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POLYCLONAL ANTIBODIES AGAINST HUMAN PLASMINOGEN KRINGLE 5

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The aim of the research was to obtain the polyclonal antibodies against a fragment of human plasminogen kringle 5 and to study their immunochemical properties. The following approaches were used: immunization of rabbits with plasminogen kringle 5, receiving of high-titer immune serum, synthesis of kringle 5-based affinity sorbent for selection of monospecific antibodies, chromatography on synthesized K 5-Sepharose, ELISA, immunoblot assay.

The obtained polyclonal antibodies reacted in ELISA with a plasminogen K 5 fragment, and to a much lesser extent with mini-plasminogen, Lys-plasminogen, K 1-3 and K 4 plasminogen fragments, Glu-plasminogen, in descending order. Based on the dissociation constant determination it was found that these antibodies had high affinity to their epitopes within K 5 fragment ($3.89 \cdot 10^{-10}$ M), mini-plasminogen ($5.46 \cdot 10^{-9}$ M) and Lys-plasminogen ($2.54 \cdot 10^{-9}$ M), and insignificant affinity to K1-3, K4 and Glu-plasminogen. These antibodies reacted in immunoblotting assay with an isolated K 5 fragment of human plasminogen, Lys-Pg and mini-Pg, and did not react with K 5 in Glu-Pg and with K 1-3 or K 4 fragments. Thus, the obtained polyclonal antibodies were monospecific and had a high affinity to kringle 5. These antibodies can be used for the development of immunochemical and immunosensory methods for the quantitative determination of angiostatin K 5 in biological materials.

Key words: human plasminogen fragments, kringle 5, polyclonal antibodies, angiostatines.

Kringle 5 is one of the structural domains of the molecule of plasminogen (Pg). The site of interaction with ligands which is located in this domain differs according to the specificity of the K 1–3 and K 4 sites. It is believed that kringle 5 interacts with the side chain radicals of lysine or arginine and provides the primary contact of Pg with polymeric fibrin [1–3]. In order to understand the molecular mechanism of fibrinolysis, it is important to investigate when the changes significant for the regulation of the fibrinolysis rate occur in the conformation of Glu-plasminogen with the participation of the K 5 plasminogen fragment, to clarify the stages of structural transformations of plasminogen and fibrin molecules critical for the regulation of the fibrinolytic process. Structural transformations of protein molecules suggest certain changes in the superposition of amino acid residues involved in intermolecular interactions. Such changes can be controlled by antibodies whose antigenic determinants (5–7 amino acid residues) are consistent with the studied polypeptide sequences, either located in their composition (sequentially or through intervals) or formed conformationally in secondary or tertiary structures. Since the antibody paratope perceives even the slight changes in its own epitope configuration, the target antibodies are used as molecular probes to detect and locate the functionally important sites of protein macromolecules [4]. Antibodies against the isolated K 5 fragment of human plasminogen can be an effective tool for studying the effect of K 5 on the interaction

of Glu-plasminogen with desAB fibrin and fibrinogen, which is activated by thrombin (polymeric fibrin), and on the activation of plasminogen by tissue plasminogen activator. Also these antibodies can be used to study the effect of the K 5 fragment on the platelet activation and how it and the products secreted during aggregation affect the activation of plasminogen.

Kringle 5 is shown to exhibit a potent antiangiogenic effect through specific inhibition of the migration and proliferation of endothelial cells in blood vessels [5, 6]. The prospect of the successful use of K 5 as a therapeutic agent in cases of oncogenesis-associated pathological processes [7, 8], and disturbed angiogenesis regulation [9] (in particular, diabetic retinopathy [10, 11]), necessitates the development of methods for detecting this angiostatin in biological material in studies of the functioning of plasminogen/plasmin system in norm and pathology.

To find out the role of this domain in the protein-protein and intercellular Pg interactions, as well as to develop approaches for the detection of angiostatin in biological material, the aim of the work was to obtain and characterize polyclonal antibodies to fragment K 5 of human Pg.

