Roles of α_2 -Macroglobulin and its Receptor in the Regulation of the Plasminogen-Plasmin System

Taro YASUKOUCHI*¹, Masahiro IEKO*², Ken-ichi SAWADA*³
and Shoki SAKURAMA*⁴

Key words: t-PA, u-PA, α_2 -M, LRP/ α_2 -MR, modulation.

Abstract

This paper focuses on the process of the formation of complexes between α_2 -macroglobulin (α_2 -M) and fibrinolytic enzymes, which play an important role in the intravascular fibrinolytic activity, and on the clearance of these complexes from the circulating blood through regulatory mechanisms, especially with reference to the mechanism involving the LDL receptor-related protein/ α_2 -Macroglobulin receptor (LRP/ α_2 -MR).

α_2 -M had previously been considered as one of the plasmin inhibitors, but is now also considered as a binding protein of certain proteases and of non-proteolytic amines, peptides, and proteins.

The proteases-like tissue-type plasminogen

activator (t-PA) and urokinase-type plasminogen activator (u-PA) showed decreased activities when they were bound to α_2 -M. But under these circumstances the activities of two-chain t-PA (tct-PA), which are more promptly suppressed by plasminogen activator inhibitor-1 (PAI-1) than are those of native single-chain t-PA, are not only preserved but also protected from this specific inhibitor. We also demonstrated in *in vivo* studies that the activities of tct-PA in the circulating blood showed a reverse correlation with the values of the PAI-1/ α_2 -M ratio, indicating that the intervention of α_2 -M in the reaction between t-PA and PAI-1 enhances fibrinolysis.

The clearance of exogenous t-PA from the circulating blood showed a biphasic mode

abbreviations PA: plasminogen activator, t-PA: tissue-type PA, u-PA: urokinase-type PA (urokinase), Plg: plasminogen, Plm: plasmin, pro-u-PA: the zymogen of u-PA, scu-PA: single-chain u-PA, tcu-PA: two-chain u-PA, u-PAR: u-PA receptor, PAI-1 and -2: PA inhibitor-1 and -2, α_2 -M: α_2 -macroglobulin, α_2 -AP: α_2 -anti-Plm, serpin: serine protease inhibitor superfamily, α_2 -MR: α_2 -M receptor, LRP: LDL receptor-related protein, MR: mannose receptor, GR: galactose receptor, IL-6: interleukin-6, sct-PA: single-chain t-PA, PDGF-BB: platelet-derived factor-BB, SK: streptokinase, tct-PA: two-chain t-PA, rtct-PA: recombinant: tct-PA, sct-PA: single-chain t-PA, C1-INAH: C1-inactivator, Lp(a): Lipoprotein(a), RAP: receptor-associated protein

*¹ Research Institute of Medical Science, Health Sciences University of Hokkaido, Kanazawa 1757, Toubetsu-Chou, Ishikari-Gun, Hokkaido 061-02, Japan

*² Department of Internal Medicine, School of Dentistry, Health Sciences University of Hokkaido

*³ Second Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo, Japan

*⁴ Medical Office, Bank of Hokkaido, Sapporo, Japan

受付: 1996. 8. 20, 受理: 1997, 7. 29.

Fig. 1 Two compartments of fibrinolytic processes.

In this schema, we omitted the details of interactions among these fibrinolytic factors. Plg: plasminogen, Plm: plasmin, u-PA: urokinase-type plasminogen activator, pro-u-PA: the zymogen of u-PA, α₂-M: α₂-macroglobulin, t-PA: tissue-type plasminogen activator; PAI-1: plasminogen activator inhibitor-1, PAI-1-t-PA: PAI-1-t-PA complex, PAI-1-u-PA: PAI-1-u-PA complex, u-PAR: u-PA receptor, PlgR: plasminogen receptor, u-PAR-pro-u-PA: u-PAR-pro-u-PA complex, u-PAR-u-PA: u-PAR-u-PA complex, PlgR-Plm: PlgR-Plm complex, LRP/α₂-MR: LDL receptor-related protein or α₂-MR, GR: galactose receptor, MR: mannose receptor. α₂-M* means the complex of α₂-M and its binding protein. u-PAR* means u-PAR-u-PA-PAI-1 complexes. Both u-PAR-bound u-PA and PlgR-bound Plm degrade extracellular matrix.

To avoid complexity, several reactions are omitted: e.g., the interaction between t-PA and C₁-INA, or α₂-AP, and that between t-PA and PlgR.

(initial phase and the terminal phase). Although the uptake of the t-PA-α₂-M complex is performed by LRP/α₂-MR, the removal of free t-PA is complicated. However, the initial phase of t-PA clearance would be mainly the responsibility of t-PA receptors (mannose and galactose receptors), and the terminal phase of that would be performed mainly by LRP/α₂-MR.

Therefore, the roles of α₂-M and LRP/α₂-MR in the entire system participating in the regulation of the plasminogen-plasmin system would be important.

Introduction

Both types of plasminogen activators (PAs), tissue-type PA (t-PA) and urokinase-type PA (u-PA), play an essential role in the fibrinolytic process by catalyzing the conversion of the zymogen plasminogen (Plg) to plasmin (Plm). Each PA has a distinct role in the Plg-Plm system in the different compartments, despite some overlap in the fibrinolytic modulation (**Fig. 1**). Endogenous u-PA appears to be the main PA in the extravascular compartment (on the extracellular matrix) and participates in cell migra-

tion and tissue remodeling, whereas t-PA plays a central role in the intravascular fibrinolysis^{1)~3)}. In the extracellular matrix, both u-PA and pro-u-PA, the latter being the zymogen of u-PA, bind to the u-PA receptor (u-PAR)^{4)~7)}. The u-PAR-bound u-PA (single-chain u-PA : scu-PA) is converted into active two-chain u-PA (tcu-PA) at the cell surface ; and the u-PAR-bound u-PA, including that activated from pro-u-PA, converts Plg, which is already bound to plasminogen receptors (PlgRs), into Plm^{8)~12)}. The activity of u-PAR-bound u-PA is attenuated by specific plasminogen activator inhibitors (PAI-1 and -2), which form complexes with u-PAR-bound u-PA^{13)~15)}. PlgR-bound Plm is resistant to inhibition by α_2 -macroglobulin (α_2 -M) and α_2 -antiplasmin (α_2 -AP), but gradually dissociates from the PlgR and is inhibited by α_2 -AP or bound to α_2 -M^{16)~17)}.

In the intravascular compartment, fibrinolysis induced by t-PA is a highly specific process. The t-PA, Plg, and fibrin form a ternary complex¹⁸⁾ that promotes Plm formation in association with the fibrin surface, where t-PA and Plm are protected from inactivation by their inhibitors (PAI-1 and α_2 -AP)^{19~24)}. However, a large amount of t-PA, which is usually exploited as a thrombolytic agent in a case of thrombosis, is immediately suppressed by PAI-1. Therefore, it is generally believed that the fibrinolytic potency of t-PA is determined by the ratio of t-PA to its immediate inhibitor, PAI-1.

Large amounts of α_2 -M, able to bind proteases, circulate in the peripheral blood²⁵⁾. Although α_2 -M is known to be an immediate inhibitor of Plm, its inhibitory mechanism differs from that of the serine protease inhibitor superfamily (serpin)^{26~28)} where, as a rule, only the reactive center of the protease is blocked. The difference is that, in the case

of α_2 -M, proteases are "trapped" in their "bait regions"^{29)~30~34)} ; and this reaction process is shown schematically in **Fig. 2**. Meanwhile, it has been shown that under these conditions protease activities are preserved^{25)~35~38)} and protected from their specific protein inhibitors^{39)~40)}. It is presumed, therefore, that the presence of α_2 -M can affect the fibrinolytic balance between t-PA and PAI-1.

Recent research has elucidated these conditions in the case of α_2 -M-protease complex formation. The α_2 -M-protease complex, once having disappeared from the circulating blood, was shown to be apprehended by α_2 -M receptors (α_2 -MR) present on the cell surface of fibroblasts, lymphocytes, macrophages, hepatocytes, and Kupffer cells of the liver, neurons and astrocytes in the central nervous system, epithelial cells of the gastrointestinal tract, glomerular mesangial cells of the kidney, vascular endothelial cells and smooth muscle cells, placenta, alveolar cells of the lung, and hepatoma HepG 2-cells^{41)~44~49)}. Following the binding of α_2 -M to α_2 -MR at the cell surface, the complex is concentrated in clathrin-coated pits and internalized into these cells^{50)~51)}.

α_2 -MR was shown to be identical to low-density lipoprotein receptor-related protein (LRP), which is known to serve as a receptor of apoprotein A (or apo E)^{52)~53)}. Therefore, the notation "LRP/ α_2 -MR" was introduced. LRP/ α_2 -MR is a multi-ligand receptor and binds with both t-PA-PAI-1 complexes formed in the intravascular compartment and u-PA-PAI-1 complexes formed in the extravascular compartment⁵⁴⁾.

Under these conditions, we especially discuss the interactions between α_2 -M and the enzymes involved in the Plg-Plm system in the intravascular compartment, and the role of LRP/ α_2 -MR in the thrombolytic process. It seems also necessary to discuss the fate of

Fig. 2 Complex formation between α_2 -M and protease

Above: a downview of the structure of α_2 -M; Below: α_2 -M as seen from the side. Solid line arrows indicate the direction of reactions between α_2 -M and protease (P); Dotted lines represent slow reactions. Solid lines connecting subunits of α_2 -M and trapped P indicate covalent binding; and dotted lines, non-covalent binding. (Courtesy: Higashi Nippon Dental Journal²⁹). (a) Native form of α_2 -M. (b) Each subunit of α_2 -M is cleaved (Mr. 185) into two fragments (Mr. 85 and 100 kDa) by a protease near or at the active center of α_2 -M. Therefore, it was proposed that a bait region in the α_2 -M is cleaved by the protease^{25(31)~34} (steps 1 and 2 in the text). (c): α_2 -M entraps the enzyme and undergoes a conformational change, with non-covalent binding between α_2 -M and the protease (steps 4 and 5). (d) Irreversible binding occurs between thioester of α_2 -M and the protease (step 6).

exogenous t-PA in the circulating blood with special respect to the roles of the mannose receptor (MR)^{55~65} and galactose receptor (GR)^{55(57)62~67} present in the liver as regulators of free t-PA.

I. α_2 -M and fibrinolytic proteases

A. Binding formula of α_2 -M

α_2 -M is a binding protein of t-PA. Human α_2 -M, which is synthesized in the liver and secreted following stimulation with interleukin-6 (IL-6)⁶⁸, is one of the acute-phase reactants⁶⁹⁽⁷⁰⁾. It is a glycoprotein of molecular weight (Mr.) of 720 kDa, consisting of 4 subunits²⁵ and representing about 20% of the total globulin in plasma⁷¹. Human α_2 -M is able to bind to 4 major proteinases (serine, metal-, thiol-, and carboxyl-proteases)⁷²⁽⁷³⁾, cytokines, growth factors⁷⁴

and nucleophilic monoamines⁷⁵.

