EXPERIMENTAL WORKS

UDC 577.151.6:616.155.2 doi: https://doi.org/10.15407/ubj96.03.013

PLASMINOGEN INFLUENCE ON THE PAI-1 RELEASE BY HUMAN PLATELETS

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Received: 06 February 2024; **Revised:** 27 March 2024; **Accepted:** 31 May 2024

PAI-1 (plasminogen activator inhibitor type 1), as a major physiological inhibitor of tissue plasminogen activator and urokinase, plays a key role in the regulation of fibrinolysis in vivo. Besides, PAI-1 suppresses plasmin formation and affects cell migration through interaction with vitronectin. PAI-1 is secreted from α-granules of platelets upon stimulation of cells by agonists. The aim of our study was to explore the effects of Glu- and Lys-forms of plasminogen on PAI-1 secretion by platelets and to evaluate the possible role of plasminogen in modulation of agonist-induced PAI-1 release. The secretion of PAI-1 by platelets was investigated by the Western blot analysis. It has been established that depending on the agonist, PAI-1 can be released from platelets in a free form, in a complex with a tissue plasminogen activator, as well as in the form of high-molecular complexes that contain a tissue activator and vitronectin molecules. The revealed induction of PAI-1 secretion under the action of Gly- and Lys-forms of plasminogen indicates their ability to activate intracellular signaling pathways that regulate the release of platelet α-granules. Our findings may be of importance for elucidating the pathogenetic mechanisms of many diseases associated with abnormally enhanced platelet function and PAI-1-related disorders.

K e y w o r d s: PAI-1, platelets, Glu- and Lys-plasminogen, vitronectin, α-granules.

The plasminogen/plasmin system is a multicomponent, finely regulated enzymatic system, which comprises the central serine protease plasmin (EC 3.4.21.7), its proenzyme plasminogen, plasminogen activators and several inhibitors. Plasminogen activator inhibitor type 1 (PAI-1) is a major endogenous inhibitor for both physiological plasminogen activators, t-PA (a tissue type activator, EC 3.4.21.68) and uPA (urinary-type activator, or urokinase, EC 3.4.21.31). PAI-1 is a member of the serpin superfamily of inhibitors. PAI-1 is a glycoprotein composed of 379 amino acid residues with apparent molecular weight of 48 kDa [1]. Besides fibrinolysis, PAI-1 is involved in the development of tissue fibrosis by suppressing the formation of plasmin. It is known that plasmin hydrolyzes proteins of the extracellular matrix (ECM), such as fibronectin, laminin, proteoglycans, type IV collagen, and activates latent forms of metalloproteinases (MMPs) and transforming growth factor β (TGF-β), mediates the release of fibroblast growth factor (FGF), and vascular endotheliocyte growth factor (VEGF) [2]. Consequently, PAI-1 can prevent ECM degradation/tissue remodeling and reduce the activation of growth factors by lowering plasmin amounts. Also, PAI-1 acts as cellular pro-migration factor, which contributes to the migration of pro-inflammatory cells and supports migration of cancer cells resulting in metastasis formation. It has been demonstrated that PAI-1 is able to promote cell migration both through uPA/uPAR-dependent manner as well as via alternative mechanism by regulating LRP-1 Jak/Stat1 signaling events that enhances cell migration [3]. PAI-1 is classified as an acute phase protein. An increase in the plasma level of PAI-1 is considered as unfavorable prognostic marker for thrombosis risk in cardiovascular and oncological diseases [4]. At the same time, absence or deficiency of inhibitor is accompanied by

the risk of hemorrhage conditions and may result in excessive bleeding [5]. It has been revealed that the level of PAI-1 in the blood plasma directly correlates with the degree of insulin resistance and can be used as a prognostic index for the development of type II diabetes mellitus [6].

PAI-1 is synthesized by several different cell types, including endotheliocytes, hepatocytes, monocytes, macrophages, adipocytes of visceral adipose tissue, and megakaryocytes, but to what extent these tissues contribute to plasma PAI-1 levels has been widely discussed [7]. However, approximately 90% of all circulating PAI-1 is stored in α-granules of platelets, which are able to deliberate inhibitor during agonist-mediated activation of platelets via distinct receptors and signaling pathways [8]. Moreover, PAI-1 is one of the proteins, which can be synthesized by platelets de novo due to the presence of megakaryocyte-derived mRNA and the machinery to translate proteins [9].