Materials and Methods

Glu-plasminogen (Glu-Pg) from donor blood plasma anticoagulated with sodium citrate and Lys-plasminogen (Lys-Pg) from the blood plasma fraction $III_{2,3}$ by Cohn (Kyiv City Blood Center, Ukraine) were chromatographed on Lys-Sepharose in the presence of Contrykal (AWD, Germany) [12]. Mini-plasminogen (mini-Pg) and K1-3 and K4 fragments were obtained by limited hydrolysis of Pg by pancreatic elastase (3.4.21.36) (Sigma, USA) for 5 hours [13]. Fragment K 5 was generated by limited proteolyzing of mini-Pg by pepsin (3.4.23.1) (Sigma, USA) for 3 hours [13]. The resulting protein preparations were stored at −20 °C and used in immunochemical studies, K 5 was also used as an antigen for the antibody production and as a ligand for the synthesis of affinity sorbent. The chemical reagents used in the work had a qualification not lower than “chemically pure”.

Gel filtration

The method of gel filtration was used to separate the proteolytic Pg fragments on TOYOPEARL HW-50 Fine Grade (TOYO SODA Manufacturing Co., Ltd., Japan).

Affinity chromatography

By affinity chromatography, Glu- and Lys-Pg and its proteolytic fragments containing Lys-binding sites were isolated and purified. Lys-Sepharose was used as an affinity sorbent. Binding of Lys to Sepharose CL-4B (GE Healthcare Bio-Science, Sweden) was carried out using the method for cyanogen bromide activation of agarose according to [14].

Affinity chromatography on aminohexyl (AH)-Sepharose 4B (Amersham Biosciences, Sweden) was used to isolate the K 5 fragment from the mini-Pg hydrolysate with pepsin.

Polyclonal antibodies to kringle 5 were isolated from the total fraction of blood serum immunoglobulins of immunized rabbits by the affinity of K 5-Sepharose. Binding of K 5 to Sepharose CL-4B (GE Healthcare Bio-Science, Sweden) was carried out using the method for cyanogen bromide activation of agarose according to [14]. For the synthesis of affinity sorbent, 0.5 g of BrCN-Sepharose dry powder was kept for 1 hour in 1 mmol HCl at 24 °C for 30 min, and was washed in 1 mmol HCl (total volume — 200 ml per 1 g powder) on a glass filter. The gel swelled in a volume of 5–10 ml of 1 mmol HCl, taking into account that 1 g of dry BrCN-Sepharose preparation becomes 3.5 ml in volume. The resulting gel was washed with buffer for binding (0.1 M $NaHCO_3$ (pH 8.3), 0.5 M NaCl) in a volume of 50 ml per 1 g of dry preparation. The preparation of protein for immobilization was carried out in a binding buffer. K5 solution was applied in the amount of 1 mg per 1 mg BrCN-Sepharose dry powder to a BrCN-Sepharose gel column. The protein-carrier binding reaction was performed for 16 hours at 4 °C with moderate mixing.

After immobilization, the sorbent was pressed on a glass filter and washed with 0.1 M $NaHCO_3$ (pH 8.3) and 0.5 M NaCl (total volume of 50 ml). Active BrCN-Sepharose groups that did not bind the protein were blocked by 1M ethanolamine (pH 8.0) for 3 hours at 24 °C with moderate mixing. The left-over ethanolamine was washed with 0.1 M $NaHCO_3$ (pH 8.3), double-distilled water and 0.1 M Na-acetate buffer (pH 4.0). The last washing of the sorbent suspension was carried out in buffered saline solution (0.05 M Tris-HCl (pH 7.4), 0.15 M NaCl).

For the obtained affinity sorbent, the ligand binding to BrCN-Sepharose was 90.2%. The received column had volume of 1.36 ml, capacity per ligand 76.6 nmol/g Sepharose, and capacity for binding antibodies of 1.19 nmol/g Sepharose.

Ultrafiltration

The protein solutions were simultaneously dialyzed and concentrated by ultrafiltration at membranes with molecular weights cut-offs of 3, 10, 30 and 100 kDa using Ultrafree-15 micro-concentrators (Millipore, USA) at 4 °C.

Determination of protein concentration

The protein concentration was determined by the difference in the optical density of solutions at 280 and 320 nm. The following values of extinction coefficients (E_{280}, 1%, 1 cm) and molecular weight were used for the calculation of concentrations: 17 and 92 kDa for Glu-Pg, 17 and 84 kDa for Lys-Pg [15], 14 and 38 kDa for mini-Pg, 18.6 and 32 kDa for kringle 1-3, 25 and 12 kDa for kringle 4 [16], 12 and 14 kDa [17] for kringle 5, and 14 and 150 kDa for the antibodies [18].