The reaction between α_2 -M and proteases is considered to consist of six steps: 1. molecular contact between α_2 -M and protease molecules, 2. dissociation of the bait region of α_2 -M, 3. formation of a reversible complex between this bait region and the protease, 4. Slowly advancing conformational change of α_2 -M, 5. rapid reactions of thioester in α_2 -M with the protease, and 6. gradual promotion of covalent binding between the protease and thioester of α_2 -M (**Figs. 2 and 3**). There is some evidence for the between the protease and thioester of α_2 -M of step six (**Figs. 2 and 3**). There is, however, evidence that the above processes in steps 4 and 5 might occur simultaneously⁷⁶.

To investigate the behavior of single chain-t-PA (sct-PA) in plasma, we mixed sct-PA

Fig. 3 Difference in the binding reaction between α_2 -M and protease and α_2 -M and non-enzymatic protein.

Above: the reaction between α_2 -M and protease (P); Below: the reaction between α_2 -M and a non-enzymatic protein such as growth factor (GF). In the latter reaction, it is necessary to utilize binding between the thioester and nucleophilic monoamine (MA) in order to separate the thiol (SH) base from the thioester of α_2 -M. (Courtesy: Higashi Nippon Dental Journal⁷⁹⁾)

(Biopool, Uppsala, Sweden) and fresh plasma obtained from healthy adults and analyzed it by gel-filtration on Sephadex G-200⁷⁷⁾. The eluted fractions showing in plasma were recognized to exist in three forms: free t-PA, t-PA-PAI-1 complexes, and t-PA bound to macromolecular protein(s). It had been reported earlier that this unknown macromolecular protein bound to t-PA in the void volume is identical to α_2 -M⁷⁸⁾. In our studies, the t-PA-macromolecular protein complex was dissociated under high ionic conditions, where we could not recognize the above-mentioned "step 2" and "6" in process of the reactions between protease and α_2 -M.

On the other hand, it has been reported that the binding reaction between α_2 -M and non-enzymatic proteins such as cytokines or growth factors is initiated by a reaction between a nucleophilic monoamine and the thioester of α_2 -M. The thiol separated then covalently binds with the cytokine in an irreversible manner (**Fig. 3**)⁷⁴⁾⁷⁹⁾⁸⁰⁾.

Another perspective emerges from studies showing that platelet-derived growth factor-BB (PDGF-BB) binds with both α_2 -M and α_2 -M-Plm, where the binding of PDGF-BB- α_2 -M is noncovalent⁸¹⁾. This indicates that complexes of α_2 -M and proteases can bind with several nonenzymatic proteins.

Fig. 4 Inhibition potency of α_2 -M towards urokinase or plasmin

The fibrinolytic activities were measured by a method of "one-dimensional diffusion", in which plasminogen-free fibrin was employed as substrate. (a) Standard curve of plasmin obtained from the mixture of stepwise diluted plasminogen (Plg) with 42.5 IU (final concentration: f.c.) of urokinase-type plasminogen activator (u-PA). The Plg was prepared by affinity-column chromatography on lysine-coupled Sepharose 4 B from human plasma obtained from healthy adults with their informed consent. (b) Fibrinolytic activities of 14.5 mg/dl Plg solution activated by addition of 42.5 IU (f.c.) of u-PA. (c) Data of the mixture of "b" when adding stepwise diluted α_2 -M solution. (d) Plg converted to plasmin (Plm) by u-PA attached to Sepharose 4 B. Data were obtained using α_2 -M added to this u-PA-free Plm solution that showed the same activity as in the case of "b". (Courtesy: Higashi Nippon Dental Journal²⁹⁾)

Electron microscopy has shown that native α_2 -M has an H-like structure and that after binding of α_2 -M and protease (activated α_2 -M), one can observe a structure like the Russian⁸²⁾. Moreover, it is known that methylamine-protease complexes change from slow to fast forms under conditions of

Fig. 5 Effects of α_2 -M on activities of t-PA. Various concentrations of α_2 -M were added to 12.5 IU/ml (f.c.) of t-PA, and residual t-PA activities were measured by a parabolic rate assay using S-2251 as substrate.

electrophoresis⁸³⁾. These two forms of α_2 -M were detected in patients with pancreatic disorders by use of a monoclonal antibody immunoadsorption assay, and it was shown that the fast form was increased significantly in these patients compared with its levels in healthy adults⁸⁴⁾.

B. Does α_2 -M act as an inhibitor?

We investigated the inhibitory activities of α_2 -M toward Plm and u-PA by using a method of one-dimensional diffusion (Fig. 4)⁸⁵⁾⁸⁶⁾. As the substrate, we employed Plg-free fibrin. α_2 -M was partially purified from healthy adults by a method described elsewhere⁷³⁾. Plg was purified from fresh plasma through affinity chromatography. In order to avoid contamination by u-PA, purified Plg was passed through a u-PA (Mochida, Pharmaceutical Co., Tokyo)-coupled Sepharose-4 B column to obtain Plm. As shown in Fig. 4, the purified Plm was inhibited completely by the addition of α_2 -M. However, α_2 -M exhibited only weak inhibitory effects with regard to a mixture of Plg and u-PA. It was claimed that Plm- α_2 -M complex could dissolve fi-

brin⁸⁷), in a study that had been performed by the clot lysis method; but the Plm employed had been prepared mixing Plg with streptokinase (SK). That is to say, the report remains problematic in that the test material was contaminated with PA.

To study the effect of α_2 -M on t-PA, we employed recombinant two-chain t-PA (rtct-PA: Duptilase, Sumitomo Pharmacia Co.) and measured its activities by the parabolic rate assay using S-2251 as a substrate⁸⁸⁾⁸⁹⁾ (Fig. 5). The rtct-PA at the final concentration (f.c.) of 12.5 IU/ml was mixed with stepwise diluted solutions of purified α_2 -M. Although 84 ± 6.4 % of the original activity of rtct-PA was preserved, 5.4 mg/ml of α_2 -M inhibited 2.0 ± 0.8 IU/ml of rtct-PA. Therefore, α_2 -M can be thought of as acting like an inhibitor of fibrinolysis.

C. Effect of α_2 -M on PAI-1

It was already reported that trypsin bound to α_2 -M was not inactivated, unlike the case when bound to soybean trypsin inhibitor³⁹⁾⁴⁰⁾.

To investigate the effect of α_2 -M on the inhibitory activity of PAI-1, we purified PAI-1 from the supernatant of the solution of washed and resolved platelets obtained from healthy adults with their informed consent. The purification was performed using gel-filtration through a Mono Q column (HR 5/5, Pharmacia AB, Uppsala, Sweden) and HPLC⁹¹⁾. The tct-PA used was obtained from Sumitomo (Osaka, Japan), and activity of tct-PA was measured by the parabolic assay with S-2251 as the substrate.

A solution of tct-PA (f.c.: 25 IU/ml) was mixed with an equal volume of PAI-1 (of the same f.c.), and then α_2 -M solution (f.c. 48 mg/dl) was added. Residual t-PA activity was determined as 15 IU/ml. This value corresponded to 60% of the original concentration of the t-PA. When the same concen-

trations of the t-PA and α_2 -M were mixed and the solution of PAI-1 was added to the mixture after 5 minutes, 73% (18 IU/ml) of the original t-PA activity was observed. These findings indicate that t-PA activity was preserved in the α_2 -M-t-PA complex formed prior to the addition of PAI-1 i.e., α_2 -M protects t-PA from the attack of PAI-1 by means of formation of a α_2 -M-tct-PA complex⁹²⁾⁹³⁾.

D. Behavior of t-PA in the circulating blood

There is evidence that the binding rate between α_2 -M and non-proteolytic proteins like insulin is extremely low and that the binding rate between of α_2 -M and TGF- β is even lower than that of α_2 -M to insulin⁷⁴⁾. From our in vitro study, as described above, the binding rate of α_2 -M to proteases seems to be relatively high in the circulating blood, where α_2 -M is exposed to both substrate and the inhibitors of the proteases.

It was already reported elsewhere that the activities of both types of t-PA, sct-PA and tct-PA, were rapidly suppressed by PAI-1²³⁾²⁴⁾. But, we found that PAI, which had been prepared from human platelets, suppress the activity of tct-PA completely within 5 min but could not completely suppress that of the single chain t-PA (sct-PA) within such a short incubation time⁹⁴⁾. It was also recognized that the kinetics of the second-order rate constant κ for the inhibition of sct-PA by PAI-1 was about three times lower than that for the inhibition of tct-PA^{23)24)95)~97)}. It was also calculated that the half-time of inhibition of sc-t-PA in plasma samples that had contained high concentrations of PAI-1 was 52-372 s (mean value of 181 s) and that the half-time of inhibition of tct-PA in undiluted plasma was 45-105 s (mean: 64 s)⁹⁵⁾. From these facts, sct-PA

Fig. 6 Typical pattern of t-PA activity in a patient with myocardial infarction during and after tct-PA administration.

A case of myocardial infarction treated with tct-PA. The area under the activity-versus-time curve (AUC: shaded) was used as a measure of the bioavailability of the administered t-PA.

seems to be more suitable than tct-PA as a thrombolytic agent. The kinetics of binding between both types of t-PA and α_2 -M would be prompt, because our investigations revealed that the binding reaction between α_2 -M and sct-PA that between α_2 -M and tct-PA equally reached equilibrium within a few minutes⁹⁸). It is assumed that the complex between sct-PA and α_2 -M is formed in larger quantities than that between tct-PA and α_2 -M in the circulating blood, and it is questionable whether the complexes formed between either type of t-PA and α_2 -M would be effective as a thrombolytic agent or not. Therefore, further investigation of the influence of α_2 -M on the interaction between tct-PA and PAI-1 is needed, because tct-PA has been employed clinically as a thrombolytic agent.

We then had a chance to administer rtct-PA to nine patients suffering from myocardial infarction (MI), and found that the total

of the t-PA activities (which were obtained from the integral calculus determination of the area under the curve {AUC} when plotting each t-PA activity measured on the time course {Fig. 6}) was not correlated with the concentration of PAI-1, α_2 -AP, or α_2 -M in the plasma. However, the AUC of t-PA activities showed a reverse correlation to the PAI-1/ α_2 -M ratio ($r = -0.78$)⁹⁹). These data on kinetics indicate that rtct-PA binding to α_2 -M competes with that of rtct-PA to PAI-1, and that α_2 -M protects tct-PA from the attack of PAI-1.

