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PLASMINOGEN FRAGMENTS K 1-3 AND K 5 BIND TO DIFFERENT SITES IN FIBRIN FRAGMENT DD

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Specific plasminogen-binding sites of fibrin molecule are located in Aα148-160 regions of C-terminal domains. Plasminogen interaction with these sites initiates the activation process of proenzyme and subsequent fibrin lysis. In this study we investigated the binding of plasminogen fragments K 1-3 and K 5 with fibrin fragment DD and their effect on Glu-plasminogen interaction with DD. It was shown that the level of Glu-plasminogen binding to fibrin fragment DD is decreased by 50-60% in the presence of K 1-3 and K 5. Fragments K 1-3 and K 5 have high affinity to fibrin fragment DD (K_d is 0.02 for K 1-3 and 0.054 μM for K 5). K 5 interaction is independent and K 1-3 is partly dependent on C-terminal lysine residues. K 1-3 interacts with complex of fragment DD-immobilized K 5 as well as K 5 with complex of fragment DD-immobilized K 1-3. The plasminogen fragments do not displace each other from binding sites located in fibrin fragment DD, but can compete for the interaction. The results indicate that fibrin fragment DD contains different binding sites for plasminogen kringle fragments K 1-3 and K 5, which can be located close to each other. The role of amino acid residues of fibrin molecule Aα148-160 region in interaction with fragments K 1-3 and K 5 is discussed.

K e y w o r d s: fibrin fragment DD, K 1-3 and K 5 plasminogen fragments, binding sites.

Plasminogen interaction with polymeric fibrin provides its activation on fibrin clot surface, selectivity of fibrin lysis by newly formed plasmin and enzyme protection from plasma inhibitor α2-antiplasmin. Plasminogen kringle domains ensure the intermolecular interactions [1, 2]. These domains contain lysine-binding sites (LBS) with different affinity to ω-amino carbonic acids and their analogs [3]. Kringles 1-3 and 4 have high affinity to ε-aminocaproic acid (ε-ACA) and C-terminal lysine residues, while kringle 5 has low affinity to ε-ACA and high affinity to lysine and arginine side chains [4, 5].

Glu-plasminogen is a native form of plasminogen that circulates in blood plasma. Glutamic acid is NH_2-terminal amino acid. Free Glu-plasminogen in solution has closed compact conformation supported by intramolecular interactions of NH_2-terminal peptide and kringle domains. Limited proteolysis by plasmin results in NH_2-terminal peptide cleavage and formation opened form Lys-plasminogen. LBS are exposed in this conformation and can interact with ligands [6]. Lys-plasminogen does not exist in blood circulation but can be formed on cell surface [7] or fibrin clot during dissolution [8].

Glu- and Lys-plasminogen differ by affinity to fibrin (K_d is 5 and 40 μM respectively) [9]. 1.0 mole of fibrin binds 0.05 mole of Glu- and 0.5 mole of Lys-plasminogen. Glu-plasminogen associates with fibrin by kringle 5 LBS [11], while Lys-plasminogen by kringle 1 [12]. All the plasminogen fragments contained kringle domains (kringles 1-3, kringle 4 and mini-plassminogen or Val442-plasminogen) which interact with fibrin [12].

It is known that the plasminogen binding sites are located in peripheral D-domains of fibrin(ogen) molecule [13, 14]. They are disposed in Aα-chains regions encompasses Aα148-160 sequence [15]. Plasminogen-binding sites are hidden in fibrinogen molecule and exposed in polymeric fibrin [16]. Plasminogen interaction with the sites induces proenzyme activation on fibrin clot surface [17, 18]. Meanwhile, it is not clear which of plasminogen kringle domains is responsible for the interaction with Aα148-160 region.

Synthetic peptide with sequence identical with Aα148-160 of human fibrinogen accelerate the plasminogen activation by tissue-type activator and do not effect mini-plasminogen activation [19]. Fibrin and purified CNBr-fragment of fibrinogen FCB-2,

which contains the Aα148-160 sequence, potentiate the activation process in much lower concentration than the peptide [19]. These findings show that amino acid residues outside the Aα148-160 region can be involved in plasminogen binding and potentiation ability of fibrin and FCB-2 fragment.

In the present study, we investigated whether fibrin fragment DD contains separate binding sites for different kringle domains.

Materials and Methods

Plasminogen with an amino-terminal glutamic acid residue (Glu-plasminogen) was prepared from citrate donor plasma using the Lysine-sepharose 4B affinity chromatography (Sigma, USA) [20].