Obtaining the immune anti-K 5 rabbit blood serum

Immunization of laboratory animals (two male chinchilla rabbits weighing 3.0–3.5 kg and fed the standard diet in vivarium of the Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine) was performed by the recommendations [19]. Rabbits were injected into six points along the paravertebral area in the skin layer with 0.5 mg K 5 of human plasminogen in 0.5 ml of PBS. The first injection contained the complete Freund's adjuvant (Sigma, USA), injections three weeks later contained the incomplete adjuvant. In 10 days, the blood serum was tested by ELISA for the presence and titre of antibodies specific to the introduced antigen. Blood was collected from the ear vein into glass centrifuge tubes and incubated at 4 °C for 16 hours for clot formation. Later the clot was removed, and the serum was centrifuged to remove the uniform blood elements at 200 g for 15 min.

The globulin fraction of the immune serum was obtained by boosting ammonium sulfate to 50% saturation at 4 °C for 16 hours. The precipitate obtained by centrifugation at 3000 g at 4 °C for 30 min was dissolved in 0.05 M Tris-HCl (pH 7.4) and 0.15 M NaCl. Dialysis in 0.05 M Tris-HCl (pH 7.4) and 0.15 M NaCl at 4 °C was performed to remove the residual ammonium sulfate. After dialysis, p-nitrophenyl-*p*-guanidine benzoate (*p*NPGB) was added to the globulin solution to the final concentration of 10^{-5} M.

Electrophoresis in polyacrylamide gel (PAG)

Homogeneity of the Glu- and Lys-Pg forms was tested by electrophoresis in 11.5% PAG with 6.25 M urea at acidic pH values [20]. 10% PAG with 0.1% sodium dodecyl sulfate (SDS) was used to determine the purity of other proteins and for pre-blotting electrophoresis [21]. Anode buffer 0.2 M Tris-HCl (pH 8.9), cathodic buffer 0.1 M Tris (pH 8.25), 0.1 M Trisine (Sigma, USA), 0.1% SDS, and gel buffer with 2 M tris-HCl (pH 8.45) and 0.1% SDS. Tris was used as Trizma® base (Sigma, USA). Acrylamide and methylenebisacrylamide were used as 30% Solution of Acryl/Bis 37.5: 1 (Sigma, USA).

The research was carried out in Mini-PROTEAN-II system for vertical gel electrophoresis (BioRad, USA) on 10×10 cm plates with a spacer of 0.75 mm. The resolution of the method is 1 μg of protein per zone. Protein zones were stained with Coomassi Brilliant Blue R-250 (Sigma, USA). The 10–260 kDa molecular weight markers were used (Spectra Multicolor Broad Range Protein Ladder, Fermentas, Lithuania).

Western blot analysis

The western blotting method [22] was used to test the binding of the polyclonal antibodies to K 5 with Glu-Pg, Lys-Pg, mini-Pg, and kringles 1–3, 4 and 5. Gel proteins were transferred to the Hycond ECL nitrocellulose membrane (Amersham Pharmacia Biothech, Sweden) using the Mini Trans-Blot® Electrophoretic Transfer Cell (BioRad, USA) electroblotter. Tris-glycine buffer was used for transfer, containing 0.048 M Tris, 0.039 M glycine (pH 8.3), 0.037% SDS, and 20% methanol. The transfer was carried out for 2 hours at a current of 0.8 mA per 1 cm^2 of nitrocellulose membrane. The proteins transferred to the membrane were stained in 0.2% Ponceau solution (Sigma, USA) in 3% trichloroacetic acid for 10 min. Then they were washed with distilled H_2O, and visualized. Next, free binding sites on nitrocellulose were blocked by 3.5% solution of dry milk (0% fat) (BioRad, USA), incubated in 0.01 M potassium phosphate buffer (pH 7.4), 0.14 M NaCl, 0.05% Tween-20 (TPBS) for 18 hours at 22 °C. Then, the membranes were washed twice for 5 min in TPBS, and incubated with the obtained polyclonal antibodies to K 5 at a concentration of 5.0 μg/ ml in TPBS for 2 hours at 37 °C. After this, the membranes were thrice washed with TPBS for 5 min, put into a solution of Goat Anti-Rabbit IgG-HRP Conjugate (Sigma, USA), diluted in TPBS 1:800 and kept on a shaker for 45 min at 37 °C. Then, the membranes were washed twice

for 5 min with 0.01 M potassium phosphate buffer (pH 7.4) and 0.14 M NaCl (PBS). The nitrocellulose membrane was transferred to the substrate solution of 0.05% 4-chloro-α-naphthol (Sigma, USA) in 0.05M potassium phosphate buffer (pH 6.0) with 0.06% H_2O_2. The reaction was monitored visually and stopped by transferring the membranes from substrate solution to distilled water.