E. Disappearance of activated α_2 -M from the circulating blood and LRP/ α_2 -MR

Offering native α_2 -M a chance to encounter proteases or nucleophilic monoamines implies that such complex formation results in α_2 -M activation and a certain conformational change. Concealed binding

Fig. 7 Molecular structure of LRP/ α_2 -MR. LRP/ α_2 -MR consists of α - and β -chains. The structure of the binding portions of LRP/ α_2 -MR to ligands represents the pattern of "complement-type repeats", which was divided into (I)-(IV)¹³⁹. u-PAR-pro-u-PA and u-PAR-u-PA-PAI-1 complexes bind to area II¹³⁶¹³⁹. The binding sites of ligands t-PA, activated α_2 -M, and apo E are different from each other, but the receptor associated protein (RAP) is able to bind to all binding sites of complement-type repeats (I-IV) and to compete with the ligands described above for their respective binding sites⁴⁹.

sequences of the native α_2 -M towards LRP/ α_2 -MR should thus be exposed, and the activated α_2 -M should be recognized and bound by LRP/ α_2 -MR expressed on the surfaces of various cells, as schematized in **Fig. 7**. The activated α_2 -M should then disappear from the circulating blood (**Fig. 8**). Activated α_2 -M bound to LRP/ α_2 -MR, therefore, should be internalized into these cells and metabolized in their lysozymes. The LRP/ α_2 -MR is recycled to be expressed on the cell

surface again⁵⁰⁵¹.

As already mentioned, LRP/ α_2 -MR is known as a receptor of apo-E, a substance which is abundantly contained in β -VLDL⁹⁹¹⁰⁰. Considering its structural properties, LRP/ α_2 -MR belongs to a family comprising LRP/ α_2 -MR, LDL receptor, VLDL receptor, and also gp 330¹⁰¹, which is known to act as an antigen in Haymann's nephritis of rats¹⁰¹¹⁰³. LRP/ α_2 -MR is a glycoprotein, a multipotential receptor, consisting of both an α -chain of Mr. 500 KDa and a transmembrane β -chain of Mr. 85 KDa¹⁰⁴ (**Fig. 7**).

The native α_2 -M in circulating blood persists at a high level for several hours after having been injected into the circulating blood¹⁰⁴¹⁰⁵, but studies using radio-labeled methylamine-activated α_2 -M injected into rats found the half-life of α_2 -M after its conformational change to be much shorter (2-4 min.)¹⁰⁴¹⁰⁷. It was reported that 50% of exogenous α_2 -M-Plm complex was removed within 5 minutes from the circulating blood in mice¹⁰⁸. These findings, however, do not yet clarify the behavior of the t-PA- α_2 -M complex, because it was claimed that the binding between components of the α_2 -M-Plm complex is irreversible and covalent¹⁸. There are investigations indicating that the complex between a protease and α_2 -M is reversible in the initial step of complex formation¹⁰¹ and that the conformational change of protease-activated α_2 -M represents a slowly advancing reaction⁷⁶. These facts further indicate the need to illuminate the role of α_2 -M bound to PAs in the circulating blood.

II. Disappearance of fibrinolytic factors from the circulating blood does not depend upon complex formation of proteases with α_2 -M.

A. Clearance mechanisms of t-PA

The forms of t-PA in the circulating blood

Fig. 8 Internalization of fibrinolytic ligands except activated α_2 -M with protease via LRP/ α_2 -MR into cells.

Each ligand is internalized into an endosome and metabolized in lysosomes in the cell. But both u-PAR and LRP/ α_2 -MR are recycled and expressed again on the cell surface. VN: vitronectin, t-PA: tissue plasminogen activator, PAI-1: plasminogen activator inhibitor-1, u-PA: urokinase-type plasminogen activator, pro-u-PA: the zymogen of u-PA, u-PAR: urokinase receptor, α_2 -M: α_2 -macroglobulin-protease complex, α_2 -AP: α_2 -anti-plasmin, u-PA-PAI-1: u-PA-PAI-1 complex, t-PA-PAI-1: t-PA-PAI-1 complex. ①, ②, and ③ represent three different routes of the internalization of t-PA into cells. ①, ②, ③, and ④ represent four different routes of the internalization of u-PA into cells. However, the modulation of both free u-PA and Plm- α_2 -AP by LRP/ α_2 -MR is controversial.

are as follows: free t-PA and complexes of t-PA-PAI-1, t-PA- α_2 -M, and/or t-PA-C1-INAH^{109,110}. The binding between C1-inactivator (C1-INAH) and t-PA occurs when active PAI-1 levels are low¹¹⁰, or when there is an overwhelming production of t-PA¹⁰⁹. α_2 -AP also neutralizes the t-PA activity, but its reaction is extremely slow (α_2 -AP inhibits sct-PA with a rate constant of $60 \text{ M}^{-1} \text{ s}^{-1}$ and tct-PA with a rate constant of $130 \text{ M}^{-1} \text{ s}^{-1}$, which corresponds to the half time ($t_{1/2}$) in plasma of 180 min or 90 min, respectively.)¹¹⁻¹¹¹. Therefore, t-PA- α_2 -AP could not be usually observed with free t-PA in the circulating blood.

There seem to be several patterns for the removal of free t-PA from the circulation; in

one of them free t-PA directly combines with MR, GR, or LRP/ α_2 -MR.

(i). *Direct regulation of free t-PA by t-PA receptors*

Recent studies have suggested that multiple mechanisms exist for t-PA clearance. The liver cells are responsible for the clearance of 80% of exogenous t-PA¹¹², and its clearance is performed mainly by both MR (a glycoprotein of Mr. 17,500⁶³) in endothelial cells and Kupffer cells and GR (a glycoprotein of Mr. 33,000-35,000^{66,67}) in parenchymal cells in the liver¹¹³. The uptake by these cells was calculated to be the following: parenchymal cells were responsible for 54%; endothelial cells for 39.5%; and Kupffer cells for 6%¹¹⁴ of the total hepatic uptake. It was also repor-

ted that the uptake per cell was two to three times higher in the non-parenchymal cells than in the parenchymal cells. But, as the number of parenchymal cells was two to three times greater than that of the other cells, it was calculated that endothelial cells were responsible for 53%, and parenchymal cells for 47% of total uptake of t-PAI and that the endocytosis of t-PA was almost absent in Kupffer cells¹¹³.

However, as it has been also reported that LRP/ α_2 -MR participates directly in the clearance of the free t-PA (PAI-1-independent t-PA)^{115,116}, it can not be denied that the uptake of a part of the free t-PA may be performed by LRP/ α_2 -MR.

Free t-PA has been demonstrated to bind to PlgRs¹¹⁷, but there is no evidence that it can be internalized and metabolized via interaction with this receptor type.

(ii). *Regulation of free t-PA by other mechanisms*

As a second route, free t-PA binds with PAI-1 fixed to vitronectin, which is a stabilizing binding protein of PAI-1 and is distributed over the entire cell surface area as a homogeneous carpet³. The t-PA-PAI-1 complex then is separated from vitronectin and becomes combined with LRP/ α_2 -MR^{30,64} (Fig. 8). Normal arterial endothelial cells do not express LRP/ α_2 -MR, but atherosclerotic arteries do exhibit it⁴⁵ on the intimal smooth muscle cells. In the extracellular matrix, t-PA interacts with PAI-1 (probably bound to vitronectin) and is regulated by LRP/ α_2 -MR⁴⁴; and this mechanism is considered to participate in a situation such as a vascular injury⁴⁸.

As a third regulatory mechanism, free t-PA forms a complex with PAI-1 in the circulating blood. The uptake of the t-PA-PAI-1 complex is performed by LRP/ α_2 -MR. A large amount of exogenous t-PA activity

would be removed by the direct uptake of free t-PA by both MR and GR, but it is assumed that the complex formation of t-PA-PAI-1 is also responsible for the removal of endogenous t-PA activity.

As for other regulatory mechanisms, the clearance of the complexes of t-PA-C1-INA and t-PA- α_2 -AP still remains obscure.

B. Modes of disappearance of t-PA from the circulating blood

Exogenous t-PA rapidly disappears from the circulating blood. Turnover studies on t-PA claimed the half life ($t_{1/2}$) is 1-3 min in rodents^{114,118}, and 3-5 min in humans^{71,119}. But, the clearance of t-PA from the circulating blood of rats was demonstrated to occur in a biphasic mode^{115,120,121}, in which the initial half life, $t_{1/2}$ (α), and the terminal phase, $t_{1/2}$ (β), were determined to be 0.5-0.6 min and 6.4-7.5 min, respectively^{113,120}. It was also demonstrated in humans that the turnover of t-PA was represented by a two-compartment model with peripheral (liver) elimination¹²². We also demonstrated that the increase in t-PA activity persisted for 15-30 min after termination of infusion of t-PA in eight of the nine patients as described above (Fig. 6).

It was also reported that the $t_{1/2}$ of the clearance of both of free t-PA and t-PA-PAI-1 complex from the circulating blood was 3.1-4.0 and 4.1-5.3 min^{123,124}, respectively.

C. Regulation of u-PA

(i). *The roles of u-PAR and PlgR*

As mentioned above, the PA in the extravascular compartment is mainly u-PA. In the extracellular matrix, u-PA combines with u-PAR and retains enzymatic activity, and converts PlgR-bound Plg into PlgR-bound Plm, as mentioned above. u-PAR is a glycoprotein of Mr of 55 kDa¹²⁵ and is ex-

pressed on the cell surface of human peripheral blood monocytes, phorbol ester-stimulated U 937 cells, neutrophils, keratinocytes, cultured endothelial cells, normal fibroblasts, A 431 carcinoma cells, and breast carcinoma^{14)(125)~130)}.

On the other hand, various types of PlgR of different Mr. were reported: Mr. 40 kDa (Annexin II) on human endothelial cells¹³⁵⁾, Mr. 45 kDa on human endothelial cell¹¹⁸⁾, Mr. 50-60 kDa on rat colon carcinoma cells¹¹⁷⁾, Mr. 45 kDa on mesencephalic neurons¹³⁶⁾, fibrinogen bound on GPIIb-GPIIIa of platelets¹³⁷⁾ and on some kinds of bacteria¹³⁸⁾. PlgRs, reviewed elsewhere, are divided in two categories, i.e., PlgRs that bind specific forms of Plg with considerably higher affinity than they bind others and those that exhibit similar affinity for the various forms of Plg¹²⁾. However, no receptor of the former category has been isolated and fully characterized. The residues of PlgR for binding to Plm are variable among different cells. In either case, the global consequence of Plg binding to cells is to enhance Plm activity. PlgR-bound Plg is converted by PAs (not only u-PA but also t-PA and SK¹¹⁾⁽¹³⁹⁾) into PlgR-bound Plm. PlgR-bound Plm is an efficient activator of cell-bound pro-u-PA to two-chain u-PA (active u-PA). PlgRs are distributed on the surface of platelets, granulocytes, monocytes, monocytoid cell lines, lymphocytes, leukaemic cells, fibrosarcoma cells, glioma cells, osteosarcoma cells, glomerular cells, and endothelial cells^{9)(117)(135)~137)(140)~143)}.

In addition, it has been shown that PlgR of Mr. 45 kDa present on endothelial cells derived from human umbilical vein interacts with t-PA¹⁴⁴⁾. This isolated PlgR was resolved into two Plg binding components. One component was found to be the 45 kDa PlgR itself, whereas the other is identified as the cytoskeletal protein actin. The actin in the isolated

PlgR (the actin seems to be bound to the PlgR) was shown to bind to Plg, t-PA, or Lipoprotein (a) (Lp (a))¹⁴⁵⁾.