Plasminogen fragments K 1-3 and mini-plasminogen were obtained using limited proteolysis of plasminogen by porcine pancreatic elastase (Sigma, USA) with subsequent size-exclusion chromatography using the Toyopearl HW-50 Fine Grade (TOYO SODA Manufacturing Co., Ltd., Japan) and affinity chromatography using the Lysine-sepharose 4B (Sigma, USA). Fragment K 5 was obtained using limited proteolysis of mini-plasminogen by pepsine (Sigma, USA) and subsequent affinity chromatography using the AH-Sepharose as described [21].

Fragments K 1-3 and K 5 were used for rabbit immunization. Monospecific antibodies against K 1-3 and K 5 plasminogen fragments were purified from blood serum by affinity chromatography using K 1-3- and K 5-Sepharose, respectively [23, 24].

Fibrinogen was purified from human plasma by fractionation with sodium sulfate [24].

desAB-fibrin was obtained by dissolving the thrombin fibrin clot formed in the presence of 50 mM ε-aminocaproic acid (ε-ACA) and sodium parahydroxy mercury benzoate (0.35 mg/ml) in 20 mM acetic acid as described elsewhere [25].

Cross-linked fibrin was obtained by thrombin-induced fibrinogen polymerization (2 NIH of thrombin per 1 mg of fibrinogen) in the presence of calcium ions (0.25 M) at 25 °C during 4 h. Concentration of fibrinogen was 16 mg/ml.

Plasmin was prepared by activation of Glu-plasminogen with urokinase (HS medac, Germany), immobilized to BrCN-activated Sepharose 4B. Proenzyme in the amount of 1 mg was incubated with 0.5 ml of urokinase-sepharose gel (1250 IU/ml) during 1 h at 37 °C in 0.05 M sodium-phosphate buffer, pH 7.4 with 25% glycerol. Plasmin was stored in 0.05 M sodium-phosphate buffer, pH 7.4 with 50% glycerol at -20°C. Activation efficiency was evaluated by plasmin caseinolytic and amidolytic activity and PAGE with SDS in the presence of 2% β-mercaptoethanol.

Fibrin fragment DD was prepared from plasmin digest of human cross-linked fibrin. Digestion was performed in 0.05 M tris buffer with 0.15 M NaCl (pH 7.4) during 16 h at 25 °C, concentration of plasmin was 0.2 CU/ml (caseinolytic units per 1 ml). Reaction was inhibited by 1000 KIU (kallicrein-inhibiting units) aprotinin (Merckle, GmbH) per 1 ml of reactive solution with ε-aminocaproic acid (ε-ACA) and EDTA in final concentrations 20 mM. Dialysis of digest was performed in 10 mM PBS H 6.0 with 10 KIU/ml aprotinin, 20 mM ε-ACA and 20 mM EDTA at 4 °C. Fragment DD was purified by ion-exchange chromatography on CM-Sephadex G-50. Fragment DD was eluted by 20 mM PBS with 300 mM NaCl (pH 7,6) and then dialyzed in 0.05 M Tris buffer solution with 0.15 M NaCl (pH 7.4) at 4 °C.

Carboxypeptidase B treatment of fragment DD was performed in 0.1 M TBS, pH 8.1 during 30 min at 37 °C in mass ratio protein to enzyme 20 : 1. Digest was then dialyzed against 0.01 M sodium-phosphate buffer solution pH 7.4 containing 0.15 NaCl.

Effect of desAB-fibrin or fibrin fragment DD on t-PA-mediated plasminogen activation was evaluated by amidolytic activity assay of newly formed plasmin, which cleaves chromogenic substrate S2251 (H-D-valyl-L-leucil-L-lysil-p-nitroanilyde). The reaction mixture contained 0.22 μM Glu-plasminogen, 0.09 nM t-PA (Actylise, Boehringer Ingelheim, Germany), 0.3 mM S2251 and 0.22 μM desAB-fibrin or fibrin fragment DD in 0.05 M Tris buffer solution, pH 7.4 with 0.15 M NaCl and 0.05% Tween 20. The assay was performed in 96-wells plate at 37 °C. The amidolytic activity was determined by measurement of the absorbance at 405 nm using Titertek Multiscan MC 96-well plate reader.

