

Plasminogen

Product Monograph 1995



CHROMOGENIX

Plasminogen

Plasminogen, Product Monograph 1995

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Plasminogen

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Chromogenic Substrate Assays for Plasminogen and their Diagnostic Relevance

Plasminogen is the inactive precursor of plasmin, a potent serine protease involved in the dissolution of fibrin blood clots. Both hereditary and acquired forms of plasminogen deficiency have been described. These are usually associated with either a thrombotic or a hyperfibrinolytic condition. In this monograph we review the plasminogen-plasmin system and describe two chromogenic-based assay kits for the photometric determination of plasminogen activity in human plasma.

Blood coagulation is a complex enzymatic event culminating in the formation of an insoluble thread-like protein called fibrin. Together with platelets, fibrin forms a hemostatic plug to prevent excessive bleeding. Fibrin blood clots are ultimately dissolved in due course in order to restore vascular patency. The enzymes involved in this physiologically important process are part of the fibrinolytic system (Figure 1).¹⁻²

The central component in the fibrinolytic system is the glycoprotein plasminogen, which is produced by the liver and is present in plasma and most extravascular fluids. Plasminogen is a precursor enzyme (zymogen) which, following partial cleavage by a plasminogen activator is converted to its active and proteolytic form, plasmin. Its primary target is fibrin, but it is also able to degrade several constituents of the extracellular matrix and to convert a number of pro-hormones and cytokine precursors to their active form. Plasmin also appears to be involved in the metastatic spread of cancer.

The generation of plasmin occurs preferentially on the fibrin surface, which offers binding sites for plasminogen and its principle activator in blood, t-PA. This binding stimulates plasminogen activation, but also localizes the action of plasmin to sites of fibrin formation which promotes efficient clot lysis. Further regulation is provided by the presence in plasma of inhibitors, primarily the plasmin inhibitor and the plasminogen activator inhibitor 1 (PAI-1).

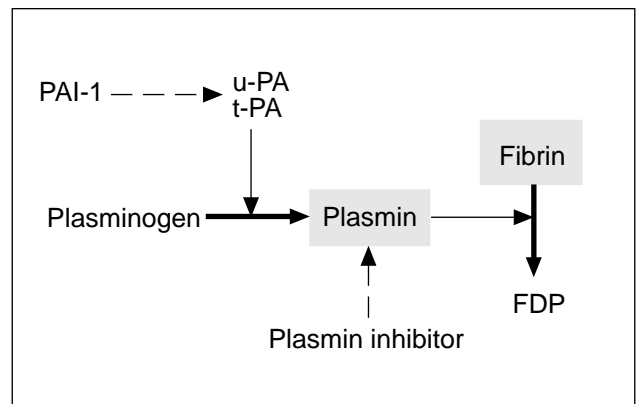


Figure 1. The fibrinolytic system

The important role of plasminogen in fibrinolysis makes it an interesting parameter to evaluate in various diseases. A decreased plasminogen level may in some situations compromise the body's ability to degrade fibrin and as such predispose to thrombosis. Hereditary plasminogen deficiency, as a cause of thrombosis, have also been reported in several cases.⁵⁻⁷ However, plasminogen deficiency is usually an acquired condition and since plasminogen is the inactive precursor of plasmin, most acquired defects are found in situations with increased fibrinolytic activity. An acquired deficiency is often seen with severe liver disease and acute disseminated intravascular coagulation (DIC), or as a result of thrombolytic therapy with plasminogen activators.^{3,4}



Introduction

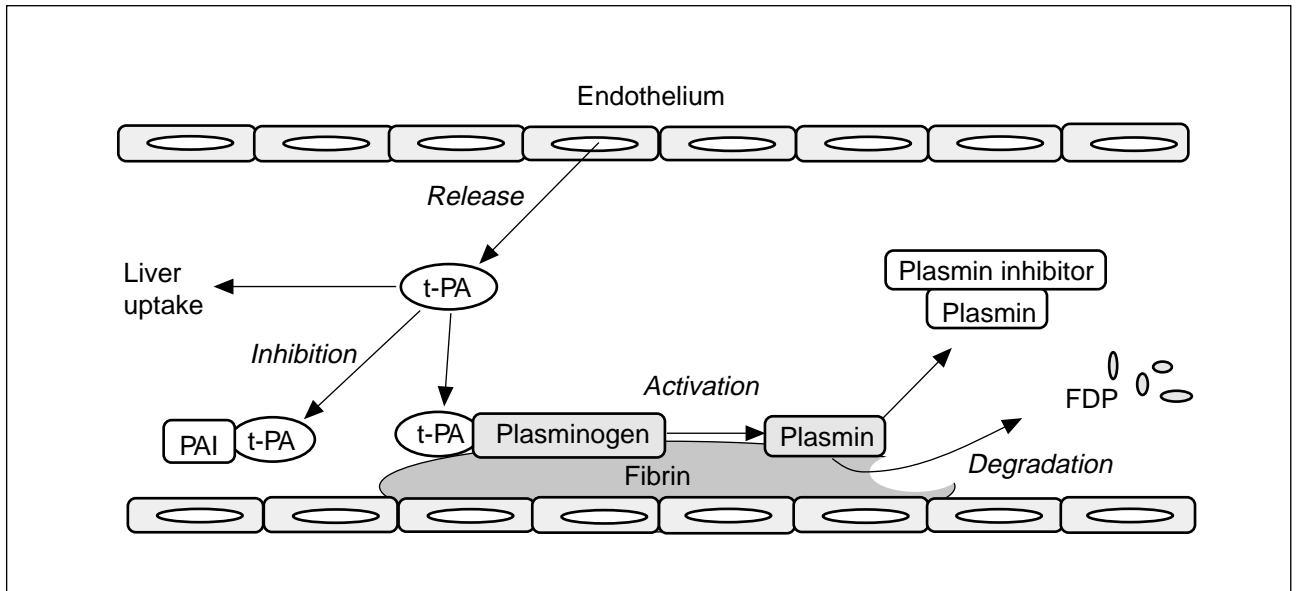


Figure 2. Schematic illustration of physiologic fibrinolysis.