Both u-PAR-bound u-PA and PlgR-bound Plm are able to degrade most components of the extracellular matrix either directly or by the activation of latent collagenase. The process stimulates the migration and invasion of cells such as occur in angiogenesis, inflammation, and wound healing.

Actin-bound t-PA or actin-bound Plg on the cellular surface might participate in the degradation of the extracellular matrix, because actin, mainly located in the intracellular compartment, is also located at the extracellular surface^{146)~148)}.

(ii) *Modulation of u-PA-u-PAR complex.*

The u-PAR-bound u-PA is neither internalized nor degraded⁶⁾⁽⁷⁾. If both u-PAR and LRP/ α_2 -MR were collocated on the same cells, the uptake of u-PA would be regulated by the latter, but the ultimate uptake of u-PAR-bound u-PA and u-PAR-bound u-PA-PAI-1 complex still remains obscure except at the cellular surface, where both of these receptors are co-located and can cooperate with each other like those on the cells of monocytoid cell lines and on trophoblast cells⁴⁶⁾. The u-PAR-bound u-PA, however, dissociates with a half life of 3-6 hours¹³¹⁾. Some speculations have been made,¹³⁷⁾ but the uptake mechanisms of u-PA complexes still remain unclear. On the other hand, when large amounts of u-PA are employed as a thrombolytic agent, u-PA in the bloodstream should exist as u-PA, u-PA-PAI-1 complex, and u-PA- α_2 -M. These u-PA compounds would be regulated by LRP/ α_2 -MR in the liver. Although pro-u-PA and u-PA are directly bound by LRP/ α_2 -MR^{5)~7)(102)}, it was also reported that the affinity of LRP/ α_2 -MR for pro-u-PA and free u-PA is 15-20 fold weaker than that for the u-PA-PAI-1 complex, i.e.

the complexes of u-PA bound to its receptor, but not free u-PA, that are readily internalized and degraded through a mechanism that involves LRP/ α_2 -MR¹⁴⁹⁾. Here, free u-PA as well as its complexes formed with PAI-1 are regulated by LRP/ α_2 -MR¹⁴⁹⁾. The u-PAR-bound u-PA is susceptible to inhibition by its specific inhibitors (PAI-1, PAI-2, and protease nexin-1:PN-1) as already mentioned. The complex formed with PAI-1 (u-PAR-u-PA-PAI-1 complex) is taken up strictly by LRP/ α_2 -MR¹⁵⁰⁾.

D. Regulation of Plg

Plg, either Glu- or Lys-Plg, Plm, and t-PA are able to bind to PlgR in the extracellular matrix. Cell-bound Plg activation is mainly performed by u-PA, but t-PA is also capable of converting Plg into Plm on rat liver cells¹⁵¹⁾. It was also reported that cell-bound Plg is able to be converted to Plm¹³⁷⁾ without PA. PlgR-bound Plm is resistant to inhibition by α_2 -M and α_2 -AP; but the bound Plm can dissociate, and free Plm immediately binds with α_2 -AP.

In the intravascular compartment, as mentioned above, t-PA, Plg, and fibrin form a ternary complex and promote Plm formation in association with the fibrin surface. If all available Plg were activated, only about one-third of the free Plm could be suppressed by α_2 -AP. This is because the total amount of α_2 -AP available for suppressing Plm activity merely corresponds to about 30% of the Plg content¹⁸⁾. The immunologically detected level of α_2 -M in the circulating blood is not easily decreased until the uncoagulable state of the blood is achieved by use of large amounts of u-PA, which has been used as a thrombolytic agent. In this situation, the immunological level of α_2 -AP is greatly decreased,¹⁵¹⁾ and then the immunological level of α_2 -M is gradually decreased. These find-

ings suggest that the Plm- α_2 -M complex is also generated after the deletion of free α_2 -AP from the circulating blood. It is well known that large quantities of Plm- α_2 -M can be observed in the liquid blood of patients suffering sudden death. Therefore, it cannot be excluded that Plm coexisting with PA also combines with α_2 -M. It was reported elsewhere that 50% of injected Plm- α_2 -M complex was removed from the plasma within 5 minutes¹¹³⁾ and this elimination of Plm- α_2 -M complex from the circulating blood would be due to uptake by LRP/ α_2 -MR.

The clearance mechanism of the Plm- α_2 -AP complex is controversial. There is a report showing binding between the Plm- α_2 -AP complex and LRP/ α_2 -MR¹²⁶⁾, but the disappearance of 50% of the Plm- α_2 -AP complexes from the blood required approximately 20 minutes. From the results, it was proposed that complexes of Plm and α_2 -AP were eliminated from the plasma by a specific and suitable pathway¹¹³⁾.

III. Competitive bindings among the ligands of LRP/ α_2 -MR and receptor-associated protein (RAP).

It has been demonstrated that LRP/ α_2 -MR offers about 76,000 high-affinity sites to t-PA ligands on one human Hep-G 2 cell⁵³⁾, but the number of LRP/ α_2 -MR per cell is controversial. Ligands t-PA-PAI-1 and u-PA-PAI-1 exhibit competitive binding for those sites⁵³⁾, and the binding to LRP/ α_2 -MR by α_2 -M and apo-E is partially competitive¹¹²⁾¹⁵²⁾. The ligand binding site on LRP/ α_2 -MR for t-PA are different from that on activated α_2 -M⁵³⁾¹⁵³⁾. It has been thought that LRP/ α_2 -MR also offers specific affinity sites to other factors, linked to fibrinolysis or not. However, RAP (Mr. 39 kDa), being produced simultaneously with the synthesis of LRP/ α_2 -MR, is known to inhibit the binding of

apo-E to b-VLDL, or of activated α_2 -M⁵³⁾¹⁵³⁾, or t-PA-PAI-1 complex⁸⁾¹²⁷⁾¹⁵³⁾ with LRP/ α_2 -MR.

Discussion

Fibrinolytic factors in the circulating blood are regulated by several mechanisms that may be classified as involving a) production, b) interaction, and c) removal. Although, in point of fact, the activities of both t-PA and PAI-1 are fairly influenced by their secretion rates¹²⁴⁾, this review is devoted only to the latter two factors, those of interaction and removal. The interactions among Plg, thrombospondin, collagen, vitronectin are also important for the process of malignancy³⁾¹⁵⁴⁾, but we have chosen not to discuss this issue here. As fibrinolytic mechanisms in the extracellular matrix were also discussed elsewhere^{1)~3)155)}, we will discuss mainly the roles of α_2 -M in the intravascular compartment.

First, the important role of α_2 -M in fibrinolysis in the circulating blood deserves attention since Plm activity is greatly influenced by α_2 -M. Large amounts of t-PA, which is extrinsic PA, are released from the vessel wall in a crisis such as a thrombotic event. t-PA is also administered for the treatment of such cases but shows a tendency to be removed rapidly from the circulating blood. However, as long as t-PA is present in the circulating blood, a part of it remains bound to Plg, PAI-1, and α_2 -M. It is appropriate to think that fibrinolytic potency (i.e., the production of Plm) depends on the balance between the quantities of t-PA and PAI-1. In fact, as the active PAI is neutralized by activated protein C, endogenous t-PA activity would be dominant under some situations of thrombotic emergency¹⁵⁶⁾. As a result, thrombolysis may occur. The immunological level of exogenous t-PA was also temporally decreased at the point of 15-30 minutes after

the initiation of t-PA infusion (**Fig. 6**). The phenomenon was also recognized in the other patients with MI, who were treated with tct-PA. We assumed that the phenomenon reflects complex formation between t-PA and PAI-1 in the circulating blood. On the other hand, the native form of α_2 -M immediately binds with t-PA and protects it from PAI-1, and so the t-PA retains its activity and continues to influence fibrinolytic potency. As a matter of fact, the sum of t-PA activities (AUC) retained in the circulating blood after the administration of t-PA (exogenous t-PA) for treatment of MI was found to be not correlated with the concentration of PAI-1, but rather with the ratio of PAI-1 to α_2 -M. When excess amounts of t-PA are contained in the bloodstream, α_2 -M acts as a weak inhibitor of t-PA, as a protective protein against the specific inhibitor PAI-1, and as a carrier protein of t-PA. However, it remains unclear whether the action of the t-PA- α_2 -M complex occurs in the solid phase of fibrin or in the fluid phase of blood.

Our study revealed that the dissociation constant (Kd) of the t-PA- α_2 -M complex was $0.7 \mu\text{M}$ ⁹⁸⁾. This indicates that the action of t-PA preserved in α_2 -M should be of the fluid-phase type. However, the possibility that t-PA bound to α_2 -M might be easily released from α_2 -M cannot be neglected, especially if the complex coexists with an excess amount of a serine protease like Plm. So, the possibility of solid-phase action could not be emphasized.

Although we have had no opportunity to use sct-PA clinically, this PA should be fairly affected by complex formation with α_2 -M; because its binding kinetics with PAI-1 does not indicate a reaction as immediate as that between tct-PA and PAI-1, the binding affinities of both forms of t-PAs to fibrin are very similar, and they have equal activity for

converting Plg into Plm¹⁵⁷⁾. However, another report showed that the binding affinity of sct-PA for fibrin was significantly greater than that of tct-PA, and the authors claimed that sct-PA showed decreased complex formation with α_2 -M¹⁵⁸⁾. The question of the binding affinities of both types of t-PAs for α_2 -M, PAI-1, and fibrin, however, still remains to be resolved.

On the other hand, both the domain deletion mutant t-PA (Reteplase)¹⁵⁹⁾, which contains only the second kringle and the protease-domain of the t-PA, and a PAI-resistant t-PA mutant might have an increased chance to encounter α_2 -M and would also form an excess amount of complexes with α_2 -M. Therefore, the reaction mechanism of the t-PA in t-PA- α_2 -M complexes in the circulating blood would be an important issue to examine in the case of treatment with t-PA mutants as the thrombolytic agents.

Thrombotic emergencies are not accompanied by an increase in u-PA, one of the main intrinsic PAs' and the thrombolytic function of u-PA is not yet fully explained. However, large amounts of u-PA are also employed for the treatment of thrombosis, and binding modes of u-PA closely resemble those of t-PA. As was shown in **Fig. 4**, α_2 -M suppressed the fibrinolytic activities of a mixture of u-PA and Plg solutions in a dose-dependent manner; but the fibrinolytic activity was not suppressed at the low concentration of about 15–20 mg/ml of α_2 -M, in spite of the fact that activities of u-PA-free Plm samples were completely suppressed at those concentrations of α_2 -M. That is to say, the fibrinolytic activities of the mixtures were weakly attenuated by α_2 -M, but the immediate anti-Plm activity of α_2 -M could not be observed in the mixture under the condition of the low concentration of α_2 -M in the mixture. This implies that the binding reac-

tion between α_2 -M and u-PA precedes conversion, the converting process of Plg into Plm, such that the residual amounts of α_2 -M to bind with the Plm converted by u-PA is decreased extremely. As mentioned above, α_2 -M may act as an anti-Plm in fibrinolysis after the production of an excess amount of Plm, but it seems not to act as the anti-Plm immediately after the injection of u-PA into the circulating blood. We assume that α_2 -M would show this phenomenon in the reaction among t-PA, Plg, and α_2 -M. On the other hand, we did not demonstrate the effect of α_2 -M on the reaction between u-PA and PAI-1, but we presume that α_2 -M would be effective in protecting u-PA against the inhibitory activity of PAI-1. Therefore, the intervention of α_2 -M in the process of the activation of Plg by PAs results in the promotion of fibrinogenolysis and/or fibrinolysis, because the action of PAs preserved in α_2 -M would be of the fluid-phase type.

