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PROTEINS OF PLASMINOGEN/PLASMIN SYSTEM: MULTIFACETED ROLES IN HEALTH AND DISEASE

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The plasminogen/plasmin (Pg/Pm) system is a cornerstone of various biological processes, encompassing roles in fibrinolysis, angiogenesis, inflammation, wound healing, and tumor biology. This review consolidates knowledge on the multifaceted functions of the Pg/Pm system proteins in health and disease, highlighting historical developments, recent advancements, and the contributions of the Department of Enzyme Chemistry and Biochemistry to the understanding of their molecular mechanisms of function. We have explored the regulation of fibrinolysis and its intricate interplay with proteins of the Pg/Pm system, delving into their pivotal role in hemostatic balance. Reciprocal interactions between Pg/Pm system proteins and platelets underscore their contribution to thrombosis, fibrinolysis, inflammation, and vascular remodeling. In oncology, Pg/Pm system proteins orchestrate tumor growth and metastasis through their involvement in extracellular matrix remodeling, angiogenesis, and cancer cell survival. However, angiostatins – proteolytically-derived fragments of Pg/Pm – emerge as multifunctional polypeptides, which are known to affect cell migration, angiogenesis, and inflammation, suppress tumor growth and metastasis. Contribution of Pg/Pm to reparative processes, including wound healing, further emphasizes their therapeutic potential in regenerative medicine. Moreover, these proteins play crucial roles in ocular health, where their dysregulation may lead to the pathogenesis of ophthalmic diseases. In conclusion, advancement of our understanding of this versatile system functions through continued research is pivotal for applications of these proteins as diagnostic and prognostic biomarkers for cardiovascular disorders, inflammatory pathologies, cancer, autoimmune conditions, and various diabetic complications, offering insights into early detection of disease and development of innovative therapeutic strategies, ultimately driving progress in personalized medicine.

K e y w o r d s: proteolysis, plasminogen/plasmin system, fibrinolysis, angiostatins, platelets, cardiovascular disorders, cancer, wound healing, ocular diseases.

Historical aspects of the department's development

The Department of Enzymes Chemistry and Biochemistry boasts a long and rich history that began with its establishment. In 1968, the Laboratory of Enzyme Biochemistry was reorganized in the Department of Enzyme Chemistry and Biochemistry. At various stages of its history, the Department achieved substantial success in different research directions. For example, in the 1970s, important discoveries were made in the field of enzymatic catalysis, forming the basis for further developments in medicine and biotechnology. Initially, this department started as a small laboratory with a group of enthusiasts dedicated to studying enzymes and their roles in biochemical processes. O. S. Tsyperovich, the first head of the Department, supervised researches focused on elucidating the mechanisms of denaturation and stabilization of globular proteins, particularly proteolytic enzymes such as pepsin, trypsin, and chymotrypsin [1]. Significant efforts of the Department's staff were directed not only to-

Abbreviations: α2-AP – α2-antiplasmin, ECM – extracellular matrix, K – kringle, LBS – lysine binding sites, MMPs – matrix metalloproteinases, PAI-1 – plasminogen activator inhibitor-1, Pg – plasminogen, Pm – plasmin, tPA – tissue type plasminogen activator, uPA – urokinase type plasminogen activator, uPAR – urokinase receptor.

wards solving theoretical questions but also towards practical tasks, including the production and application of animal and microbial enzymes for the needs of medicine, agriculture, and industry. The guiding principle for the Department's scientists became encapsulated in the phrase, "It is not enough to know, one must also be able to apply". The main scientific and technical innovations of the Department during those years included "Method for Producing the Preparation 'Gastric Juice'", "Method for Producing Pepsin Preparation", "Highly Active and Crystalline Preparations of Trypsin and Alpha-Chymotrypsin", "Pronase Enzyme – Its Production and Application", and "Crystalline Alpha-Amylase". The implementation of these and other enzyme preparations in the national economy generated multimillion economic benefits [2].

From 1976 to 2009, the Department was headed by Dr. Sci., Prof. S. O. Kudinov. Until 1980, the Department's staff continued to study microbial enzymes. However, in 1980, a new scientific direction was approved: the study of mechanisms of functioning and regulation of the blood fibrinolytic system (Fig. 1) [3]. This focus remained for several decades and continues to be one of the leading research directions of the Department to this day. In the 1990s, the Department actively collaborated with international scientific institutions, significantly broadening the scope of research and improving the scientific infrastructure. Through diligent work and significant scientific achievements, the Department gradually expanded and gained international recognition. From 2009 to 2019, the Department was under the direction of Dr. Sci. T. V. Grynenko, during whose tenure the research into the biological function of Pg/Pm system proteins was significantly expanded [4]. Since 2020, the Department has been managed by Dr. Sci. Artem Tykhomyrov, who has further advanced the Department's scientific research into the biological functions of the Pg/Pm system and its role in the development of various diseases.

The staff of the Department underwent fellowships at leading foreign scientific institutions, including Oxford Bioresearch (Great Britain), Juntendo University (Tokyo, Japan), Alexander-Friedrich University (Erlangen-Nuremberg, Germany), University of Santiago de Compostela (Spain), Bingöl University (Turkey), and others.

Today, the Department of Enzyme Chemistry and Biochemistry is a leading research unit engaged in studying enzymes, developing new biopreparations and diagnosticums, and investigating their effects on human health. A key milestone in the Department's development was the implementation of modern technologies and research methods, enabling new heights in scientific advancements.

