



Adaptation Strategies of the Living Systems 2014

ESC "Institute of Biology" of Taras Shevchenko National University of Kyiv  
Faculty of Natural Sciences of Vilnius University  
O.V. Palladin Institute of Biochemistry of National Academy of Sciences of Ukraine  
Institute of Bioorganic Chemistry and Petrochemistry National Academy of Sciences of Ukraine  
V.I. Vernadsky Taurida National University  
Russian State Hydrometeorological University  
Institute University of Research Santa Rita  
Ukrainian Biophysical Society  
Ukrainian Biochemical Society  
Ukrainian Physiological Society

**Interdisciplinary Scientific Conference**  
**ADAPTATION STRATEGIES OF THE LIVING SYSTEMS**

**Novy Svet, AR Crimea, Ukraine**  
**May 12–17, 2014**

**Proceedings of the Conference**

mavis  
  
PUBLISHER  
Kyiv  
2014

**УДК 577:54.05**  
**ББК 20.1я 43 + 26.23я 43**  
**Т 29**

**Матеріали Міжнародної міждисциплінарної наукової конференції "Адаптаційні стратегії живих систем», 12-17 травня 2014, Новий Світ, Україна. - Київ: Видавець В.С. Мартинюк, 2014. – 90 с. ISBN 978-966-2727-03-6**

Збірник матеріалів доповідей періодичної Міжнародної міждисциплінарної наукової конференції "Адаптаційні стратегії живих систем». Розглянуто широке коло міждисциплінарних питань щодо механізмів функціонування живих систем в умовах впливу різноманітних факторів. Розрахований на учасників конференції та широке коло читачів, які працюють у сфері фундаментальних біологічних наук, медицини, біотехнології та суміжних наук.

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**The proceedings of the International Interdisciplinary Conference "Adaptation Strategy of the Living Systems", 12-17 May 2014, Novy Svet, Ukraine . - Kyiv : VS Martynyuk Publisher, 2014 . – 90 p . ISBN 978-966-2727-03-6**

The proceedings of the periodic International Interdisciplinary Conference "Adaptation strategy of living systems". Wide range of interdisciplinary issues of mechanisms of living systems under the influence of various factors are discussed. The proceedings are addressed for participants of the conference and also a wide range of readers who work in the basic biological sciences, medicine, biotechnology and related sciences.

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## CONCERNING THE MECHANISM OF INHIBITORY EFFECT OF Lys-PLASMINOGEN ON THE AGGREGATION OF HUMAN PLATELETS

**Bilous V.L.<sup>1,2</sup>, Roka-Moya Y.M<sup>2</sup>, Zhernossekov D.D.<sup>2</sup>**

<sup>1</sup> Taras Shevchenko National university of Kyiv, ESC «Institute of Biology», Kyiv, Ukraine;

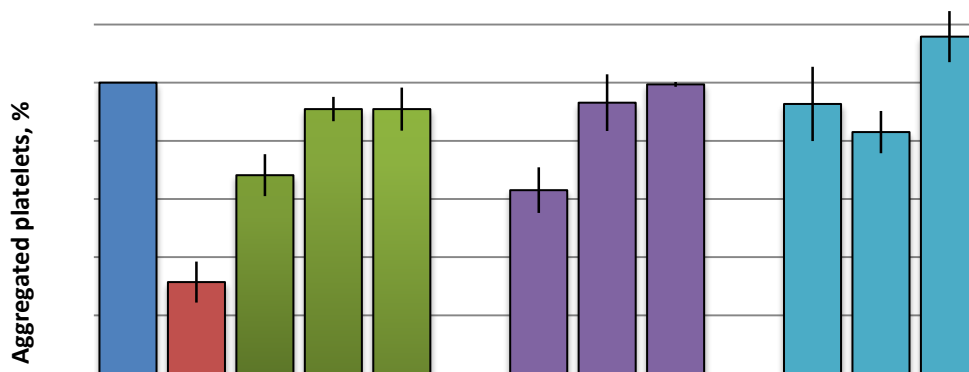
<sup>2</sup> Palladin Institute of Biochemistry of the National Academy of Science of Ukraine, Kyiv, Ukraine.

e-mail: basil.bilous@yandex.ru

**PURPOSE:** Plasminogen is a zymogen of plasmin, the key enzyme of fibrinolytic system. Circulating in plasma form, Glu-plasminogen is composed of 791 amino acids and divided into seven structural domains: N-terminal peptide domain, five kringle domains and C-terminal serine protease domain. Four of five kringle domains (K1, K2, K4 and K5) contain lysine-binding sites (LBS). It was found that K1-LBS is the only LBS for ligand binding in circulating plasminogen, whereas all other LBS are engaged in intra-molecular interactions (1).

