrinolytic enzyme (AhV_TL-I) (0.917) < nereis kinase (SAK) (0.958) < earthworm kinase (EFEa) (0.970) < urechis unicinctus fibrinolytic enzyme (UFE-II) (0.975) < yellow mealworm fibrinolytic enzyme (YFP) (1.030) < human fibrinolytic enzyme (PL) (1.524) < streptokinase (SK) (3.527) < hirudin (H) (11.544) < natto kinase (NK) (18.719). These results indicate that MK is highly similar, in terms of spatial structure, to UK, snake venom fibrinolytic enzyme, nereis kinase, earthworm kinase, and urechis unicinctus, yellow mealworm, and human fibrinolytic enzymes. As shown in Figure 12, MK has overlapping portions with all the other fibrinolytic enzymes, particularly with UK. Conversely, significant differences in spatial structure were observed between MK and SK, hirudin, and NK.

3. ACTIVE CENTER AND CATALYTIC ACTIVITY OF FIBRINOLYTIC ENZYMES

As shown in Table 2, the active center of fibrinolytic enzymes comprises three amino acid residues, His, Asp, and Ser, which constitute a conserved catalytic triad for serine proteases. The amino acid residues of the triad are organized in the same sequence, with His, Asp, and Ser located in the front, middle, and back positions of the peptide chain, respectively. Moreover, the primary structures of His, Asp, and Ser are relatively distant from each other. However, after folding of the peptide, these amino acid residues become close to each other, thereby constituting the active center of the enzyme. The imidazole group of His can function as a nucleophilic group and a generalized acid−base functional group. Moreover, His forms a hydrogen bond with Asp and Ser, building a bridge between the carboxyl group of Asp and the hydroxyl group of Ser. These three residues build a charge relay network in the active center of the protein which plays an important role in

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Table 1. Clinical Uses of Thrombolytic Proteins

Name of thrombolytic protein	Clinical use	Advantages in clinical use	Disadvantages in clinical use
Lumbrokinase	Treatment of ischemic cardiovascular and cerebrovascular diseases in patients with increased fibrinogen levels and increased platelet aggregation rate in a context of ischemic cerebrovascular disease.[63−65]	Lumbrokinase can effectively improve whole blood viscosity and reduce fibrinogen content, thereby reducing plasma viscosity and platelet aggregation rate. It is associated with good thrombolytic activity, less toxic side effects, and a low complication rate. Lumbrokinase is a protein-hydrolyzing enzyme that can activate the fibrinolytic system, as well as plasminogen and plasmin formation.[66−68]	A very small number of patients may experience mild headaches, dizziness, constipation, nausea, and other adverse reactions that do not require special treatment.[69−71]
Urokinase	Thrombolytic therapy for thromboembolic diseases.[72−74]	Urokinase can act on the fibrinolytic system and activate plasminogen. It exhibits no antigenicity and has a good safety profile.[75−77]	Although it exhibits no antigenicity, individual patients treated with the urokinase may develop mild allergic reactions, such as rash, bronchospasm, and fever.[78−80]
Snake venom thrombolytic enzymes	Suitable for the treatment of cerebral thromboses, thromboembolic phlebitis, and venous thromboses.[81−83]	Snake venom thrombolytic enzyme is characterized by fast onset of action and mild adverse effects. It is also characterized by high thermal stability and low hemorrhagic activity, and can directly dissolve fibrinogen and fibrin.[84−88]	Increased bleeding tendency and allergic reactions in a small number of patients.[89−91]
Hirudin	Treatment of various thrombotic disorders, especially venous thrombosis and diffuse vascular coagulation; it is also used to prevent the development of arterial thromboses after surgical procedures.[92−94]	Hirudin exhibits very potent anticoagulant and antithrombotic effects.[95−97]	High doses of hirudin may cause bleeding; to ensure patient safety, they should be monitored through regular prothrombin time measurements during treatment.[98−100]
Streptokinase	Treatment of thrombotic diseases such as acute myocardial infarction.[101−103]	Streptokinase induces plasminogen activation into fibrinolytic enzymes, which can dissolve blood clots and treat diseases with thrombus formation as main pathologic change.[104−106]	May cause fever, chills, nausea, vomiting, and bleeding.[107−109]
Natto kinase	Most natto kinase-containing products on the market are used as health supplements for the prevention of thrombotic diseases, and are not used as clinical drugs.[110−113]	Effective in thrombolysis, antiplatelet aggregation, and anticoagulation.[114−117]	Natto kinase is very sensitive to acids, heat, and oxides.[118,119]