Fig. 1. Fragment of the research report "Study of Protein-Protein Interactions of Fibrinolytic System Components" of the Department of Enzyme Chemistry and Biochemistry for 1987 (supervisor: Prof. S. O. Kudinov, D.Sc.; performers: Senior Researcher, Ph.D. Y. M. Makohonenko, Researcher, Ph.D. T. V. Grynenko, Junior Researcher, Ph.D. V.V. Novokhatny, Junior Researcher, Ph.D. L. A. Kolesnyk, Junior Researcher S. V. Verevka, Junior Researcher T. I. Lejen, Junior Researcher L. D. Taran)

Molecular mechanisms of fibrinolysis regulation involving proteins of Pg/Pm system

Fibrinolysis is a fundamental physiological process essential for life, as it ensures the removal of blood clots, maintains vascular integrity, and preserves the continuous flow of blood necessary for tissue survival and homeostasis. Fibrinolysis is regulated by the selective degradation of fibrin by the Pg/Pm system. Pm activity, driven by tPA-mediated activation of Pg on the fibrin surface, remains confined to the fibrin clot, while circulating Pm is neutralized by α2-AP [5, 6]. Since the end of the 1970s, the Department has conducted a comprehensive investigation of the fibrinolytic system. Using affinity chromatography and limited proteolysis, highly purified plasma-derived proteins of the fibrinolytic system were isolated, including Pg, tPA, and α2-AP. Various Pg fragments were also obtained, such as K1-3, K4, K5 (corresponding to specific kringle domains), mini-Pg (comprising K5 and the catalytic domain), and micro-Pg. A significant achievement was determining the ligand specificity of all kringle domains, which enabled the study of their interactions with fibrinogen/fibrin, activators (e.g., streptokinase), and α2-AP, as well as their role in Pm-mediated fibrinolysis [7].

Remarkable studies have been conducted on uncovering the protein-protein interactions between Pg and fibrinogen/fibrin, as well as the molecular mechanisms underlying fibrinolysis. It was found that fragments such as desAB-fibrin, DDE complexes, DD, D, and E interact with Pg, stimulating its conversion into active proteinase. Kinetic parameters of this activation process have been established [8]. However, questions remain regarding the molecular mechanisms initiating Pg activation during fibrin polymerization and the localization of binding sites for different Pg domains. Structural rearrangements of fibrinogen/fibrin molecules involved in fibrinolysis activation are not fully understood. Modified experimental approaches, such as sandwich ELISA, revealed independent binding of K1-3 and K5 to the DD fragment of fibrin [9]. K5-mediated interaction of circulating Glu-Pg with fibrin triggers fibrinolysis. Identifying K5 binding sites is pivotal for understanding fibrinolysis mechanisms and developing targeted therapeutic strategies. Addressing these shortcomings, a method for isolation of the functionally active K5 fragment of human Pg was developed, and high-affinity polyclonal antibodies specific to K5 were obtained. [10]. Binding sites for K5 were identified in the α- and γ-chains of the DD fragment within the sequences α148-198 and γ266-302, respectively. Key residues, α171Arg and/or α176Lys, mediate K5 interaction, while complementary binding regions for Pg were localized to the sequences α168-183 in the α-chain and γ266-302 in the γ-chain (unpublished data).

The conversion of fibrinogen into fibrin exposes Pg and tPA binding sites, enabling efficient Pg activation on the fibrin surface (Fig. 2). Structural changes in the β- and γ-modules of the D region during fibrin polymerization uncover these interaction sites. For the first time, the sequence α168-183 was identified as responsible for K5 binding, while α581-610 mediates complex formation between Pg and the αC regions of fibrin(ogen). These interactions are crucial for initiating fibrinolysis. Research has shown that Pg and its fragments K1-3, K4, and K5 interact in a dose-dependent manner with the αC region of fibrinogen, specifically the sequence α581-610 [11]. This interaction is essential during fibrinogen conversion to fibrin and highlights the importance of conformational changes in fibrinogen.

In conclusion, these findings highlight the critical role of Pg activation and its interaction with fibrin in the fibrinolytic process, emphasizing the importance of conformational changes in fibrinogen for efficient Pg binding. The identification of specific binding sites, particularly those involving the K5 domain, provides valuable insights into the molecular mechanisms of fibrinolysis and offers a foundation for developing targeted strategies to regulate this process.

Angiostatins as multifunctional components of the Pg/Pm system

Angiostatins, naturally occurring Pg-derived polypeptides, are potent inhibitors of angiogenesis, the process of forming new blood vessels. These proteins, integral to the Pg/Pm system, regulate vascular growth through their kringle domains, structural motifs responsible for their biological activity. Angiostatins are generated by proteolytic cleavage of Pg by enzymes like MMPs, elastase, and plasmin itself, and usually are composed of the first 1 to 4, or occasionally 4.5, K domains of the precursor protein (K1-3, K1-4, K1-4.5) [12]. Discovered in the 1990s, angiostatins garnered attention for their ability to inhibit tumor growth by disrupting the tumor blood supply [13, 14]. They act by binding to