Plasminogen is the proenzyme of plasmin, whose primary target is the degradation of fibrin in the vasculature. t-PA is the principle activator of plasminogen in blood, while u-PA is the predominant activator outside the bloodstream in the extracellular matrix. t-PA is produced by the vascular endothelial cells and is released into the circulation after stimulation. Fibrin regulates its own destruction by providing receptors or binding sites for plasminogen and t-PA, thus localizing the action of plasmin. Inhibition of the system can occur at the level of plasminogen activation (PAI-1) or at the level of plasmin (plasmin inhibitor). Free t-PA as well as complexed t-PA/PAI-1 is cleared from the circulation by receptors in the liver. Abbreviations: t-PA= tissue-type plasminogen activator, PAI-1= plasminogen activator inhibitor 1, FDP= fibrin degradation products

Compound	Size [kDa]	Amino acids	Plasma concentration	Half-life in circulation	Location of synthesis	Function
Glu-plasminogen	92	791	200 µg/ml	2.2 days	Liver	Proenzyme of plasmin
sc.u-PA	54	411	8 ng/ml	5-10 min	Kidney, lung	Plg. activator
sc.t-PA	68	527	5 ng/ml	3-4 min	Endothelium	Plg. activator
Plasmin inhibitor	70	452	70 µg/ml	2.6 days	Liver	Plasmin inhibitor
PAI-1	52	379	20 ng/ml	2-3 hours	Endothelium	Plg. activator inhibitor
PAI-2	60	393	*250 ng/ml	24 hours	Placenta	Plg. activator inhibitor

Table 1. The major components of the fibrinolytic system. * 30th week of pregnancy



The fibrinolytic system

Evolution of fibrinolytic enzymes

Plasminogen and its natural activators (t-PA, u-PA) belong to a large family of enzymes considered to have evolved from an ancestral protease similar to trypsin- a serine protease of broad specificity that breaks down dietary proteins. The kinship between the fibrinolytic enzymes in question and trypsin is attributed to their similar protease moiety, which cleaves proteins on the C-terminal side of arginyl and lysyl residues. Stretches of high homology are generally found in the active site pocket composed of serine, histidine and aspartic acid.

During evolution, several types of homologous units or domain structures (generally coded by individual exons) have been added to the trypsin-like protease, enabling the fibrinolytic proteases to gradually fulfill more specialized tasks. The addition of five kringle domains gave rise to plasminogen, whereas the addition of two kringles, one finger and one EGF (epidermal growth factor) domain gave rise to t-PA. In similar fashion, the addition of one kringle and EGF domain gave rise to u-PA (Figures 2–3).

Plasminogen

Human plasminogen is a single-chain glycoprotein containing 791 amino acid residues and 2% carbohydrate. Its molecular mass is about 92,000 daltons. The plasminogen molecule contains a total of six structural domains, each with different properties.⁸⁻¹⁰ The N-terminal portion of the molecule consists of five kringle domains with the capacity to bind to fibrin. The kringle domain was first described by Magnussen et al (1975) who compared the structure with Danish pastry. Together with the preactivation peptide, the kringles control the ability of plasminogen to adopt different conformations. The protease domain resembles that of other serine proteases and contains the active site pocket His⁶⁰³, Asp⁶⁴⁶ and Ser⁷⁴¹. This region also contains Ala⁶⁰¹ which appears to be essential for the normal function of plasminogen, since mutation to Thr⁶⁰¹ leads to an increased risk of thrombosis.¹⁰

Plasminogen facts

Name:	Plasminogen
Synonym:	Profibrinolysin
History:	The existence of plg. postulated by Dastre in 1938, identified by Christensen in 1945 purified by Kline 1953
Biosynthesis:	Liver
Concentration:	200 µg/ml
Half-life:	2.2 days (Glu-plg.) 0.8 days (Lys-plg.)
Molecular weight:	92.000 daltons
Carbohydrate:	2%
Primary structure:	791 amino acids
Function:	Inactive precursor of plasmin
Type:	Serine protease
Gene:	Located on chromosome 6, position q26-q27, length 53.5 kb containing 19 exons
Importance:	Hereditary defects of plasminogen is a predisposing risk factor for thromboembolic disease

Several forms of plasminogen in plasma are known and can be separated by affinity chromatography.¹¹ The native form of plasminogen in plasma has glutamic acid at the N-terminal and is termed Glu-plasminogen. Other plasminogen forms generated by the catalytic cleavage by plasmin and containing mostly lysine at the N-terminal position, are termed Lys-plasminogen.

Glu-plasminogen exists in a closed conformation that becomes extended when binding to lysine residues on a fibrin surface. A similar conformational change is believed to take place when Glu-plasminogen is converted to Lys-plasminogen. The physiological role of these conformational changes is not well known although the general effect is believed to be an increased plasminogen activation rate catalyzed by t-PA.^{12,13} The opposite effect is observed in the presence of anions, in particular with Cl⁻, which stabilizes the closed form of Glu-plasminogen rendering plasminogen poorly activatable.¹⁴



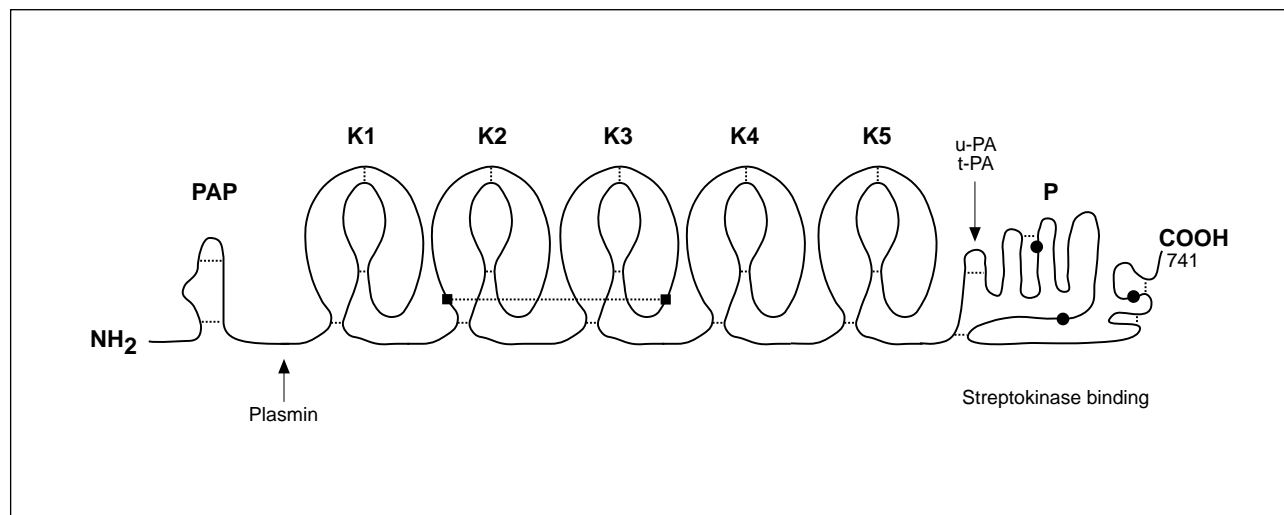


Figure 2. Domain structure of the human plasminogen molecule.

