A C T A U N I V E R S I T A T I S L O D Z I E N S I S
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VERTEBRATE PLASMINOGEN

Native plasminogen from different species of vertebrates (mammals, birds, amphibia and fish) was isolated by affinity chromatography on L-lysine *Sepharose* 4B and then characterized by SDS-PAGE. Remarkable similarities of the analysed plasminogens in respect to their concentration in plasma, molecular weight and chain protein structure to human plasminogen were found despite some differences in biological properties. In contrast to human plasminogen none of studied animal plasminogens was activated by streptokinase.

INTRODUCTION

Plasminogen (Plg) is a precursor of plasmin, a major fibrinolytic blood enzyme which plays an important role in the degradation of fibrin and the resolution of thrombus. Human plasminogen has been widely studied by a variety of investigators and its properties seem to be well known. It is a single chain glycoprotein with a molecular weight of about 90 000. The molecule consists of 790 amino acid residues and 24 disulfide bridges that build five homologous kringles [1, 2]. Native plasminogen contains glutamic acid as the amino-terminal residue and is referred to as Glu-plasminogen (Glu-Plg). An amino-terminal peptide (MW 8000) is often lost from Glu-Plg during the activation or purification procedure mainly in the absence of protease inhibitors. The resulting plasminogen having lysine, methionine or valine as the amino-terminal residue is termed Lys-plasminogen (Lys-Plg, MW 84 000) [3]. Lys-Plg is known to be more susceptible

to activation by plasminogen activators and has a higher affinity for fibrin than Glu-Plg [4, 5], but now it is unclear whether or not Lys-Plg always exists in the normal circulating blood [3, 6, 7] and should be considered as an intermediate in alteration process of plasminogen to plasmin.

Physiological fibrinolysis is believed to be exerted by tissue -type plasminogen activator (tPA) although plasminogen activation for therapeutic purposes or laboratory investigations may be also achieved by urokinase (UK) or streptokinase (SK). It has been established [8] that all of the mammalian plasminogens are able to be activated by human urokinase, whereas only human, cat and monkey plasminogens can be converted into plasmin even by low concentrations of streptokinase while dog, rabbit and horse plasminogens needed high concentrations of streptokinase [8, 9].

Comparative studies of some mammalian plasminogen species showed remarkable similarity to human plasminogen both in respect to molecular weight and amino acid composition [8]. Little work has been done to examine the fibrinolytic system in other than mammalian vertebrates.

The present work was undertaken to isolate native plasminogens from different species of vertebrates plasma (mammalian, birds, amphibia and fish) and determine its molecular weights as well as to study activation process of plasminogens by streptokinase.

MATERIALS AND METHODS

Animal blood was collected by cardiac puncture from surgically open-chested animals (hamster, frog, carp) or drawn from the wing vein (chicken, duck). Fresh blood making up to 3.8% trisodium citrate (9 parts of blood were mixed with 1 part of trisodium solution) and centrifuged for 20 minutes at 500 g. Human plasma was obtained from Blood Donnation Center in Łódź.

In all cases plasminogen was isolated from plasma pools by affinity chromatography on lysine *Sepharose* 4B according to the method of Deutsch and Mertz [10]. The plasma was three times diluted with PBS buffer (10 mM sodium phosphate buffer, pH 7.1 with

0.14 M NaCl) and passed through an L-lysine-substituted *Sepharose* column (0.5 x 15 cm). The column was washed with the same buffer to eliminate non-specific bounded proteins and plasminogen was then eluted with 0.2 M ε-aminocaproic acid (EACA) in PBS. A 2.5 ml fractions were collected and protein for the elution profiles was monitored at 280 nm. The purification procedure was carried out at 4°C and in the presence of the plasmin inhibitor-Trasylol.

The plasminogen in the eluate was precipitated with ammonium sulfate to final concentration 0.4 g/ml, centrifuged and redissolved in PBS buffer. The final step was the dialysis of protein against the same buffer overnight. The purified plasminogen solutions were stored at -20°C until required.

The protein was determined by measuring the absorption at 280 nm employing $A^{1\%}_{1cm}$ of 16.1 for purified plasminogen or by the procedure of L o w r y et al. [12].

SDS-polyacrylamide rod gel electrophoresis (SDS-PAGE) was performed as described by W e b e r and O s b o r n [11]. 40--60 µg of the preparation were applied to 5% or 7.5% polyacrylamide gel in the presence of 2-mercaptoethanol.