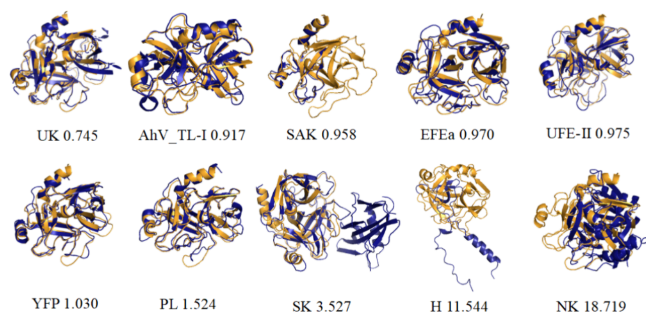

Figure 12. Comparison of the structure of MK with that of other fibrinolytic enzymes.

Table 2. Active Centers of Fibrinolytic Enzymes

Fibrinolytic enzyme	Active center
Maggot kinase (MK)	His41, Asp86, Ser180
Lumbrokinase (EFEa)	His44, Asp93, Ser191
Plasmin (PL)	His603, Asp646, Ser741
Fibrolase (AhV_TL-I)	His43, Asp88, Ser184
Urokinase (UK)	His224, Asp275, Ser376
Natto kinase (NK)	His138, Asp170, Ser327
Urechis unicinctus fibrinolytic enzyme (UFE-II)	His72, Asp119, Ser214
Yellow mealworm fibrinolyric protein (YFP)	His72, Asp117, Asp213
Streptokinase (SK)	-

hydrolyzing peptide bonds.[121] As shown in Figure 13, the process by which serine family proteases catalyze the hydrolysis of peptide substrates can be divided into two phases;

(1) Acylation phase: The serine protease active center binds to the peptide substrate to form a transition intermediate. The hydroxyl group of Ser functions as a nucleophilic group that attacks the carbonyl carbon atom on the peptide bond (A). Subsequently, the hydrogen atom on the hydroxyl group of Ser is transferred to the His imidazole group to form a tetrahedral transition intermediate (B), in which the amine part of the substrate is linked to the His imidazole group via hydrogen bonding. The proton on His drives the cleavage of the peptide bond of the substrate molecule into two parts, and the product amine is dissociated from the imidazole ring. The carboxyl portion of the substrate esterifies with the hydroxyl group of Ser to form the "acylase" covalent intermediate (C), while the carboxyl group on the side chain of Asp in the active center is always in the ionic state, and contributes to the progress of the reaction.[122]

(2) Deacylation phase: The proton dissociates and is accepted by His, while H_2O attacks the carboxyl carbon atom of the substrate attached to the hydroxyl group of Ser (D) to form a tetrahedral intermediate (E). Subsequently, His transfers the proton to the oxygen atom on Ser, the C−O bond breaks, "acylase" is deacylated, and the carboxyl-containing peptide (product) is dissociated from the Ser residue of the enzyme molecule (F).[123] Thus, the peptide bond of the substrate is hydrolyzed, and the enzyme molecule returns to its initial state.

The active centers of other fibrinolytic enzymes comprise the His, Asp, and Ser triplet, characteristic of the serine family of proteases. However, SK is a nonenzymatic protein the active site of which does not contain Ser. It forms a complex with plasminogen at a 1:1 molecular ratio through its three key α (residues 1−150), β (residues 151−287), and γ (residues 288−414)[38] regions, thereby promoting plasminogen activation and exerting thrombolytic effects.