The plasminogen molecule consists of a preactivation peptide (PAP), five kringle domains and a protease domain. The preactivation peptide is generated by plasmin cleavage giving rise to a slightly shorter plasminogen molecule called Lys-plasminogen. Activation of plasminogen into plasmin occurs when plasminogen activators (t-PA, u-PA) cleave a unique bond in the serine protease domain resulting in two polypeptide chains, linked to each other via two disulphide bonds. The three amino acid residues His⁶⁰³, Asp⁶⁴⁶ and Ser⁷⁴¹ (indicated by circles) are part of the active site pocket. Kringles 2 and 3 are connected by an inter-kringle disulfide bond.

Plasmin

Activation of plasminogen by its natural activators, t-PA and u-PA, involves a bond cleavage at a specific site in the plasminogen molecule, which gives rise to a two-chain molecule linked by two disulphide bonds. The plasmin formed may degrade fibrin(ogen) in a variety of ways resulting in soluble fibrin degradation products or fragments called X, Y, D and E. Plasmin is a relatively non-specific protease and can degrade not only fibrin but also many proteins in both plasma and extracellular spaces. In the coagulation pathway factors V, VIII, and von Willebrand factor are known targets of plasmin.

Plasmin activity is inhibited mainly by binding to the plasmin inhibitor, which forms a stable complex with plasmin devoid of proteolytic activity.

Plasminogen activators

Plasminogen activators,^{8,10,15-16} can be divided into two groups: endogenous activators (t-PA and u-PA), present in blood and other body fluids, and exogenous activators (e.g. streptokinase). Plasminogen

activators are used clinically as clot-dissolving (thrombolytic) agents for the treatment of pulmonary embolism and acute myocardial infarction.

t-PA

Tissue-type plasminogen activator (t-PA) is the principal endogenous activator of plasminogen in blood. It is produced as a single-chain molecule by the vascular endothelial cells and is secreted into the plasma continuously or by an acute release reaction following stimulation of certain endothelial cell receptors. Rapid fluctuations in t-PA concentration can be observed in response to exercise, venous occlusion, alcohol and drugs, such as DDAVP and anabolic steroids. Individuals who do not show increased t-PA activity when exposed to some of these stimuli, may be a risk group for deep vein thrombosis.

Plasmin cleavage of t-PA produces a more active two-chain molecule. However, unlike many other serine proteases, t-PA is active in its single-chain form, especially in the presence of fibrin or fibrin(ogen) fragments.



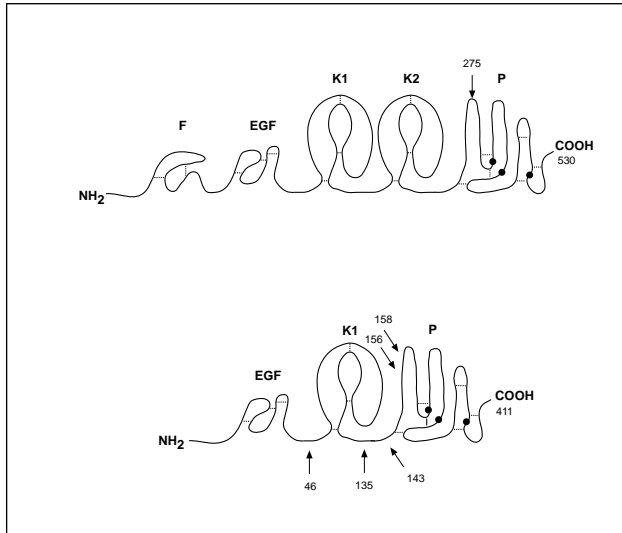


Figure 3. Domain structures of t-PA and u-PA.

The t-PA molecule is composed of at least five domains: a finger domain, the epidermal growth factor domain (EGF), two kringle domains and the protease domain. The u-PA molecule consists of a EGF domain, one kringle and the protease domain. The finger domain is homologous to structures found in fibrinectin. In the t-PA molecule this domain is implicated with fibrin binding. The EGF domain often confers affinity to specific receptors on cell surfaces.

Single-chain t-PA is a 68 kDa glycoprotein, consisting of 530 amino acids and containing 7-13% carbohydrate. In human plasma, t-PA occurs mainly as a complex together with its principal inhibitor PAI-1. The level of t-PA antigen is about 5 µg/ml, whereas the concentration of free t-PA is only about 1 µg/l or 0.5 IU/ml (specific activity range 500,000 to 700,000 U/mg). The single-chain t-PA molecule is converted by plasmin to a two-chain form by cleavage of the Arg²⁷⁵-Ile²⁷⁶ peptide bond. Binding to fibrin concentrates and correctly orientates t-PA and plasminogen, as well as inducing conformational changes in the molecules that promote efficient clot lysis.

Urokinase

Urokinase-type plasminogen activator (u-PA) is mainly produced in the kidneys as an inactive single-chain molecule (scu-PA). u-PA has its major function in tissue-related proteolysis and is believed to play only a secondary role to t-PA as a physiological activator in blood.

The activation of scu-PA by catalytic amounts of plasmin results in a two-chain structure with increased activity towards plasminogen. Through this mechanism, initial traces of plasmin may catalyze the production of active u-PA, leading to the formation of more plasmin. u-PA can only activate plasminogen in the presence of fibrin. However, it does

not bind to fibrin and is not activated by fibrin. In human plasma, u-PA antigen concentrations range from 2 to 7 ng/ml. Higher values are often found in patients with liver cirrhosis and hepatoma.