Second, the clearance of t-PA from the circulating blood showed a biphasic mode even in humans, as mentioned above. However, analysis of the mode of clearance is quite difficult to resolve, because several factors participate in the clearance of t-PA from the circulating blood. As mentioned above, the t-PA clearance by MR and GR is thought to be responsible for the initial phase of clearance, and 50% clearance of t-PA-PAI-1 was relatively prompt. The participation of α_2 -MR/LRP in the initial phase of free t-PA clearance can not be denied. The study of the clearance of the deletion mutant t-PA (Reteplase) from the circulation in the rat revealed that $t_{1/2}$ in the initial phase and terminal phase was about 1 minute and 20–27 minutes, respectively, and that the amount cleared in the initial phase and the terminal phase was 28% and 72%, respectively¹⁵⁹⁾. The authors speculated that the slower plasma

clearance of the t-PA mutant might be explained by the fact that the mutant lacked the mannose-rich carbohydrate chain in the first kringle domain, which may be involved in the mannose-specific t-PA uptake in liver parenchymal cells, and indicated that the longer plasma half life for the mutant is also in agreement with data on other t-PA mutants that suggested that the finger and/or growth factor domain was associated with the rapid plasma clearance of t-PA¹⁵⁹⁾. This study indicated that the terminal phase of tct-PA clearance would be also performed mainly by LRP/ α_2 -MR. The slowness of clearance of the mutant t-PA compared with that of native t-PA would result in enhanced fibrinolytic potency. However, fibrinolytic potency might also be increased due to formation of the t-PA- α_2 -M complex because native α_2 -M is rapidly produced as an acute reactant protein, and increased t-PA- α_2 -M complex formation might strengthen the effect of fibrinogenolysis.

LRP/ α_2 -MR plays a role in the removal of various complexes such as t-PA- α_2 -M or Plm- α_2 -M in the blood stream and t-PA-PAI-1, which is formed in the circulating blood through the process of binding of free t-PA with vitronectin on the extracellular matrix. This removal mechanism is essential to suppress the excess formation of activated products in cases of thrombotic accidents and inflammation. LRP/ α_2 -MR eliminates these products from the circulation; and thus α_2 -M, as seen from this viewpoint, acts as a scavenger of the promoting factors of fibrinolysis.

In addition, α_2 -M is not only able to bind to four major enzymes, but also has the ability to bind to about 37 different kinds of non-proteolytic proteins, e.g., proteases, cytokines, hormones, etc.³¹⁾. Therefore, α_2 -M activated by these factors acts as a multi-potential protein (as a binding and carrier

protein) and would be expected to participate in numerous other reactions.

In conclusion

1. α_2 -M is considered to act as one of the immediate anti-Plms in fibrinolysis, but not in fibrinogenolysis, because Plm- α_2 -M complex can resolve fibrinogen.

2. This anti-Plm activity of α_2 -M could not be revealed immediately after the infusion of PAs into the bloodstream.

3. The reasons why α_2 -AP acts as the first anti-Plm, and α_2 -M does as the secondary, are as follows: when Plg converted into Plm by PAs in the circulating blood, the large amounts of native α_2 -M might be decreased by the complex formation of PA- α_2 -M, but the amounts of α_2 -AP would almost remain intact without the complex formation with t-PA since this complex formation is a time-consumed reaction.

4. α_2 -M slightly attenuates the activities of PAs by the PA- α_2 -M complex formation, but these PAs reserve their activities.

5. α_2 -M protects the activities of PAs from the inhibitory activity of PAI-1. Therefore, the intervention of α_2 -M in the reaction between PA and PAI-1 would enhance fibrinolysis.

6. It is assumed that t-PA- α_2 -M complex acts in the fluid-phase; thus large amounts of the formed complex may induce fibrinogenolysis.

7. The clearance of t-PA from the circulating blood is a biphasic mode: the initial phase is largely dependent on the uptake of free t-PA by the specific t-PA receptors (MR and GR), and terminal phase is mainly dependent on the uptake of the complexes between t-PA and α_2 -M and those between such complexes by LRP/ α_2 -MR.

8. The clinical use of a mutant t-PA, of which uptake of t-PA by the specific t-PA

receptors is suppressed, results in retention of t-PA in the bloodstream for long time, and thus an increase in the role of the elimination due to the terminal phase. The slowness of the mutant t-PA clearance compared with that of native t-PA would promote fibrinolysis, but may also enhance fibrinogenolysis by the relatively large amount of t-PA- α_2 -M complex produced.

References

- 1) Danø K, Andreasean PA, Grøndal-Hansen J, Kristensen P, Nielsen LS and Skriver L: Plasminogen activators, tissue degradation, and cancer. Eds.: Klein, G. and Weinhouse: *Advances in Cancer Research*. **44**: pp. 139-266, Academic Press, Inc., Orlando, San Diego, New York, London, Toronto, Montreal, Sydney and Tokyo, 1985.
- 2) Danø K, Behrendt N, Lund LR, Rønne E, Pöllänen J, Salonen E-M, Stephens RW, Tapiovaara H and Vaheri A: In "Cancer Metastasis". Eds.: Schirrmacher V and Schwartz-Albeitz R: *Molecular and cellular biology, host immune responses and perspectives for treatment* **5**: pp. 98-107, Springer-Verlag, Berlin, 1989.
- 3) Pöllänen J, Stephens RW and Vaheri A: Detected plasminogen activation at surface of normal and malignant cells. Eds.: Woude GEV and Klein G: *Advances in Cancer Research*. **57**: pp. 273-328, Academic Press, Inc., San Diego, New York, Boston, London, Sydney, Tokyo, and Tronto, 1991.
- 4) Ploug M, Rønne E, Behrendt N, Jensen AL, Blasi F and Danø K: Cellular receptor for urokinase plasminogen activator. *J. Biol. Chem.* **266**: 1926-1933, 1991.
- 5) Ploug M, Kjalke M, Rønne E, Weide U, Høyer-Hansen, G., and Danø, K.: Localization of the disulfide bonds in the NH₂-terminal domain of the cellular receptor for human urokinase-type plasminogen activator. *J. Biol. Chem.* **268**: 17539-17546, 1993.
- 6) Vassalli JD, Baccino D and Belin D: A cellular binding site for Mr. 55,000 form of human plasminogen activator, urokinase. *J. Cell Biol.* **100**: 86-92, 1985.
- 7) Stoppelli MP, Corti A, Soffientini A, Cassani G, Blasi F and Assosian RK: Differentiation enhanced binding of aminoterminal fragment of human urokinase plasminogen activator to a specific receptor on U 937 monocytes. *Proc. Natl. Acad. Sci. USA.* **49**: 4939-4943, 1985.
- 8) Plow EF, Freaney DE, Plesia J and Miles LA: The plasminogen system and cell surfaces: Evidence for plasminogen and urokinase receptors on the same cell type. *J. Cell Biol.* **103**: 2411-2420, 1986.
- 9) Gonias SL, Braud LL, Geary WA and VandenBerg SR: Plasminogen binding to rat hepatocytes in primary culture and to thin slices of rat liver. *Blood* **74**: 729-736, 1989.
- 10) Ellis V, Behrendt N and Danø K: Plasminogen activation by receptor-bound urokinase. *J. Biol. Chem.* **266**: 12752-12758, 1991.
- 11) Gonzalez-Gronow M, Stack S and Pizzo SV: Plasmin binding to plasminogen receptor enhances catalytic efficacy and activates the receptor for subsequent ligand binding. *Arch. Biochem. Biophys.* **286**: 625-628, 1991.
- 12) Redlitz A and Plow EF: Receptors for plasminogen and t-PA: an update. Eds.: Lijinen and Colle, D. *Baillière Clinical Haematology*. **8**: pp. 313-327. Fibrinolysis. Baillière Tindall, London and Tokyo. 1995
- 13) Cubellis MV, Anreassen P, Rango P, Mayer M, Danø K and Blasi F: Accessibility of receptor-bound urokinase to type-1 plasminogen activator inhibitor. *Proc. Natl. Acad. Sci. USA* **86**: 4838-4832, 1989.
- 14) Estreicher A, Mühlhauser J, Carpentier J-L, Orci L and Vassalli J-D: The receptor for urokinase type plasminogen activator polarizes expression of the protease to the leading edge of migrating monocytes and promotes degradation of enzyme inhibitor complexes. *J. Cell Biol.* **111**: 783-792, 1990.
- 15) Ellis V, Wun T-C, Behrendt N, Rønne E and Danø K: Inhibition of receptor-bound urokinase by plasminogen-activator inhibitors. *J. Biol.*