Binding assay was performed using avidin-biotin system (Sigma, USA). The wells of high binding polysterene microtitrate plates (Nunc MaxiSorp) were coated with 1 μg protein (fibrin fragment DD or plasminogen fragments) in 0.1 ml of 0.01 M sodium-phosphate buffer solution (pH 7.4) with 0.15 M NaCl at 4 °C overnight. After binding the excess of proteins was removed by washing buffer with 0.1% Tween 20. To avoid nonspecific sorption, 2% BSA was pippeted into the plate wells, incubated for 2 h at 37 °C and washed out. Another protein

(Glu-plasminogen, plasminogen fragments or fibrin fragment DD) was biotin-labeled in accordance to [26]. After washing procedures, bound proteins in each well were incubated with 0.1 ml of biotinylated protein for 20 h at 4 °C and washed out. Then 0.1 ml avidin-phosphatase (prepared as manufacturer recommends) was added into the wells and washed out after 30 min incubation. The 0.1 ml alkaline phosphatase substrate (1 mg/ml p-nitrophenyl phosphate in 10% diethanolamine, pH 9.8) was pippeted in the wells and incubated for 1 hour at 37 °C. Binding of biotin labeled proteins with fragments immobilized on well surface was estimated by measurement of *p*-nitrophenol absorbance level at 405 nm using Titertek Multiscan reader.

In several experiments plasminogen fragments binding to fibrin fragment DD was estimated using polyclonal monospecific antibodies against them.

Dissociation constant K_d of protein-protein complexes formation was calculated using plots of concentration/level of ligand binding to immobilized protein relation in accordance to equation:

$$A = A_{max}/(1 + K_d/[L]),$$

where A is a *p*-nitrophenol absorbtion level that is proportional to bound ligand amount on a linear section of curve; A_{max} is an absorption at ligand saturated concentration; [L] is a molar concentration of ligand at A; K_d is a dissociation constant [27].

The investigation of different binding sites for different plasminogen kringle domains presence in fibrin fragment DD was performed using bisite enzyme-linked solid-phase immunoassay (ELISA). K 1-3 or K 5 were immobilized on high sorption microplate wells (0.1 ml of 10 μg/ml fragments in 0.01 M PBS, pH 7.4, with 0.15 M NaCl). To avoid nonspecific sorption wells were incubated with 2% BSA. Fibrin fragment DD solution (1 μg in 0.1 ml of working buffer) was added into wells with immobilized protein. Procedure was performed as described above, after each step plates were washed by working buffer with 0.05% Tween 20. Than K 1-3 and K 5 were added into the wells coated with complex of fragment DD and immobilized K 5 and K 1-3, respectively. Concentration of added fragments was in 1.25-50 μg range. Incubation lasted 2 h at 37 °C. After washing procedure, plates were incubated with 0.1 ml of 5 μg/ml rabbit polyclonal monospecific antibodies against K 1-3 and K 5, respectively, in working buffer with 0.05% Tween 20 during 1 h at 37 °C. On the next step wells were incubated with 0.1 ml of alkaline-phosphatase conjugated anti-rabbit IgG (Sigma, USA) during 1 h at 37 °C. Than alkaline phosphatase substrate (1 mg/ml *p*-nitrophenyl phosphate in 10% diethanolamine, pH 9.8) was pippeted in the wells and incubated for 1 h at 37 °C. Binding of proteins with complexes immobilized on the well surface was estimated by measurement of *p*-nitrophenol absorbance level at 405 nm using Titertek Multiscan reader.

As control the level of nonspecific binding of kringle fragments with BSA and anti-K 1-3 antibodies with immobilized K 5, as well as anti-K 5 antibodies with immobilized K 1-3, was estimated. In all cases $E_{405-492}$ was lower than 0.1. Fibrin fragment DD ability to bind with K 1-3 and K 5 immobilized on microplate surface was demonstrated in control experiments using biotinylated DD.

All proteins were tested for homogeneity by 8 to 12% SDS-PAGE. Glu-plasminogen and plasmin purity was tested by PAGE in acidic conditions.

Protein concentrations were calculated by measurement of the absorbance at 280 and 320 nm using $E_{1\%}^{280}$ and molecular weights.

Statistical analysis of the data was performed using "MSO Excel 2007" program and "Ascent Software" microreader program. *P*-value of obtained data was lower than 5%. Kinetic curves demonstrated below are typical of repeated experiment series (three and more in each series).

Results and Discussion

desAB-fibrin and fibrinogen/fibrin fragments contain plasminogen and tissue-type activator binding sites and have accelerating effect on Glu-plasminogen activation by t-PA [17, 18]. Fibrin fragment DD obtained from plasmin hydrolysate of fXIIIa cross-linked fibrin also can potentiate the plasminogen activation process (Fig. 1). The rate of plasmin formation in fragment DD and desAB-fibrin mediated activation was the same at 1 : 1 molar ratio of plasminogen to stimulator. This result meant that plasminogen and t-PA interaction sites are exposed in the studied fragment.