ELISA

The specificity of the obtained polyclonal antibodies to K 5 was studied by ELISA. To this end, 0.11 ml of antigens (Glu-Pg, Lys-Pg, mini-Pg or kringles 1–3, 4, or 5) were added to the wells of 96 well plates (Nunc, Denmark) at a concentration of 10 μg/ml in 0.02 M bicarbonate buffer (pH 9.5). Adsorption of proteins was carried out for 18 hours at 4 °C. The unbound protein was removed. The wells were then several times washed with TPBS. This operation was carried out–after each subsequent stage. To prevent nonspecific sorption, 0.15 ml of 2.5% bovine serum albumin was added to the wells and incubated for 1 h at 37 °C. After this, the wells were washed three times with TPBS and 0.1 ml of a solution of polyclonal antibodies to K 5 was added to them at a concentration of 6.25–50 μg/ml in TPBS. (To determine the antibody titer at this stage, 0.1 ml of immune anti-K5 rabbit serum diluted 1500 times was added to the wells. The soluton was prepared out in TPBS with 5% dry milk (0% fat) (BioRad, USA) and 0.38% sodium citrate. When introduced to the wells, they were titrated with 1: 3 dilution factor). They were incubated at 37 °C for 1 hour, and then thrice washed with TPBS. Then, 0.1 ml of Goat Anti-Rabbit IgG-HRP Conjugate solution (Sigma, USA), diluted in TPBS 1: 2500, was added to the wells. They were incubated at 37 °C for 1 hour, and then washed three times with TPBS and 0.1 ml of 0.05 M potassium phosphate buffer (pH 6.0) with 0.03% H_2O_2 and 0.04% of o-Phenylenediamine (Synbias, Ukraine) added to wells. The reaction was stopped by adding 50 μl of 2 M H_2SO_4 to each well. The optical density of the solutions was measured on a plate reader (Titertek Multiskan MC, Finland) in dual-beam mode at 492 and 630 nm.

Determination of dissociation constants

The dissociation constants (K_d) of the resulting polyclonal antibodies to K 5 in the binding reactions of Glu-Pg, Lys-Pg, mini-Pg, and kringles 1–3, 4, and 5 were determined using an indirect competitive ELISA by the Friguet method with correction on the bivalence of antibodies by Stevens [23]. To the microplate wells with Glu-Pg, Lys-Pg, mini-Pg or kringles 1–3, 4 or 5 (immobilized as described previously) was injected 0.1 ml of polyclonal antibodies with a concentration of 16 μg/ml in PBS, pre-incubated at 8 °C for 18 hours, respectively, with Glu-Pg, Lys-Pg, mini-Pg or kringles 1–3, 4 or 5 at concentrations of 0.5 to 50 μg/ml. Tightly closed plates were incubated at 22 °C for 2 hours on a shaker, and washed three times with TPBS. The amount of polyclonal antibodies which reacted with the immobilized protein was determined using Goat Anti-Rabbit IgG-HRP Conjugate (Sigma, USA) as described previously.

Statistical processing of results

Statistical processing of experimental data was performed using Prism 5 software (GraphPad Software Inc., USA). Only the experimental results with the error not exceeding 5% ($P < 0.05$) are included in the work. The curves shown in the figures are typical for a series of repeated studies (at least three in each series). Electrophoregrams and blot results presented in the figures are typical for repeated experiments (at least three repetitions). The table contains information in the form of average arithmetic values (M) and standard mean errors (m).

Results and Discussion

Glu-Pg from the blood donation plasma anti-coagulated with sodium citrate, and Lys-Pg from the blood plasma fraction $III_{2,3}$ by Cohn were obtained by chromatography at Lys-Sepharose in the presence of a contrykal proteinase inhibitor. Pg- kringle 1–3, kringle 4 and mini-Pg fragments were obtained by limited hydrolysis of Pg by elastase with subsequent gel filtration on TOYOPEARL HW-50, and Lys-Sepharose chromatography [13]. K 5 fragment was obtained by limited proteolysis of mini-Pg with pepsin followed by chromatography on AH-Sepharose 4B as described in [13]. The obtained electrophoretically pure Glu-Pg (Fig. 5, track 2) and Lys-Pg, mini-Pg and Pg K1-3 and K4 fragments (Fig. 1, *A*) were used for immunochemical studies, K 5 (Fig. 1, *B*) was also used as a ligand for the synthesis of affinity sorbent and an antigen for animal immunization for the purpose of obtaining polyclonal antibodies.

