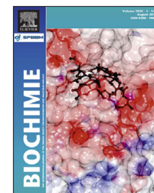
Biochimie 107 (2014) 216–222

Contents lists available at ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi



Research paper

How does association process affect fibrinogen hydrolysis by thrombin?

Elena Zavyalova*, Alexey Kopylov

Chemistry Department, M.V. Lomonosov Moscow State University and LTD 'APTO-PHARM', Leninskie gory 1-3, Moscow 119991, Russian Federation

A R T I C L E I N F O

Article history:
Received 24 April 2014
Accepted 4 September 2014
Available online 16 September 2014

Keywords:
Thrombin
Fibrinogen
Enzyme
Kinetics

A B S T R A C T

Thrombin, a key enzyme in the blood coagulation cascade, hydrolyzes fibrinogen into fibrin, which specifically associates into the fibers that build up a thrombus scaffold. The assembly of fibrin involves a set of stepwise reactions, for which a complete and detailed kinetic portrait is needed. Existing kinetic models focus on particular parts of the process, for example the mechanism of enzyme action itself or the kinetics of formation of fibrin assemblies.

The current study considers a thorough model of the process from fibrinogen hydrolysis to the assembly of fibrin. Composing the model requires taking into account several reaction intermediates, stepwise removal of fibrinopeptides, and association of partially hydrolyzed fibrin, in particular desAA fibrin. The model is versatile enough to adopt new data both on fibrinogen hydrolysis and fibrin association. In addition, the model could be considered as an example of a kinetic description of other complex enzyme systems having several intermediates and feedbacks, such as the blood coagulation cascade and signal transduction.

© 2014 Elsevier B.V. and Société française de biochimie et biologie Moléculaire (SFBBM). All rights reserved.

1. Introduction

Thrombin is a key enzyme in the blood coagulation cascade. It hydrolyzes four bonds in fibrinogen to produce fibrinopeptides and fibrin, the latter of which self assembles to form the fibers constituting the thrombus scaffold [1,2]. Thrombin has been thoroughly studied because it plays a crucial role in hemostasis. Low-molecular weight chromogenic substrates have been used to study some key features of the enzyme such as pH-dependence [3], cation activation [4], and the allosteric connection between its active site and its fibrinogen-binding site [5].

Several kinetic models for the hydrolysis of fibrinogen by thrombin have been proposed [6–8], but they are all rather simple. These models consider fibrinogen as a monomeric protein, but it is of course a symmetric crosslinked dimer of three nonidentical polypeptide chains [9]. Moreover, fibrinopeptide cleavage is a sequential process with several intermediate products. The cleavage of two fibrinopeptides A initiates formation of fibrin protofibrils, whereas the cleavage of two fibrinopeptides B occurs in large part from early protofibril and fibril assemblies of an intermediate fibrin form (called "desAA fibrin") in which the two fibrinopeptides A have already been cleaved [10]. Therefore, a more detailed model of the association of fibrin intermediates is required for describing fibrinopeptide B cleavage. Weisel and Nagaswami [11] provided the first example of a model of fibrin association, assuming that the association is an irreversible process and oversimplifying the kinetics of the hydrolysis.

This work aims to provide a flexible model of fibrinogen hydrolysis by thrombin that allows considering subtle details of the reaction, including several reaction intermediates and the details of the association process. The development of this model is a good example of a kinetic description of complex enzyme systems, such as the enzyme cascades in blood coagulation and signal transduction.

2. Experimental procedures

Inorganic salts and Tris were obtained from MP Biomedicals (France). Recombinant human thrombin with a specific activity of 3.6 kIU/mg (HTI, USA), fibrinogen from human plasma (Calbiochem, Germany), PPACK·2 HCl, and fibrinogen E-domain (HTI, USA) were also used. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, *N*-hydroxysuccinimide, and ethanolamine (Sigma–Aldrich, USA) were used in surface plasmon resonance

* Corresponding author. Tel.: +7 (495) 939 31 49; fax: +7 (495) 939 31 81.
E-mail address: zlenka2006@gmail.com (E. Zavyalova).

http://dx.doi.org/10.1016/j.biochi.2014.09.009
0300-9084/© 2014 Elsevier B.V. and Société française de biochimie et biologie Moléculaire (SFBBM). All rights reserved.

experiments. Guanidine hydrochloride (Helicon, Russia), acetonitrile, trifluoroacetic acid, and acetone of chromatography-grade purity (Baza No. 1 Khimreactivov, Russia) were used in the HPLC analysis. All reactions were carried out at 27 °C in a reaction buffer containing 20 mM Tris–acetate (pH 7.4), 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$.

2.1. Estimation of apparent kinetic constants for the thrombin–fibrin complex

The fibrinogen E domain was immobilized on a CM-5 Biacore sensor chip in the Biacore X apparatus (GE Healthcare, USA) according to the manufacturer's guidelines. Namely, the CM-5 chip was treated with a mixture of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-hydroxysuccinimide mixture, followed by addition of a solution of the fibrinogen E domain (pH 3.0, 10 mM citrate buffer), and the residual reaction groups were deactivated with an ethanolamine solution In total, 1900 RU of fibrinogen E domain were immobilized.