To study the plasminogen kringle domains involvement in the interaction with fibrin fragment DD we investigated the effect of proenzyme kringle-containing fragments on biotin-labeled Glu-plasminogen binding to immobilized on plate wells fibrin fragment DD. Concentration of Glu-plasminogen and fragment DD in the reaction medium was 0.25 and 0.05 μM, respectively. At this concentration ratio

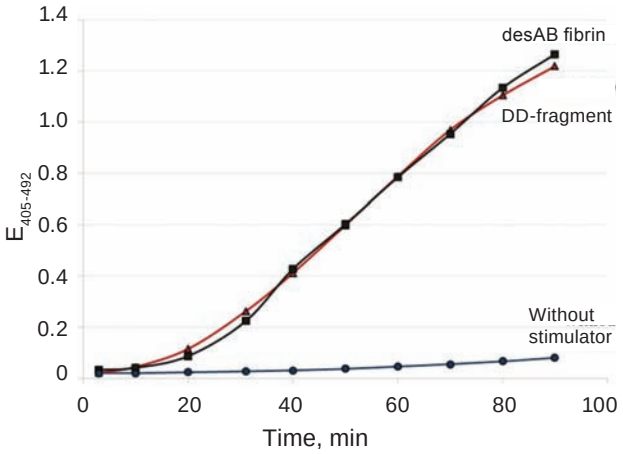

Fig. 1. Glu-plasminogen activation by t-PA in the presence of desAB-fibrin and fibrin fragment DD. Kinetic curves were typical of three experiment series

plasminogen saturates the binding sites in DD molecule, and proenzyme binding level is maximum [29]. Simultaneous addition of kringle-containing fragments K 1-3 and K 5 with Glu-plasminogen (molar ratio fragment/proenzyme was 1, 1.25, 5 and 10) into reaction decreased plasminogen binding level with fibrin fragment DD (Fig. 2). Five-fold molar excess of fragments was most effective and binding level was lowered to 62 and 57% in the presence of K 1-3 and K 5. This effect of kringle fragments indicates their involvement in Glu-plasminogen interaction with fibrin fragment DD.

The possible reason of plasminogen molecule K 1-3 involvement in fibrin fragment DD binding is a conformational change of proenzyme from native form to similar to Lys-plasminogen form, which occurs during the interaction. The change of plasminogen conformation leads to exposition of K 1-3 ligand-binding sites and their accessibility for protein-protein interactions. As previously demonstrated, Glu-plasminogen acquires the Lys-plasminogen-like properties during interaction with partly hydrolysed fibrin [30] and fibrinogen fragments X and E [31], which contain C-terminal lysine residues. This data suggested conformational changes in proenzyme molecule.

In the next set of experiments using polyclonal monospecific antibodies against plasminogen fragments K 1-3 and K 5 or their biotinylated derivatives we showed the binding of isolated fragments K 1-3 and K 5 to immobilized fibrin fragment DD. Both of approaches provide the same data. Fig. 3 demonstrates the relation between biotin-libeled fragments K 1-3 and K 5 binding to fragment DD and their concentration. Concentration increase of both fragments results in saturation of binding sites in fibrin fragment DD. K_d for K 1-3 was 0.02 μM and 0.054 μM for K 5. Thus both of fragments have high affinity to fibrin fragment DD and K 1-3 affinity is higher than of K 5.

Plasmin catalyzes hydrolytic cleavage of peptide bond formed by lysine and arginine carboxyl group and, as a result, these amino acid residues become C-terminal residues of substrate fragments of polypeptide chains. The endpoint products of fXIIIa cross-linked fibrin hydrolysis are fragments E and DD. Fragment E contains lysine residues on all six polypeptide chains C-termini. Fragment DD contains two C-terminal lysine residues in each α-chain after fibrin αC-domains removal. C-terminal lysine residues meet structural requirement of kringle 1 LBS, which is a structural part of fragment K 1-3. Therefore we assessed the role of C-terminal lysine residues in plasminogen fragments K 1-3 and K 5 interaction with fibrin fragment DD. For this reason, we used carboxypeptidase B-treated fragment DD. Carboxypeptidase B is a metal-dependent proteinase, which catalyze specific peptide cleavage and removal of positive-charged C-terminal amino acids, such as lysine or arginine. Carboxypeptidase B treatment was performed at 1/20 enzyme/substrate mass ratio. Previously we have shown that carboxypeptidase B-treated (mass ratio was 1/50) early fibrinogen fragment EE completely loses its ability to potentiate