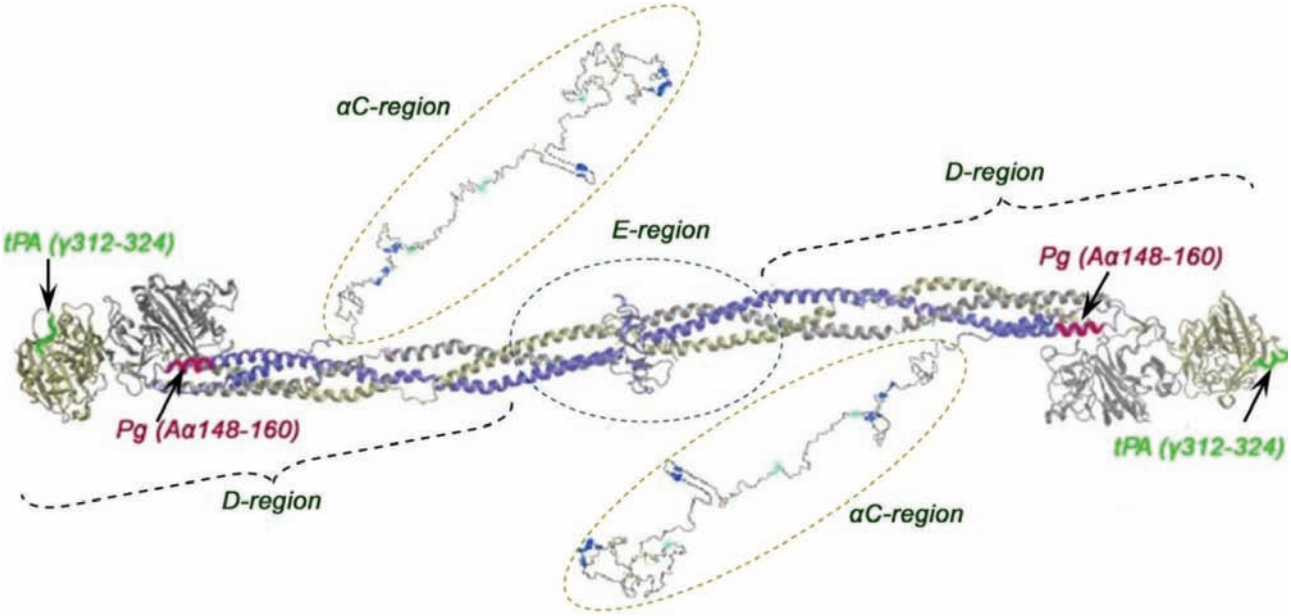

Fig. 2. Plasminogen (Pg) and tissue Pg activator (tPA) binding sites in the fibrinogen molecule [11]. The precise localization of the binding sites for Pg and tPA in the fibrinogen αC-regions, which are cryptic in the fibrinogen molecule and presumably exposed during fibrin polymerization, remains undefined

receptors on endothelial cells, such as integrins, annexin II, and ATP synthase, which suppresses cell migration, proliferation, and promotes apoptosis [15]. For example, the single kringle 5 (K5) fragment is particularly effective in curbing endothelial cell proliferation and migration [16]. By inhibiting angiogenesis, angiostatins contribute to the suppression of pathological processes like tumor growth and metastasis. They also limit ECM remodeling and counteract pro-angiogenic factors like VEGF and basic fibroblast growth factor (bFGF), emphasizing their therapeutic value in cancer treatment [15, 17]. Angiostatins interfere with hepatocyte growth factor (HGF) signaling by targeting its receptor, c-Met. This disrupts the phosphorylation of c-Met, a pivotal step in activating signaling pathways like Akt and ERK1/2, which are essential for endothelial cell survival, proliferation, and migration. Moreover, angiostatins competitively inhibit HGF by binding directly to c-Met, forming a complex that blocks HGF interaction [18, 19]. This novel mechanism enhances angiostatin anti-angiogenic effects. Angiostatin also interacts with the voltage-dependent anion channel (VDAC) exposed on the endothelial cell surface and in mitochondria, modulating its function to inhibit ATP production and contribute to its anti-angiogenic effects [20].

Beyond oncology, angiostatins are relevant in conditions like diabetic retinopathy [21], rheumatoid arthritis [22], and cardiovascular diseases [23], where they regulate abnormal angiogenesis and preserve tissue integrity. However, excessive production of angiostatins in ischemic tissues can suppress the development of reparative vessel collaterals, ultimately hindering the restoration of blood supply to the affected organ [24]. Platelets, as reservoirs of angiostatins, can produce and release these Pg fragments during aggregation, underscoring their physiological importance [25]. Research has also linked angiostatins to brain physiology and pathology, as astrocytes produce these proteins in specific brain regions [26].

Additionally, angiostatins suppress inflammation by reducing leukocyte infiltration and cytokine release [27]. They impair the formation of actin filopodia and lamellipodia, immobilizing monocytes and macrophages, and inhibiting their migration [28]. This mechanism highlights their therapeutic potential in diseases where immune cell recruitment drives progression, such as cancer and atherosclerosis.

The K5 fragment stands out as a promising therapeutic agent due to its selective cytotoxicity toward proliferating endothelial and tumor cells, sparing normal tissues (Fig. 3).

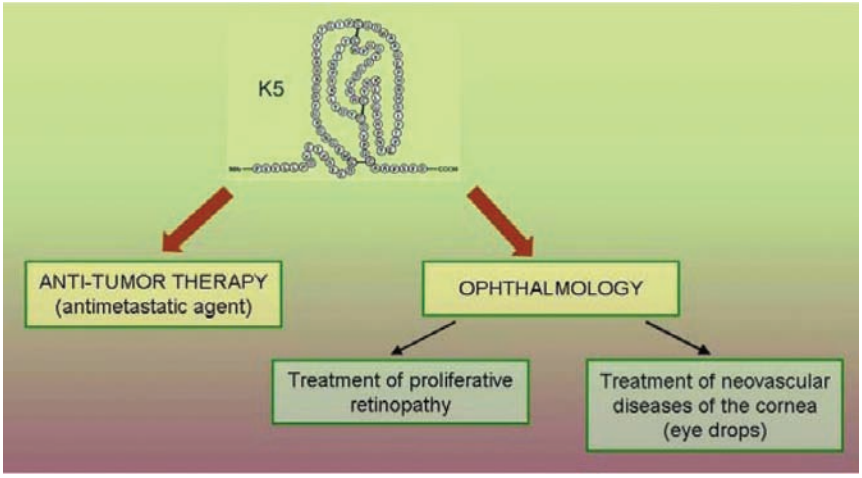

Fig. 3. Potential application of angiostatin K5 in biomedicine [29]

As an endogenous protein, K5 does not elicit immune responses, making it a safe option. Its small, stable polypeptide structure allows efficient recombinant production in *E. coli* cells, facilitating targeted delivery and sustained release [29].

