Development of Marine Fibrinolytic Enzymes as a Resource for Novel Proteases and Their Roles in Fibrinolysis and Thrombolysis

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Introduction

Thrombosis is a serious disease of human health and life, such as acute myocardial infarction (AMI) and venous thrombus embolism (VTE). Nowadays, thrombolytic therapy is a common treatment method for the heart muscle infarction and other thrombotic disease.

Thrombosis is a small clot of blood that is formed on the surface of the vessel lining or the repair cardiovascular system. In variable flow dependent patterns, thrombus is composed of insoluble fibrinous proteins, deposited platelets, accumulated white blood cells and trapped red blood cells. Thrombosis is a multivariate process of interaction and interaction between a set of genetic and environmental factors. There are three main mechanisms of thrombosis: Firstly, damage of intima of heart and blood vessel. (1) When the lining is damaged, the endothelial cells are transgendered and necrotic and the skin is exposed to collagen. To activate the endogenous clotting system, endogenous coagulation system is activated. (2) The tissue coagulation factor which releases by damaged intima can activate exogenous coagulation system. (3) The damaged intima becomes coarse, making platelets easy to aggregate. Secondly, hemodynamic change. The blood flow slows and the blood forms vortex. Thirdly, blood change. Increased coagulation of blood was found in the increase of platelets and coagulation factors.

Thrombolysis refers to degrade thrombosis by enzymatic reaction. When a clot is formed in the body, the plasminogen (Pg) in the blood is partially enriched to the thrombus because of its affinity for fibrin. In vivo. Plasminogen activation (pA) can activate Pg to plasmin (Pm) and Pm with the activity of serine protease can degrade the fibrin that forms the thrombus skeleton. Thus it can degrade the thrombosis. There are three types of drugs that can prevent clots. The first is anticoagulants whose main function is to prevent blood clotting. The second is anti-platelet aggregation drugs in which a receptor antagonist of platelet glycoprotein II b/III a is an important milestone of anti-platelet therapy. The third is thrombolytic drugs which can act on Arg-Lys to decompose fibrinous protein into a soluble product. It contains two main kinds, streptokinase (SK) and urokinase (UK). SK can combine with Pg to form SK-Pg which can activate Pg into Pm and degrade thrombosis finally. UK can immediately act on Pg and form Pm to hydrolyze for fn.

Streptokinase[1-3] as a classical fibrinolytic enzyme from Streptococcus

Streptokinase, a protein secreted by several species of streptococci can bind and activate human plasminogen. Streptokinase is used as an effective an inexpensive thrombolysis medication in some cases of myocardial infarction (heart attack) and pulmonary embolism. SK belongs to a group of medications known as fibrinolytic, and complexes of streptokinase with human plasmin can hydrolytically activate other unbound plasminogen by activating through bond cleavage to produce plasmin.

Streptokinase is an extracellular bacterial protein produced by several strains of Streptococcus haemolyticus group C, and it is also secreted by Streptococcus equisimilis, pathogenic strains of streptococcus. Streptokinase, an important thrombolytic protein, displays a molecular mass in the region of 45–48 kD and an isoelectric point of 4.7. Streptococcus equisimilis streptokinase is a single-chain protein of 414 residues. Streptokinase is composed of three structurally autonomous domains, which are homologous, independently folded, and connected by flexible linking segments, with a C-terminal “tail” that is relatively unstructured. In solution, free streptokinase is highly flexible, with the three domains able to assume many different conformations due to the linking sequences. The three domains of streptokinase are denoted α (residues 1–150), β (residues 151–287), and γ (residues 288–4140). Each domain binds plasminogen, although none can activate plasminogen independently. All of streptokinase’s three domains are essential for native-like streptokinase activity. The central and C-terminal domains mediate plasminogen-binding and active site-generating function, whereas the N-terminal domain mediates an activity-potentiating function. Streptokinase is used extensively in the clinical treatment of acute myocardial infarction due to its ability to
activate human plasminogen.