Membrane of blood cell is a place of localization of proteins which belong to the plasminogen activation system: plasminogen activators and plasmin. On the surface of endothelial cells and monocytes Glu-plasminogen is transformed into Lys-form by the limited proteolysis. Lys-plasminogen possesses open conformation and can be more easily activated with the plasmin formation (2). However, Lys-form of plasminogen is not detected in the blood of healthy donors. So, physiologic role of Lys-plasminogen is unclear. We have shown that exogenous Lys-plasminogen but not its native Glu-form inhibits platelet aggregation stimulated by ADP, thrombin and collagen in both: platelet rich plasma and suspension of washed platelets (3). The aim of this work is to define the role of certain structural domains of plasminogen molecule in the observed inhibitory effect. One of the possible mechanisms of plasminogen interaction with the surface receptors is the binding of carboxyl-terminal lysines by LBS of plasminogen molecule. The lysine analogue, 6-aminohexanoic acid (6-AHA), prevents this binding. In our experiments 6-AHA in concentration from 0.05 to 1mM abolishes above mentioned inhibitory effect. Taking into consideration the range of the used 6-AHA concentrations we can conclude that LBS of low and high affinity, which are located in kringle domains of plasminogen, are involved in the interaction of Lys-plasminogen with platelet surface proteins. Serine protease inhibitor, aprotinin (5.5 IU/ml) added to aggregation mixture does not make any influence on the inhibitory effect of Lys-plasminogen during thrombin-induced platelet aggregation. It has to be noted that this concentration of aprotinin has no effect on platelet aggregation.

**METHODS:** The influence of plasminogen kringles (K1-3, K4, K5) and mini-plasminogen, which contains K5 and serine protease domain, on platelet aggregation has been studied. K1-3, K4 fragments and mini-plasminogen were obtained by limited hydrolysis of plasminogen by pancreas elastase according to (4). K5 was obtained from mini-plasminogen by pepsin limited proteolysis as described (5, 6). We have shown that K1-3, K4 and their combination have no influence on thrombin-induced aggregation of washed platelets. It also was not observed any changes in platelet aggregation in case of mini-plasminogen, where catalytic activity was inhibited by aprotinin or p-nitrophenyl-p'-guanidine benzoate. However, preincubation of washed platelets with K1-3, K4 and K5 taken into equimolar concentration with Lys-plasminogen completely abolishes inhibitory effect of the last one (Fig. 1, points 5, 8, 11). The plasminogen kringle K5 added to the reaction mixture (washed platelets and 1.2 μM Lys-plasminogen) even at concentration 0.12 μM recovers platelet aggregation till 80%, whereas K1-3 and K4 at this concentration reach only near 60% as compared as control aggregation level (Fig. 1, points 9, 3, 6, respectively).



**Fig 1.** The influence of kringle-containing plasminogen fragments on the inhibitory effect of Lys-plasminogen on platelet aggregation induced by thrombin (1 unit NIH/ml): **1** – control aggregation; **2** – 1.2 μM Lys-plasminogen; **3-5** – plasminogen fragment K1-3, at concentrations 0.12, 0.6 and 1.2 μM, respectively; **6-8** – plasminogen fragment K4 at concentrations 0.12, 0.6 and 1.2 μM, respectively; **9-11** – plasminogen fragment K5 at concentrations 0.12, 0.6 and 1.2 μM, respectively.

**RESULTS:** The presented data let us conclude that kringle domains of plasminogen may occupy the plasminogen binding sites on the platelet membrane. It cannot be excluded that efficient inhibitory effect on platelet aggregation, which we can observe in case of Lys-plasminogen but not Glu-form is provided by multicenter character its interaction with simultaneous involving of LBS of certain kringle domains.

The observed effect of plasminogen kringles on the inhibition of platelet aggregation by Lys-plasminogen let us suggest that LBS of plasminogen kringles provide the plasminogen binding to the adhesive proteins of platelet surface. There are several candidates on the role of Lys-plasminogen receptors on the surface of activated platelets (7, 8). These proteins are secreted from α-granules and remain bound to platelet surface. One of them is thrombospondin, which was proposed to bridge platelets via binding to fibrinogen bound to the platelet integrin αIIbβ3 or by binding directly to this integrin (9). Thrombospondin has two binding sites for fibrinogen, one of which coincides with plasminogen binding site. It was suggested that the binding site of plasminogen to thrombospondin is located within the kringle 5 domain (10). So, we can suggest that in our experiments Lys-plasminogen can probably impede the effective binding of thrombospondin to fibrinogen and as a result the platelet aggregation can be less effective. The peculiarity of kringle 5 which we have shown is in accordance with this suggestion. On the other hand, actin can also be considered as a possible candidate for plasminogen binding. It is known that actin is exposed on the platelet surface after thrombin-induced secretion (11). Plasminogen is able to bind actin with high affinity (12). We can not also exclude fibrinogen from the list of possible candidates for plasminogen binding. As it was shown before, fibrinogen adsorbed on the surface undergoes conformational changes and acquires the properties of fibrin [13], which possesses good binding abilities towards Lys-plasminogen (14).

**CONCLUSION:** So, the obtained effects of plasminogen kringles showed that LBS of plasminogen kringles can bind with some adhesive proteins on the surface of activated platelets. This binding may lead to the disturbance of protein-protein interaction which is the necessary condition for efficient platelet aggregation.

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