It has been described that plasminogen can be harbored by platelets, stored within their α-granules, released and exposed on the surface of agonist-stimulated platelets via binding with several receptors [10]. Plasminogen activation rates by t-PA are enhanced by binding with platelet surface to drive local fibrinolysis. However, our recent data suggest that plasminogen may, in turn, reciprocally regulate the functional activity of platelets, reducing their susceptibility to agonists and, thus, limiting their ability to aggregate. It has been shown that Lys-plasminogen (a partially degraded form of zymogen) in contrast to Glu-form (the native form of zymogen), selectively suppresses aggregation of human platelets induced by ADP, thrombin, or collagen, but does not affect ristomycin-dependent aggregation [11]. It has been established for the first time that Lys-plasminogen prevents agonist-induced reorganization of the actin cytoskeleton of platelets and reduces thrombin-induced secretion of P-selectin from α-granules [12]. These data prompted our group to explore if plasminogen can be involved in the regulation of PAI-1 levels in plasma by modulating its release from platelets. Thus, the aim of our study was to explore the effects of Glu- and Lys-forms of plasminogen on the secretion of PAI-1 from platelets and evaluate the possible role of plasminogen in modulating agonist-induced platelet-derived PAI-1 release.

Materials and Methods

Glu-plasminogen was isolated from fresh citrated donor blood plasma, Lys-plasminogen was purified from blood plasma fraction $III_{2,3}$ by Cohn with the use of affinity chromatography on Lysine-Sepharose as described elsewhere [13]. To inactivate spontaneous proteolytic activity, Glu- and Lys-plasminogen preparations were treated with the proteinase inhibitor 4-nitrophenyl-4-guanidinobenzoate hydrochloride (10^{-4} M). The reaction mixture, V~2.0 ml, was incubated at 4°C overnight. Then, dialysis was carried out against 0.05 M Tris HCl buffer, pH 7.4, with 0.15 M NaCl with seven times replacement of the buffer solution. The presence of residual spontaneous activity was monitored using chromogenic substrate for plasmin – S2251 (H-D-Val-L-Leu-L-Lys-p-nitroanilide). The following reagents were added to the wells of a microplate (Greiner Bio One, Germany): 0.75 μg Glu- either Lys-plasminogen (10 μl), 0.05 M tris-HCl buffer, pH 7.4, with 0.15 M NaCl containing 3 mM S2251 (0.24 ml). The reaction was developed at 37°C. Since 4 h after adding the substrate, the absorption level of released p-nitroaniline was measured with the use of a microplate reader (Thermo Multiskan EX, China) at 405 and 495 nm. It was less than 0.05 o.u.

DesAB fibrin was prepared from bovine fibrinogen in the presence of a factor XIIIa inhibitor (4-(hydroxymercury) sodium benzoate) and 0.1 M 6-aminohexanoic acid as described earlier [14]. Rabbit polyclonal specific antibodies against t-PA were produced according to the described protocol [15]. The purity of the resulting protein preparations was controlled using SDS-PAGE [16].

Platelet-rich plasma (PRP) was isolated from donor whole blood containing sodium citrate as an anticoagulant (3.8%) in a 9:1 ratio. The plasma was centrifuged at 160 g for 20 min at 20°C. Aliquots of PRP were removed, and the remainder was centrifuged at 200 g and room temperature for 20 min to obtain washed platelets. The cell pellet was resuspended in platelet washing buffer (20 mM HEPES, pH 6.8, 0.137 M NaCl, 4 μM KCl, 0.2 μM $MgCl_2$ containing 0.2% glucose and 0.2% BSA). The cell suspension was centrifuged again under the same conditions and resuspended in platelet washing buffer the volume of which was ½ the volume of PRP. The number of cells and their aggregation properties

were determined using a "SOLAR AT-02"(Republic of Belarus) aggregometer. For aggregation assay, the samples containing 300-350.000 platelets per µl were taken. Informed written consent was obtained from all donors before their participation in the study. The investigation conforms to the principles outlined in the latest revision of the Helsinki Declaration.