Blood serum titers of immunized rabbits were tested by ELISA with the use of sorbed

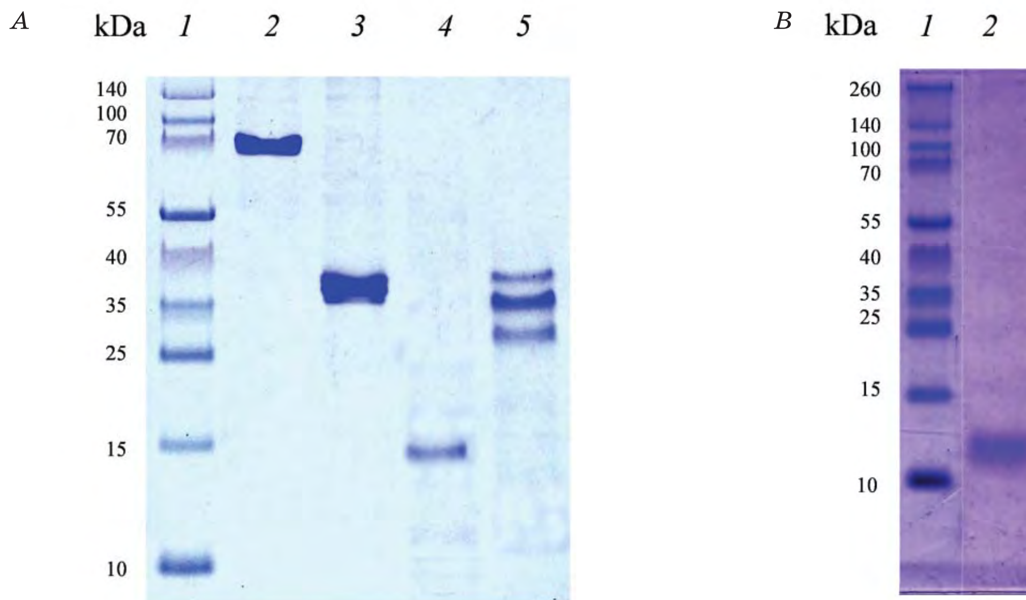

Fig. 1. ***A*** **— typical electrophoregram of Pg and its fragments К 1–3, К 4 and mini-Pg:**
1 — molecular weight markers; *2* — Pg (Lys-form); *3* — mini-Pg; *4* — kringle К 4; *5* — kringle К 1–3 [13];
B **— typical electrophoregram of human plasminogen fragment kringle 5:**
1 — molecular weight markers; *2* — kringle 5

native antigens. The applied antigen apparently induces specific antibodies with a titer up to 1: 40500 (Fig. 2). The high serum titer indicates the active induction of B-lymphocyte clones that produce antibodies of a given specificity, which allows using them for the receiving of polyclonal antibodies, highly specific to the antigen determinant. As a negative control, serum from non-immunized rabbits was used.

From the general IgG fraction, polyclonal antibodies against K5 were isolated by affinity chromatography on the obtained sorbent. The IgG mixture was applied to a balanced in PBS column of K 5-Sepharose. Polyclonal antibodies that bound to K 5-Sepharose were eluted with 0.2 M glycine-HCl buffer (pH 2.8). The collected fractions were immediately titrated with 1 M K_2HPO_4 solution to pH 7.2. The protein concentration in the fractions was monitored spectrophotometrically at λ = 280 nm. Fractions containing IgG that specifically bound to K 5-Sepharose were combined and dialyzed against 0.05 M Tris-HCl (pH 7.4), 0.15 M NaCl, and simultaneously concentrated using Ultrafree-15 microconcentrators (Millipore, USA) with a cut-off limit of 100 kDa. The resulting antibodies were stored in a mixture of glycerol (1:1) at −20 °C. The content of the obtained immunoglobulins in preparations evaluated by electrophoresis was no less than 95%, the output of polyclonal antibodies was no less than 1 mg per 1 ml of immune serum.