Streptokinase is a potent activator of the fibrinolytic enzyme system, in humans streptokinase binds to plasminogen and generates a streptokinase-plasminogen activator complex. This complex catalyzes the conversion of plasminogen to plasmin, an active enzyme that degrades the fibrin component of thrombin: binding specifically and tightly to plasminogen; inducing conformational change in the plasminogen molecule; reordering it proteolytically active.

Streptokinase secreted by Streptococcus equisimilis activates the human fibrinolytic system by converting the zymogen, plasminogen (Pgl) into the clot-dissolving proteinase, plasmin (Pm). This activity is the basis for the use of streptokinase as a thrombolytic drug for treatment of myocardial infarction. Streptokinase activates Pgl by a unique mechanism initiated by rapid and reversible formation of an SK-Pg.

### Marine fibrinolytic enzymes from marine organism

Bacteria, fungi and actinomycetes have the ability to produce fibrinolytic enzymes, especially bacteria which have many kinds. The streptokinase secreted by Streptococcus hemolyticus and staphylokinase (SaK) secreted by Staphylococcus aureus are found earlier which is produced through bacteria. In recent years, there are more and more kinds of fibrinolytic bacteria found. Most of them are marine microorganism. Kim et al. separated and purified a kind of plasm in which molecular weight is 28.2 kD and secreted by Bacillus Sp.CK-114. Oaks Walton et al. extracted a medium-type endo-dermal fibrinolytic enzyme streptocococcus faceum, which molecular weight is 19 kD. Poonam et al. reported a low molecular weight chymotrypsin-like novel fibrinolytic enzyme from Streptomyces sp. CS624. Wang et al. also extracted a kind of plasmin SW-1 which molecular weight is 30 kD. It is important to note that the same fermentation is often caused by the diversity of bacterial strains and the diversity of fibrinolytic enzymes. In China, the Bacillus amyoliquificaciens DC-4 can produce DFE. Bacillus subtilis DC3 can secrete FS33. In Korea, the Bacillus sp.CK11-4 can secrete CK, and the Bacillus villismortis Ace02 can extract Ace02. Liu et al. reported on a marine Pseudomonas sp., which can produce a strong alkaline protease MPAP that can directly disolve fibrin protein and antithrombogenic formation. Ac et al. studied on the characterization of a 27 kDa fibrinolytic enzyme from Bacillus amyoliquificaciens CHH6-1 isolated from Cheonggukjang. Li et al. reported a marine microorganism B5815, which can produce plasmin. Han et al. detected a unique fibrinolytic enzyme in Aeromonas sp. JHI. Huang et al. found a marine strain IQS-3 with high fibrinolytic enzyme activity.

Won et al. showed biochemical analysis of a fibrinolytic en-zyme purified from bacillus subtilis strain A1. Wang et al. studied on anticoagulant and thrombolytic effects of a novel marine fibrinol-ytic enzyme in vitro. The results showed that there were apparent anticoagulant effect as well as thrombolytic effects in the self-made marine fibrinolytic enzymes, and the status of the released blood cells from thrombus using this enzyme was also better than that of using lumbrukiase. Aungkawipa et al. reported novel fibrinolytic en-zymes from Vibobacillus halodenitrificans SK1-3-7 isolated from fish sauce fermentation. Li et al. reported a marine Bacillus subtilis which produces fibrinolytic enzyme was mutagenesis by ultravio-let (UV). A mutant (Y-22) contained high fibrinolytic activity was obtained. It was stable after several generations, Ramakat et al. studied on production, purification, and biochemical characterization of a fibrinolytic enzyme from Thermophilic Streptomyces sp. MCMB-379. Liu et al. studied a kind of plasm in which extracted from Urechis Unicinctus. The result of fibrinolytic test shows that UFE owns kinase activity and apparent fibrinolytic effection and conclusion was achieved that UFE1 possesses significant effect on anticoagulability and anti thrombotic activity, which induces a temporary rise of specific antibodies in mice, but does not affect adversely on animal bodies. Chen et al. did the research on purification and biochemical properties of a fibrinolytic enzyme from Bacil-lus subtilis fermented red bean. Wu et al. reported that three small molecular compounds with fibrinolytic facilitation were obtained from the metabolites of algae and microorganisms. The compound is not the enzyme, but it has showed excellent fibrinolytic facilitation in the simulated reaction system, and its thrombolytic promoter was confirmed from animal models. Jo et al. introduced purification and characterization of a major fibrinolytic enzyme from Bacillus amyoliquificaciens MJ-54 isolated from Meju.