In conclusion, angiostatins are multifunctional components of the Pg/Pm system with significant implications for angiogenesis, tumor biology, and inflammatory processes. Their diverse roles and therapeutic potential make them a focal point of research in the fields of oncology and vascular biology.

Reciprocal interactions between proteins of Pg/Pm system and platelets in the regulation of thrombosis, fibrinolysis, and angiogenesis

The interplay between the Pg/Pm system and platelets is critical in regulating thrombosis, fibrinolysis, and angiogenesis. While platelets are traditionally recognized for their role in hemostasis, they also modulate clot dynamics through interactions with the Pg/Pm system. Activated platelets expose binding sites for Pg, promoting its activation into Pm on their surface, which contributes to clot dissolution and tissue remodeling. Conversely, Pm can activate platelets by cleaving surface receptors, enhancing thrombus formation. Platelets, in turn, regulate Pm activity through the release of inhibitors and cofactors, maintaining a delicate balance between clot formation and resolution essential for vascular integrity [30]. Platelets also release PAI-1, which suppresses fibrinolysis and underscores their dual role in stabilizing and breaking down clots [31, 32].

The Department conducts extensive, long-term studies aimed at uncovering the biochemical mechanisms of reciprocal interactions between the Pg/Pm functional activity and platelets. This complex study holds promise for addressing challenges related to thrombogenesis, inflammation, and angiopathies. The department's team has obtained the following scientific findings. According to the current concept, platelets are able to play a dual role in regulating both coagulation and fibrinolysis [33]. To investigate these interconnected processes, we examined the impact of platelets on fibrin clot formation, maturation, and degradation [34]. Our findings demonstrated that platelets coordinate coagulation and fibrinolysis via components of the intrinsic coagulation pathway and the Pg/Pm system. Specifically, inactive prothrombinase complex (PTC) proteins compete with Glu-Pg for platelet binding. Meanwhile, Factor Xa stimulates tPA-dependent Pm formation in platelet suspensions, as confirmed through the colocalization of platelet-bound labeled tPA and anti-FX(a) antibodies (unpublished data). Moreover, a subpopulation of platelets with accumulated labeled Glu-Pg is formed only after simultaneous contact of platelets with thrombin and tPA in the presence of PTC, i.e., due to the joint activation of PTC proteins and Pg. Apparently, newly formed Pm enhances the fragmentation of Factor Xa, generating Xa33/13 fragments that act as cofactors for tPA and Glu-Pg [35]. This mechanism suggests a Pm autoactivation loop regulated by platelet-associated thrombin-generating systems within the PTC. These findings provide valuable insight into the reciprocal regulation of coagulation and fibrinolysis by platelets, highlighting their pivotal role in maintaining vascular integrity.

Furthermore, the Lys-form of Pg (Lys-Pg), but not the Glu-form, has been shown for the first time to suppress platelet responses to agonist stimulation [36]. Lys-Pg inhibited thrombin- and collagen-induced platelet aggregation and reduced P-selectin exposition on the platelet membrane, possibly by impairing actin cytoskeleton reorganization, thus disrupting secretion and aggregation [37, 38].

Beyond fibrinolysis, Pg/Pm interactions with platelets impact angiogenesis. Pm-mediated degradation of the extracellular matrix releases angiogenic factors, while platelets secrete pro-angiogenic molecules such as VEGF [39]. However, Lys-Pg inhibits the agonist-induced release of VEGF from platelets, indicating the possible role of this Pg form in suppressing angiogenesis [40]. Additionally, platelets regulate angiogenesis by forming and releasing angiostatins. Angiostatins are generated through the enzymatic cleavage of Pg upon platelet activation and released during aggregation. These inhibitors are crucial in maintaining vascular homeostasis and preventing pathological angiogenesis, such as in tumor growth or chronic inflammation [41]. The temporal dynamics of angiostatin release are also significant. Angiostatins stored in platelet α-granules are released during later stages of aggregation, balancing pro- and anti-angiogenic factors [25, 42]. This balance is vital for regulating angiogenesis in both physiological and pathological conditions. We proposed that actin exposed on activated platelet membranes serves as one of the binding sites for Pg and likely plays a role in Pg fragmentation on the platelet surface, leading to angiostatin formation [40, 43] (Fig. 4).

In conclusion, the Pg/Pm system and platelets exhibit intricate reciprocal interactions, which are essential for regulating thrombosis, fibrinolysis, and angiogenesis, thus ensuring vascular homeostasis. Their balanced interplay is essential for vascular health, and its disruption contributes to disease pathogenesis. Moreover, aberrant angiogenesis, influenced by these systems, underlies various disorders, including cancer and chronic inflammation. The described findings have significant implications for developing targeted therapeutic strategies for thrombotic and angiogenic disorders, highlighting the importance of understanding the spatial and temporal dynamics of these interactions for developing precise interventions.

