Fibrinolytic Bacterial Enzymes with Thrombolytic Activity

Abstract This book describes the fibrinolytic enzymes of microbial origin that are able to dissolve endogenous thrombi in vivo. The fibrinolytic enzyme streptokinase for example, is produced by β-hemolytic streptococci and exerts its enzyme action indirectly by activating plasminogen. On the other hand, staphylokinase is produced by *Staphylococcus aureus* by stoichiometric complexation with plasmin(ogen) that activates other plasminogen molecules. Serrapeptase is a different fibrinolytic enzyme produced by enterobacterium *Serratia* sp. E-15 with multiple functions including fibrin degradation. In addition, nattokinase is a very promising enzyme produced by *Bacillus natto* in fermented soybean in the Japanese diet providing them with the lowest rate of thrombosis disorders all over the world. For each fibrinolytic enzyme there is a special focus on the enzyme structure and mechanism of action. In Sect. 6 the methods used for assessment of clot lysis in vitro are discussed: Fibrin plate methods, streptokinase lysis methods, nephelometric methods, dilute blood clot lysis time, euglobulin lysis time, esterolytic, and fluorimetric assays. Finally, hemostasis screening tests are discussed, such as CBC, PT, PTT, TT, fibrinogen, D-dimer, and BT assays. They should be done regularly to check the physiological and fibrinolytic activity of blood to reduce the onset of endogenous thrombi.

Keywords Fibrinolytic enzymes · Microorganisms · Thrombosis · Hemostasis · Blood clots · Streptokinase · Staphylokinase · Serrapeptase · Nattokinase · Assays

1 Introduction

Enzyme therapies are becoming more prevalent in medicine today, with many manufacturers targeting their advantages in disease treatment. In the last 100 years, enzymes have been increasingly used to treat various diseases.

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Early observations of *Bacillus pyocyaneus* revealed that its secretions could destroy anthrax bacilli and protect mice from inoculation with this deadly bacterium. Scientists deduced that the secretions were able to destroy anthrax via enzymatic degradation. This early observation paved the way for the use of enzymes in medicine. Today, enzymes are used as anticoagulants, oncolytics, thrombolytics, anti-inflammatories, fibrinolytics, mucolytics, antimicrobials, and digestive aids.

Enzymes are found throughout the natural world; the number of uses for them in various fields of industry in addition to medicine is staggering. Enzymes are found in animal and plant sources. Enzymes can be thought of as protein molecules with a specific mission—to initiate and regulate countless biologic reactions in living organisms.

Enzymes are used for metabolic and digestive processes. Metabolic enzymes greatly increase the speed at which chemical processes take place within the body; without enzymes, cells could not perform their multiple functions. Every aspect of life depends on the energetic stimulus that enzymes provide.

Perhaps therapeutic enzymes are used most often for enhancing digestive function. Enzymes help break down food into its smallest components. Enzymes secreted by humans include pepsin and protease for breakdown of proteins, lipase for fats, and amylase for carbohydrates. Cellulase, which helps with digestion of plant cells, is not produced by humans but is extracted from plant tissues as they are mechanically broken down. Plant-based foods are often cooked, but heat destroys enzymes; a plant food in its raw, fresh state produces considerably more enzyme activity than one that has been cooked.

Enzymes, like their application in medicine, exert their effects in a multitude of ways. One primary focus of enzymatic action is on the protein fibrin. Fibrin is an insoluble protein involved in blood clotting. In the many steps of the clotting cascade, fibrin is the final product. It is derived from its soluble protein precursor, fibrinogen. Fibrin is laid down inside blood vessels that have been compromised by disease or injury. Fibrin forms minuscule strands that eventually dry and harden, capturing blood vessel components effectively.

Certainly, fibrin occupies a vital role in health and healing; however, fibrin may also be responsible for an overzealous propensity to form inappropriate clots in the body. Inappropriate clotting, of course, is a major risk factor for myocardial infarction and stroke.

When correctly balanced, deposition and removal of fibrin maintains avoidance of blood loss and adverse viscosity in the vascular system. A balance tipped in favor of fibrin overproduction leads to dangerous clotting.

Various types of thrombosis are responsible for an increasing number of deaths each year. In the USA alone, lung blood clots affect an estimated 1,000,000 patients annually (Lopez-Sendon et al. 1995). According to a report published by the World Health Organization (WHO) in 2001, 17 million people die every year of cardiovascular diseases (CVDs).

The formation of a blood clot in a blood vessel (intravascular thrombosis) is one of the main causes of CVDs. The major protein component of blood clots, fibrin, is

formed from fibrinogen via proteolysis by thrombin. Meanwhile, fibrin clots can be hydrolyzed by plasmin to avoid thrombosis in blood vessels. In an unbalanced situation due to some disorders, the clots are not hydrolyzed, and thus thrombosis occurs (Lopez-Sendon et al. 1995).

So, several investigations are being pursued to enhance the efficacy and specificity of fibrinolytic therapy, and microbial fibrinolytic enzymes have attracted much more medical interest in recent decades (Goldhaber and Bounameaux 2001; Tough 2005).

Based on their different working mechanisms, thrombolytic agents are classified into two types. One is plasminogen activators, such as tissue-type plasminogen activator (t-PA) (Collen and Lijnen 2004) and urokinase (Duffy 2002), which activate plasminogen into active plasmin to degrade fibrin. The other type is plasmin-like proteins, which directly degrade fibrin, thereby dissolving thrombi rapidly and completely.

Although plasminogen activators and urokinase are still widely used in thrombolytic therapy today, their expensive prices and undesirable side-effects, such as the risk for internal hemorrhage within the intestinal tract when orally administrated, have prompted researchers to search for cheaper and safer resources (Ismail 1981; Nakajima et al. 1993; Bode et al. 1996). Therefore, microbial fibrinolytic enzymes have also attracted much more medical interest during recent decades (Bode et al. 1996).

Fibrinolytic enzymes were successively discovered from different microorganisms, the most important among which is the genus *Bacillus* from traditional fermented foods (Mine et al. 2005).

The physiochemical properties of these enzymes have been characterized, and their effectiveness in thrombolysis in vivo has been further identified. Therefore, microbial fibrinolytic enzymes, especially those from food-grade microorganisms, have potential to be developed as functional food additives and drugs to prevent or cure thrombosis and other related diseases (Ambrus et al. 1979; Sumi et al. 1987, 1990; Kim et al. 1996a, b; Hwang et al. 2002).

Fibrinolytic enzymes are mainly proteases. These catalyze total hydrolysis of proteins and specifically act on interior peptide bonds (Bayoudh et al. 2000).

All living cells produce different types of proteases, but the majority are produced by microorganisms. Many workers have reported that bacteria are high protease producers (Kalisz 1988).

Proteases are grossly subdivided into two major groups, namely exopeptidases and endopeptidases, depending on their site of action. Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate, whereas endopeptidases cleave peptide bonds distant from the termini of the substrate (International Union of Biochemistry and Molecular Biology 1992).

Based on the functional group present at the active site, proteases are further classified into four families: serine proteases, aspartic proteases, cysteine proteases, and metalloproteases (Hartley 1960).

Fibrinolytic proteases are mainly serine or metalloproteases (Sharma et al. 2004) and are also of major importance in food, leather, detergent, pharmaceutical, and

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Kotb, E.

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