Streptokinase

Streptokinase (SK) is an exogenous plasminogen activator of 47 kDa, derived from streptococci bacteria. It is not an enzyme and functions by forming a stoichiometric 1:1 complex with human plasminogen. This complex can function as an activator of other plasminogen molecules. Complex formation is accompanied by a conformational change in the plasminogen molecule, exposing the active site to activate a second plasminogen molecule and is followed by the conversion of the SK-plasminogen into a SK-plasmin complex. Both types of SK-complexes are equally efficient activators of plasminogen (Figure 4).

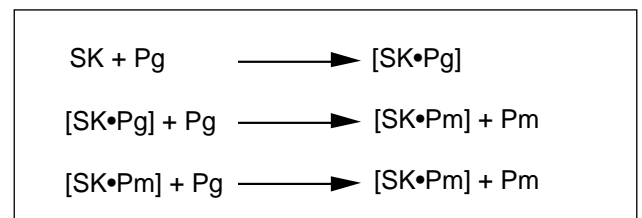


Figure 4. The sequence of streptokinase (SK), human plasminogen (Pg) and plasmin (Pm) reactions.⁸



Plasminogen levels

Plasminogen is synthesized in the liver¹⁷ and is maintained in plasma at a stable concentration of around 200 mg/l.¹⁸ The reference interval for plasminogen activity is 75% to 135%. In full-term neonates the plasminogen concentrations are about half those of adults,^{19,20} with levels gradually rising to normal by 6 months.²¹ Plasminogen levels vary little through adult life in relation to age, sex and smoking habits.²² There is no diurnal variation and levels are not affected by exercise.⁵

Histidine-rich glycoprotein (HRG) and plasmin inhibitor are two plasma proteins that form reversible complexes with the lysine-binding sites of plasminogen. Approximately 50% of plasminogen is bound to histidine-rich glycoprotein and about 15% to the plasmin inhibitor.²³ Complexing with these proteins has an inhibitory effect on the binding of plasminogen to fibrin.²⁴ This means that the plasma concentration of free plasminogen is determined not only by plasminogen levels but also by HRG and plasmin inhibitor. In some cases increased HRG levels may be associated with the increased risk of thrombosis.^{25,26}

Elevated plasminogen

Elevated levels have been reported in conjunction with pregnancy (around 140% in the 2nd and 3rd trimester),²⁷ hormonal contraceptives,²² obesity,²⁸ anabolic steroids,²⁹ in Africans,³⁰ hypothyroidism,³¹ and in liver or kidney transplant patients.³² Increased levels are usually found 3 days before any clinical signs of transplant rejection are noticed.

Decreased plasminogen

Decreased levels have been shown in several clinical conditions, including disseminated intravascular coagulation (DIC),^{33,53} sepsis,^{34,35} leukemia,³⁶ hyaline membrane disease,³⁷ liver disease,³⁸ Argentine hemorrhagic fever,³⁹ hyperthyroidism,³¹ and after L-asparaginase therapy, thrombolytic therapy and surgery. The decrease in plasminogen in some of these conditions may be a negative prognostic sign.

There are several mechanisms that may cause an acquired plasminogen deficiency. Increased consumption as well as depressed synthesis may be the reason for the deficiency observed in severe liver disease. An excessive release of natural plasminogen activators induced by massive stimuli (extensive tissue damage, stress, shock, certain drugs, etc.) could possibly lead to a depleted plasminogen level. The degradation of plasminogen into low molecular-plasminogen by leukocyte elastase is believed to be the reason for the reduction in functional plasminogen observed in septic patients and Argentine hemorrhagic fever.⁴⁰

Thrombolytic therapy

During thrombolytic therapy with high doses of streptokinase or urokinase there is a depletion of plasminogen that may terminate the efficacy of the thrombolytic drugs (Figure 5).^{20,41,42} The use of thrombolytic agents should therefore always involve close monitoring of the components of the plasminogen-plasmin system, especially during long-term thrombolytic treatments.

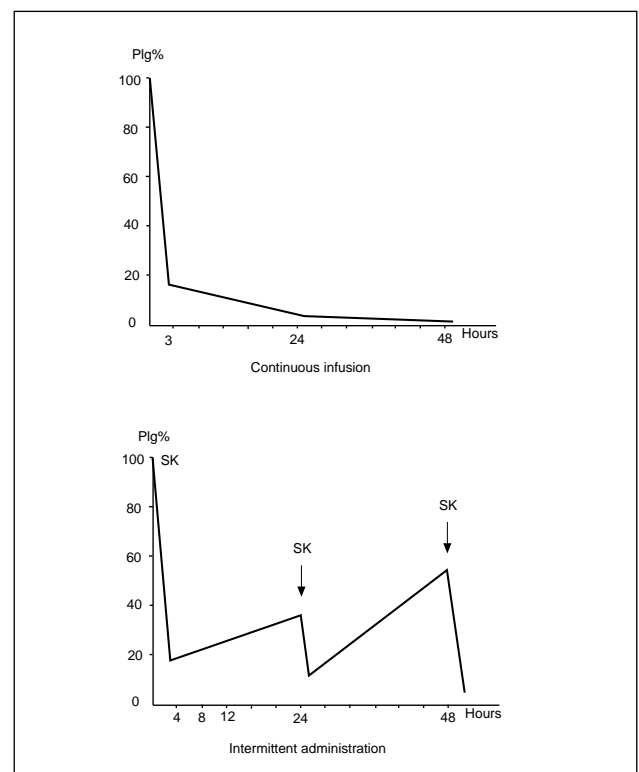


Figure 5. Plasminogen profile of a patient undergoing streptokinase treatment.⁴²



Hereditary plasminogen deficiency

There are two types of hereditary plasminogen deficiencies— *hypoplasminogenemias*, characterized by a parallel reduction of both plasminogen antigen and activity to about 50% of normal, and *dysplasminogenemia*, with a reduced activity/antigen ratio.⁵⁻⁷

Hypoplasminogenemia

Hypoplasminogenemia or type I plasminogen deficiency was first reported by Hasegawa et al in 1982. Up to now, about 15 families with this disorder have been identified. Several reports indicate that patients with hereditary plasminogen deficiency may have a higher risk of developing thrombosis.^{43,44,45} However, the correlation between type I deficiency and the risk of developing thrombosis has not yet been fully confirmed.^{45,46}

Dysplasminogenemia

The first case of hereditary plasminogen deficiency due to abnormalities in the plasminogen molecule and associated with venous thrombosis was reported by Aoki in 1978,⁴⁷ and has since then been followed by similar reports.⁷

Dysplasminogenemia or type II plasminogen deficiency has been observed mainly in young males who suffer from thromboembolic disorders. In contrast to the experience with recognized thrombophilias, family members with the same abnormality do not often present with thrombosis. This fact has raised significant doubt as to whether type II plasminogen deficiency alone is a sufficient risk factor for thrombosis or whether it is only a predisposing factor in the development of thrombophilia.