From the blood serum of immunized rabbits, polyclonal antibodies to the K5 fragment were isolated using affinity chromatography, and their immunochemical characteristics were determined. To investigate the specificity of affinity-isolated polyclonal antibodies, an indirect ELISA was implemented. Thus using that method, binding of polyclonal antibodies against K 5 with Lys-Pg, Glu-Pg, mini-Pg, plasminogen K

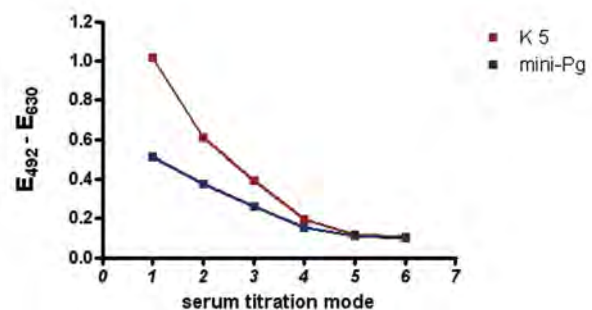

Fig. 2. **Interaction of anti-К 5 rabbit blood serum with kringle 5 and mini-Pg which are immobilized at the well plate. The dilution factors of the immune anti-К 5 rabbit serum:**
1 — 1/1500; *2* — 1/4500; *3* — 1/13500; *4* — 1/40500; *5* — 1/121500; *6* — 1/364500. Averaged (n = 3) curves are typical of the serial research

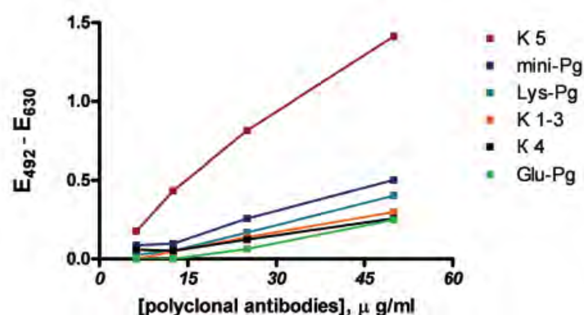

Fig. 3. **Binding of polyclonal antibodies to K 5 (6.25–50 μg/ml) with Pg and its fragments, immobilized at a well plate. Averaged (n = 3) curves are typical for a series of repeated studies**

1-3, and K 4, and K 5 fragments, immobilized on a plate, was studied depending on the concentration antibodies. As can be seen from Fig. 3, polyclonal antibodies to K 5 react with K 5 plasminogen fragment, and to a lesser extent with mini-Pg, Lys-Pg and Glu-Pg, and K 1-3 and K 4 plasminogen fragments.

The dissociation constants for polyclonal antibodies against K5 in the reactions of binding of K5, mini-Pg and Lys-Pg were determined using indirect competitive ELISA as described above. Dissociation constants K_d [M] were calculated as the tangent of the angle of the curve incline, linearized in coordinates {Sgrt ($A_0/(A_0$-A)); 1/Ag}, as described in [23]. Optical density of the reaction medium (as determined by the difference in the optical density of

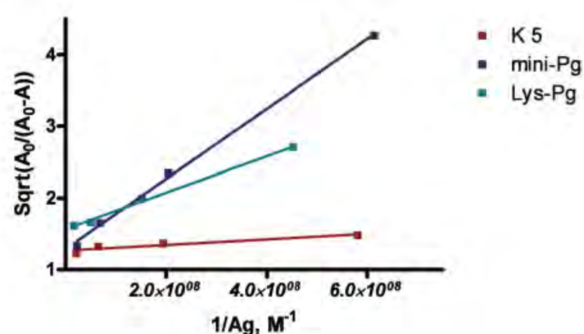

Fig. 4. **Binding of polyclonal antibodies against K 5 with Lys-Pg, mini-Pg and K5 immobilized at a well plate in an indirect competitive ELISA.** Averaged lines (n = 3) are typical for a series of repeated studies

solutions at 492 and 630 nm): A0 without pre-incubation of antibodies with antigen, A under conditions of pre-incubation of antibodies with antigen at Ag concentration. The obtained results are presented in Fig. 4 and in Table.

The constant values indicate a high affinity of produced polyclonal antibodies to their epitopes in K 5 fragment, mini-Pg and Lys-Pg. It should be noted that in the conducted experiment, the reactions of binding of polyclonal antibodies against K 5 with K 1-3, K 4 and Glu-Pg, dissociation constants (K_d) were not determined indicating an insignificant affinity of such interaction. Thus the obtained antibodies against K 5 antigen are apparently monospecific.