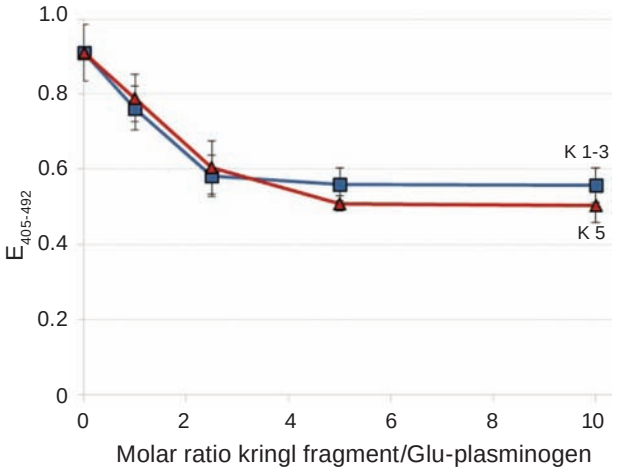

Fig. 2. Effect of plasminogen fragments K 1-3 and K 5 on Glu-plasminogen binding with fibrin fragment DD. Fragment DD was immobilized on high-binding microplate surface. Glu-plasminogen was biotinylated

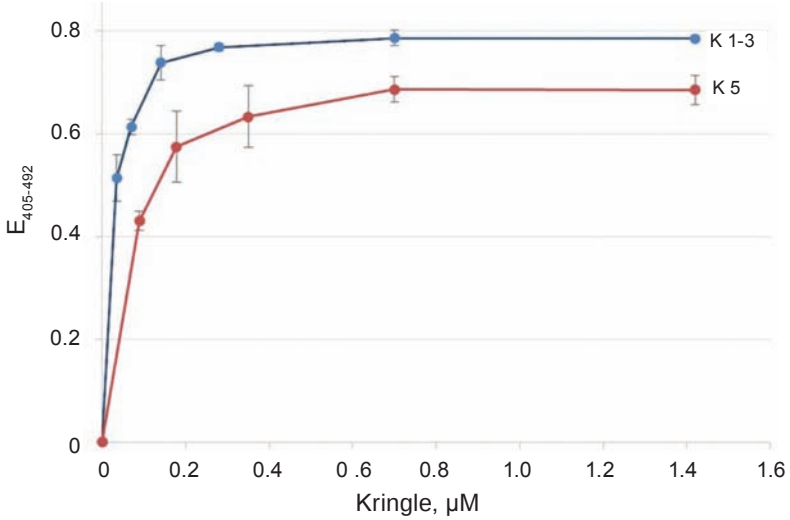

Fig. 3. Binding of kringle fragments K 1-3 and K 5 to fibrin fragment DD. Fragment DD was immobilized on high-binding microplate surface. Kringle fragments were biotinylated

Glu-plasminogen activation by tissue-type activator [28]. Fibrin fragment DDE also loses the potentiation ability after carboxypeptidase B treatment at much lower enzyme/substrate mass ratio [32].

Fig. 4 demonstrates the interaction levels of fragments K 1-3 and K 5 with immobilized fragment DD before and after C-terminal lysines removal. Concentrations of kringle fragments and fibrin fragment DD were 0.5 and 0.05 μM, respectively. The level of K 5 binding with fragment DD was changeless, while K 1-3 binding with carboxypeptidase B-treated DD was 25% lower than with untreated. Thus, we conclude that K 5 binding to fibrin fragment DD is C-terminal lysine-independent process, whereas in K 1-3 binding these amino acid residues are involved but are not critical.

To find out whether different binding sites for plasminogen fragments K 1-3 and K 5 are in fibrin fragment DD we used the bisite ELISA approach. At the first step fragment DD complexes were formed with immobilized K 1-3 and K 5 at saturation of kringle fragment binding sites in DD. Molar ratio of added fragment DD to immobilized K 1-3 and K 5 was 1/5 and 1/14, respectively. On the next stage, we assessed the triple complex formation. For this reason, we analyzed the K 1-3 binding to K 5-DD complex and K 5 binding to K 1-3-DD complex on added plasminogen fragments concentration using polyclonal monospesific antibodies.

K 1-3 and K 5 binding to fibrin fragment DD adsorbed on immobilized K 5 and K 1-3, respectively, is concentration-dependent (Fig. 5). Meanwhile K 1-3 affinity is next-higher order than K 5. K_d value is 0.01 μM for K 1-3 and 0.2 μM for K 5. This result supports the hypothesis that fibrin fragment DD contains individual sites for plasminogen fragments K 1-3 and K 5 binding. Similar dissociation constant values of K 1-3 binding with immobilized fragment DD and fragment DD adsorbed on immobilized K 5 suggests that K 1-3-binding sites in both cases are identically exposed. In contrast, K 5 affinity to fragment DD complex with immobilized K 1-3 fragment is lower than its affinity to immobilized DD. The probable reason is the occupation or partial shielding by K 1-3 some of K 5 binding sites.