Prevalence

It has been estimated that type I and II deficiency accounts for 2-3% of unexplained deep venous thrombosis in young patients (<45 years).⁴

The incidence of plasminogen deficiency in 1,479 healthy Japanese blood donors was found to be 5.4% (plasminogen activity <60%).⁴⁸ 3.3% were

Decreased plasminogen levels

- Argentine hemorrhagic fever
- DIC
- Elevated HRG
- Hereditary plasminogen deficiencies
- Hyaline membrane disease
- Hyperthyroidism
- Leukemia
- Liver disease
- Neonates
- Sepsis
- Thrombolytic therapy

Elevated plasminogen levels

- African males
- Anabolic steroids
- Hypothyroidism
- Hormonal contraceptives
- L-asparaginase therapy
- Obesity
- Post-operative surgery
- Pregnancy
- Transplant rejection

Table 2. Situations associated with decreased or elevated plasminogen levels.

diagnosed as type II deficiency as they had activity/antigen ratios less than 0.66. It was calculated from this data that the total number of type II deficiency in Japan was approximately 4 million out of 120 million inhabitants. In spite of this high incidence the occurrence of venous thrombosis in the Japanese population is very rare compared to Caucasians. In a recent study of 9,611 healthy Scottish blood donors, a wide range of plasminogen (25-200%) was identified.²² Plasminogen activity below 65% was recorded in 0.6% (61 cases), although none appeared to have a history of thrombosis. These studies suggest that plasminogen in the majority of individuals is present in excess of physiological requirements and that plasminogen deficiency is not an independent risk factor for thrombosis.



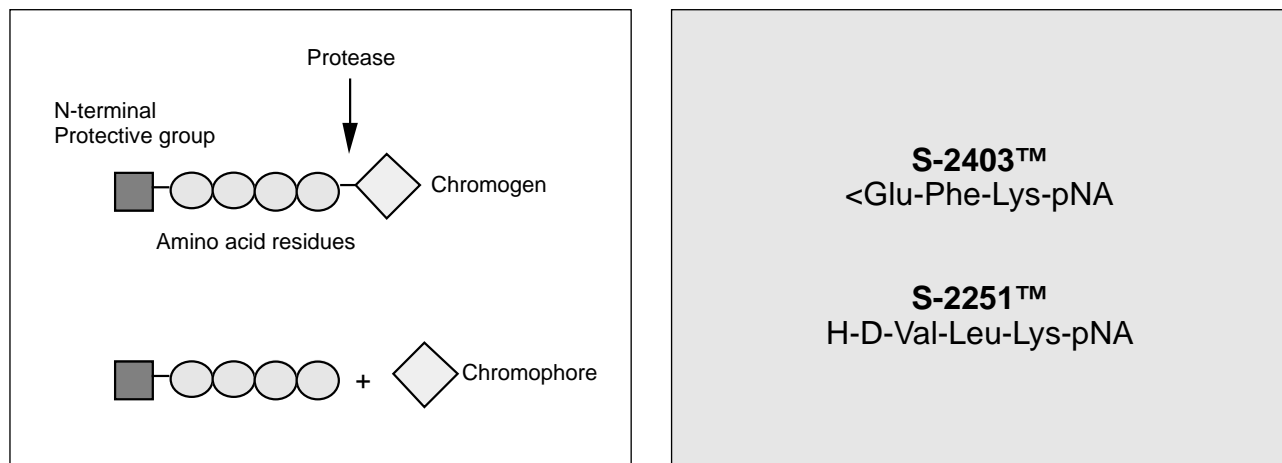


Figure 8. Left: Principle structure of synthetic peptide substrates. Right: Chromogenic substrates for plasmin and streptokinase-activated plasminogen. Abbreviations: pNA; 4-nitroaniline.

Plasminogen assays

The concentration of plasminogen in a sample may be assayed by means of an immunoassay such as radial immunodiffusion, immunoelectrophoresis or radioimmunoassay. A common approach when screening for hereditary plasminogen deficiency is to employ a functional assay as a first-line test and, in the event of a subnormal result, follow this with an immunological assay.⁴ These two results together help distinguish between type I deficiency, when both activity and antigen levels are reduced in parallel, and type II deficiency when antigen levels are normal although activity levels are reduced. For routine clinical purposes spectrophotometric assays based on chromogenic substrates are preferred, as they measure the functional activity of activated plasminogen and are quick to perform.^{3,49}

Chromogenic substrates

Chromogenic substrates for coagulation and fibrinolytic enzymes were first synthesized in the early 1970s and were soon utilized in assays for a variety of clinical applications including plasminogen determination.⁵⁰⁻⁵¹

A chromogenic substrate is composed of 3-5 amino acids that mimic the cleavage site of the natural protein substrate and has the chromophore

4-nitroaniline (pNA) attached to its end (Figure 8). When pNA is attached to the peptide chain it is colourless but when liberated by enzymatic cleavage the free pNA is yellow.

Chromogenic plasminogen assays

The determination of plasminogen using chromogenic substrates requires all plasminogen in the sample to be activated. Most assays use the streptokinase-plasminogen complex as an activator, which is formed in plasma when streptokinase is added in excess.^{52,53} The major advantage using this activator is that it is not inhibited by the plasmin inhibitor. A widely used chromogenic substrate for plasminogen determination is S-2251, which is a substrate for both plasmin and the streptokinase-plasminogen complex.

When a plasmin-sensitive chromogenic substrate is incubated with the streptokinase-plasminogen complex it is cleaved and pNA is liberated. The release is measured at 405 nm, either continuously during the reaction in a photometer cuvette, or after stopping the reaction with acetic or citric acid. The photometric signal is directly proportional to the plasmin activity, which reflects the amount of functional plasminogen originally present in the sample.