Using Western blotting we studied the binding of the resulting polyclonal antibodies against K 5 to Glu-Pg, Lys-Pg, mini-Pg, and kringles 1-3, 4 and 5. It was found that the antibodies react with the isolated K 5 plasminogen fragment (Fig. 5, track *11*), Lys-Pg and mini-Pg (Fig. 5, tracks *9* and *10*, respectively), but do not react at all with K 5 in Glu-Pg and with K 1–3 and K 4 fragments (Fig. 5, tracks *6, 8* and *7*). Hence, it becomes clear that there is no specific interaction (which is also characterized by K_d) between the polyclonal antibodies against K5 with Glu-Pg and K 1–3 and K 4 fragments. The lack of binding of the resulting antibodies to Glu-Pg can be explained by the fact that the closed conformation of Glu-Pg does not exhibit kringle 5. This quality of the antibodies makes it possible to differentiate Glu- and Lys-forms of Pg in immunochemical studies. The lack of cross-linking with the K 1–3 and K 4 kringles is a useful feature and demonstrates the monospecificity of the resulting antibodies to the K5 plasminogen fragment.

Laboratory animals (rabbits) are immunized with electrophoretically pure K 5 fragment of a human plasminogen molecule. Affinity sorbent based on the K 5 fragment was synthesized for the selection of monospecific antibodies from rabbit immune serum. Polyclonal antibodies to the K 5 fragment are obtained, with established monospecificity and high affinity to their antigen. These antibodies are suitable for the creation of immunochemical and immunosensory methods for the quantitative determination of angiostatin K5 in a biological material.

Table. **Values of Kd in reaction of binding of polyclonal antibodies with K5, mini-Pg and Lys-Pg, with Pierson correlation coefficient, *r***

Antigen	K_d, [M]	*r*
K 5	$(3.89 \pm 0.17)\cdot10^{-10}$	0.91
Mini-Pg	$(5.46 \pm 0.23)\cdot10^{-9}$	0.94
Lys-Pg	$(2.54 \pm 0.11)\cdot10^{-9}$	0.89

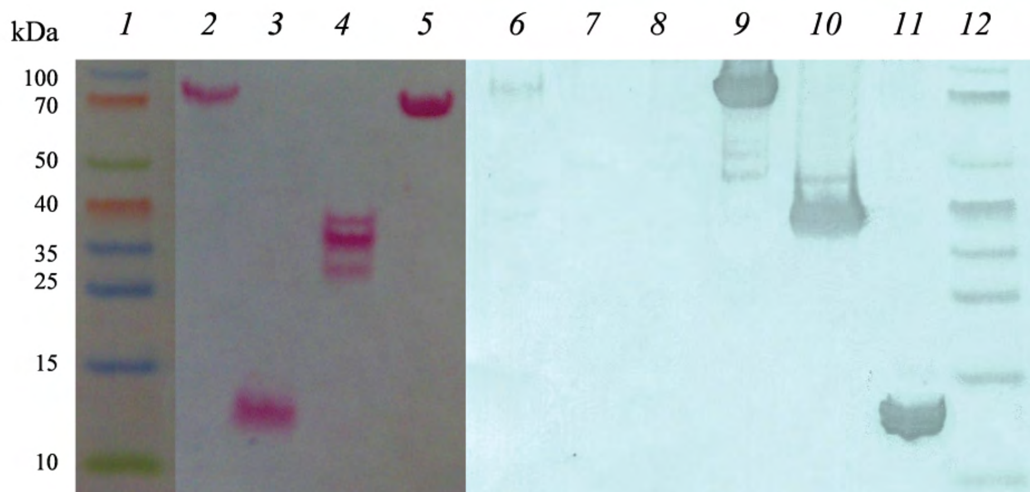

***Fig. 5*. Binding of polyclonal antibodies against K 5 to Pg and its fragments, immobilized at nitrocellulose membrane:**
1–5 typical blot pictures (Ponceau staining);
6–12 — typical immunoblotogram with polyclonal antibodies to kringle 5;
1, 12 — molecular weight markers;
2, 6 — Glu-Pg;
3, 7 — K 4;
4, 8 — K 1–3;
5, 9 — Lys-Pg;
10 — mini-Pg;
11 — K 5

The received polyclonal antibodies against K 5 are widely used in current research at the Palladin Institute of Biochemistry of NAS of Ukraine by scientists of our department of chemistry and biochemistry of enzymes, and several related departments. These antibodies were used to obtain results that allow us to conclude that there are different binding sites for the K 1–3 and K 5 kringles in the peripheral domains of the fibrin molecule, and several binding sites for K 1–3 kringle. It is found that the presence of C-terminal lysines in the fibrin D-dimer molecule does not affect the binding of the K 5 kringle, while for the K 1–3 kringle it matters though not decisively [24]. The obtained results are important for determining the role of certain plasminogen kringle domains at the stage of the activation with a tissue activator, which is a fibrinolysis process trigger and can be used to develop some approaches to fibrinolysis regulation.