To determine the activity of PAI-1 in PRP before and after stimulation of platelets with thrombin, agonist (1 NIH unit/ml, Sigma, USA) was added to PRP, which contained 10^7 cells in the volume of 0.3 ml, and incubated for 10 min at 37°C. After incubation, platelets were separated by centrifugation for 20 min at 200 g at room temperature. Then, the supernatant was used for analysis. For PAI-1 activity evaluation, the following reagents were added to the wells of a microplate (Greiner Bio One, Germany): t-PA (Actilyse®, Boeringer Ingelheim GmbH, Germany) – 10 µl (0.5 IU); PRP collected before and after incubation with thrombin (10 µl). Each sample was measured in triplicate. The reaction mixture was kept for 10 min at 25°C. Then, 12.5 µg of Glu-plasminogen (10 µl), 30 µg of desAB fibrin (10 µl), and 0.05 M tris-HCl buffer, pH 7.4, containing 3 mM chromogenic substrate for plasmin S2251 (H-D-Val-L-Leu-L-Lys-p-nitroanilide) (210 µl) were added. No plasma was added to the control wells. The total volume of the reaction medium was 0.25 ml. The reaction was developed at 37°C, and 60 min after adding the substrate, the absorption level of released p-nitroaniline was measured with the use of a microplate reader (Thermo Multiskan EX, China) at 405 and 495 nm.

Immunochemical detection of PAI-1 both in a free form and as a part of protein complexes in platelet releases and lysates was made by western blot analysis with the use of primary polyclonal antibodies to PAI-1 (Thermo Fischer scientific, USA, catalog no. 13801-1-AP), vitronectin (Thermo Fischer scientific, USA, catalog no. 720229) and t-PA, which were obtained previously [15]. Electrophoretic separation of proteins was carried out in 8% SDS-PAGE. Samples were transferred onto nitrocellulose membranes (GE Healthcare Amersham, UK) by electroblot in 0.0125 M Tris-HCl and 0.198 M glycine buffer, pH 8.3, containing 20% methanol at a current of 180 mA for 90 min. The membranes were washed twice in 0.05 M Na-phosphate buffer with 0.13 M NaCl, pH 7.4, containing 0.05% Tween-20 (PBST). Sites of nonspecific sorption of antibodies were blocked during overnight incubation of membranes in 3.5% solution of non-fat skim milk (Carnation, USA) in PBST at 4°C. After blocking, the blots were incubated with primary antibodies diluted 1:3,000 in PBST containing 3.5% skim milk overnight at 4°C. After washing five times in PBS, the membranes were probed with the appropriate anti-species antibodies (goat anti-rabbit IgG, conjugated with horseradish peroxidase - HRP, Sigma, USA, catalog no. A0545) 1:7,500 diluted in PBST containing 3.5% skim milk for 90 min at 37°C. After incubation, non-specifically adsorbed antibodies were washed out five times in the same buffer solution. Immunoreactive bands were developed on X-ray films by enhanced chemiluminiscence (ECL) approach, using 0.25 M solution of luminol in dimethyl sulfoxide, 0.09 M solution of coumaric acid in dimethyl sulfoxide, and 0.0035% H_2O_2 mixed in 0.1 M Tris-HCl, pH 8.5, or using 0.05 M Tris-HCl buffered saline, which contained a HRP substrate and chromogen (0.01% H_2O_2 and 0.05% diaminobenzidine tetrahydrochloride, respectively). After immunostaining was developed, the films or membranes were scanned to obtain a digital image.

Data of quantification analysis were expressed as mean (M) ± standard error of the mean (m). Differences between mean values were evaluated by Student *t*-test and considered statistically significant at $P < 0.05$. All statistical calculations and graphic design were made with the use of OriginPro software version 9.0.

Results and Discussion

Free form of PAI-1 can exist in active or latent forms, but only active form of PAI-1 is able to form a complex with t-PA, thus regulating its activity and rate of fibrinolysis. The active form of PAI-1 is unstable and spontaneously turns into a stable latent form, the half-life of which is 1-2 h at 37°C. Platelets have a mechanism, which allows them preserving PAI-1 in the active configuration for a longer time. Several lines of evidence indicate that at least 50-70% of platelet-derived PAI-1 are retained in a functionally active state on the membrane of stimulated platelets via a fibrin/integrin $\alpha_{IIb}\beta_3$-mediated mechanism and are able to bind t-PA [17]. This process has an outstanding significance for normal hemostasis function due to direct participation of platelets in preserving fibrin clot from premature lysis by plasmin to maintain thrombus integrity. On the other hand, excessive production of PAI-1 by platelets enhances resistance of platelet-rich blood clot to both