An important aspect to be observed when measuring the plasminogen concentration using



streptokinase as an activator, is the overestimation that may occur in patients with elevated fibrin(ogen) degradation products (FDP) which stimulate the assay reaction.⁵⁴ The source of error can be overcome by the addition of plasminogen-free human fibrinogen in excess, thereby achieving the maximum stimulation of the streptokinase-plasminogen complex for all samples.⁵⁵ This approach is employed successfully in the COAMATIC® Plasminogen kit.

The two major forms of plasminogen in plasma (i.e. Glu-plasminogen and Lys-plasminogen) can be measured using a chromogenic method based on the different rate of activation of the two forms of urokinase.⁵⁶

Clot lysis time

The activity of plasminogen can also be determined by means of various coagulation tests with casein or fibrin as the substrate (Figure 9). A typical assay would involve activation of plasminogen to plasmin by streptokinase or u-PA, the addition of plasminogen-free thrombin and measuring the lysis time. The principle is very simple although it has some major disadvantages, due mainly to the interference from the plasmin inhibitor which rapidly inactivates plasmin.

Immunological assays

The concentration of plasminogen can be measured by its antigenicity. Since the normal antigen concentration of plasminogen is between 150 and 250 ng/l the test can be performed using simple methods with moderate sensitivity such as radial immunodiffusion (Figure 10).⁵⁷ The main disadvantage is the 24-hour time lapse before results can be obtained.

More rapid and sensitive methods are immunoelectrophoresis⁵⁸ or laser nephelometry,⁵⁹ with results obtained within 2 hours. A radioimmunoassay can also be used, which has a very high level of sensitivity but requires the handling of radioactive substances.⁶⁰

1. Plasminogen $\xrightarrow{\text{Activator}}$ Plasmin
2. Addition of thrombin for clot formation
3. Measuring lysis time

Figure 9. Principle of a plasminogen clot lysis time assay

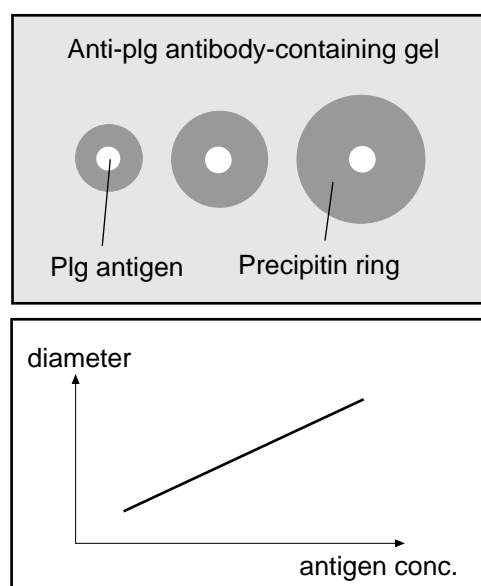


Figure 10. Radial immunodiffusion.

Wells are punched in plates with agar gel containing anti-plasminogen antibody. Standard volumes of test antigen of different concentration are put in the wells. The plates are left for 24 hours, during which time the antigen diffuses out of the wells to form soluble complexes with the antibody. These continue to diffuse outwards, binding more antibody until an equivalence point is reached and the complexes precipitate in a ring. The area within the ring is proportional to the antigen concentration. Unknowns are derived by interpolation from the standard curve.



Diagnostic kits from Chromogenix

We invented the technology

The development of the first chromogenic peptide substrate S-2160 in the early Seventies, initiated the introduction of photometry in hematology.⁵⁰ Today, there are many tests based on chromogenic substrates for coagulation factors, fibrinolytic factors as well as for inhibitors of both enzyme systems and endotoxin (Table 8).^{51,52} The tests can be performed manually or on automated analytical systems with high specificity, sensitivity and accuracy. Important, non-chromogenic-based products include kits for APC resistance (APTT-test) and several ELISA kits (Table 9).

Plasminogen kits

Chromogenic plasminogen assays have considerably simplified the evaluation of plasminogen and, as a consequence, increased our understanding of the fibrinolytic system and its role in pathogenesis.

COAMATIC® Plasminogen and COATEST® Plasminogen are two chromogenic kits designed for the specific determination of plasminogen activity in human plasma. Both kits are adaptable to the majority of leading clinical chemistry analysers and automated coagulation systems on the market.

A major feature of the COAMATIC® kit is that it is not influenced by elevated levels of fibrinogen degradation products and/or fibrinogen, which in other commercial products may cause the over-estimation of plasminogen activity.

HEMOSTASIS

Prekallikrein
Factor VII
Factor VIII
Factor X
Soluble fibrin
Antithrombin
Heparin/LMW heparin
Protein C
 α_2 -macroglobulin
 α_1 -antitrypsin
Plasminogen
t-PA
PAI-1
Plasmin inhibitor

ENDOTOXIN

Endotoxin

HEMOSTASIS

APC resistance
Anti-Cardiolipin IgG, IgM
D-dimer
Lipoprotein(a)
t-PA
PAI-1

INFLAMMATION/

SEPSIS

EndoCAb
IL-6
TFNa
Endotoxin

Table 6. Substances that can be determined with diagnostic kits based on synthetic peptide substrates (Examples from Chromogenix product range 1995).

Table 7. Substances that can be determined with kits from Chromogenix, based on either ELISA or clotting techniques.

Plasminogen Diagnostic indications

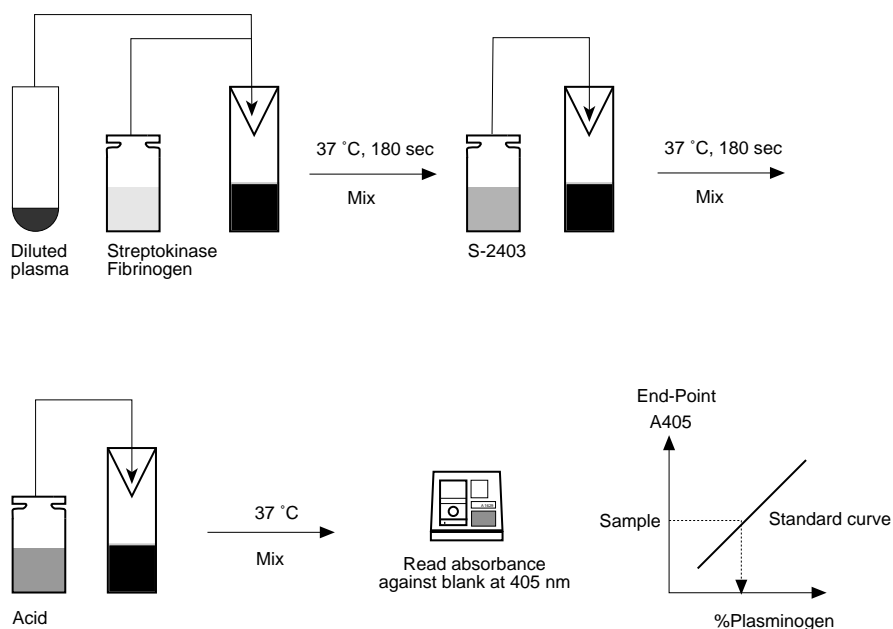
- Diagnosing hereditary thrombophilia
- Control of thrombolytic therapy
- Prognostic/diagnostic aid in patients with:
 - sepsis
 - severe liver damage
 - DIC



