Regulation of Streptokinase-Human Plasmin Complex by the Plasma Proteinase Inhibitors $\alpha_2$-Antiplasmin and $\alpha_2$-Macroglobulin Is Species Specific and Temperature Dependent

By Steven L. Gonia, Nancy L. Figler, and Lela L. Braud

Streptokinase-plasmin complex (SkPI) was prepared with human plasminogen. Regulation of SkPI and plasmin by the plasma proteinase inhibitors, $\alpha_2$-antiplasmin ($\alpha_2$AP) and $\alpha_2$-macroglobulin ($\alpha_2$M), was studied as a function of temperature in plasminogen-depleted human plasma, mouse plasma, and solutions of purified proteins. The reaction of plasmin with proteinase inhibitors in human plasma was complete. $\alpha_2$AP was the predominant inhibitor. The fraction of $\alpha_2$M-plasmin recovered was not affected significantly by incubation temperature. In contrast, the reaction of SkPI with human proteinase inhibitors was markedly temperature dependent. The apparent second-order rate constant for the reaction of SkPI with purified $\alpha_2$AP at 37°C ($1.5 \times 10^5$ mol/L·s$^{-1}$) was >150-fold higher than the constant derived at 4°C. In human plasma and in solutions containing mixtures of purified human proteins, $\alpha_2$AP was the principal inhibitor of SkPI. Elevating the temperature enhanced the reaction of SkPI with $\alpha_2$AP and $\alpha_2$M comparably. Equivalent results were obtained when incubations were performed in platelet-rich plasma (PRP) or whole blood. In murine plasma, SkPI reacted readily with the proteinase inhibitors. The principal inhibitor of SkPI was $\alpha_2$M. Maximum reaction between SkPI and murine $\alpha_2$M was observed at 37°C; however, significant reaction also occurred at 4°C. $\alpha_2$AP was the predominant inhibitor of plasmin in mouse plasma. Reaction of $\alpha_2$AP with SkPI in murine plasma was significant only after the $\alpha_2$M was inactivated with methylamine. These results were not affected by platelets or whole blood cells. We conclude that the thrombolytic efficacy of streptokinase reflects not only the nature of the plasminogen activator complex but also the function of the proteinase inhibitors.

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TREPTOKINASE is a bacterial protein that binds human plasminogen rapidly and with high affinity, forming a stoichiometric complex of 1:1. The bound plasminogen undergoes conformational change that involves the active site so that the substrate specificity of streptokinase-plasminogen complex is distinct from that of plasmin. Streptokinase-plasminogen (SkPI) rapidly activate human plasminogen by catalyzing the hydrolysis of a specific Arg-Val peptide bond. This reaction is species specific since plasminogens from many species including mouse are not activated by streptokinase unless human plasminogen is present to form SkPI. Solution components influence the rate of reaction of streptokinase-plasminogen complex with plasminogen. Fibrinogen, fibrin monomer, and fibrinogen fragment D enhance the reaction, whereas chloride has an adverse effect. Naturally occurring mammalian extracellular proteinases are closely regulated by plasma proteinase inhibitors. The use of streptokinase as a thrombolytic agent generated considerable interest in the regulation of SkPI since the complex is an unnatural proteinase in the circulation. Initial attention focused on $\alpha_2$-antiplasmin ($\alpha_2$AP), the principal inhibitor of plasmin in the plasma. In vitro, the reaction of $\alpha_2$AP with SkPI was slow compared with the reaction of $\alpha_2$AP with plasmin. At 25°C, the second-order rate constant was decreased by a factor of 10. In similar in vitro studies, $\alpha_2$-macroglobulin ($\alpha_2$M), the second major plasmin inhibitor, also failed to react significantly with SkPI. This was in marked contrast to the results obtained when the regulation of human SkPI was studied in vivo in the mouse. Murine plasma possesses an $\alpha_2$AP and an $\alpha_2$M that are homologous with the human proteins. When human SkPI was injected into the murine circulation, most of the complex reacted rapidly with $\alpha_2$M and was cleared from the plasma within ten minutes. The discrepancy between this in vivo study and previous in vitro studies was partially explained by the large temperature coefficient for the reaction of SkPI with human $\alpha_2$M. At 37°C, the rate of reaction was greatly increased as compared with 4 or 25°C, the temperatures at which previous studies had been performed. Nevertheless, the in vivo reaction was still significantly faster than what was predicted based on in vitro results. In all the investigations cited above, dissociation of SkPI either was not detected or was considered negligible in the interpretation of the data.