Plasminogen activation studies were carried out in PBS buffer, at 37°C, for 30 minutes, using 2000 units of streptokinase per mg of protein. The reaction was stopped by addition of 8 M urine in sodium phosphate buffer, pH 7.1 with 0.2 M EACA.

RESULTS AND DISCUSSION

Affinity chromatography method used for isolation of plasminogen from different species allowed to evaluate the level of this protein in plasma i.e. 5.7 mg of plasminogen in 100 ml of human plasma, 9.5 mg/100 ml (hamster), 11.1 mg/100 ml (duck), 7.2 mg/ /100 ml (chicken), 4.4 mg/100 ml (frog) and 5.0 mg/100 ml for carp. Figure 1 shows the representative elution profile of duck plasminogen from the L-lysine *Sepharose* column. General patterns for all examined species were very similar to each other (not shown). SDS-polyacrylamide gel analysis of the plasminogens also

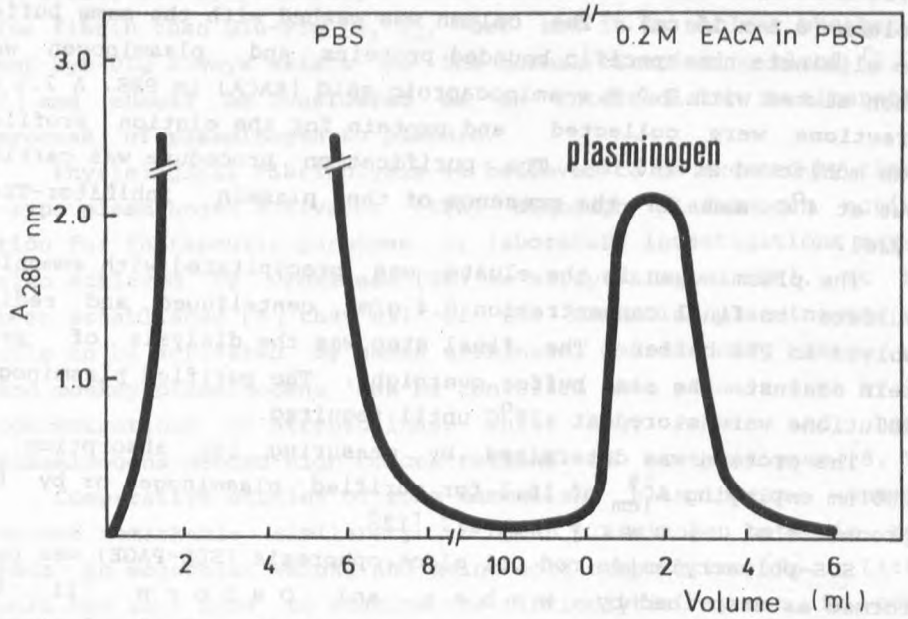

Fig. 1. Affinity chromatography of the duck plasminogen on Lys *Sepharose* 4B. Non-adsorbed material was eluated with PBS solution. Specifically adsorbed plasminogen was eluted with 0.2 M EACA in PBS. A 2.5-ml fractions were collected

Table 1

Molecular weights of plasminogens from different sources estimated by 7.5% PAGE-SDS

Plasminogen species		Molecular weight
Human	(Glu-Plg)	91 000
	(Lys-Plg)	83 000
Hamster		90 000
Chicken		91 000
Duck		96 000
Frog		100 000
Carp		93 000

