Send Orders for Reprints to reprints@benthamscience.net

Current Pharmaceutical Biotechnology, 2020, *21*, 000-000

LETTER ARTICLE

Characterizations and Fibrinolytic Activity of Serine Protease from *Bacillus subtilis* C10

Nguyen T.A. Thu[1,2], Nguyen T.M. Khue[1], Nguyen D. Huy[3], Nguyen Q.D. Tien[1], Do T.B. Thuy[4] and Nguyen H. Loc[1*]

[1]*Institute of Bioactive Compounds and Department of Biology, University of Sciences, Hue University, Hue, Vietnam;* [2]*University of Medicine and Pharmacy, Hue University, Hue, Vietnam;* [3]*Institute of Biotechnology, Hue University, Vietnam;* [4]*University of Agriculture and Forestry, Hue University, Hue, Vietnam*

Abstract: ***Background*:** Fibrinolytic enzymes, such as Nattokinases from *Bacillus* species are known to degrade the fibrin blood clots. They belong to serine protease group having commercial applications, such as therapeutic agents and functional food formulation.

***Objective*:** The present study reports some characteristics and fibrinolytic activity of serine protease from *B. subtilis* C10 strain that was isolated from shrimp shell.

***Methods*:** Extracellular enzyme from *B. subtilis* C10 culture was harvested and partially purified by ammonium sulphate precipitation. Fibrinolytic activity of the enzyme was determined by zymography and measured by spectrophotometry with fibrinogen and thrombin used as substrates. The optimal temperature and pH for fibrinolytic activity were studied in the range of 31-43°C and 5-10, respectively. The thermal and pH stability of enzyme was studied by incubating enzyme for 30 min in the same range of temperature and pH as above. The effect of some metal ions and reagents on fibrinolytic activity of enzyme was evaluated by concentrations of 5 mM and 5%, respectively.

***Results*:** Zymogram analysis indicated the presence of four fibrinolytic enzymes with molecular weights of approximately 69, 67, 39 and 36 kDa. The optimal temperature and pH for enzyme activity were 37°C and 9, respectively. The thermal and pH stability ranged from 35-39°C and 8-10, respectively. Fibrinolytic activity reached a maximum value of about 400 U/mg protein after 16 h of C10 strain culture. Enzyme has been drastically inhibited by PMSF and SDS, and partially inhibited by EDTA, while Triton X-100 has significantly increased enzyme activity. Effects of ions such as Mg^{2+}, Ca^{2+} and Mn^{2+} on enzyme were negligible, except Cu^{2+} and Zn^{2+} have strongly decreased its activity.

***Conclusion*:** Results from the present study suggested that enzyme obtained from *B. subtilis* C10 could be serine protease that has a high fibrinolytic activity up to about 400 U/mg protein at the most appropriate temperature and pH of 37°C and 9. This activity can be improved up to 142% by incubating enzyme with 5% Triton X-100 for 30 min.

ARTICLE HISTORY

Received: June 17, 2019
Revised: August 26, 2019
Accepted: September 11, 2019

DOI:
10.2174/1389201020666191002145415

Keywords: *Bacillus subtilis*, fibrinolytic activity, nattokinase, serine protease.

1. INTRODUCTION

Serine proteases, as a subclass of protease, are proteolytic enzymes with a serine residue (Ser) on the active site. These enzymes are grouped into two broad categories based on their structure: chymotrypsin-like (trypsin-like) or subtilisin-like [1]. Fibrinolytic proteases (*e.g.* tPA) belonging to serine proteases usually operate at neutral and alkaline pH, with an optimum between pH 8 and 10 [2]. Fibrinolytic proteases (also known as nattokinase) have an ability to degrade fibrin which is normally formed from fibrinogen by the action of thrombin [3]. In human, the tPA catalyzes the conversion of plasminogen to plasmin for slicing fibrin to avoid thrombosis in blood vessels [4]. According to Di Cera [5], a typical genome contains 2-4% of genes encoding for proteolytic enzymes. Among these, serine proteases were considered as the most abundant and functionally diverse group.

On the basis of the catalytic mechanism, fibrinolytic proteases from microorganism are classified into three types, serine protease (*e.g.* nattokinase from *Bacillus*), metallo protease (*e.g. Armillaria mellea* metallo protease), and mixture of both types of proteases above (*e.g.* protease from *Streptomyces*) [6]. Some fibrinolytic proteases produced by *Bacillus*

*Address correspondence to this author at the Institute of Bioactive Compounds and Department of Biology, University of Sciences, Hue University, Hue, Vietnam; E-mail: nhloc@hucuni.edu.vn

1389-2010/20 $65.00+.00