endogenous and pharmacological thrombolysis that increases a risk for cardiovascular events [18]. The mechanisms of PAI-1 release from α-granules and its interactions with other plasma or platelet-derived proteins are controversial. Thus, the results of the previous studies [19, 20] showed that only 5-10% of the total platelet pool of PAI-1 effectively binds and inhibits t-PA in plasma. At the same time, Brogren et al., using the methods of Western blot analysis and scintigraphy, established that more than 50% of platelet PAI-1 exists in the active conformation [21]. Taking into account the given data, at the first stage of our study, we evaluated if thrombin stimulation of platelets might change the level of PAI-1 in blood plasma. This investigation was performed with the use of amidolytic method for the evaluation of functionally active PAI-1. Briefly, an excessive amount of exogenous t-PA was added to the plasma sample containing the inhibitor. After the formation of an equimolar PAI-1–t-PA complex, the activator remaining outside the complex converts plasminogen into plasmin on the surface of desAB fibrin. The resulting enzyme hydrolyzes S2251 with the release of p-nitroaniline, the amount of which is detected spectrophotometrically. The inhibitory activity of PAI-1 is determined with the help of calibration curve. A calibration curve is constructed based on the activity of the tissue activator (0–75 IU/ml). Based on the final activity of t-PA, the activity of PAI-1 is calculated as equivalent to the activity of inhibited t-PA [22].

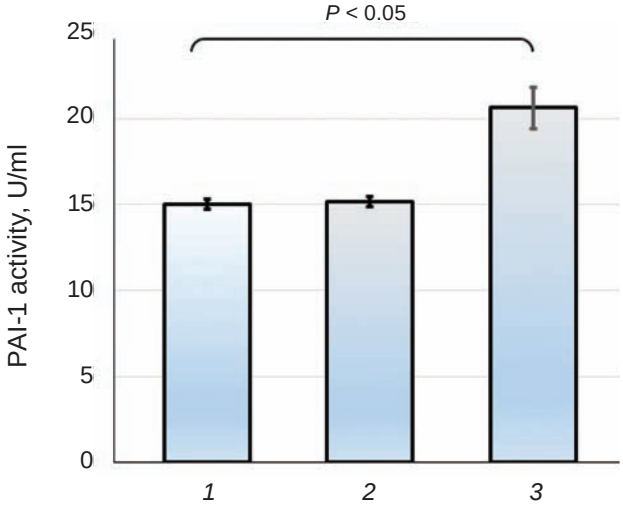

Fig. 1. Activity of PAI-1 in donor blood plasma: 1 – platelet-poor plasma, 2 – platelet-rich plasma, 3 – platelet rich plasma after the addition of thrombin (n = 3 in each group)

According to the literature data, the level of PAI-1 activity in plasma varies from 11 to 15 units/ml [23]. In the plasma samples analyzed in our study (n = 3), the mean PAI-1 activity was about 15 IU/ml. In the samples of platelet rich plasma (PRP), inhibitor activity was found at the same level. However, incubation of PRP with thrombin (1.0 units of NIH/ml per 0.3 ml of plasma containing 1×10^7 platelets), which causes platelet stimulation, promoting secretion of PAI-1, resulted in an increase in inhibitory activity by 27% ($P < 0.05$) (Fig. 1).

The obtained results indicate that platelet activation leads to a significant elevation of inhibitory activity in the blood plasma in relation to the plasminogen tissue activator. It can be hypothesized that the release of PAI-1 by agonist-stimulated platelets can be one of the mechanisms for the physiological and pharmacological resistance of thrombi enriched by platelets against proteolysis. Western blot analysis was used to determine active PAI-1 in the releasates of thrombin-activated platelets, which is secreted by platelets upon stimulation and capable of forming complexes with t-PA and vitronectin. Vitronectin is one of the major adhesive plasma proteins and plays a role as a component of the extracellular matrix [24]. Like most adhesive proteins, vitronectin has an RGD sequence that is responsible for binding ligands to integrin receptors [25]. It is known that PAI-1 is stored in the α-granules of platelets in a complex with vitronectin [26]. Using electron microscopy and FITC-labeled proteins, it has been earlier shown that after the activation of platelets and the release of their α-granules, this complex is located on the platelet membrane, where vitronectin interacts with the cytoskeletal protein vimentin, which is exposed on the surface of activated platelets [27]. For the detection of PAI-1 complexes with t-PA or vitronectin in our experiment, washed platelets were activated by thrombin in the presence of exogenous t-PA, taken in an equimolar amount to platelet PAI-1, with or without the addition of blood plasma as a source of vitronectin. Plasma (10 µl) or an equal volume of 0.05 M Tris-HCl buffer, pH 7.4, with 0.13 M NaCl, t-PA (400 ng) and thrombin (1 NIH unit/ml) were added to the suspension of platelets (4×10^8). The final volume of the incubation mixture was 0.08 ml. After 10 min of incubation at 37°C, platelets were sedimented by centrifugation at 250 g for 20 min at 37°C. The supernatant (platelet releasate), which contains proteins released during agonist-induced cell degranulation, was collected and immuno-