- Chem. **265**: 9904-9908, 1990.
- 16) Hall SW, Humphries JE and Gonias SL: Inhibition of cell surface receptor-bound urokinase by plasminogen-activator inhibitors. *J. Biol. Chem.* **266**: 12329-12336, 1991.
 - 17) Humphries JE, Vasudevan J and Gonias SL: Fibrinolytic and fibrinolytic activity of cell-associated plasmin. *Arterioscl. Thrombos.* **13**: 48-55, 1993.
 - 18) Aoki N and Harpel PC: Inhibitors of the fibrinolytic enzyme system. *Sem. Thrombos. Haemostas.* **10**: 24-41, 1984.
 - 19) Hoylaerts M, Rijken DC, Lijnen HR and Collen, D: Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. *J. Biol. Chem.* **257**: 2912-2919, 1982.
 - 20) Wiman B, Lijnen HR and Collen D: On the specific interaction between the lysine-binding site in plasmin and complementary sites in α_2 -antiplasmin and in fibrinogen. *Biochim. Biophys. Acta.* **579**: 142-154, 1979.
 - 21) Chmielewska J Rånby M, Wiman B: Kinetics of inhibition of plasminogen activators by plasminogen-activator inhibitor. Evidence for 'second-site' interactions. *Biochem. J.* **251**: 321-332, 1988.
 - 22) Masson C and Angles-Cano E: Kinetic analysis of the interaction between plasminogen activator inhibitor-1 and tissue-type plasminogen activator. *Biochem. J.* **256**: 237-244, 1988.
 - 23) Thorsen S, Røder M, Philips M, Suenson I, Lecander I and Astedt B: The influence of fibrin on the kinetics of inhibition of plasminogen activators by their specific inhibitors. Eds.: Takada, A., Sammama, M.M., and Collen, D.: *Protease inhibitors. International Congress Series.* **873**: 69-77, 1990.
 - 24) Thorsen S: The mechanism of plasminogen activation and variability of the fibrin effector during tissue-type plasminogen activator-mediated fibrinolysis. Eds.: Brakmann, P., and Kluft, C.: *Plasminogen activation in fibrinolysis, in tissue remodeling, and in development.* Ann. N.Y. Acad. Sci., **667**: 52-63, 1992.
 - 25) Harpel PC and Brower MS: α_2 -Macroglobulin: an introduction, chemistry and biology of α_2 -macroglobulin. Ed.: Feinman, R.D.: *Chemistry and biology of α_2 -macroglobulin.* Ann. N.Y. Acad. Sci., **421**: 1-9, 1983.
 - 26) Carrel RW and Boswell DR: *Serpins: The superfamily of serine protease inhibitors.* Ed: Barrett, A.J., and Salveen, G: *Protease inhibitors* pp 403-420. Elsevier, Amsterdam and New York, 1986.
 - 27) Huber R and Carrell RW: Implications of the three dimensional structure of α_1 -anti-trypsin for structure and function of serpins, *Biochem.* **28**: 8951-8966, 1989.
 - 28) Gettins P, Patson PA and Shapira M: Structure and mechanism of action of serpins. *Hematol. Oncol. Clin. North. Am.* **6**: 1393-1408, 1992.
 - 29) Yasukouchi T, Satoh M, Ieko M and Sawada K: Regulation of fibrinolytic factors...with special reference to α_2 -macroglobulin and its receptor. *Higashi Nippon Dent. J.* **14**: 1-12, 1995.
 - 30) Harpel PC: Studies on human plasma α_2 -macroglobulin enzyme interactions. Evidence for proteolytic modification of the subunit chain structure. *J. Exp. Med.* **138**: 508-521, 1973.
 - 31) Chu CT, Howard GC, Misra UK and Pizzo SV: α_2 -Macroglobulin: A sensor for proteolysis. Eds.: Borth W, Feinman RD, Gonias SL, Qugley JP and Strickland DK: *Biology of α_2 -macroglobulin, its receptor, and related proteins.* Ann. N.Y. Acad. Sci., **737**: 291-307, 1994.
 - 32) Barrett AJ and Starkey PM: The interaction of α_2 -macroglobulin with proteases. *Biochem. J.* **133**: 709-724, 1973.
 - 33) Wang D, Yuan I and Feinman D: Structure of α_2 -macroglobulin-protease complex. Ann. N.Y. Acad. Sci., **421**: 90-97, 1983.
 - 34) Chandler WL, Levy WC and Stratton JR: The circulatory regulation of TPA and UPA secretion, clearance, and phenylephrine. *Circulation.* **92**: 2984-2994, 1995.
 - 35) Harpel PC and Mosesson MW: Degradation of human fibrinogen by plasma α_2 -macroglobulin-enzyme complexes. *J. Clin. Invest.* **52**: 2175-2184, 1973.
 - 36) Riderknecht H and Geokas MC: Role for α_2 -macroglobulin in haemostatic balance. *Nature*

- New Biol. **239**: 116-117, 1972.
- 37) Rinderknecht H and Geokas MC : On the physiological role of α_2 -macroglobulin. *Biochim. Biophys. Acta* **295**: 233-244, 1973.
- 38) Heimbürger N, Haupt H and Schwick HG : Proteinase inhibitors of human plasma. Eds.: Fritz, H. and Tschesche, H.: Proceeding of the internal research conference on protease inhibitors. pp. 1-12. Walter de Gruyter, New York, N. Y., 1971.
- 39) Ganrot PO : Determination of α_2 -macroglobulin trypsin-protein esterase. *Clin. Chim. Acta*. **14**: 493-495, 1979.
- 40) Swenson R and Howard JB : Structural characterization of human α_2 -macro-globulin subunits. *J. Biol. Chem.* **254**: 4452-4456, 1979.
- 41) Moestrup SK, Gliemann J and Pallesen G : Distribution of the α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res.* **269**: 17401-17404, 1992.
- 42) Bu G, Maksymovitch EA, Nerbonne JM and Schwartz AL : Expression and function of low density lipoprotein receptor-related protein (LRP) in mammalian central neurons. *J. Biol. Chem.* **269**: 18521-18528, 1994.
- 43) Moestrup SK, Glieman J and Pallesen G : Distribution of the α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res.* **269**: 375-382, 1992.
- 44) Luoma J, Hiltunen T, Sarkioja S, Moestrup SK, Glieman J and Kodama T, Nikkari T and Ylä-Herttuala, S.: Expression of α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein and scavenger receptor in human atherosclerotic lesions. *J.Clin. Invest.* **93**: 2014-2021, 1994.
- 45) Lupu F, Heim D, Bachmann F and Kruiythof EK : LDL-receptor-related protein in human normal and atherosclerotic arteries. *Arterioscl. Thrombos.* **14**: 1438-1444, 1994.
- 46) Teesalu T, Blasi F and Talarico D : Embryo implantation in mouse : fetomaternal coordination in the pattern of expression of uPA, UPAR, PAI-1 and α_2 -MR/LRP genes. *Mechanism. Develop.* **56**: 103-116, 1996.
- 47) Grobmyer SR, Kuo A, Orishimo M, Okada SS, Cines DB and Determinants of binding and internalization of tissue-type plasminogen activator by human vascular smooth muscle and endothelial cells. *J. Biol. Chem.* **268**: 13291-13300, 1993.
- 48) Bu G, Maksymovitch A and Schwartz AL : Receptor-mediated endocytosis of tissue-type plasminogen activator by low density lipoprotein receptor-related protein on human hepatoma Hep G2 cells. *J. Biol. Chem.* **268**: 13002-13009, 1993.
- 49) Van Leuven F, Cassiman J J and Van den Berghe H : Functional modifications of α_2 -macroglobulin primary amines. I. Characterization of α_2 -M after derivatization by methylamine and by factor XIII. *J. Biol. Chem.* **256**: 9016-9022, 1981.
- 50) Hanover JA, Willingham MC and Pastan I : Receptor-mediated endocytosis of α_2 -macroglobulin : Solubilization and partial purification of the fibroblast α_2 -macroglobulin receptor. Ed.: Feinman, R.D. Chemistry and biology of α_2 -macroglobulin. *Ann. N. Y. Acad. Sci.*, pp. **421**: 410-423, 1983.
- 51) Strickland DK, Ashcom JD, Williams S, Burgess, WH, Migliorini M and Argraves WS : Sequence identity between the α_2 -macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. *J. Biol. Chem.* **256**: 17401-17404, 1990.
- 52) Kristensen T, Moestrup SK, Gliemann J, Bendtsen L, Sand O and Sottrup-Jensen L : Evidence that the newly cloned LRP is the α_2 -macroglobulin receptor. *FEBS Lett.* **276**: 151-155, 1990.
- 53) Warshawsky I, Bu G and Schwartz AL : LRP and receptor-mediated endocytosis of plasminogen activators. Eds.: Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P. and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. *Ann. N.Y. Acad. Sci.* **737**: 70-87, 1994.

- 54) Hildenbrandt GR and Aronson Jr, NN: Endocytosis of bovine lactoperoxidase by two carbohydrate-specific receptors in rat liver. *Arch. Biochem. Biophys.* **237**: 1-10, 1985.
- 55) Lennartz MR, Cole FS, Shepherd VL, Wileman TE and Stahl PD: Isolation and characterization of a mannose-specific endocytosis receptor from human placenta. *J. Biol. Chem.* **262**: 9942-9944, 1987.
- 56) Stephenson JD and Shepherd VL: Purification of human alveolar macrophage mannose receptor. *Biochem. Biophys. Res. Commun.* **148**: 883-889, 1987. 46).
- 57) Smedsrød B, Einarsson M and Pertoft H: Tissue plasminogen activator is endocytosed by mannose and galactose receptors of rat liver cells. *Thrombos. Haemostas.* **59**: 480-484, 1988.
- 58) Muller CD and Schuber F: Neo-mannosylated liposomes: synthesis and interaction with mouse Kupffer cells and resident peritoneal macrophages. *Biochim. Biophys. Acta.* **986**: 97-105, 1989.
- 59) Taylor ME, Conary JT, Lennart MR, Stahl PD, and Drickamer K: Primary structure of the mannose receptor contains multiple resembling carbohydrate-recognition domains. *J. Biol. Chem.* **265**: 12156-12162, 1990.
- 60) Seydel W, Stang E, Rops N and Krause J: Endocytosis of the recombinant tissue plasminogen activator alteplase by hepatic endothelial cells. *Arznei.-Forsch./Drug Res.* **41**: 182-18, 1991.
- 61) Stang E, Krause J, Kindberg GM and Roos N: Hepatic endocytosis of tissue-type plasminogen activator with special reference to the sinusoidal cells. Eds.: Wisse, E., Knock, R.S., and McCuskey, R.S.: Kupffer cell foundation, The Netherlands Cells of Hepatic Sinusoid. **3**: pp.542-545, Leiden. 1991.
- 62) Otter M, Barrett-Bergshoeff MM and Rijken DC: Binding of tissue-type plasminogen activator by mannose receptor. *J. Biol. Chem.* **266**: 13931-13935, 1991.
- 63) Bu G, Morton PA and Schwartz AL: Identification and partial characterization by chemical cross-linking of a binding protein for tissue-type plasminogen activator (t-PA) on rat hepatoma cells. *J. Biol. Chem.* **267**: 15595-15602, 1992. 53).
- Anraku, Y.: Transport of sugar and aminoacids in bacteria. *J. Biol. Chem.* **243**: 3116-3122, 1968.
- 64) Anraku Y: Transport of sugar and aminoacids in bacteria. *J. Biol. Chem.* **243**: 3123-3127, 1968.
- 65) Weber K and Osborn M: The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244**: 4406-4412, 1969.
- 66) Zukin RS, Strange PG, Heavey LR and Koshland Jr, DE: Properties of the galactose binding protein of *Salmonella typhimurium* and *Escherichia coli*. *Biocnem.* **16**: 381-386, 1977.
- 67) Kichler A and Schuber F: Versatile synthesis of bi- and tri-antennary galactose ligands: interaction with the Gal/GalNAc receptor of human hepatoma cells. *Glycoconjugate J.* **12**: 275-281, 1995.
- 68) Horn F, Wegenka UM, Luetticken C, Yaun J, Buschmann J and Heinrich PC: Regulation of α_2 -macroglobulin gene expression by interleukin-6. Eds.: Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P. and Strickland, D.K.: Biology of α_2 -macroglobulin, its receptor, and related proteins. Eds.: Borth, W., Feinman, R.D., Gonias, S. L., Quigley, J.P., and Strickland, D.K.: Biology of α_2 -macroglobulin, its receptor, and related proteins. *Ann. N.Y. Acad. Sci.* **737**: 308-323, 1994.
- 69) Kushner I: The phenomenon of the acute phase response. Eds.: Boland, B., Hitchcock, J.M., and Simon, W.: C-reactive protein and the plasma protein response to tissue injury. *Ann. N. Y. Sci.* **389**: 39-49, 1982.
- 70) Gordon AH and Koj A.: The acute phase response to injury and infection, Eds.: Gordon, A. H., and Koj, A. Research monographs in cell and tissue physiology. **10**: Elsevier. Amsterdam, New York, 1985.
- 71) Sottrup-Jensen L: α_2 -Macroglobulin structure, shape, and mechanism of proteinase complex formation. *J. Biol. Chem.* **264**: 11539-11542, 1989.
- 72) Lorand L, Downey T, Gotoh T, Jacobsen, A. and Tokura S: The transpeptidase system which crosslinks fibrin by γ -glutamyl- ϵ -lysine bonds.