The fibrinolytic molecular weight of discovered from microbial is generally between 21 and 40 kD. The molecular weight of Bacillus is concentrated in about 28 kD. Occasionally the enzyme has a higher molecular weight, such as Joot-Ga, whose molecular weight is 41 kD, SK006 whose molecular weight is 43–46 kD and the plasmin of Cordyceps militar is 52 kD. Most of these fibrinolytic enzyme in less than 40 °C and weak alkaline environment is stable and high-est energy. The fibrinolytic enzyme produced by Streptomyces megasporus SDX which is isolated from hot spring has highest en-zyme activity in 55 °C and in 70 °C it can has half. Ca plays an important role in keeping the stability of the enzyme. The origin and properties of some fibrinolytic enzymes are shown in Table 1.
FGFC1 showed excellent thrombolysis effects. Qu et al.\(^\text{[9]}\) reported dissolving effect of marine pseudomonas alkaline protease on the arterial thrombus of experimental rabbits. The results showed MPAP has certain solubility to the experimental rabbit femoral artery thrombosis and has obvious anticoagulant function. Wang et al.\(^\text{[21]}\) also studied on thrombolytic effects of a novel marine fibrinolytic enzyme in vivo. The results showed that there were apparent anticoagulant effect as well as thrombolytic effects in the self-made marine fibrinolytic enzymes, and the status of the released blood cells from thrombus using this enzyme was also better than that of using lumbrukinase. It has better thrombolysis activity. Chu et al.\(^\text{[7]}\) studied on UFE-I which extracted from Urechis unicinctus. The results showed it has excellent thrombolytic action. Through the above study, we find more and more thrombolytic drugs which are secreted by marine organism and they have excellent thrombolysis activity. Currently, thrombolysis drugs produced by marine organism (especially marine microorganism) have become a research hotspot.

### Table 1 Comparison of species and characteristics of fibrinolytic compound\(^\text{[32]}\)

| Organism          | Producing strain | Enzyme       | Molecular weight | N-terminal AA | Ref.  |
|-------------------|------------------|--------------|------------------|---------------|-------|
| Actinomycetes     |                   |              |                  |               |       |
| Streptomyces sp.  | SRF 1950         |              | 33 kD            | GLCSGGAIAQGGA | [45]  |
| Streptomyces megasporus |          |              | 35 kD            | No data       | [46]  |
| Rhizopus chinesis 129 |            |              | 18 kD            | SYEIQLMHNGL  | [47]  |
| Fungi             |                   |              |                  |               |       |
| Cordyceps militaris | CMas            | 27.3 kD      | IVGGVSV AIE      | [48]         |
| Armillaria mellea  | AMMP             | 21 kD        | MFSSLRFYTLCSLAVA | [49]         |
| Flammulina velutipes | FVP-1            | 37 kD        | LTVRVIPTKQAVTEG-TELL | [50]         |
| Fusarium sp. BLB  | Fusarium enzyme  | 27 kD        | IVGVTAAAGGDFPFIHVS | [9]       |
| Stachybotrys longispora FG216 | GFGC1       | 869Da        | No data          | [10]         |
| Other             | Urechis unicinctus | UFE-II      | 26kD             | ICGGSPADIT   | [7]   |