chemical detection of proteins was carried out by probing with monospecific antibodies against t-PA in Western blot. As depicted in Fig. 2, polypeptide bands with a molecular weight of about 110 kDa are identified by antibodies to t-PA in platelet releasates (line 3), which may correspond to the t-PA-PAI-1 complex. In platelet lysates obtained in the presence of plasma (line 4), an additional band with a molecular weight of about 180 kDa was discovered, which may correspond to the t-PA-PAI-1-vitronectin complex. Thus, with the help of antibodies to t-PA, it was shown that PAI-1 is secreted in an active form upon stimulation of platelets by thrombin and can form complexes with exogenous t-PA and plasma vitronectin.

At the next step, we studied the release of PAI-1 and its complexes with other proteins during the activation of platelets by various agonists and verified the ability of plasminogen to affect the secretion of the inhibitor. To do this, t-PA (150 ng) was added to the incubation mixture containing platelets (0.3 ml, 3×10^8 platelets) to detect functionally active PAI-1. Next, one of the agonists was added as follows, thrombin (1 NIH unit/ml), collagen (1.25 mg/ml), or ADP (5 mM). After 10 min of incubation at 37°C, the supernatant (platelet releasates) was separated from the cells by centrifugation at 250 g and 37°C for 20 min. Immunochemical detection of proteins in platelet releases was performed by immunoblotting using monospecific polyclonal antibodies to t-PA or

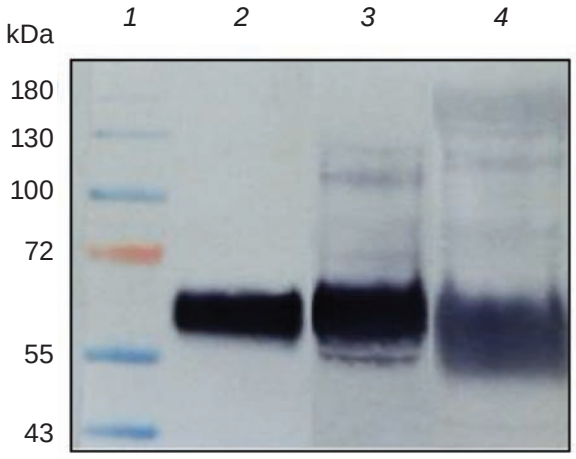

Fig. 2. Typical blotogram of platelet proteins released by thrombin-stimulated platelets ($3\cdot10^8$ in each sample) in the presence of t-PA (polyclonal antibodies against t-PA): 1 – markers of molecular weights, 2 – t-PA standard, 3 – platelet releasates, 4 – platelet releasates in the presence of blood plasma

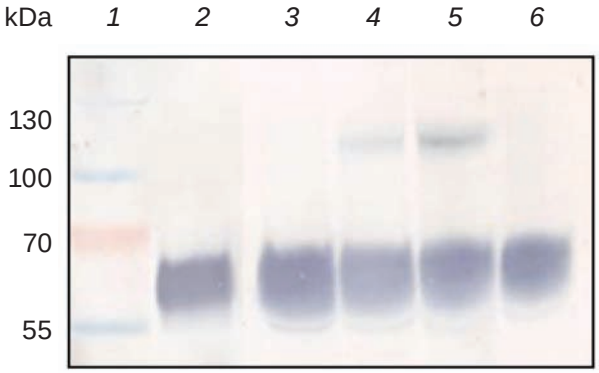

Fig. 3. Western blot analysis of platelet proteins released by agonist-stimulated platelets ($3\cdot10^8$ in each sample) in the presence of t-PA (polyclonal antibodies against t-PA): 1 – markers of molecular weights, 2 – t-PA standard, 3 – resting platelets, 4 – thrombin-activated platelets, 5 – collagen-activated platelets, 6 – ATP-activated platelet