- Biochem. Biophys. Res. Commun. **31** : 220-230, 1968.
- 73) Hapel PC, : Human α_2 -macroglobulin. Ed. : Lorand, L., Method in enzymol. **45** : pp. 639-652, Academic Press, New York, San Francisco and London, 1975.
- 74) Gettins PGW and Crews BC : Binding of epidermal growth factor to human α_2 -macroglobulin : significance for cytokine α_2 -macroglobulin interactions. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann.N.Y. Acad. Sci. **737** : 383-398, 1994.
- 75) Swenson R and Howard JB : Characterization of a lysine sensitive site in α_2 -macroglobulin. Proc. Natl. Acad. Sci. USA. **76** : 4313-4316, 1979.
- 76) Feinman DR : The protease-binding reaction with α_2 -macroglobulin Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann. N.Y. Acad. Sci. **737** : 246-266, 1994.
- 77) Yasukouchi T, Sakurama S, Satoh M, Sawada K, Fujie T, Ieko M, Thoma Y, Handa H, Mita, M Sato N and Nakagawa S : The behavior of tissue plasminogen activator in human plasma. Eds. : Gaffney PG, Castellino FJ, Plow EF and Takada, A : Fibrinolysis, current prospects, pp. 185-190, John Libbey, London and Paris, 1988.
- 78) Sakurama S and Wiman B : Reversible binding protein of functional tissue-plasminogen activator in human plasma : (1) α_2 -macroglobulin identified as purified binding protein. Thrombos. Haemostas. **62** : 393, 1985.
- 79) McCaffrey TA, Falcone DJ, Borth W and Weksler BB : α_2 -Macroglobulin/transforming growth factor- β 1 interactions. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann. N.Y. Acad. Sci. **737** : 368-382, 1994.
- 80) Gonias SL, LaMarre J, Crookston K P, Webb D J, Wolf BB, Lopes MS, Moses HL and Hayes MA : α_2 -Macroglobulin receptor/LRP. A growth regulatory axis. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann. N.Y. Acad. Sci. **737** : 273-290, 1994.
- 81) Bonner JC, Badgett A, Hoffman M, Lindroos P. M : Inhibition of platelet-derived growth factor-BB-induced fibroblast proliferation by plasmin-activated α_2 -macroglobulin is mediated via an α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein-dependent mechanism. J.Biol. Chem. **270** : 6389-6395, 1995.
- 82) Schramm W : Computer averaging of single molecules of two forms of α_2 -macroglobulin and the α_2 -macroglobulin/typsin complex. Ed. : Feinman, R.D. Chemistry and biology of α_2 -macroglobulin. Ann. N.Y. Acad. Sci. **421** : 149-159. 1983.
- 83) Barrett AJ, Brown MA and Sayers CA : The electrophoretically "slow" and "fast" forms of the α_2 -macroglobulin molecule. Biochem. J. **181** : 401-418, 1979.
- 84) Birkenmeier G, Schuke HL, Briest S and Kopperschläger G : Determination of total and transformed α_2 -macroglobulin by a monoclonal antibody immunosorption assay in patients with different diseases. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann. N.Y. Acad. Sci. **737** : 425-427, 1994.
- 85) Yasukouchi T and Watanabe T : A new method based on one dimensional diffusion using small glass tubes for the measurement of fibrinolytic activity. I. Fibrinolytic activity of plasmin and "euglobulin + streptokinase". Thrombos. Diathes. Haemorrh (Stuttg). **28** : 329-341, 1972.
- 86) Yasukouchi T, Fujie M, Kohn S, Sakurama S, Morioka A, Iju M and Nakagawa S : A method for the measurement of fibrinolytic activity based on one dimensional diffusion using small glass tubes. II. With special reference to the difference between the use of plasminogen-rich fibrinogen and that of plasminogen-free fibrinogen as the substrates. Thrombos. Diathes. Haemorrh (Stuttg). **37** : 456-463, 1977.

- 87) Veremeenko K.N and Kizim AI : Selective action of α_2 -macroglobulin-plasmin complex on fibrin. *Thrombo. Res.* **23** : 317-322, 1981.
- 88) Rånby M and Wallén P : A sensitive parabolic rate assay for tissue plasminogen activator. Ed. : Davidsson JF, Nilson IM and Åstedt B : Progression fibrinolysis. Vol 5, pp. 233-235, Churchill Livingstone, Edinburgh, 1981.
- 89) Rånby M, Norman B and Wallén P : A sensitive assay for plasminogen activator. *Thrombos. Res.* **27** : 743-749, 1982.
- 90) Wiman B, Mellbring B and Rånby M : Plasminogen activator release during venous stasis and exercise and as determined by a new specific assay. *Clin. Chim. Acta.* **127** : 279-288, 1983.
- 91) Fay WP and Owen WG : Platelet plasminogen activator inhibitor : Purification and characterization of interaction with plasminogen activator and activated protein C. *Biochem.* **28** : 5773-5778, 1989.
- 92) Ieko M, Sakurama S, Thoma Y, Fujie T, Satoh M, Yasukouchi T, Sato N, Handa H and Nakagawa S : Reversible binding protein of functional tissue-type plasminogen activator in human plasma. (III) Its protective effect on t-PA activity inhibition caused by plasminogen activator inhibitor. *Thrombos. Haemostas.* **62** : 1245, 1989.
- 93) Ieko M, Sawada K, Yasukouchi T, Sakurama S, Tohma Y, Shiroshita K, Kurosawa S, Ohmoto A, Kohno M, Satoh M and Koike T : Protection by α_2 -macroglobulin of tissue plasminogen activator against inhibition by plasminogen activator inhibitor-1. *Brit. J. Haematol.* **97** : 214-216, 1997.
- 94) Yasukouchi T, Fujie T, Sakurama S, Satoh M, Ieko M and Nakagawa S : The effects of the PAI derived from human platelets on two different types of t-PA. *Thrombo. Haemostas.* **58** : 446, 1987.
- 95) Jørgensen M, Philip M, Thorsen S, Selmer J, and Zeuthen J : Plasminogen activator inhibitor of tissue-type plasminogen activator in pregnancy plasma. *Thrombos. Haemostas.* **58** : 872-878, 1987.
- 96) Thorsen S, Philips, M, Selmer J, Lecander I, and Åstedt B : Kinetics of inhibition of tissue-type and urokinase-type plasminogen activator inhibitor type-1 and type-2. *Eur. J. Biochem.* **175** : 33-39, 1988.
- 97) Hekman CM and Loskutoff DJ : Kinetic analysis of the interactions between plasminogen activator inhibitor 1 and both urokinase and tissue plasminogen activators. *Arch. Biochem. Biophys.* **262** : 199-210, 1988.
- 98) Tohma Y, Sakurama S, Wiman B, Ieko M, Satoh N, Handa H, Fujie T, Nakagawa S, Satoh M, and Yasukouchi T : Reversible binding protein of functional tissue-type plasminogen activator in human plasma : (II) Interaction between t-PA and α_2 -macroglobulin identified as binding protein. *Thromb. Haemostas.* **62** : 310, 1989.
- 99) Kowal RC, Herz J, Goldstein JL, Esser V and Brown MS : Low density lipoprotein receptor-related protein mediates uptake of cholesterol esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86** : 5810-5814, 1989.
- 100) Beisel U, Weber W, Ihrke G, Herz J and Stanley KK : The LDL-receptor-related protein, LRP, is an apoprotein E-binding protein. *Nature.* **341** : 162-164, 1989.
- 101) Kerjarschki D and Earquhar MG : Identification of membrane glycoprotein from kidney brush border as pathogenic antigen of Heyman's nephritis. *Proc. Natl. Acad. Sci. USA* **79** : 557-5561, 1982.
- 102) Williams SE, Kounnas KM, Argraves WS and Stickland DK : The α_2 -macro-globulin receptor/low density lipoprotein and receptor-associated protein. An overview. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. *Ann. N.Y. Acad. Sci.* **737** : 1-13, 1994.
- 103) Kerjarschki D and Farouhar MG : Immunological localization of the Heymann's nephritis antigen (gp 330) in glomerular epithelial cells of normal Lewis rats. *J. Exp. Med.* **157** : 667-686, 1983.
- 104) Boers W, Linthorst C, van Dijk MM and van

- Berkel TJC : Uptake of methylamine-activated α_2 -macroglobulin by rat liver. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann. N.Y. Acad. Sci. **737** : 428-430, 1994.
- 105) Blatrix C, Amouch P, Drouet J and Steinbuch M : Study on the plasmatic elimination of the α_2 -macroglobulin/proteinase complexes. Pathol. Biol. **21** : 11-14, 1973.
- 106) Gliemann J, Nyljaer CM, Petersen CM, Joregenesen KE, Andeasen PA, Christensen EI, Lookene A, Olivecroma G and Moestrup SK : The multiligand α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein (α_2 -MR/LRP), binding and endocytosis of fluid phase and membrane-associated ligands. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann. N.Y. Acad. Sci. **737** : 20-38, 1994.
- 107) Cu CT, Howard GC, Misra UK and Pizzo SV : α_2 -Macroglobulin : A sensor for proteolysis. Eds. : Borth, W., Feinman, R.D., Gonias, S.L., Quigley, J.P., and Strickland, D.K. : Biology of α_2 -macroglobulin, its receptor, and related proteins. Ann. N.Y. Acad. Sci. **737** : 291-307, 1994.
- 108) Gonias SL, Einarsson M and Pizzo S : Catabolic pathways for streptokinase, plasmin, and streptokinase activator complex in mice. J. Clin. Invest. **70** : 412-423, 1982.
- 109) Bennett B, Croll A, Ferguson K and Booth NA : Compleing of tissue plasminogen activator with PAI-1, α_2 -macroglobulin, and C1-inhibitor : Studies in patients with defibrination and a fibrinolytic state after electroshock or complicated labor. Blood. **75** : 671-676, 1990.
- 110) Chandler WL, Levy WC and Stratton JR : The circulatory regulation of TPA and UPA secretion, clearance, and inhibition during exercise and during the infusion of isoproterenol and phenylephrine. Circulation. **92** : 2984-2994, 1995.
- 111) Korninger C, Collen D : Neutralization of human extrinsic (tissue-type) plasminogen activator in human plasma : no evidence for a specific inhibitor. Thromb. Haemostas (Stuttgart) **46** : 662-665, 1981.
- 112) Kuiper J, Otter, M, Rijken DC and van Berkel TJC : Characterization of the interaction *in vivo* of tissue-type plasminogen activator with liver cells. J. Biol. Chem. **263** : 18220-18224, 1988.
- 113) Einarsson, M., Smedsrød, B and Pertoft H : Uptake and degradation of tissue plasminogen activator in rat liver. Thrombos. Haemostas. **59** : 474-479, 1988.
- 114) Korniger C, Rijken DC, Linjen HR and Collen D : Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. Thrombos. Haemostas. **46** : 658-661, 1981.
- 115) Bu G, Williams S, Strickland DK and Schwartz AL : Low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. Proc. Natl. Acad. Sci. USA **89** : 7427-7431, 1992.
- 116) Warshawsky I, Bu G and Schwartz AL : Sites within the 39-kDa protein important for regulating ligand binding to the low-density lipoprotein receptor-related protein. Biochem. **34** : 3404-3415, 1995.
- 117) Dudani AK, Pluskota A and Ganz PR : Interaction of tissue plasminogen activator with a human endothelial cell 45-kilodalton plasminogen receptor. Biochem. Cell. Biol. **72** : 126-131, 1994.
- 118) Nilsson S, Einarsson M, Ekvärn S, Häggroth L and Mattsson, Ch. : Turnover of tissue plasminogen activator in normal and hepatectomized rabbits. Thromb. Res. **39** : 511-22, 1985.
- 119) Nilsson T, Wallén P and Mellbring G : *In vivo* metabolism of human tissue-type plasminogen activator. Scand. J. Haematol. **33** : 49-53, 1984.
- 120) Smedsrød, B and Einarsson M : Clearance of tissue plasminogen activator by mannose and galactose receptors in the liver. Thrombos. Haemostas. **63** : 60-66, 1990.
- 121) Narita M, Bu G, Herz J and Schwartz AL : Two receptor systems are involved in the plasma clearance of tissue-type plasminogen activator (t-PA) *in vivo*. J. Clin. Invest. **96** : 1164-1168, 1995.