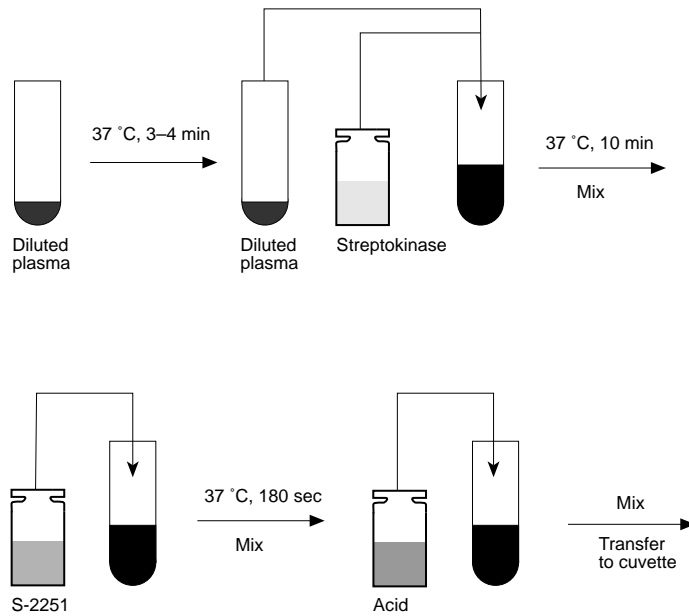
Assay Procedures

Product	Activator	Substrate	Plasma
COAMATIC® Plasminogen	Streptokinase/Fibrinogen	S-2403	Diluted
COATEST® Plasminogen	Streptokinase	S-2251	Diluted

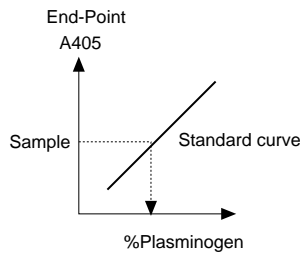
COAMATIC® Plasminogen



COATEST® Plasminogen




Read absorbance
against blank at 405 nm



COAMATIC® Plasminogen

COAMATIC® Plasminogen is a chromogenic test for the specific determination of plasminogen activity in human plasma. By adding plasminogen-free fibrinogen to the streptokinase reagent in this test, the overestimation of plasminogen concentrations resulting from elevated levels of FDP or fibrinogen is overcome.

Measurement principle

Plasminogen in the diluted test plasma is converted into active plasminogen-streptokinase complex (Plg-Sk), when an excess of streptokinase is added. The Plg-Sk complex catalyzes the cleavage of p-NA from the chromogenic substrate, S-2403. The rate at which pNA is released, measured photometrically at 405 nm, is proportional to the plasminogen concentration. This can be followed on a recorder (kinetic method) or read after stopping the reaction with acid (end-point method).

Article: 82 24 52

The kit contains

S-2403	2 vials
Streptokinase/fibrinogen	2 vials

Storage and stability in solution

S-2403	2–8 °C, 6 months
Streptokinase	2–8 °C, 1 month

Measuring range

0-130% of normal plasma

Detection limit

5%

Repeatability

Performed on Cobas Mira:

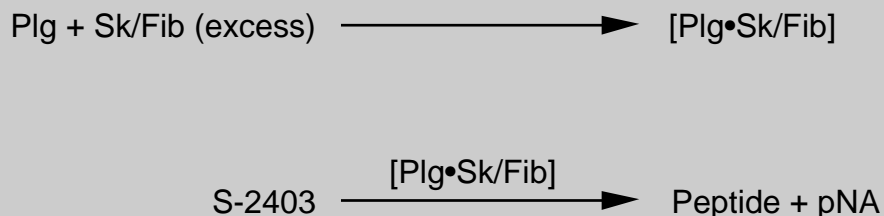
Plg.	CV within series	CV between series
50%	2.2%	1.2%
100%	1.8%	1.2%

Specificity and interfering factors

No drug interference reported. The assay is not influenced by varying concentrations of fibrinogen and/or FDP

Determinations per kit

Manual method 50, microplate 200, automated methods 200



The measurement principle of COAMATIC® Plasminogen. Abbreviations: Plg; plasminogen, Sk; streptokinase, Fib; fibrinogen



COATEST® Plasminogen

COATEST® Plasminogen is a chromogenic test for the specific determination of the plasminogen activity in human plasma after activation with an excess of streptokinase.

Measurement principle

Plasminogen in the diluted test plasma is converted into an active plasminogen streptokinase complex when an excess of streptokinase is added. The Plasminogen-Streptokinase complex catalyzes the cleavage of pNA from the substrate S-2251. The rate at which pNA is released, measured photometrically at 405 nm, is proportional to the plasminogen concentration. This can be followed on a recorder (kinetic method) or read after stopping the reaction with acetic acid (end-point method).