PAI-1 as described above. Visualization of protein zones was done in a solution of horseradish peroxidase substrate (0.05% 4-chloro-α-naphthol) in 0.05 M potassium phosphate buffer (pH 6.0) with 0.06% H_2O_2. To study the modulating effect of plasminogen on the agonist-stimulated secretion of PAI-1, Glu- or Lys-plasminogen (1 μM) was added to the suspension of platelets prior to obtaining releasates. A blotogram of proteins identified by antibodies to t-PA in platelet releasates obtained in the presence of t-PA when cells were activated by different agonists is presented in Fig. 3. No protein immunoreactive bands are observed in the releasates of non-activated (resting) platelets, which served as a control (line 3). When platelets were activated by thrombin and collagen (lines 4 and 5, respectively), polypeptide bands with a molecular weight of about 70 and 110 kDa are identified, which corresponds to the molecular weight of the tissue activator and the PAI-1–t-PA complex. However, in the releasates produced by ADP-stimulated platelets, single band of ~ 70 kDa is visualized (line 6).

Further, platelet releasates obtained during cell activation by thrombin or collagen alone or after pretreatment of platelets by Glu- or Lys-plasminogen, were analyzed by western blot with the use of antibodies against PAI-1. In parallel, proteins from platelet supernatant samples obtained after 10 min incubation of cells with Glu- or Lys-plasminogen (1 μM) without any stimulation with agonists were also examined. Fig. 4 presents a blotogram of proteins recognized by antibodies to PAI-1 in releasa-

tes of platelets exposed to Glu- or Lys-plasminogen (lines 3 and 4, respectively), stimulated by thrombin (line 5), or activated by thrombin after pretreatment with Glu- or Lys-plasminogen (line 6 and 7, respectively).

The blotogram shows that upon platelet activation by thrombin (line 5), PAI-1 is detected in free form (~50 kDa) and as a ternary complex of PAI-1–t-PA–vitronectin (~180 kDa). On the contrary, PAI-1 is detected only in the composition of high-molecular complexes (~180 kDa) in the releasates of collagen-stimulated platelet (Fig. 5). The obtained results support the paradigm that platelet activation by different agonists triggers various intracellular signaling pathways and induces diverse cellular responses depending on activation of specific receptors. It is known that collagen activates platelets through glycoprotein VI, ADP interacts with platelets via their purinergic receptors P2Y1 and P2Y12, while thrombin acts by cleaving proteinase-activated receptors (in humans, PAR1 and PAR4) [28].

The effect of plasminogen on the platelet secretion appeared to be to some extent unexpected. Both the native zymogen, or Glu-form, and its partially truncated (Lys-) form induced the release of PAI-1 even in the absence of agonists. This fact is supported by the presence of protein band, which is correspondent to PAI-1 with M_m of about 50 kDa, visualized on the blotogram (Fig. 4, lines 3 and 4). In addition, plausible complexes of the inhibitor

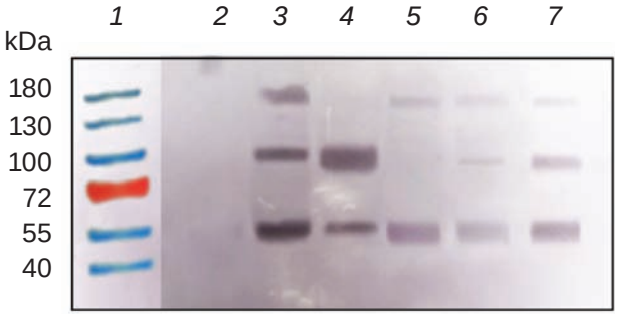

Fig. 4. Effects of Glu- and Lys-forms of plasminogen on the release of PAI-1 by thrombin-stimulated platelets ($2 \cdot 10^8$ in each sample) (results of western blot analysis of platelet releasates): 1 – markers of molecular weights, 2 – resting platelets, 3 – Glu-plasminogen-treated platelets, 4 – Lys-plasminogen-treated platelets, 5 – thrombin-stimulated platelets, 6 – Glu-plasminogen-pretreated thrombin-stimulated platelets, 7 – Lys-plasminogen-pretreated thrombin-stimulated platelets