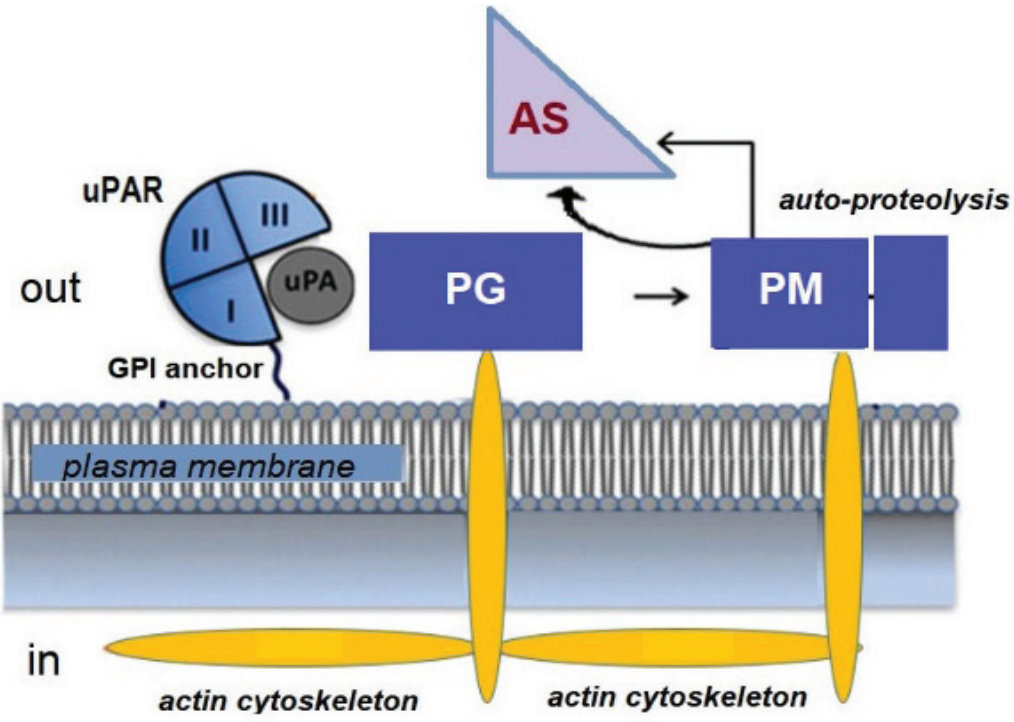

Fig. 4. A hypothetical role of surface-exposed actin in angiostatin (AS) generation on the platelet plasma membrane (Pg – plasminogen, Pm – plasmin, uPA – urokinase-type plasminogen activator; uPAR – urokinase-type plasminogen activator receptor; GPI anchor – glycosylphosphatidylinositol anchor)

Proteins of the Pg/Pm system as diagnostic-prognostic markers of cardiovascular diseases and inflammatory processes

The Pg/Pm system plays a crucial role in cardiovascular health and inflammatory processes, with its components serving as valuable diagnostic and prognostic markers. Dysregulation of this system is associated with thrombotic disorders, atherosclerosis, and chronic inflammation [44]. For example, PAI-1, as the key regulator of blood fibrinolytic potential, emerges as a critical biochemical marker reflecting hemostatic balance. Elevated PAI-1 levels are linked to heightened cardiovascular risks, including myocardial infarction, stroke, arterial and venous thrombosis [45]. Moreover, inflammatory conditions such as obesity, diabetes, and metabolic syndrome influence Pg/Pm components, with increased PAI-1 levels frequently observed [46]. During acute myocardial infarction, a progressive rise in PAI-1 is considered an unfavourable prognostic marker indicative of endothelial dysfunction [47]. PAI-1 plays dual roles in thrombotic conditions and malignant neoplasms. Elevated levels contribute to vascular diseases, while PAI-1 deficiency or reduced activity leads to hyperactivation of fibrinolysis, increasing hemorrhagic risk. At the Department of Enzyme Chemistry and Biochemistry, a sensitive method was developed to analyze functionally active PAI-1 based on its inhibition of the amidolytic activity of the tPA-Pg system [48]. Unlike the closest analogues, our method utilizes the D-fragment of fibrinogen, which serves as a storage-stable activator of the activation reaction and offers greater stability compared to desAB-fibrin. Additionally, it eliminates the need for highly toxic substances, such as cyanogen bromide-derived fibrin fragments, making the approach safer and more efficient. This approach proved effective for monitoring treatment dynamics in prostate hyperplasia with chronic heart failure and assessing endothelial dysfunction risk in post-COVID syndrome [49]. As a marker implicated in inflammation, endothelial dysfunction, and tissue fibrosis, PAI-1 is also associated with severe courses of infectious diseases. In COVID-19 studies, elevated PAI-1 and reduced urokinase/PAI-1 complex levels correlated with disease severity [50].

Genetic polymorphisms significantly influence PAI-1 activity. PAI-1 4G/5G genetic polymorphisms affect the expression levels of PAI-1, with the 4G allele typically linked to elevated PAI-1 concentrations. This increase in PAI-1 levels can disrupt fibrinolysis and enhance thrombotic potential, raising the risk of cardiovascular and inflammatory diseases. Our investigations of the 5G/4G polymorphism in the PAI-1 promoter region revealed associations with pro-inflammatory cytokines and endothelial dysfunction. The 5G/5G genotype emerged as a risk factor for inflammation-induced endothelial dysfunction, whereas the 4G/4G genotype showed protective effects in inflammation. Cellular studies confirmed the anti-inflammatory role of PAI-1, demonstrated using 4G/5G human endothelial cells stimulated by IL-1β [51]. Therapeutic targeting of the Pg/Pm system is under exploration, with PAI-1 and uPA showing promise in preclinical studies [52].