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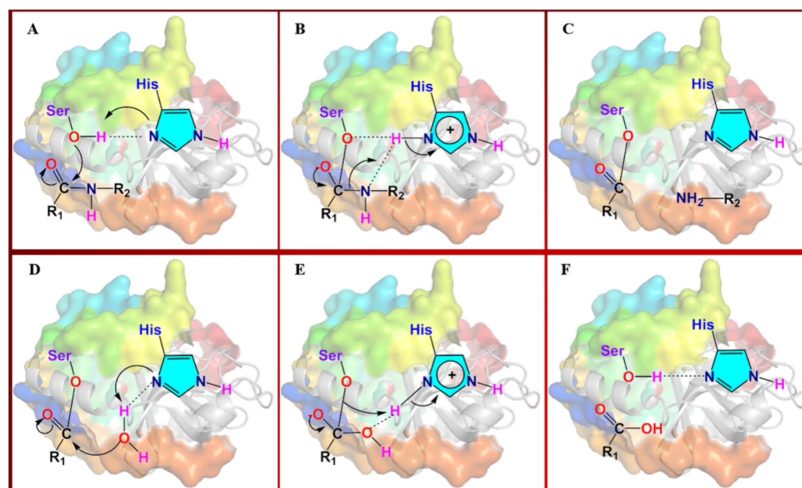

Figure 13. Catalytic mechanisms of serine family fibrinolytic enzymes. (A) The serine protease active center binds to the peptide substrate to form a transition intermediate. (B) The hydrogen atom on the hydroxyl group of Ser is transferred to the His imidazole group to form a tetrahedral transition intermediate. (C) The carboxyl portion of the substrate esterifies with the hydroxyl group of Ser to form the "acylase" covalent intermediate. (D) The proton dissociates and is accepted by His, while H_2O attacks the carboxyl carbon atom of the substrate attached to the hydroxyl group of Ser. (E) Form of a tetrahedral intermediate. (F) Product is dissociated from the Ser residue of the enzyme molecule.

4. ACTIVE CENTER AND CATALYTIC ACTIVITY OF FIBRINOLYTIC ENZYMES

The amino acid sequence of MK was compared with that of each fibrinolytic enzyme extracted from different species (Figure 14). The homology, in descending order, was as follows: YFP (49.2%), UFE II (34.70%), EFEa (29.60%), plasmin (27.82%), UK (25.34%), AhV_TL-I (23.13%), NK (12.83%), SAK (11.28%), SK (5.88%), and hirudin (H) (5.81%). The primary structure of a protein determines its spatial structure, and a higher percentage of identity indicates a closer homology between sequences. The results of the primary structure comparison showed that among the 10 selected fibrinolytic enzymes, MK had the closest sequence homology with the yellow mealworm fibrinolytic enzyme (YFP), SK belonged to the nonserine protease family, and MK exhibited the highest difference in sequence homology between its primary structure and that of SK.

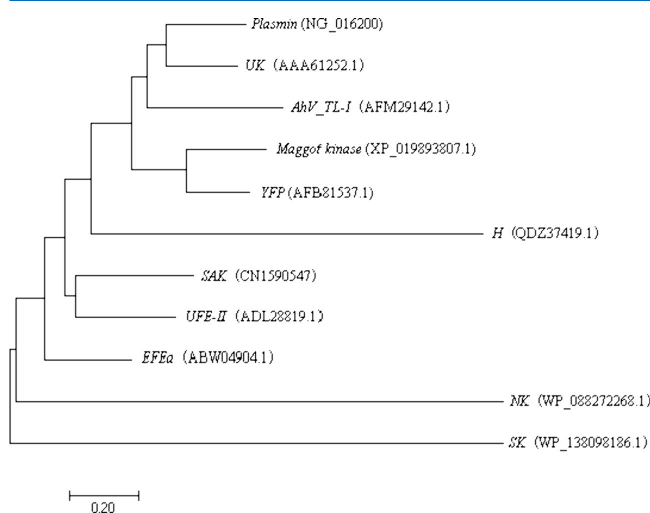

Figure 14. Evolutionary tree of different fibrinolytic enzymes.