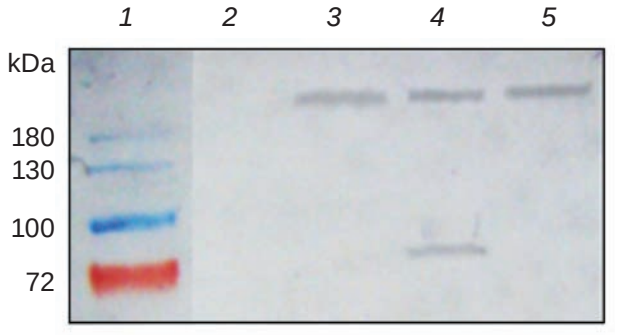

Fig. 5. Effects of Glu- and Lys-forms of plasminogen on the release of PAI-1 by collagen-stimulated platelets ($4 \cdot 10^8$ in each sample) (results of western blot analysis of platelet releasates): 1 – markers of molecular weights, 2 – resting platelets, 3 – collagen-stimulated platelets, 4 – Glu-plasminogen-pretreated collagen-stimulated platelets, 5 – Lys-plasminogen-pretreated collagen-stimulated platelets

with tissue activator (M_m ~110 kDa) and vitronectin (M_m ~180 kDa) are detected in the platelet releasates obtained during the incubation of platelets with Glu-plasminogen, while treatment of platelets with Lys-plasminogen produced complexes of the inhibitor only with t-PA. Plasminogen also enhanced the secretion of PAI-1 upon activation of platelets by thrombin (Fig. 4, lines 6 and 7) that may indicate the synergism of the action of the agonist and proenzyme. These results are in good agreement with the data obtained earlier in our laboratory [29]. Using flow cytometry with application of antibodies to vitronectin, it was shown that treatment of washed resting platelets by Lys-plasminogen increased the number of vitronectin-positive cells.

Typical blotograms of vitronectin detection in platelets lysates and releasates are depicted in Fig. 6. The polypeptide band of 180 kDa, which corresponds to the complex of vitronectin with PAI-1 and t-PA, was revealed in platelet releasates as a result of action of all tested agonists (Fig. 6, *A*). In the samples of platelet lysates, the corresponding band is observed in the case of action of Glu- and Lys-plasminogen, but is absent after the activation of platelets by thrombin or collagen, that indicates a complete deliberation of the complex under the action of strong agonists and only a partial release by plasminogen-exposed platelet (Fig. 6, *B*). It should be noted that vitronectin (75 kDa) is detected in the releasates of platelets treated by Glu- or Lys-plasminogen, while this protein is absent in platelet

lysates, which indicates its complete exocytosis from α-granules due to the action of both forms of plasminogen.

It is known that plasminogen molecule consists of several structural domains, including NH2-terminal PAN/apple domain, five kringle domains (K1-5), and a serine proteinase domain, which contains a catalytic triad of amino acid residues of His603, Asp646, and Ser741 [30]. Plasmin may cleave one of the three peptide bonds formed by Arg68, Lys77 or Lys 78 in Glu-plasminogen molecule, resulting in the appearance of a partially truncated form of zymogen, which is referred to as Lys-plasminogen. The NH2-terminal domain of plasminogen shares structural homology with the PAN/apple domain of hepatocyte growth factor, in the molecule of which it binds with the kringle 1. The interaction of the PAN/apple domain of this growth factor with heparin leads to its activation and binding to c-met receptor [31]. Earlier obtained data and the results of our experiments suggest that the NH_2-terminal domain of plasminogen may play a special biological role in the regulation of PAI-1 secretion by platelets.

Therefore, our study uncovered for the first time a novel function of plasminogen, which may selectively modulate the release of the functionally active pool of PAI-1 from platelets. If these effects have a functional relevance for thrombi stabilization and regulation of thrombolysis needs to be further elucidated. Nevertheless, platelets are largely responsible for the elevation of PAI-1 levels in the elderly and in a variety of clinical conditions that are typical for the aging process (e.g., obesity, insulin resistance, psychosocial stress, decreased immune responses, increased inflammation, vascular sclerosis/remodeling) [32]. Thus, PAI-1 release from platelets may represent a promising pharmacological target for the herapy of numerous pathological conditions associated with abnormally enhanced platelet activation and formation of prothrombotic states.