This study was undertaken to explore possible reasons for the enhanced reaction of human SkPI with $\alpha_2$M in the murine circulation. The results reconcile the many previous studies by demonstrating heretofore undescribed differences in the function of the proteinase inhibitors across species line. In addition, this investigation shows that the activity of streptokinase in a given species is not only a function of ability to bind plasminogen, as has been described in detail, but a function of the reactivity of the proteinase inhibitors as well.

MATERIALS AND METHODS

Experimental Procedures

Reagents. H-D-Val-t-Leu-t-Lys-p-nitroanilide-HCl (S-2251) was purchased from Helena Laboratories (Beaumont, TX). p-Nitrophenyl-p'guanidinobenzoate (PNPGB) and N-benzoyl-DL-arginine-p-nitroanilide-HCl were from Sigma (St Louis). Na$^{123}$I was...
from Amersham (Arlington, IL), and iodobeads were from Pierce (Rockford, IL). All other reagents were of the best grade commercially available.

Proteins. Streptokinase was purified from Kabikinase (Kabi, Stockholm) by the method of Castellino et al.14 The final preparation demonstrated a single band with an apparent mol wt of 50,000 by sodium dodecyl sulfate SDS-gel electrophoresis. Plasminogen was prepared from human plasma as described by Deutsch and Mertz18 and activated with catalytic quantities of streptokinase with 4% glycerol added or with <10 CTA U urokinase. α2AP was purified from human plasma by the method of Wiman.19 Human α2M was purified as previously described23 and was free of "reacted form" as judged by native gel electrophoresis.22 Anti-urokinase antibody was a gift of Dr Salvatore Pizzo (Duke University Medical Center). Fibrinogen, L grade, was purchased from Kabi and further purified by chromatography on lysine-Sepharose. Soybean trypsin inhibitor and trypsin were from Sigma. The trypsin was 60% active as determined by active site titration.23

Activity assays. Plasmin activity after activation of purified plasminogen was determined by hydrolysis of S-2251 (0.8 mmol/L) under previously specified conditions.14 A Hewlett Packard 8450A temperature-controlled diode-array spectrophotometer was used (Hewlett Packard, Sunnydale, CA). Percentage of activity was calculated based on published steady-state kinetic parameters,21,22 using a molar extinction coefficient of 9,950 for nitroaniline at 405 nm. Plasminogen in plasma preparations was assayed by a similar method after acid precipitation to remove inhibitors.22 α2M in solutions containing purified proteins and in plasma was assayed at 22°C with S-2251 by the method of Gang et al.21 α2AP activity was determined based on the depletion of S-2251 amidase activity in plasma or solutions containing plasmin. In these studies, plasminogen was activated with urokinase. The urokinase was then inactivated with a 400-fold molar excess of antiurokinase antibody before incubation with a2AP. Urokinase was activated with urokinase. The urokinase was then activated with a 400-fold molar excess of antiurokinase antibody before incubation with a2AP. α2AP in 20 mmol/L sodium phosphate, I 50 mmol/L NaCl, pH 7.4, and incubated for 20 minutes at 37°C for variable temperatures. At various times, aliquots of the reaction mixture were removed and the SkP1 was inactivated with 0.1 mol/L PNPGB. Samples were denatured without reductant and subjected to SDS-polyacrylamide gel electrophoresis on 5% slabs using the imidazole-HEPES (pH 7.3) buffer system described by McLeiinan22 as modified by Gonias et al.20 All gels were autoradiographed, and each lane of the dried gel was sliced into 3-mm sections and counted in an LKB model 1275 Minigamma γ counter (counting efficiency for 125I > 75%). This procedure quantitates the plasmin in each gel band. Apparent second-order rate constants (kapp) were calculated using the basic equation:

\[ k_{app} = \frac{1}{[I]^0 - [E]^0} \ln \frac{[E]^0([I]^0 - [E]^0 + [E])}{[I]^0[E]} \]

with [I]^0 and [E]^0 the initial concentrations of inhibitor and enzyme, respectively, and [E] the concentration of enzyme at time t. All experiments were performed in duplicate, and the results were averaged.