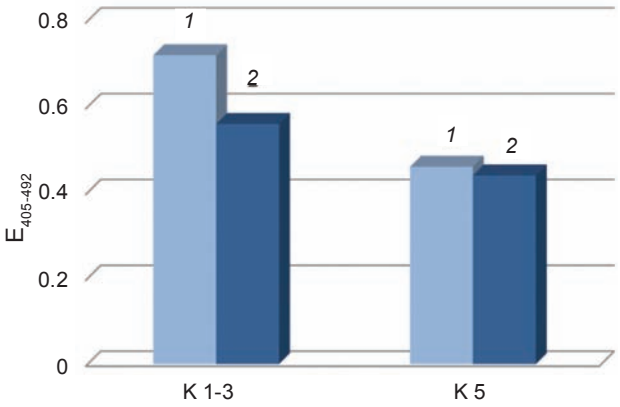

Fig. 4. Fragment K 1-3 and K 5 binding to fibrin fragment DD before (1) and after (2) carboxypeptidase B treatment. Plasminogen fragments binding was monitored using anti-K 1-3 and anti-K 5 polyclonal monospecific antibodies

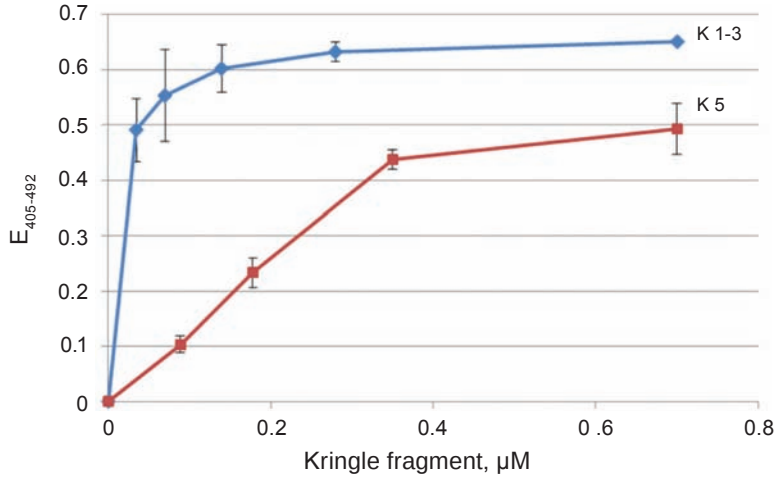

Fig. 5. Binding of kringle fragments K 1-3 and K 5 to complexes between fibrin fragment DD and immobilized K 5 and K 1-3, respectively. The binding level was detected using polyclonal monospecific antibodies against each fragment

Since both of plasminogen fragments can simultaneously bind to fibrin fragment DD, we next sought the ability of K 1-3 and K 5 to displace each other from binding sites. Biotinylated fragments K 1-3 and K 5 were bound to immobilized DD as described above. After removal of non-bound proteins non-labeled K 5 and K 1-3 were added into the wells with K 1-3 and K 5, respectively, adsorbed on immobilized fragment DD, and incubated for 2 h at 37 °C. Fig. 6 shows that addition of K 1-3 and K 5 in the concentration range 1.25-25 μg/ml does not change the amount of DD-bound biotin-labeled K 5 and K 1-3 respectively. Thus, kringle-containing plasminogen fragments do not displace each other from fibrin fragment DD.

This data confirms the above results (Fig. 5) and supports the hypothesis that fibrin fragment DD contains individual sites for plasminogen fragments K 1-3 and K 5 binding.

In contrast, in the presence of one non-labeled kringle fragment (K 1-3 or K 5) the binding of other biotin-labeled kringle fragment (K 5 or K 1-3, respectively) with immobilized fibrin fragment DD was decreased in non-labeled protein concentration-dependent manner (Fig. 7). This indicates that kringle fragments of plasminogen compete for the binding sites in fibrin fragment DD.

K 1-3 binding level in the presence of K 5 in saturating concentrations was lowered to 50%, while K 5 binding in the presence of K 1-3 in saturating