Conclusions. Here, the secretion of PAI-1 by platelets activated by thrombin, collagen, or ADP, and modulating effects of Gly- and Lys-forms of plasminogen on platelet exocytosis were studied with the use of immunochemical assay. It was shown that PAI-1 can be secreted by platelets in a free form, as a complex with tissue activator (t-PA), and as a part of high-molecular complexes that consist of t-PA and vitronectin. It was found that such complexes differ in composition depending on the nature of agonist. It was demonstrated that, in addition to PAI-1, platelet α-granules are loaded with t-PA and vitronectin. Induction of PAI-1 deliberation from platelets by Gly- and Lys-forms of plasminogen indicates its ability to activate intracellular signaling pathways that regulate platelet exocytosis. These findings may have significant relevance to the pathogenesis of numerous diseases associated with abnormally enhanced platelet function and PAI-1-related disorders.

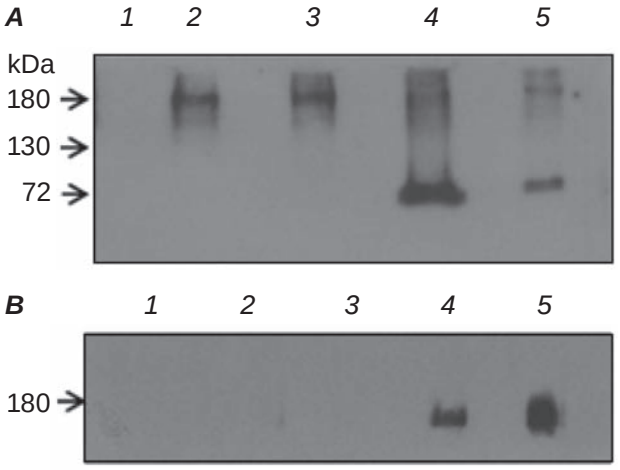

*Fig. 6. Western blot analysis of vitronectin in platelet releasates (**A**) and lysates (**B**): 1 – resting platelets, 2 – thrombin-stimulated platelets, 3 – collagen-stimulated platelets, 4 – Glu-plasminogen-treated platelets, 5 – Lys-plasminogen-treated platelets ($4 \cdot 10^8$ in each sample)*

Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. This study was funded by the basic theme "Investigation of the functional role of plasminogen/plasmin system proteins in regulation of molecular and cellular interactions in fibrinolysis and reparative processes" of Palladin Institute of Biochemistry of NAS of Ukraine (state registration number 0123U100516).

ВПЛИВ ПЛАЗМІНОГЕНУ НА ВИВІЛЬНЕННЯ PAI-1 ТРОМБОЦИТАМИ ЛЮДИНИ

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PAI-1 (інгібітор активатора плазміногену типу 1) як основний фізіологічний інгібітор активатора плазміногену та урокінази відіграє ключову роль у регулюванні фібринолізу *in vivo*. Крім того, PAI-1 пригнічує утворення плазміну та впливає на міграцію клітин шляхом взаємодії з вітронектином. PAI-1 секретується з α-гранул тромбоцитів при їх стимулюванні агоністами. Метою роботи було дослідити вплив Glu- і Lys-форм плазміногену на секрецію PAI-1 тромбоцитами та з'ясувати можливу роль плазміногену в модулюванні індукованого агоністами вивільнення PAI-1. Секрецію PAI-1 тромбоцитами визначали методом Вестерн-блот аналізу. Встановлено, що в залежності від типу агоніста, PAI-1 може вивільнятися з тромбоцитів у вільній формі, в комплексі з тканинним активатором плазміногену, а також у вигляді високомолекулярних комплексів, що містять тканинний активатор і молекули вітронектину. Виявлена індукція секреції PAI-1 під дією Glu- та Lys-форм плазміногену вказує на здатність цих протеїнів активувати внутрішньоклітинні сигнальні шляхи, які регулюють вивільнення α-гранул тромбоцитів. Отримані результати можуть мати важливе значення для з'ясування механізмів патогенезу різних захворювань, які пов'язані з надактивацією тромбоцитів і розладами, асоційованими з вивільненням PAI-1.

Ключові слова: PAI-1, тромбоцити, Glu- і Lys-плазміноген, вітронектин, α-гранули.

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