Proteinase inhibitor distribution experiments. SKPI or plasmin was prepared with radiolabeled plasminogen and incubated with various combinations of purified inhibitors, plasma, or modified plasma. Components were diluted in 20 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4, and incubated for 20 minutes unless otherwise specified. The temperature was varied. Reactions were terminated with 0.1 mol/L PNPGB and analyzed using the electrophoresis-autoradiography procedure described above. All experiments were performed at least in duplicate.

RESULTS

Effect of Temperature on the Regulation of Plasmin in Human Plasma

Plasmin was incubated with variably diluted PPP at 4, 22, and 37°C. Solutions were then equilibrated at 22°C, and residual plasmin activity was determined using 0.8 mmol/L S-2251. In this system, substrate hydrolysis is catalyzed by the free proteinase and by plasmin bound to α2M at nearly equal rates; the kcat and Km values are 11.8 s\(^{-1}\) and 0.18 mmol/L for plasmin and 8.5 s\(^{-1}\) and 0.21 mmol/L for α2M-plasmin under these exact conditions.24 Figure 1 shows that when the quantity of α2AP in the PPP was sufficient to inhibit the added plasmin, amidase activity was decreased by 90% to 95%. The residual activity most likely represented plasmin bound to α2M. Temperature did not affect the distribution of the plasmin between α2AP and α2M as determined by this method.

Equivalent experiments were performed with radioiodinated plasmin. Reaction with proteinase inhibitors in PPP was studied at different temperatures using the somewhat more sensitive electrophoresis procedure (Fig 2). The relative mobilities of plasminogen, α2AP-plasmin, and α2M-plasmin are shown in the insets (Fig 2). Approximately 80% to 90% of the plasmin that reacts with α2M forms a covalent bond.24 This material distributes into two bands representing plasmin bound to two α2M subunits or four α2M subunits. A small correction factor was applied uniformly to the data to account for the fraction of noncovalent α2M-plasmin complex. At each of the temperatures studied, all of the active form characteristic of plasmin.28 This result indicates that the SkP1 studied in this investigation was uniformly streptokinase-plasmin.
invariably diluted PPP (active enzyme concentration, 75 nmol/L) was incubated with 2251. was calculated assuming that the plasma concentration of a2AP is 25°C (0) or 37°C (D). Estimated molar ratio of a2AP to plasmin l5

compete for proteinase was observed at 4°C; however, the substrate assay. A slight decrease in the ability of a,M to a2AP was uniformly predominant, as was detected by sub-

strate assay. A slight decrease in the ability of a,M to a2AP was uniformly predominant, as was detected by sub-

ister was determined with 5-

pH 7.4, for ten minutes. Incubation temperature was 4°C

/NaCl.

Temperatures.

The reaction of radioiodinated SkPl with proteinase inhibitors was studied in partially diluted PDP (Fig 3). The extent of reaction was markedly temperature dependent. At all temperatures, the major inhibitor of SkPl was a2AP. The distribution of radiolabeled proteinase between a2AP and a2M after incubation at 37°C approximated that observed in the plasmin experiments.

Platelets or whole blood cells were reconstituted in PDP as described. The radioiodinated SkPl distribution experiments were then repeated. The rate of reaction of the SkPl with the proteinase inhibitors and the fractions associated with a2AP and a2M remained unchanged.

Experiments performed with purified proteins confirmed that SkPl reacts preferentially with a2AP at 37°C (Fig 4). a2M–Proteinase complex was recovered at significant levels only when a2AP was not included in the incubation. At 4°C, most of the SkPl did not react with either proteinase inhibitor. Table 2 summarizes the data. Equivalent results were obtained when SkPl was formed with unpurified Kabikinase mixture.