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ПОЛІКЛОНАЛЬНІ АНТИТІЛА ПРОТИ КРИНГЛА 5 ПЛАЗМІНОГЕНУ ЛЮДИНИ

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Метою роботи було одержати поліклональні антитіла проти фрагмента плазміногену людини крингла 5 та дослідити їхні імунохімічні властивості. Для цього було застосовано такі підходи: імунізація кролів кринглом 5, отримання імунної сироватки з високим титром, синтез афінного сорбенту на основі кringла 5 для селекції моноспецифічних антитіл, хроматографія на синтезованій К 5-сефарозі, імуноензимний аналіз, ELISA, імуноблотаналіз.

Отримані поліклональні антитіла реагували за ELISA із фрагментом плазміногену К 5, значно меншою мірою (у порядку зменшення) — з міні-плазміногеном, Lys-плазміногеном, фрагментами К 1–3 та К 4 плазміногену та Glu-плазміногеном. На основі даних визначення констант дисоціації одержаних антитіл із цими протеїнами встановлено високу афінність антитіл до своїх епітопів у складі фрагмента К 5 ($3{,}89\cdot10^{-10}$ М), міні-плазміногену ($5{,}46\cdot10^{-9}$ М) та Lys-плазміногену ($2{,}54\cdot10^{-9}$ М), а також несуттєву афінність до К 1-3, К 4 та Glu-плазміногену. Ці антитіла реагують за імуноблотаналізу з ізольованим фрагментом К 5 плазміногену людини, Lys-Pg та mini-Pg і не реагують із К 5 у складі Glu-Pg та з фрагментами К 1-3 і К 4. Отже, отримані поліклональні антитіла є моноспецифічними та високоафінними до кringла 5. Такі антитіла є придатними для розроблення імунохімічних та імуносенсорних методів кількісного визначення ангіостатину К 5 у біологічному матеріалі.

Ключові слова: фрагменти плазміногену людини, крингл 5, поліклональні антитіла, ангіостатини.

ПОЛИКЛОНАЛЬНЫЕ АНТИТЕЛА ПРОТИВ КРИНГЛА 5 ПЛАЗМИНОГЕНА ЧЕЛОВЕКА

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Целью работы было получить поликлональные антитела против фрагмента плазминогена человека крингла 5 и исследовать их иммунохимические свойства. Для этого применены следующие подходы: иммунизация кролей кринглом 5, получение иммунной сыворотки с высоким титром, синтез аффинного сорбента на основе крингла 5 для селекции моноспецифических антител, хроматография на синтезированной К 5-сефарозе, иммуноэнзимный анализ, ELISA, иммуноблотанализ.

Получены поликлональные антитела, реагирующие в ELISA с фрагментом плазминогена К 5, в значительно меньшей степени (в убывающем порядке) — с мини-плазминогеном, Lys-плазминогеном, фрагментами К 1–3 и К 4 плазминогена и Glu-плазминогеном. На основе данных определения констант диссоциации полученных антител с этими протеинами установлена высокая аффинность антител к своим эпитопам в составе фрагмента К 5 ($3{,}89\cdot10^{-10}$ М), мини-плазминогена ($5{,}46\cdot10^{-9}$ М) и Lys-плазминогена ($2{,}54\cdot10^{-9}$ М), а также несущественная аффинность к К 1-3, К 4 и Glu-плазминогену. Данные антитела реагируют при иммуноблотанализе с изолированным фрагментом К 5 плазминогена человека, Lys-Pg и mini-Pg и не реагируют с К 5 в составе Glu-Pg и с фрагментами К 1–3 и К 4. Таким образом, полученные поликлональные антитела являются моноспецифическими и высокоаффинными к кринглу 5. Такие антитела пригодны для разработки иммунохимических и иммуносенсорных методов количественного определения ангиостатина К 5 в биологическом материале.

Ключевые слова: фрагменты плазминогена человека, крингл 5, поликлональные антитела, ангиостатины.