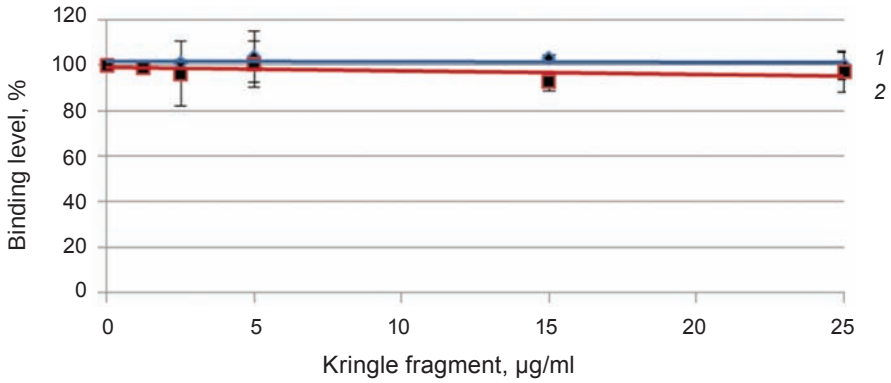

Fig. 6. Effect of different concentration of K 5 (1) and K 1-3 (2) on the amount of biotinylated K 1-3 and K 5, respectively which are bound to fragment DD. Fragment DD was immobilized on high-binding microplate surface. Level of kringle fragments binding to DD without effectors was taken as 100%

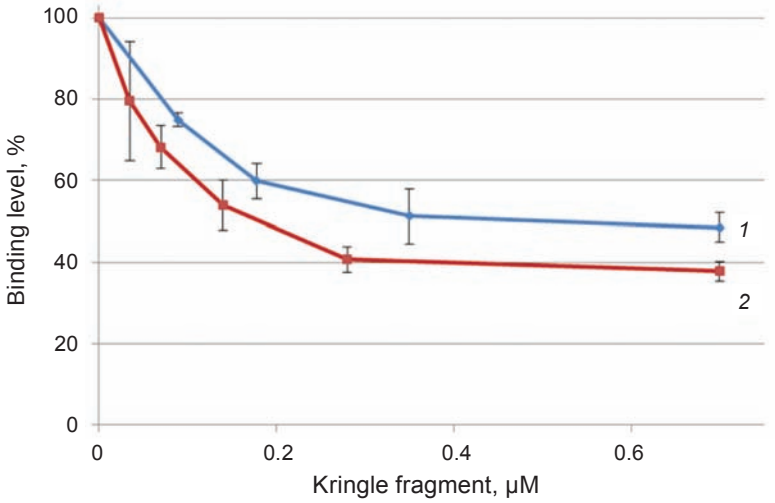

Fig. 7. Binding of biotinylated kringle fragments K 1-3 (1) and K 5 (2) with fibrin fragment DD in the presence of K 5 and K 1-3, respectively. Fragment DD was immobilized on high-binding microplate surface. The level of kringle fragments binding to DD without effectors was taken as 100%

concentrations was decreased to 40%. C_{50} value for K 1-3 is 0.07 μM and for K 5 is 0.13 μM. Our result indicates that K 1-3 and K 5 binding sites in fibrin fragment DD closely set or partly overlap.

Localization of plasminogen binding sites in fibrin molecule peripheral D-domains in Aα148-160-regions is widely known [15]. This region contains unusual sequence of charged amino acids:

148 160

Lys-Arg-Leu-Glu-Val-Asp-Leu-Asp-Leu-Lys-Leu-Arg-Ser

\+ + 0 – 0 – 0 – 0 + 0 + 0

Negative charged and neutral amino acids alteration disposed between two sets of positively charged amino acids. Synthetic peptide with the same structure as Aα148-160 demonstrates acceleration effect toward plasminogen activation by tissue-type activator [35]. AαLys157 of this sequence plays a critical role for the peptide acceleration activity [19]. It is consider that kringle 5 binding to side chain of AαLys157 provides Glu-plasminogen interaction with polymeric fibrin [33]. Replacement of this lysine residue by Ala or Glu does not alter the peptide potentiation effect, while Val or Asp residues in this position result in total loss of the effect. Aα154-159 sequence, which contains Asp155, Lys157 and Arg159, is the shortest active region of the peptide [34]. Investigation of plasminogen kringle domains LBS ligand specificity showed similar interaction efficiency of the first kringle towards lysil-carboxyl pair and arginil-carboxyl pair, whereas the fourth kringle demonstrates ligand specificity only to lysil-carboxyl pair. Binding site of the fifth kringle is single-component and interacts with lysyl or arginyl groups without carboxyl group [5, 35]. Crystallographic study revealed fine differences of LBS structure and organization reasoning the ligand specificity and functional activity differences of kringle domains [36].

Described structural characteristics of kringle domains strongly correspond to our results of the investigation of plasminogen fragments K 1-3 and K 5 interaction with fibrin fragment DD. Side chains of Lys157 or Arg159 in Aα 148-160 region can provide the interaction with the fifth kringle LBS whereas positively and negatively charged amino acid residues of Arg149-Glu151 or Asp155-Lys157 provide the binding of the first kringle LBS in the fragment K 1-3. Probably different K 1-3 and K 5 binding sites are located in common region Aα 148-160 in fibrin D-domain.