Temperature-dependent dissociation of SkPl to regenerate active plasmin was considered as a possible explanation for the reaction of the enzyme with a2AP. Many previous studies indicate that dissociation does not occur.11,12 Studies of the murine system presented below strongly support non-dissociation. As additional evidence, fibrinogenolysis experiments

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>( k_{\text{app}} ) (mol/L−1·s−1)</th>
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<tbody>
<tr>
<td>37</td>
<td>( 1.5 \times 10^7 )</td>
</tr>
<tr>
<td>25</td>
<td>( 2.3 \times 10^7 )</td>
</tr>
<tr>
<td>15</td>
<td>3.4</td>
</tr>
<tr>
<td>4</td>
<td>&lt;1.0</td>
</tr>
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</table>

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Fig 1. Plasmin activity after addition to human plasma. Plasmin (active enzyme concentration, 75 nmol/L) was incubated with variably diluted PPP in 20 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4, for ten minutes. Incubation temperature was 4°C (△), 25°C (○), or 37°C (□). Estimated molar ratio of a2AP to plasmin was calculated assuming that the plasma concentration of a2AP is 1.0 μmol/L. Residual plasmin activity was determined with S-2251.

Fig 2. Covalent binding of plasmin to proteinase inhibitors in human plasma. 125I-Plasminogen was activated with 5 U urokinase for ten minutes at room temperature and then treated with antiiurokinase antibody. Activation was 47% to 58% complete. The plasmin (0.17 μmol/L) was incubated with variably diluted PPP for ten minutes at 4°C (○, △), 25°C (□, ■), and 37°C (△, △). Samples were analyzed by electrophoresis, autoradiography, and gel slicing. Closed symbols: percentages of a2AP-125I-plasmin. Open symbols: percentages of a2M-125I-plasmin. Insets a and b show autoradiography of unreacted plasmin (P1) and plasmin incubated at 37°C with PPP at an estimated molar ratio of a2AP to plasmin of 0.8, respectively.

Fig 3. Reaction of SkPl with plasma proteinase inhibitors in human plasma. SkPl (0.17 μmol/L) was formed with 125I-plasminogen and incubated with PDP diluted into 20 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4, for 30 minutes at 4, 25, and 37°C. Estimated molar ratios of a2AP to SkPl were calculated for each incubation mixture assuming that the concentration of a2AP in plasma is 1.0 μmol/L. Reactions were terminated with PNPG and analyzed by electrophoresis. Percentage of recovery of total radioactive plasmin is shown for a2AP-plasmin (○) and a2M-plasmin (△) after incubation at 37°C; a2AP-plasmin (○) and a2M-plasmin (△) after incubation at 25°C; and a2AP-plasmin (□) after incubation at 4°C.
REGULATION OF SkPI

Fig 4. Reaction of SkPI with purified human α2M and α2AP. SkPI (0.2 μmol/L) was formed with 125I-plasminogen and incubated for 30 minutes in 20 mmol/L sodium phosphate, 150 mmol/L NaCl, pH 7.4, at 37°C. Included in the incubation mixture was: (a) no other protein; (b) a threefold molar excess of α2M; (c) a threefold molar excess of α2M and a threefold molar excess of α2AP; (d) a threefold molar excess of α2M and a sixfold molar excess of α2AP; and (e) a threefold molar excess of α2M and a ninefold molar excess of α2AP. Samples were subjected to electrophoresis and analyzed by autoradiography.

Fig 5. Fibrinogenolysis by SkPI in vitro. Fibrinogen was depleted of plasminogen by chromatography on lysine-Sepharose and subjected to electrophoresis in lane a. The same fibrinogen (1.3 μmol/L) was incubated with SkPI (0.1 μmol/L) at 4°C (lane b) or 37°C (lane d) for 30 minutes. Lane C shows fibrinogen after incubation with 0.1 μmol/L plasmin. Band. Because these data are not corrected for the fraction of α2M-plasmin that lacks a covalent bond, the reaction at 37°C may have been essentially complete.

α2AP competed poorly with α2M when SkPI was added to the murine plasma. This was independent of temperature. Extraction of the plasminogen from murine plasma by chromatography on lysine-Sepharose did not affect either the extent of reaction of SkPI with the inhibitors or the distribution between α2AP and α2M. Equivalent results were also obtained when SkPI was incubated with murine PRP or whole blood.