Thrombin was inactivated with the active site covalent inhibitor PPACK. Specifically, 1.4 μM thrombin was incubated with 160 μM PPACK for 1 h at 37 °C. For surface plasmon resonance experiments, the inactivated thrombin was diluted in the reaction buffer (described above) and passed over the chip at a speed of 10 μl/min. The experimental data were treated manually using the conventional model for bimolecular complex formation. All experiments were conducted at least three times and averaged.

2.2. HPLC-separation of fibrinopeptides

Typically, the reaction mixture contained 2 μM fibrinogen and 3.6 nM thrombin as well as reaction buffer. Three volumes of chilled

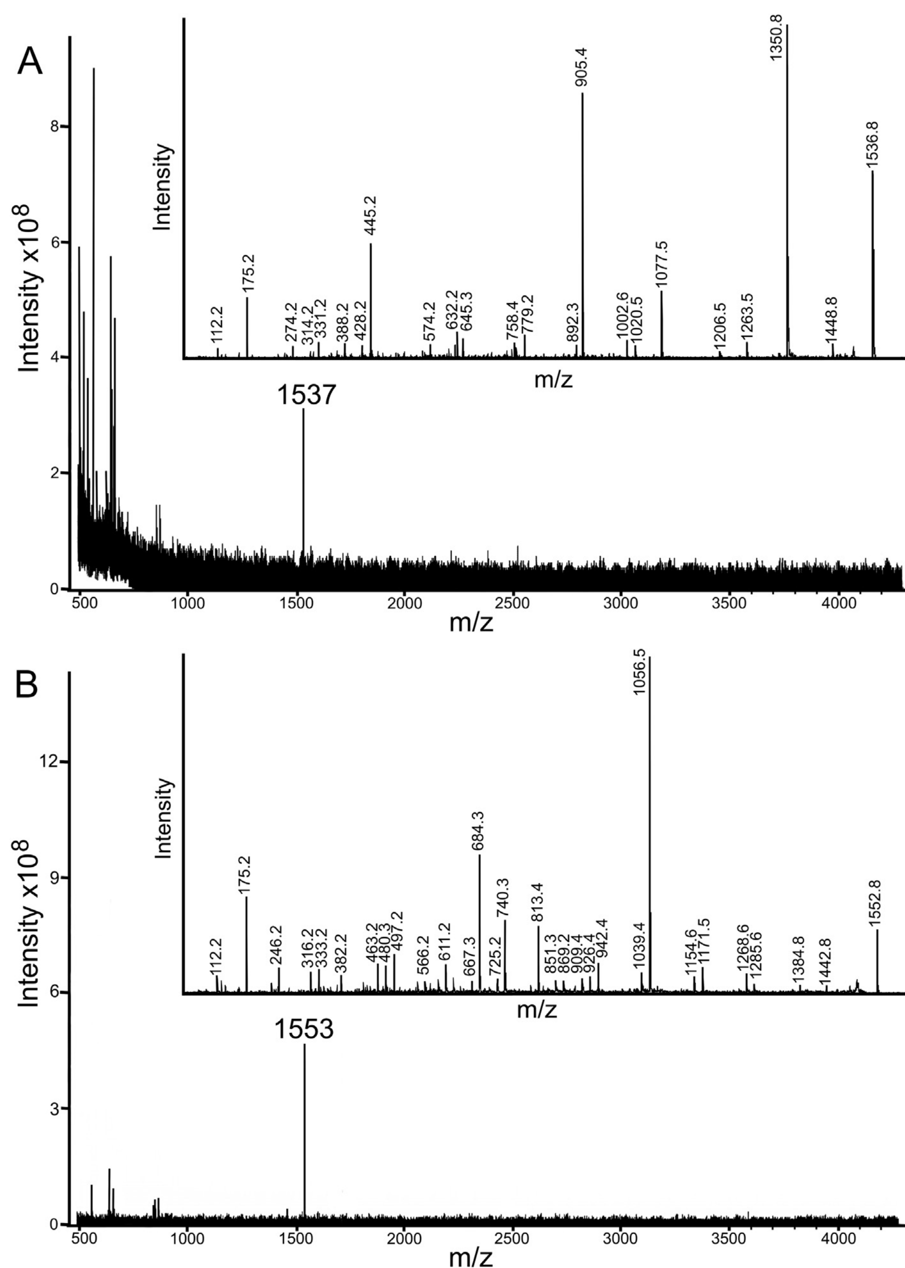


Fig. 1. The MALDI-TOF spectra of fibrinopeptides A (A) and B (B); the tandem MALDI–TOF/TOF spectra of labeled peaks are shown in the insets.

Download English Version:

https://daneshyari.com/en/article/10803603

Download Persian Version:

https://daneshyari.com/article/10803603

Daneshyari.com