Given the unpredictable circulatory changes in pathological states, comprehensive methods for evaluating coagulation and fibrinolysis imbalances are necessary. Clot waveform analysis (CWA) offers a promising alternative to traditional coagulation tests, such as PT and APTT [53]. Adaptations of the CWA assay for assessing hemostatic balance during fibrin clot formation and lysis in platelet-free and platelet-rich plasma [34], demonstrated effectiveness in predicting hemostatic shifts in cardiovascular patients, evaluating rt-PA thrombolytic efficacy, and managing clotting disorders caused by immune checkpoint inhibitors [54, 55]. Analysis of fibrinolytic potential revealed imbalances in tPA and PAI-1 levels in chronic heart failure (CHF) complicated by severe vascular inflammation, alongside reduced protein C levels, indicating impaired fibrinolytic and anticoagulant capacity. Stable angina pectoris was associated with increased hs-CRP, tPA, and Glu-Pg levels and protein C deficiency. Simvastatin improved fibrinolytic potential but failed to correct protein C deficiency, suggesting independent regulation of the protein C system [56, 57]. Our adaptation of the CWA test for PRP demonstrates promising potential for the clinical diagnosis of hemostatic disorders, as confirmed by these findings.

Emerging evidence highlights angiostatins as biomarkers for disease severity and therapeutic response. For example, evaluation of Pg and angiostatin levels in benign breast lesions, such as mastopathy and fibroadenomas, revealed angiostatins' potential role in non-cancerous breast disease pathophysiology [58]. A pilot clinical study examined circulating biomarkers, including angiostatin, in patients with atrial fibrillation (AF) and chronic heart failure. Findings indicated elevated angiostatin levels in post-stroke patients with persistent and

permanent AF types, emphasizing its potential for stroke risk stratification and disease progression [59]. Angiostatin levels emerged as a significant marker in studies evaluating vascular health interventions. During various cardiovascular disease treatments, citicoline reduced angiostatin levels by 40% as compared with untreated control [60], simvastatin decreased angiostatin alongside improving lipid metabolism and reducing inflammation [61], and L-arginine counteracted angiostatin's anti-angiogenic effects by enhancing nitric oxide production [62], collectively highlighting their roles in endothelial function and recovery in ischemic conditions.

In conclusion, the Pg/Pm system represents a critical axis in cardiovascular and inflammatory diseases. Its components offer valuable insights into disease mechanisms and hold potential as diagnostic-prognostic markers and therapeutic targets.

Involvement of Pg/Pm system proteins in the regulation of tumor growth and metastasis

The Pg/Pm system is a central regulator of tumor growth and metastasis, influencing ECM remodeling, angiogenesis, immune modulation, and metastatic dissemination. Pm degrades ECM components, enabling tumor invasion, and activates MMPs, amplifying ECM breakdown and aiding tumor dissemination [63]. Angiogenesis, driven by Pm-mediated release of pro-angiogenic factors like VEGF, is vital for tumor growth, while angiostatins, Pg-derived fragments, suppress not only angiogenesis by inhibiting endothelial cell proliferation and migration activities, but also may directly target cancer cells, acting as antitumorigenic agents [64]. Together with colleagues from the Department of Cell Signaling Mechanisms, we showed that angiostatins K1-3 and K5 have potential in inhibiting the migration of lung adenocarcinoma cells, highlighting their therapeutic promise in limiting cancer spread (unpublished data).

The uPA/uPAR axis is pivotal in localized Pg activation. Binding of uPA to uPAR on tumor and stromal cells enhances Pm activity, facilitating ECM degradation and activating latent growth factors like TGF-β, which stimulate tumor proliferation and angiogenesis [44, 65]. The Pg/Pm system also aids tumor cell intravasation and extravasation by breaking down vascular barriers, providing pathways for metastatic dissemination. Additionally, Pg receptors, such as S100A10, play a pivotal role in promoting tumor cell survival and chemoresistance by modulating signaling pathways involved in cell death and immune evasion [66]. These findings underscore the importance of targeting the Pg activation system as a potential therapeutic strategy in cancer treatment.

We also demonstrated that lung adenocarcinoma cells are able to convert Pg into Pm, which is responsible for disrupting adhesive contacts and inducing autophagy marked by elevated LC3 and beclin-1 levels. It was assumed that autophagy likely contributes to temporary survival under anoikis-induced stress. Furthermore, Pg/Pm increases TIGAR expression, which induces signaling metabolic shifts that enhance oxidative stress resistance, DNA repair, and pentose phosphate pathway activity, thus supporting cancer cell survival [67].

PAI-1, a key regulator of Pg activation, exhibits paradoxical effects in cancer. While primarily inhibiting Pm activity, it promotes tumor growth by enhancing angiogenesis and aiding cancer cell survival through apoptosis inhibition. PAI-1 interacts with vitronectin and integrins to support tumor cell migration, invasion, and adhesion, facilitating metastasis by enabling cells to escape primary tumors and establish secondary growths [68]. Elevated PAI-1 levels are associated with poor prognosis and increased metastatic potential across various cancers. As a biomarker, PAI-1 provides insights into tumor aggressiveness [69]. However, its multifaceted functions pose challenges for therapeutic targeting, as its inhibition may disrupt both pro-tumor and anti-tumor mechanisms.

In conclusion, the components of Pg/Pm system serve as potential biomarkers for cancer prognosis and therapeutic targets, offering opportunities for innovative cancer treatments. For instance, targeting the Pg/Pm system has shown promise in preclinical models, with inhibitors of uPA, uPAR, and PAI-1 reducing tumor invasion and metastasis [52, 70]. Understanding the spatial and temporal dynamics of Pg/Pm activity in the tumor microenvironment remains essential for developing effective interventions.

The proteins of the Pg/Pm system in reparative processes and wound healing

The Pg/Pm system is crucial for wound healing and reparative processes. At sites of tissue damage, Pg accumulates and converts into Pm, facilitating fibrin degradation, wound clearance, and enabling keratinocyte and fibroblast migration. Pm further ac-

tivates growth factors and MMPs essential for ECM remodeling. Pg also directly stimulates macrophage and keratinocyte migration and acts as a mitogen for fibroblasts, accelerating angiogenesis, fibroplasia, and epithelialization, key steps in wound closure [71]. Its multifaceted roles establish Pg as a "master regulator" of wound healing [72].