5. SUMMARY AND PROSPECTS

Cardiovascular and cerebrovascular diseases are characterized by high morbidity, disability, mortality, recurrence, and complication rates. In rural and urban China, cardiovascular disease is one of the most common causes of death, and the demand for related drugs is increasing. Thrombolytic drugs are important agents for the treatment of thrombotic diseases as they have potent thrombolytic effects, and can directly dissolve formed thrombi, quickly unblocking blocked blood vessels. In addition, they have a great market prospect.

Natural resources can be used for the development of new drugs, and the extraction of natural fibrinolytic enzymes has gained significant research attention in recent years. With the increase in the incidence of thrombotic diseases, the cardiovascular drug market has significant potential as companies are constantly developing new thrombolytic drugs. Understanding thrombolytic enzyme mechanisms is important for the development of these drugs. Research has focused on the development of effective, safe, and inexpensive natural thrombolytic enzymes from various species. The primary mechanisms of thrombolytic proteins include: (1) Direct activation of plasmin to induce fibrinolysis through the hydrolysis of the amide bond between the Arg561 and Val562 residues of plasminogen; (2) indirect induction of the conversion of plasminogen to plasmin; (3) direct degradation of fibrin and fibrinogen; (4) inhibition of thrombin activity (5) inhibition of platelet activation and aggregation. Currently, earthworms, pit vipers, urine, and hemolytic *Streptococci* are used to purify fibrinolytic components and develop thrombolytic drugs. Upon circulation in the pharmaceutical market, these drugs are used to cure illnesses and save lives. Except for SK, which is a nonenzymatic protein, all thrombolytic enzymes are structurally serine proteins, with His, Asp, and Ser constituting their active centers, through which they carry out enzyme binding and substrate catalysis. Sequence and structural comparisons revealed that thrombolytic proteins share a certain amino acid sequence homology, and a high degree of similarity in spatial structure.

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The demand for thrombolytic proteins is high, and earthworms are the raw material for the extraction of earthworm kinases. They are currently hunted and killed in large quantities, with serious nefarious consequences on the ecological balance, as acknowledged in the statement, "Strictly cracking down on the destruction of the soil such as electrocution of earthworms, etc.," published in the 2023 Central Document No. 1 in China. The collection of urine and pit viper venom is also challenging, indirectly increasing the price of thrombolytic drugs. Therefore, developing thrombolytic drugs with high efficiency and low cost is necessary. Fly maggots, which are insect larvae, have the advantage of a short breeding cycle and low growing environmental requirements. They could be used to solve the problem of raw material shortage for thrombolytic enzyme production and provide a new direction for the development of highly efficient and inexpensive thrombolytic drugs.

Furthermore, biosynthesis is an important prospective direction for thrombolytic enzyme development.[124] Synthesis of thrombolysis proteins using the technology of microbial synthesis can circumvent the limitation associated with the production of thrombolysis proteins from natural raw materials.[125] In addition, biosynthesis technology is traceable and can simultaneously improve product purity, reduce cost of production, and reduce the dependence on natural raw materials.[126,127] At present, marketed urokinase is mostly synthesized through genetic engineering, using *Escherichia coli*[128,129] or hamster ovary cells[130,131] for expression. Streptokinase is produced mainly through microbial synthesis;[132] the biosynthesis technology for recombinant streptokinase is highly robust, and can allow for its expression in *E. coli*, with improvements in its production yield and activity.[133−135] At present, the earthworm kinase gene has been successfully expressed in fungi,[136] bacteria,[137] and animal cells;[138] however, its expression is low and its effect is unsatisfactory.[136] The earthworm kinase on the market is mainly extracted from *Eisenia fetida*.[139,140] The yeast expression system has become the method of choice for the expression of snake venom thrombolytic enzymes;[141,142] however, the expression of recombinant snake venom thrombolytic enzymes in this system is low,[143] with the snake venom thrombolytic enzymes available on the market mainly extracted from snakes.[144] With the continuous development of biosynthesis technology, synthesis of more active thrombolytic enzymes using microorganisms will become a reality.

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Notes

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