In summary, we have shown high-affinity interaction of plasminogen fragments K 1-3 and K 5 with fibrin DD-fragment and 50-60% decrease of native plasminogen binding to this fibrin fragment in the presence of kringle-containing proenzyme fragments. K 1-3 and K 5 ability to bind with complexes DD-K 5 and DD-K 1-3, respectively, infers the existence of different binding sites for different kringle fragments. These sites possibly dispose in Aα 148-160 region of peripheral fibrin domains.

ВЗАЄМОДІЯ ФРАГМЕНТІВ ПЛАЗМІНОГЕНУ K 1-3 ТА K 5 З РІЗНИМИ ДІЛЯНКАМИ DD-ФРАГМЕНТА ФІБРИНУ

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У молекулі фібрину специфічні сайти зв'язування плазміногену локалізовані в периферичних доменах на ділянках Aα148-160. Взаємодія плазміногену з ними ініціює процес активації зимогену з утворенням плазміну і наступний лізис фібрину. У представленій роботі досліджували зв'язування фрагментів плазміногену K 1-3 і K 5 з DD-фрагментом фібрину та їх вплив на взаємодію Glu-плазміногену з цим фрагментом. Показано, що в присутності фрагментів K 1-3 і K 5 величина зв'язування Glu-плазміногену з DD-фрагментом фібрину знижується на 50–60%. Фрагменти K 1-3 та K 5 зв'язуються з високою афінністю із DD-фрагментом фібрину, K_d дорівнює 0,02 і 0,054 мкМ відповідно. Взаємодія K 5 не залежить, тоді як K 1-3 частково залежить від наявності в α-ланцюгах DD-фрагмента С-кінцевих залишків лізину. Встановлено, що K 1-3 взаємодіє з комплексом DD-фрагмент-іммобілізований K 5, також як K 5 – з комплексом DD-фрагмент-іммобілізований K 1-3. Досліджувані фрагменти плазміногену не витісняють один одного із сайтів зв'язування, локалізованих в DD-фрагменті фібрину, але можуть конкурувати між собою за взаємодію з ними. Одержані результати свідчать про існування в DD-фрагменті фібрину різних сайтів зв'язування для кринглових фрагментів плазміногену K 1-3 та K 5, які можуть бути розташовані поблизу один одного. Обговорюється роль окремих амінокислотних залишків послідовності Aα148-160 молекули фібрину у взаємодії з фрагментами плазміногену K 1-3 та K 5.

Ключові слова: DD-фрагмент фібрину, K 1-3 і K 5 фрагменти плазміногену, сайти зв'язування.

ВЗАИМОДЕЙСТВИЕ ФРАГМЕНТОВ ПЛАЗМИНОГЕНА K 1-3 И K 5 С РАЗНЫМИ УЧАСТКАМИ DD-ФРАГМЕНТА ФИБРИНА

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В молекуле фибрина специфические сайты связывания плазминогена локализованы в периферических доменах на участках Aα148-160. Взаимодействие плазминогена с ними инициирует процесс активации зимогена с образованием плазмина и последующий лизис фибрина. В данной работе исследовали связывание фрагментов плазминогена K 1-3 и K 5 с DD-фрагментом фибрина и их влияние на взаимодействие Glu-плазминогена с этим фрагментом. Показано, что в присутствии фрагментов K 1-3 и K 5 величина связывания Glu-плазминогена с DD-фрагментом фибрина снижается на 50–60%. Фрагменты K 1-3 и K 5 связываются с DD-фрагментом фибрина с высоким сродством, K_d составляет 0,02 и 0,054 мкМ соответственно. Взаимодействие K 5 не зависит, а K 1-3 частично зависит от наличия в α-цепях DD-фрагмента С-концевых остатков лизина. Установлено, что K 1-3 взаимодействует с комплексом DD-фрагмент-иммобилизованный K 5, также как K 5 – с комплексом DD-фрагмент-иммобилизованный K 1-3. Исследуемые фрагменты плазминогена не вытесняют друг друга с сайтов связывания, локализованных в DD-фрагменте фибрина, однако могут конкурировать между собой за взаимодействие с ними. Полученные результаты свидетельствуют о существовании в DD-фрагменте фибрина различных сайтов связывания для кринговых фрагментов плазминогена K 1-3 и K 5, которые могут быть расположены близко друг от друга. Обсуждается роль отдельных аминокислотных остатков последовательности Aα148-160 молекулы фибрина во взаимодействии с фрагментами плазминогена K 1-3 и K 5.

Ключевые слова: DD-фрагмент фибрина, K 1-3 и K 5 фрагменты плазминогена, сайты связывания.

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