Disruptions in the Pg/Pm system, such as mutations in PLG (the Pg-encoding gene), contribute to delayed healing and chronic wounds [73]. In diabetes mellitus, reduced Pg function impairs fibrinolysis, increases fibrin deposition, and delays cell migration, creating a pro-inflammatory microenvironment that hinders tissue repair. Animal studies reveal Pg administration accelerates wound closure, underscoring its therapeutic potential [74]. Pg-based matrices have also demonstrated efficacy in diabetes-related wound models [75]. In 2024, we showed for the first time that local application of autologous Pg enhances healing of chronic foot ulcers in type II diabetes patients [76]. Plasma-derived Pg has improved outcomes in acute and chronic wounds in hypoplasminogenemia cases, further highlighting its reparative benefits [77]. Current research focuses on formulating Pg-based ointments for clinical use.

Chronic hyperglycemia in diabetes triggers Pg glycation, altering its structure and reducing Pm generation, enzymatic activity, and fibrinolysis, leading to thrombotic risks and delayed healing. A novel hypothesis involves auto-antibodies to Pg (auto-Pg Abs) in diabetes. These auto-Pg Abs may impair Pg conversion to Pm, exacerbate impaired wound healing through chronic inflammation, persistent fibrin deposition, and delayed cell migration (unpublished data). The Pg/Pm system proteins play a crucial role in modulating inflammatory responses, making them promising targets for autoimmune disease therapy. Developing an ELISA-based kits for detecting anti-Pg autoantibodies could enable precise monitoring of disease progression and treatment efficacy, facilitating personalized therapeutic strategies. This approach may improve early diagnosis and intervention, ultimately enhancing the management of autoimmune conditions by targeting dysregulated plasminogen activation pathways. Targeting auto-Pg Abs could offer new therapeutic avenues, such as immunomodulatory treatments, to enhance diabetic wound repair.

Angiostatins, proteolytic fragments of Pg, exhibit dual roles in wound healing. While Pg promotes tissue repair, angiostatins inhibit angiogenesis by suppressing endothelial cell proliferation, migration, and blood vessel formation, resulting in hypoxia and impaired tissue regeneration, which are hallmarks of chronic wounds [78]. Excessive angiostatin production, observed in diabetes and conditions like Martorell's syndrome, disrupts the pro-angiogenic balance necessary for effective healing [79]. Although angiostatin's anti-inflammatory properties are beneficial in specific contexts, they can impede the inflammatory response essential for initiating tissue repair. Modulation of angiostatin levels may represent a promising strategy for restoring balance in chronic wound management.

High levels of PAI-1 disrupt the delicate balance required for the Pg/Pm system to function effectively, which in turn impairs wound healing. Excessive PAI-1 hinders the breakdown of fibrin clots, preventing the proper clearance of damaged tissue and delaying the migration of essential cells like keratinocytes and fibroblasts [45, 80]. This imbalance underscores the necessity of tightly regulating PAI-1 levels to ensure efficient tissue repair and prevent complications associated with chronic or non-healing wounds.

The Pg/Pm system also contributes to other reparative processes, including vascular injury recovery and myocardial ischemia repair [81], bone fracture healing [82], and tympanic membrane regeneration [83]. Its involvement in cell migration and ECM remodeling makes it a valuable target for innovative therapies.

In conclusion, the Pg/Pm system is indispensable for efficient wound healing and tissue repair. Its multifaceted roles in fibrin clearance, cell migration, and ECM remodeling make it a cornerstone of reparative biology. Future research should focus on harnessing its therapeutic potential while addressing the challenges of its regulation.

Role of the Pg/Pm system proteins in ocular function and disease pathogenesis

The Pg/Pm system is vital for ocular health and plays a significant role in the pathogenesis of various eye diseases. Its involvement in fibrinolysis, ECM remodeling, and cell migration is essential for maintaining the proper function of ocular structures [84]. In the cornea, Pg facilitates wound healing by promoting epithelial cell migration and degrading fibrin deposits. Dysregulation can lead to delayed healing or excessive tissue damage. Elevated levels of Pg activators in allergic conjunctivitis highlight the sys-

tem's role in leukocyte migration and collagen degradation [85]. Pg-based teardrops, which leverage its fibrinolytic and anti-inflammatory properties, show promise as a treatment for ligneous conjunctivitis by mitigating inflammation and enhancing tissue repair [86].

In the retina, the Pg/Pm system governs angiogenesis and vascular remodeling, crucial in diseases like diabetic retinopathy and retinal vein occlusion. Abnormal angiogenesis and fibrin deposition are hallmarks of these conditions, and imbalances in Pg activators may worsen disease progression. Excessive Pg/Pm activity has been linked to retinal detachment and proliferative vitreoretinopathy, where matrix degradation and cell migration contribute to pathological changes [87]. Targeting the Pg/Pm system offers therapeutic potential for these conditions.

The Pg/Pm system also influences glaucoma pathogenesis. In the trabecular meshwork, ECM remodeling affects intraocular pressure regulation. Elevated PAI-1 levels can hinder matrix degradation, increasing resistance to aqueous humor outflow [88]. Microplasmin (ocriplasmin) has been used therapeutically in intravitreal injections to treat vitreomacular adhesion and vitreomacular traction. By enzymatically disrupting abnormal vitreoretinal adhesions, it provides a less invasive alternative to vitrectomy, particularly in cases uncomplicated by epiretinal membranes [89, 90].