Table 2: Reaction of SkPI With Purified Human α2AP and α2M

<table>
<thead>
<tr>
<th>Concentration (μmol/L)</th>
<th>Recovered 125I-plasmin (%)</th>
<th>Free</th>
<th>α2AP-Pl</th>
<th>α2M-Pl</th>
</tr>
</thead>
<tbody>
<tr>
<td>SkPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 0 0</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.2 0.6 0</td>
<td>75</td>
<td>0</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>0.2 0.6 0.6</td>
<td>49</td>
<td>44</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>0.2 0.6 1.2</td>
<td>48</td>
<td>48</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>0.2 0.6 1.8</td>
<td>47</td>
<td>50</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>0.2 0 0</td>
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<tr>
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<td>3</td>
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<tr>
<td>0.2 0.6 1.2</td>
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<tr>
<td>0.2 0.6 1.8</td>
<td>96</td>
<td>3</td>
<td>1</td>
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</tbody>
</table>

Incubation time 30 minutes.

Fig 6. Reaction of SkPI with proteinase inhibitors in murine plasma. SkPI was formed with 125I-human plasminogen and incubated with diluted murine plasma for 20 minutes. The concentration of SkPI was 0.1 μmol/L. The incubation temperatures and plasma dilution factors were lane b, 4°C, 1:10; lane c, 4°C, 2:5; lane d, 25°C, 1:10; lane e, 25°C, 2:5; lane f, 37°C, 1:10; lane g, 37°C, 2:5. Lane a shows purified human α2M reacted with SkPI at 37°C as a control. Reactions were analyzed by electrophoresis and autoradiography.

Regulation of SkPI by Murine Plasma Proteinase Inhibitors

When human SkPI was incubated with murine plasma, significant reaction with α2M was observed (Fig 6). Radioactive α2M-plasmin was recovered in bands with apparent mol wts of 420,000 and >700,000, which corresponded closely with the human α2M-plasmin bands. The reaction was temperature dependent; however, even at 4°C, nearly 40% of the SkPI formed complex with α2M (Table 3). At 37°C, <20% of the radioactivity was recovered in the free plasmin band. Because these data are not corrected for the fraction of α2M-plasmin that lacks a covalent bond, the reaction at 37°C may have been essentially complete.

α2AP competed poorly with α2M when SkPI was added to the murine plasma. This result was independent of temperature. Extraction of the plasminogen from murine plasma by chromatography on lysine-Sepharose did not affect either the extent of reaction of SkPI with the inhibitors or the distribution between α2AP and α2M. Equivalent results were also obtained when SkPI was incubated with murine PRP or whole blood.
Table 3. Reaction of SkPI With Proteinase Inhibitors in Murine Plasma

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Plasma Dilution Factor</th>
<th>Recovered 125I-plasmin (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Free</td>
</tr>
<tr>
<td>4</td>
<td>1:10</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>2:5</td>
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<td>1:10</td>
<td>20</td>
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<td>37</td>
<td>2:5</td>
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Concentration of SkPI was 0.1 μmol/L. All incubations were for 20 minutes.

†Values are not corrected for fraction of noncovalent α2M-plasmin.

Experiments were performed to evaluate the role of α2AP as a fibrinolysis inhibitor in murine plasma (Fig 7). Human 125I-plasminogen was activated with catalytic amounts of urokinase or streptokinase and incubated with PPP. Reaction occurred nearly exclusively with α2AP (>95%) confirming previous in vivo studies. Murine plasma was then diluted 1:2 with a solution of 400 mmol/L methylamine, 20 mmol/L Tris-HCl, titrated to pH 8.0, and incubated at room temperature for 40 minutes to inactivate the α2M. A second aliquot of plasma was treated similarly except that methylamine was omitted. SkPI reacted readily with α2M and minimally with α2AP in the control plasma. In methylation-treated plasma, significantly enhanced reaction of SkPI with α2AP was detected. These data suggest that the predominant role of murine α2M in regulation of human SkPI reflects enhanced reactivity with α2M and not decreased reactivity with α2AP.

DISCUSSION

α2AP is a member of the serpin proteinase inhibitor family that includes α1-proteinase inhibitor and antithrombin-III. The reaction of α2AP with plasmin involves at least two steps: a very rapid noncovalent association step followed by covalent bond formation. The active-site serine in plasmin is unable to deacylate after reaction with the α2AP active-site Arg-Met peptide bond.