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### References

1. Ding, H.; Zhu, S. S. Chin. J. Biotechnol. 1994, 10, 56.
2. Gulga, C.; Bode, M. S.; Runge, K. H. Fibrinolysis & Proteolysis 1998, 12, 39.
3. Wang, C. X. BIOLOGICS, Beijing, 2014, pp. 207–209.
4. Fu, S. Q.; Yan, T.; Wu, W. H. Pharm. Care Res. 2015, 15, 99.
5. Sun, X. Y.; Song H. X. C. S. P. 2016, 24, 2797.
6. Liu, C. G.; Wang, P. Chin. J. Biochem. Pharm. 2002, 1, 34.
7. Chu, J. X.; Cai, W. D.; Han, B. Q.; Liu, C. G.; Liu, W. S. Pharm. Biotechnol. 2010, 4, 331.
8. Qu, Z. L.; Liu S.; Dong, H.; Liu, C. G.; Liu, W. S. Chin. J. Mar. Drugs 2005, 3, 10.
9. Ueda, M.; Kubo, T.; Miyatake, K. Appl. Microbiol. Biotechnol. 2007, 74, 331.
10. Wang, C.; Guo, R. H.; Bao, B. Chin. J. Chem. 2015, 8, 1.
11. Wonkeuk, K.; Keehyun, C. AEM 1996, 62, 2482.
12. Richard, A. G. ABB 1980, 202, 629.
13. Wang, M.; Wang, J. Acta Pharm. Sin. 1997, 31, 481.
14. Peng, Y.; Huang, Q.; Zhang, R. H. Biochem. Mol. Biol. 2003, 434, 45.
15. Wang, C. T.; Ji, B. P.; Li, B. Ind Microbiol. Biotechnol. 2006, 33, 750.
16. Kim, W.; Choi, K.; Kim, Y. AEM 1996, 62, 2482.
17. Kim, J. B.; Jung, W. H.; Ryu, J. M. Biotechnol. Lett. 2007, 29, 605.
18. Liu, C. G.; Wei, X.; Liu, W. S. J. Ocean Univ. China 2001, 31, 730.