Article: 82 11 32

The kit contains

S-2251	1 vial
Streptokinase	1 vial
Buffer	1 vial
Normal plasma	2 vials

Storage and stability in solution

Substrate	2–8 °C, 6 months
Streptokinase	2–8 °C, 1 month -20 °C, 6 months
Buffer	2–8 °C, 1 month
Normal plasma	2–8 °C, 1 week -20 °C, 1 month

Measuring range

10–130% of normal plasma

Detection limit

10%

Repeatability

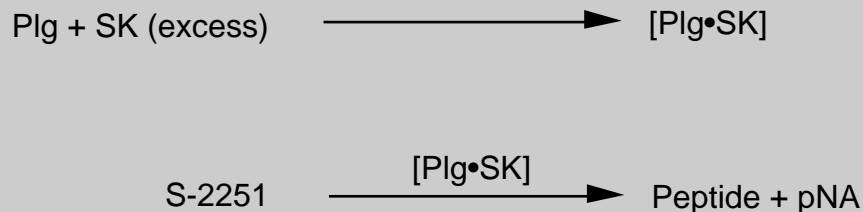
Plg.	CV within series	CV between series
25%	1.6%	4.6%
100%	0.9%	2.9%

Specificity and interfering factors

No drug interference reported. Fibrin(ogen) degradation products obtained during fibrinolysis treatment and in acute DIC increase the activity of the Plg-SK complex on S-2251.

Determinations per kit

Manual method 50, microplate method 140, automated methods up to 200



The measurement principle of COATEST® Plasminogen



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Glossary

Allele. One of an array of possible mutational forms of a gene at a specific locus

Amino acids. Basic building blocks of all proteins

Antibody. A molecule produced by animals in response to antigen.

Antigen. A molecule which induces the formation of antibody.

APC resistance. An hereditary defect caused by a point mutation in the gene coding for factor V and characterized by a poor anticoagulant response to activated protein C.

Argentine hemorrhagic fever. Viral disease caused by the infection with Junin virus, a member of the Arenaviridae family.

Autosome. A chromosome other than a sex chromosome.

Chromosome. The darkly staining bodies within the cells made up of a large number of genes and a centromere region.

Embolism. Obstruction or occlusion of a vessel by a transported clot.

Endothelium. Cells lining blood vessels and lymphatics which control the passage of materials into and out of the bloodstream.

Enzymes. A protein with catalytic power.

Exon. Gene segment encoding protein.

Fibrin. An elastic filamentous protein derived from fibrinogen by the action of thrombin, which releases fibrinopeptides A and B from fibrinogen.

Fibrinogen. Factor I; a globulin of the blood plasma that is converted into the coagulated protein, fibrin, by the action of thrombin in the presence of calcium ions.

Fibrinolysis. The hydrolysis of fibrin by plasmin.

Gene. The unit of inheritance, located at a specific region on the chromosome.

Glycoprotein. One of a group of protein-carbohydrate compounds

Hemostasis. Process which arrests the escape of blood from injured vessels.

Homozygous. Condition of having identical alleles at one or more loci under consideration

Heparin cofactor II. Serpin with heparin cofactor abilities. Specific inhibitor of thrombin.

Hepatocytes. Cells in the liver that are arranged in folded sheets. They produce many of the blood proteins.

Heterozygous. Having a dissimilar alleles at one or more loci.

Hyperthyroidism. An abnormality of the thyroid gland in which thyroid secretion is usually increased.

Hypothyroid disease. Diminished production of thyroid hormone, leading to thyroid insufficiency.

Intron. Gene segment between exons not encoding protein.

Locus. The position on a chromosome at which a particular gene is found.

Platelets. A little disk-shaped blood cell, containing granules in the central part and peripherally, clear protoplasm, but no nucleus. Numbering 200,000 to 300,000/ μ l.

Proteases, proteinases. Enzymes hydrolyzing native protein, or polypeptides, making internal cleavages; they include pepsin, chymosin, trypsin, papain etc.

Proteins. A class of macromolecules that are built from a repertoire of twenty amino acids.

Proteoglycan. A macromolecular glycoconjugate composed of sulfated glycosaminoglycans covalently linked to a protein core.

Proteolysis. Enzymatic cleavage of protein.

Prothrombin. Factor II, zymogen of thrombin; a glycoprotein formed and stored in the parenchymal cells of the liver. Present in blood at approximately 100 μ g/ml.

Receptor. A cell surface molecule which binds specifically to particular proteins or peptides in the fluid phase.

Sepsis. A clinical syndrome of serious bacterial infection.

Serine protease. Proteolytic enzyme with a serine residue at its enzymatically active site.

Serpin. Serine protease inhibitor.

Serum. The watery portion of blood remaining after fibrinogen has been removed from the plasma

Thrombocyte. Blood platelet

Thrombocytopenia. A condition in which there is an abnormally small number of platelets in the circulating blood (usually less than 150,000/ μ l).

Thromboembolism. Refers to either thrombosis or embolism or a combination of both.

Thrombolytics. Biological and synthetic substances capable of activating the fibrinolytic system in plasma.

Thrombin. Active protease deriving from prothrombin (factor II). Induces conversion of fibrinogen into clot-forming fibrin monomers resulting in the coagulation of blood.

Thrombophilia. A disorder in which there is a tendency to develop thrombosis.

Thrombosis. The formation of a thrombus (blood clot).

Thrombotic. Relating to, caused by, or characterized by thrombosis.

Zymogens. The enzymatically inactive precursors of proteolytic enzymes.



Notes



Plasminogen

Chromogenix Product Monographs

Antithrombin

COAMATIC® Antithrombin
 COAMATIC® AT 400
 COAMATIC® LR Antithrombin
 COATEST® Antithrombin
 COACUTE® Antithrombin R

APC resistance

COATEST® APC Resistance
 COATEST® APC Resistance – C
 COATEST® APC Resistance – S
 COATEST® APC Resistance – SC
 COATEST® APC Resistance V
 COATEST® APC Resistance V-S
 COASET® FV-506

D-dimer

SimpliRED® D-dimer
 DIMERTEST® GOLD EIA

Factor VIII

COAMATIC® Factor VIII
 COATEST® Factor VIII
 COATEST® VIII:C/4

Heparin

COATEST® Heparin
 COATEST® LMWHeparin/Heparin
 COACUTE® Heparin

Plasminogen

COAMATIC® Plasminogen
 COATEST® Plasminogen

Protein C

COAMATIC® Protein C

t-PA

COASET® t-PA
 COALIZA® t-PA

COAMATIC®

The latest techniques adapted specifically for the use with automated laboratory equipment.

COATEST®

Innovative and well-documented products with a range of applications for automated instruments.

COALIZA®

Complete enzyme-immunoassay (ELISA)-based kits for antigen determinations.

COACUTE®

For a small number of tests. All the reagents are freeze-dried in a single test cuvette.

COASET®

A group of products aimed for research applications.

COAMAB®

Monoclonal antibodies for research purpose.

CHROMOGENIX