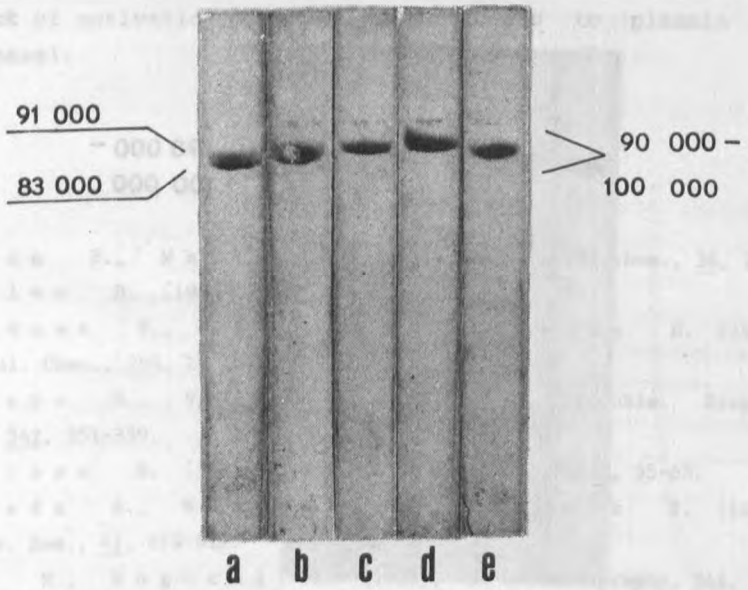

Fig. 2. SDS-polyacrylamide gel (7.5%) electrophoresis of human plasminogen a), hamster b), duck c), frog d) and carp e) after reduction with 2-mercaptoethanol. For molecular weight determinations a calibration curve was used obtained with phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme (all from BioRad)

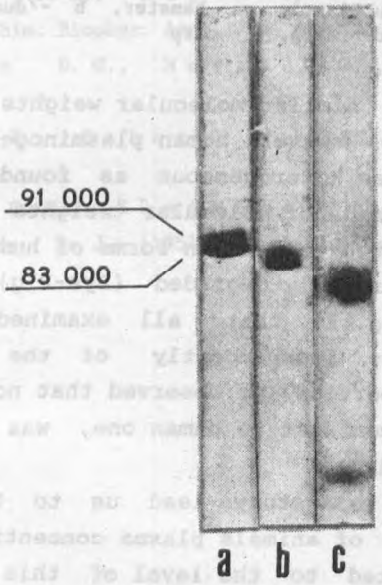

Fig. 3. Streptokinase activation products of human plasminogen (5% PAGE-SDS); a - plasminogen, b - plasmin, c - heavy and light chains of plasmin after reduction with 2-mercaptoethanol

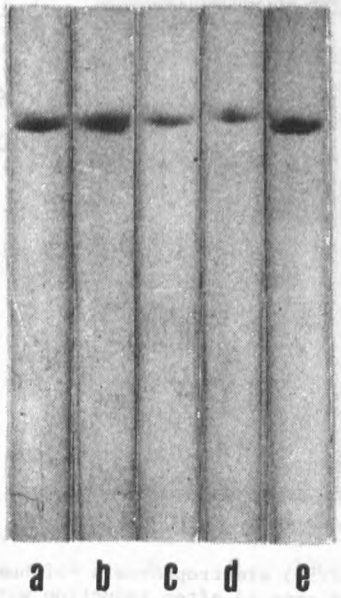

Fig. 4. Activation of animal plasminogens by streptokinase (7.5% PAGE-SDS, analyse of proteins after reductions); a - hamster, b - duck, c - chicken, d - frog, e - carp

demonstrated more or less similar molecular weights ranging from 90 000-100 000 (Table 1). However, human plasminogen contrary to other analysed animals was heterogeneous as found by SDS-PAGE analysis. Two visible bands of molecular weights 90 000 and 83 000 seem to represent two well-known forms of human plasminogen, native (Glu-Plg) and partially degraded (Lys-Plg) (Fig. 2). It was also demonstrated (Fig. 2) that all examined plasminogens were single chain proteins independently of the origin. With regard to biological properties we observed that none of studied animal plasminogens in contrast to human one, was activated by streptokinase (Figs 3 and 4).

The data obtained in this study lead us to the conclusion that in analysed classes of animals plasma concentration of plasminogens is closely related to the level of this protein in human being, but there are some differences in biological proper-

ties (lack of activation of animal plasminogens to plasmin by streptokinase).

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PLAZMINOGEN KRĘGOWCÓW

Metodą chromatografii powinowactwa na złożu Lys *Sepharose* 4B wyizolowano natywny plasminogen z osocza różnych grup kręgowców (ssaki, ptaki, płazy i ryby), a następnie scharakteryzowano elektroforetycznie. Wykazano, że istnieją znaczne podobieństwa między plazminogenem człowieka a plazminogenami analizowanych zwierząt pod względem zawartości w osoczu, masy cząsteczkowej i łańcuchowej struktury białka, chociaż znaleziono i pewne różnice w biologicznych właściwościach. W przeciwieństwie do plazminogenu człowieka żaden z badanych plazminogenów zwierzęcych nie ulegał aktywacji pod działaniem streptokinazy.