Angiostatins, generated by corneal cells on the healthy ocular surface and presenting in the tear fluid, inhibit excessive angiogenesis and maintain corneal transparency [91, 92]. These molecules have shown promise in treating diseases with abnormal blood vessel growth, such as diabetic retinopathy and corneal neovascularization after trauma or autoimmune-related ocular diseases [93, 94]. In our experimental model of alkali-induced burn, angiostatins inhibited corneal neovascularization, reduced macrophage activity, and normalized markers related to hypoxia, fibrosis, and autophagy. They also stabilized intercellular junctions, suggesting protective effects [95, 96]. Notably, angiostatins have been shown to reduce the expression of ACE2, a receptor for SARS-CoV-2, in burn-damaged corneas, highlighting their relevance in pandemic-related complications [97]. Recombinant angiostatins present a promising avenue for ophthalmologic disease therapy, offering a more affordable alternative to native angiostatins while maintaining their anti-angiogenic efficacy. Their lower production costs and scalability could facilitate broader clinical application, making targeted treatments for retinal neovascular disorders more accessible.

Several angiostatin variants and their genetic constructs offer therapeutic benefits in retinopathy by inhibiting abnormal angiogenesis, a key factor in disease progression, thereby protecting retinal cells from further damage and blindness [98, 99]. These approaches have demonstrated potential in reducing vascular complications and enhancing the overall stability of retinal tissue in experimental studies. Additionally, our research on experimental hyperglycemia in rats revealed that PARP-1 inhibitors elevated angiostatin levels in diabetic rats, thus enhancing anti-angiogenic effects and reducing the risk of retinal detachment [100].

These and other findings collectively underscore the Pg/Pm system's broad therapeutic potential in addressing ocular diseases, from wound healing and inflammation to abnormal angiogenesis and tissue remodeling.

Conclusions. In summary, the Pg/Pm system plays a crucial role in both the maintenance of ocular health and the pathogenesis of ophthalmic diseases. Dysregulation of this system can lead to a range of ocular disorders, emphasizing the need for a deeper understanding. Future research should prioritize developing targeted therapies to modulate the Pg/Pm system for more effective treatments. New data on the molecular mechanisms of Pg/Pm function can be integrated into the development of innovative pharmaceutical drugs. This integration may enhance therapeutic efficacy and lead to more advanced treatment strategies. For example, recent findings on the molecular mechanisms of Pg/Pm function, as well as the role of structural reorganization in the fibrinogen molecule during fibrin formation in initiation and fibrinolysis, can be used for the development of innovative targeted pharmaceutical drugs. These drugs will enable precise regulation of thrombus formation and effective treatment of thrombotic diseases. Further, our current discoveries offer valuable insights into the intricate interplay between coagulation and fibrinolysis mediated by platelets, shedding new light on their reciprocal regulation. Particularly, findings on the influence of Pg on platelet thrombus formation and secretory functions provide a foundation for developing novel therapeutic strategies aimed at regulating thrombogenesis and thrombolysis. This knowledge paves the way for novel approaches in the development of innovative therapeutic agents de-

signed to correct hemostatic imbalances and prevent thrombotic or hemorrhagic complications.

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

ПРОТЕЇНИ ПЛАЗМІНОГЕН/ ПЛАЗМІНОВОЇ СИСТЕМИ: РІЗНОМАНІТТЯ ФУНКЦІЙ В НОРМІ ТА ЗА ПАТОЛОГІЧНИХ СТАНІВ

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Плазміноген/плазмінова (Pg/Pm) система є ключовою ланкою різних біологічних процесів, таких як фібриноліз, ангіогенез, запалення, загоєння ран та пухлинний ріст. У представленому огляді узагальнено відомості про багатоаспектні функції протеїнів цієї системи за норми та патологічних станів, акцентовано увагу на історичних досягненнях, новітніх здобутках та внеску відділу хімії та біохімії ферментів у розуміння молекулярних механізмів їх функціонування. Нами досліджено механізми регулювання фібринолізу в контексті складних міжпротеїнових взаємодій за участі компонентів системи Pg/Pm, що поглиблює знання їх ключової ролі в підтриманні гемостатичного балансу. Реципрокні взаємодії між протеїнами системи Pg/Pm та тромбоцитами відіграють важливу роль у регулюванні тромбоутворення і фібринолізу, а також роблять внесок до розвитку запалення та ремоделювання судин. За онкологічних процесів протеїни системи Pg/Pm відіграють ключову роль у стимулюванні росту пухлин та метастазування, сприяючи ремоделюванню позаклітинного матриксу, ангіогенезу та виживанню ракових клітин. Проте, ангіостатини – протеолітичні фрагменти Pg/Pm – відомі як поліфункціональні поліпептиди, що пригнічують міграцію клітин, інгібують ангіогенез, запальні процеси, пухлинний ріст і метастазування. Залучення Pg/Pm у репаративні процеси, зокрема, загоєння ран, підкреслює їх значний терапевтичний потенціал у галузі регенеративної медицини. Крім того, досліджувані протеїни відіграють важливу роль у забезпеченні нормального функціонування ока, а порушення регуляції їх роботи може призводити до розвитку низки офтальмологічних захворювань. Отже, подальше дослідження функціонування протеїнів цієї універсальної системи має вирішальне значення для їх використання як діагностичних і прогностичних біомаркерів серцево-судинних захворювань, запальних процесів, злоякісних новоутворень, аутоімунних станів та різних ускладнень цукрового діабету й відкриває нові перспективи для раннього виявлення хвороб, розробки інноваційних терапевтичних стратегій і розвитку персоналізованої медицини.

Ключові слова: протеоліз, плазміноген/плазмінова система, фібриноліз, ангіостатини, тромбоцити, серцево-судинні захворювання, злоякісні новоутворення, загоєння ран, офтальмологічні хвороби.

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