The reaction of α2M with proteinase involves a mechanism very different from other inhibitors. Each of the four identical α2M subunits has a centrally located sequence of peptide bonds that is highly susceptible to proteolysis. Reaction of a proteinase with one of these peptide bonds proceeds unhindered through the step of deacylation; however, the cleavage causes a major conformational change in the α2M that traps the proteinase. Covalent bonds form variably between proteinase lysine groups and specialized thioester bonds in each α2M subunit. The α2M-proteinase complex is irreversible, even when no covalent bonds form.

Based on the difference in reaction mechanism, different factors evidently affect the reactivity of a proteinase with α2AP and α2M. One important consideration in α2M reactions is proteinase size. Reaction of SkPI with human α2M occurs through facilitated transfer of the plasmin from streptokinase to the inhibitor. The dissociation of the streptokinase in this reaction is probably necessary because the α2M proteinase binding sites cannot accommodate the extensive mass of the entire SkPI complex. A high-energy intermediate involving a temporarily strained SkPI–α2M tertiary complex was proposed to explain the temperature dependence of the SkPI–α2M reaction.

The importance of temperature helped reconcile results in vitro studies of the SkPI–human α2M reaction; however, the extremely fast reaction of human SkPI with murine α2M in vivo remained incompletely explained. The data we present demonstrate markedly enhanced reactivity of SkPI with murine α2M as compared with human α2M. This enhanced reactivity was evident in plasma and did not require cell-surface cofactor activity. The SkPI–murine α2M reaction was temperature dependent; however, significant levels of reaction were detected even at 4°C.

With an electrophoresis procedure, second-order rate constants were derived for the reaction of purified human α2AP with SkPI. The value derived at 25°C (23 mol/L·1 s−1) was midway between two previously reported values determined at the same temperature with a substrate assay. The kinetics experiments were performed to demonstrate the marked effect of temperature on the SkPI–α2AP reaction. The constants were calculated based on covalent product which actually forms in the second step of the reaction following noncovalent association. The previously used substrate assays did not require covalent binding to detect complex. Because the rate constants we report are not significantly lower than the previous values, formation of an SDS-stable covalent bond is not the rate-limiting step in the α2AP–SkPI reaction.
REGULATION OF SKPI

The ratio of recovered $\alpha_2$AP-125I-plasmin to $\alpha_2$M-125I-plasmin remained nearly constant when SKPI was incubated in human plasma at different temperatures. This result suggests that increased temperature enhances the rate of reaction of SKPI with both inhibitors comparably.

The reaction of SKPI with $\alpha_2$M in mouse plasma was essentially complete. The reaction of SKPI with human $\alpha_2$AP was not. This result was observed in experiments with purified inhibitors and in human plasma. The initial rates of reaction between human $\alpha_2$AP and SKPl followed the principles of second-order kinetics closely. After slightly more than 50% of the SKPl had reacted with $\alpha_2$AP, the rate of complex formation decreased. These data may reflect heterogeneity in the SKPl preparation. Such heterogeneity could result from the internal digestion of streptokinase in streptokinase-plasmin complex. The data we present suggest that murine $\alpha_2$M is unique in its ability to bridge any heterogeneity in the SKPl and react with the entire preparation.

Muninoglobulin is a proteinase inhibitor found in murine plasma but not in human plasma. Proteinases that bind to murinoglobulin retain esterase activity, a property attributed to the $\alpha$-macroglobulin class of inhibitors. The mol wt of murinoglobulin is 180,000. An SDS-stable complex of murinoglobulin with plasmin would have a maximum mol wt of 260,000. The complexes formed during incubation of SKPl with murine plasma were significantly larger than the hypothetical murinoglobulin–plasmin complex. We therefore conclude that murinoglobulin does not contribute to the regulation of SKPl in the mouse.

Plasminogens from different species differ significantly in ability to bind streptokinase and form activator complex. The SKPl complexes that do form demonstrate widely variable activity. In contrast, studies of the proteinase inhibitors, $\alpha_2$M and $\alpha_2$AP, have generally demonstrated conservation of function across species lines. The reaction of $\alpha_2$M with SKPl is an exception to the rule, perhaps because SKPl is not a natural mammalian proteinase. We conclude that the thrombolytic activity of streptokinase in a given species can reflect variability in proteinase inhibitors as well as plasminogen.

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Regulation of streptokinase-human plasmin complex by the plasma proteinase inhibitors alpha 2-antiplasmin and alpha 2-macroglobulin is species specific and temperature dependent

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