- 122) Verstrete M, Bounameaux H, De Cock F, Van de Werf F and Collen D: Pharmacokinetics and systemic fibrinolytic effects of recombinant human tissue-type plasminogen activator (rt-PA) in humans. *J. Pharm. Exp. Ther.* **235**: 506-512, 1985.
- 123) Brommer EJP, Derkx FHM, Schalekamp MADH and Doijewaard G: Renal and hepatic handling of endogeneous tissue-type plasminogen activator (t-PA) and its inhibitor in man. *Thrombos. Haemostas.* **59**: 404-411, 1988.
- 124) Chandler WL: A kinetic model of the circulatory regulation of tissue plasminogen activator. *Thrombos. Haemostas.* **66**: 321-328, 1991.
- 125) Nielsen LS, Kellerman GM, Behrendt N, Picone R, Danø, K and Blasi F: A 55,000-60,000 Mr receptor protein for urokinase-type plasminogen activator. Identification in human tumor cell lines and partial purification. *J. Biol. Chem.* **263**: 2358-2363, 1988.
- 126) Wing LR, Combe CJ, Bennett B and Booth NA: A receptor on primary human hepatocytes for tissue plasminogen activator inhibitor 1 and plasmin- α_2 -antiplasmin complexes. *Fibrinolysis* **6**: 2, 1992.
- 127) Kuiper J, Otter M, Voorschuur AH, van Zonneveld AJ, Reijken DC and van Berkel TJC: Characterization of the interaction of a complex of tissue-type plasminogen activator and plasminogen activator inhibitor type 1 with rat liver cells. *Thrombos. Haemostas.* **74**: 1298-1304, 1995.
- 128) Bajpai A and Baker JB: Cryptic urokinase binding sites on human foreskin fibroblasts. *Biochem. Biophys. Res. Commun.* **133**: 475-482, 1985.
- 129) Stoppelli P, Tacchetti C, Cubellis V, Corti A, Hearing VJ, Cassani G, Appella E and Blasi F: Autocrine saturation of pro-urokinase receptors on human A431 cells. *Cell* **45**: 675-684, 1986.
- 130) Needham GK, Sherbet GC, Farndon JR and Harris AL: Binding of urokinase to specific receptor sites on human breast cancer membranes. *Br. J. Cancer* **55**: 13-16, 1987.
- 131) Blasi F: Surface receptor for urokinase plasminogen activator. *Fibrinolysis* **2**: 73-84, 1988.
- 132) Nykjær A, Petersen CM, Christensen EI, David-son O and Gliemann J: Urokinase receptors in human monocytes. *Biochim. Biophys. Acta.* **1052**: 399-407, 1990.
- 133) Miles LA and Plow EF: Receptor mediated binding of the fibrinolytic components, plasminogen and urokinase, to peripheral blood cells. *Thrombos. Haemostas.* **58**: 936-942, 1990.
- 134) McNeill H and Jensen PJ: A high-affinity receptor for urokinase plasminogen activator on human keratinocytes: characterization and potential modulation during migration. *Cell Regul.* **1**: 843-852, 1990.
- 135) Hajjar KA and Jacovina A: Molecular cloning of an endothelial cell tissue plasminogen activator/plasminogen receptor: Annexin II-related protein. *Thrombos. Haemostas.* **69**: 989, 1993.
- 136) Nakajima K, Nagata K and Kohsaka S: Plasminogen mediates an interaction between microglia and dopaminergic neurons. *Eur. Neurol.* **34**(suppl 3): 10-16, 1994.
- 137) Plow EF, Felez J and Miles LA: Cellular regulation of fibrinolysis. *Thrombos. Haemostas.* **66**: 32-36, 1991.
- 138) Ullberg M, Wiman B and Kronvall G: Binding of tissue-type plasminogen activator (t-PA) to *Neisseria meningitidis* and *Haemophilus influenzae*. *FEMS Immunol. Med. Microbiol.* **9**: 171-178, 1994.
- 139) Gonzalez-Gronow M, Stack S and Pizzo S: Plasmin binding to the plasminogen receptor enhances catalytic efficiency and activates the receptor for subsequent ligand binding. *Arch. Biochem. Biophys.* **286**: 625-628, 1991.
- 140) Hall SW, VandenBerg SR and Gonias SL: Plasminogen carbohydrate side chains in receptor binding and enzyme activation: A study of C6 glioma cells and primary cultures of rat hepatocytes. *J. Cell. Biochem.* **43**: 213-227, 1990.
- 141) Tam SP, Zang X and Koschinsky ML: Interaction of a recombinant form of apolipoprotein (a) with human fibroblasts and with the human hepatoma cell line HepG2. *J. Lipid Res.* **37**: 518-533, 1996.

- 142) Burtin P and Fondaneche M-C : Receptor for plasmin on human carcinoma cells. *J. Nation. Cancer Inst.* **80** : 762-765, 1988.
- 143) Yoshida M, Shirouzu M, Tanaka M, Semba K, and Fibiger, H.C. : Dopaminergic neurons in the nucleus raphe dorsalis innervate the prefrontal cortex in the rat : a combined retrograde tracing and immunohistochemical study using anti-dopamine serum. *Brain Res.* **496** : 373-376, 1989.
- 144) Dudani AK, Pluskota A and Ganz PR : Interaction of tissue plasminogen activator with a human endothelial cell 45-kilodalton plasminogen receptor. *Biochem. Cell Biol.* **72** : 126-131, 1994.
- 145) Dudani AK and Ganz PR : Endothelial cell surface actin serves as a binding site for plasminogen, tissue plasminogen activator and lipoprotein (a). *Br. J. Haematol.* **95** : 168-178, 1996.
- 146) Sanders SK and Craig SW : A lymphocyte cell surface molecule that is antigenically related to actin. *J. Immunol.* **131** : 370-377, 1983.
- 147) Pardridge WM, Nowlin DM, Choi TB, Yang J and Calaycay J : Brain capillary 46,000 Dalton protein is cytoplasmic actin and is localized to endothelial plasma membrane. *J. Cereb. Blood Flow Metab.* **9** : 675-680, 1989.
- 148) Moroianu J, Fett JW, Riodan JF and Vallee BL : Actin is a surface component of calf pulmonary artery endothelial cells in culture. *Proc. Natl. Acad. Sci. USA.* **90** : 3815-3819, 1993.
- 149) Conese M and Blasi F : Urokinase/urokinase receptor system : internalization/degradation of urokinase-serpin complexes : mechanism and regulation. *Biol. Chem. Hoppe-Seyler.* **376** : 143-155, 1995.
- 150) Nykjær A, Kjøller L, Cohen RL, Lawrence, D. A, Carni-Wagner BA, Todd III RF, van Zon-nveld A-J, Gliemann J and Andreasen PA : Regions involved in binding of urokinase-type-1 inhibitor complex and pro-urokinase to the endocytic α_2 -macroglobulin receptor/low density lipoprotein receptor-related protein. *J. Biol. Chem.* **269** : 25668-25676, 1994.
- 151) Yasukouchi T, Uemura K, Sato M, Morioka T, Sawada K, Sakurama S, Kohn S, Nakagawa S and Kondoh M : An approach to the theoretical urokinase treatment-With special reference to the anti-plasmin activity level as one of the indicators of urokinase-treatment. *J. Med. Enzym.* **3** : 571-580, 1980. (in Japanese).
- 152) Hussain MM, Maxfield FR, Mas-Oliva J, Tabas L, Ji Z-S and Innerarity TL and Maley RW : Clearance of chylomicron remnants by the low density lipoprotein receptor-related protein/ α_2 -macroglobulin receptor. *J. Biol. Chem.* **266** : 13936-13940, 1991.
- 153) Willow TE, Orth K and Herz J : Molecular dissection of ligand binding sites of the low density lipoprotein receptor-related protein. *J. Biol. Chem.* **269** : 15827-15832, 1994.
- 154) Albo D, Arnoletti JP, Castiglioni A, Granick MP, Solomon VI, Rothman VL and Tuszynski GP : Thrombospondin (TSP) and transforming growth factor beta 1 (TGF- β) promote human A549 lung carcinoma cell plasminogen activator inhibitor type 1 (PAI-1) production and stimulate tumor cell attachment *in vitro*. *Biochem. Biophys. Res. Comm.* **203** : 857-865, 1994.
- 155) Sakata Y : Blood vessel and fibrinolysis. *Jpn J. Thrombos. Haemostas.* **2** : 103-111, 1991. (in Japanese)
- 156) Sakata Y, Loskutoff DJ, Glasdson CL, Hekman CM, Griffin JH : Mechanism of protein C-dependent clot lysis : role of plasminogen activator inhibitor. *Blood* **68** : 1218-1223, 1986.
- 157) Rijken DC, Hoylaerts M and Collen D : Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue type) plasminogen activator. *J. Biol. Chem.* **257** : 2920-2925, 1982.
- 158) Tate KM, Higgins DL, Holmes WE, Winker, ME, Heyneker HL and Vehar GA : Functional role of proteolytic cleavage at Arginine-275 of human tissue plasminogen activator as assessed by site-directed mutagenesis. *Biochem.* **26** : 338-343, 1987.
- 159) Kuiper J, van de Bilt H, Martin U and van Berkel Th JC : Uptake, internalization and degradation of the novel plasminogen activator Reteplase (BM 06.022) in the rat. *Thrombos. Haemostas.* **74** : 1501-1510, 1995.