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[19] Li, Z. Q.; Hu, J. C.; Pan, H. Q.; Wang, S. J. *Microbiol. 2009*, **9**, 29, 41.
[20] Huang, S.; Li, J. Q.; Zhang, Y. K. *Food Sci. Technol.* **2009**, **34**, 19.
[21] Wang, D. L.; Liu, W. S.; Han, B. Q. *Chin. J. Mar. Drugs* **2006**, **25**, 37.
[22] Li, Y.; Zhang, Z. F.; Tian, M. *China Brewing* **2012**, **31**, 33.
[23] Liu, W. S.; Cheng, H. Z.; Han, B. Q. *J. Ocean Univ. China* **2012**, **4**, 88.
[24] Wu, W. H.; Bao, B. J. *Food Sci.* **2005**, **26**, 34.
[25] Zhang, Y.; Wu, W. H.; Zhou, P. G.; Bao, B. *Chin. J. Mar. Drugs* **2008**, **27**, 39.
[26] Wen, W.; Rituko, N.; Bunnkenn, M.; Hasumi, K. *Biosci. Biotechnol. Biochem.* **2004**, **68**, 1549.
[27] Wu, W. H.; Keiji, H.; Peng, H.; Wang, X. C.; Bao, B. J. *Chin. Mar. Drugs* **2009**, **7**, 85.
[28] Kim, H. K.; Kim, G T.; Kim, D. K. *J. Ferment Bioing* **1997**, **84**, 307.
[29] Hua, Y.; Jiang, B.; Mine, Y. J. *Agric. Food Chem.* **2008**, **36**, 1451.
[30] Kim, J. S.; Sapkota, K.; Park, S. E. *J. Microbiol.* **2006**, **44**, 622.
[31] Chitte, R. R.; Dey, S. *Appl. Microbiol.* **2000**, **31**, 405.
[32] Weng, Y. H.; Yang, S. T.; Yang, M. J.; Yang, Q. Y. *Prog. Modern Biomed.* **2010**, **10**, 1562.
[33] Wang, S. H.; Hu, K. H.; Sha, L. *Food Sci. Technol.* **2006**, **22**, 174.
[34] Tai, M. W.; Sweet, B. V. *Am. J. Heal System Pharm.* **2006**, **63**, 1121.
[35] Qiu, Z. L.; Liu, S.; Liu, C. G. *Acta Academiae Medicinae Qingdao Universitas* **2003**, **39**, 156.
[36] Kim, J. B.; Jung, W. H.; Ryu, J. M. *Biotechnol. Lett.* **2007**, **29**, 605.
[37] Jeong, Y. K.; Yang, W. S.; Kim, K. H. *Biotechnol. Lett.* **2004**, **26**, 393.
[38] Choi, N. S.; Song, J. J.; Chung, D. M. *J. Ind. Microbiol. Biotechnol.* **2009**, **36**, 417.
[39] Wang, S. L.; Chen, H. J.; Liang, T. W. *Process Biochem.* **2009**, **44**, 70.
[40] Kong, J. H.; Yan, J. P. *Zhu, L. Toxicol. Pharmacol.* **2004**, **137**, 65.
[41] Kim, S. H.; Choi, N. S. *Biosci. Biotechnol. Biochem.* **2000**, **64**, 1722.
[42] Fujita, M.; Nomura, K.; Hong, K. *Biochem. Biophys. Res. Commun.* **1993**, **197**, 1340.
[43] Kim, J. B.; Jung, W. H.; Ryu, J. M. *Biotechnol. Lett.* **2007**, **29**, 605.
[44] Wang, J.; Wang, M.; Wang, Y. G. *Chin. J. Biotechnol.* **1999**, **15**, 147.
[45] Bono, F.; Savl, P.; Tuong, A. *Fems Microbiol. Lett.* **2010**, **141**, 213.
[46] Chitte, R. R.; Dey, S. *Appl. Microbiol.* **2000**, **31**, 405.
[47] Liu, X. L.; Du, L. X.; Lu, F. P. *Appl. Microbiol. Biotechnol.* **2005**, **67**, 209.
[48] Cui, L.; Dong, M. S.; Chen, X. H. *World J. Microbiol. Biotechnol.* **2008**, **24**, 483.
[49] Lee, S. Y.; Kim, J. S.; Kim, J. E. *Protein Express Purif.* **2005**, **43**, 10.
[50] Park, S. E.; Li, M. H.; Kim, J. S. *Biosci. Biotechnol. Biochem.* **2007**, **71**, 2214.
[51] Poonam, M.; Seung, S. C.; Jaya, R. S.; Yun, H. C.; Jin, C. Y. *Process Biochem.* **2011**, **46**, 1449.
[52] Ae, R. L.; Gyoung, M. K.; Jae, Y. P.; Hyeon, D. J.; Jaeho, C.; Y, S. S.; Jeong, H. K. *J. Korean Soc. Appl. Biol. Chem.* **2010**, **53**, 56.
[53] Han, Y. C.; Min, J. S.; Jeong, U. P.; Y, H. C.; Young, C.; Young, S. C.; Yong, K. J. *J. Microbiol.* **2011**, **49**, 1018.
[54] Won, S. K.; Min, J. S.; Min, J. K.; Hye, H. L.; Byoung, W. K.; Jeong, U. P.; Yang, J. C.; Yong, K. J. *Microbiol.* **2011**, **49**, 376.
[55] Aungkawipa, M.; Susitsorn, K.; Sureelak, R.; Sittirak, R.; Jirawat, Y. *Process Biochem.* **2012**, **47**, 2379.
[56] Ratnakar, R. C.; Siddharath, V. D.; Pradnya, P. K. *Appl. Biochem. Biotechnol.* **2011**, **165**, 1406.
[57] Chen, T. C.; Pei, M. W.; Ya, F. H.; Yun, C. C. *Food Chem.* **2012**, **133**, 1611.
[58] Jo, H. D.; Huang, A. L.; Seon, J. J.; Jeong, H. K. *J. Microbiol.* **2011**, **21**, 1166.
[59] Madhubani, A. K.; Jason, M.; Christopher, J. L.; George, J. S.; Mitchell, S. V. E. *Curr. Neurovas. Res.* **2012**, **9**, 207.
[60] Yuan, J.; Yang, J.; Zhuang, Z. H.; Yang, Y. L. *BMC Biotechnol.* **2012**, **12**, 36.