A Comparison of the Urokinase and Streptokinase Activation Properties of the Native and Lower Molecular Weight Forms of Sheep Plasminogen*

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Native sheep plasminogen (SPg-a), of molecular weight 80,000 to 90,000, in the presence of sheep plasminogen (SPm), is rapidly and specifically degraded to a plasminogen (SPg-b), of molecular weight 80,000 to 82,000, by loss of a peptide(s) from the NH₂ terminus of SPg-a. More extensive treatment of SPg-b with SPm results in further loss of a single peptide (P) of molecular weight 29,000 to 32,000 from the NH₂ terminus of SPg-b, yielding a much lower molecular weight (50,000 to 52,000) plasminogen (SPg-c) which is fully activatable to SPm.

The two affinity chromatography forms of SPg-a (Paoni, N., Violand, B. N., and Castellino, F. J. (1977) J. Biol. Chem. 252, 7725-7732) are activated to SPm by urokinase at approximately the same rate and to the same extent. Although SPg-b is activated to SPm in a manner similar to that of SPg-a, SPg-c free of P appears to be activated significantly more rapidly by urokinase when compared to SPg-b and SPg-a. Addition of P to SPg-c restores the SPg-b and SPg-a activation rates to SPg-c. We show SPg-a, SPg-b, and SPg-c to be insensitive to activation to SPm by streptokinase. However, all sheep plasminogen forms are fully activated by catalytic levels of a 1:1 molar complex of streptokinase and human plasmin. A major reason for the insensitivity of SPg-a, SPg-b, and SPg-c to streptokinase activation results from the rapid degradation of streptokinase to inactive fragments by small amounts of SPm initially formed in the activation.

Plasminogen is the inactive form of the proteolytic enzyme plasmin and has been found in the plasma of all mammalian species tested to date. This protein can be readily purified from any species by affinity chromatography (1, 2) and detailed analyses of the properties of plasminogen have been forwarded for the rabbit (3-8), human (2, 8-13), and sheep (14) systems. There are many similarities in the plasminogens derived from several species. It has been established that a high degree of multiplicity exists in human, rabbit, and sheep plasminogen. At least two forms of this protein can be resolved by affinity chromatography (2, 14) and each major form consists of several subforms (3, 13, 14). In addition, human, rabbit, and sheep plasminogen undergo dramatic alterations in conformation as a consequence of their binding small molecules of the 6-Ahx¹ class (2, 8, 14, 15).

Notable differences also exist between the various plasminogens. The ability of the bacterial protein streptokinase to activate plasminogen differs from species to species. Human plasminogen is highly sensitive, while rabbit plasminogen is only weakly sensitive to activation by this agent (16-18). The observation that sheep eglobulin, when treated with crude streptokinase, did not develop further proteolytic activity suggested that sheep plasminogen was not activated by streptokinase (16). The above species of plasminogen also differ in the nature of the products obtained as a result of plasminolysis. It has been previously shown that plasminolysis of human (19) and rabbit (20-22) plasminogen leads to loss of an Mₜ = 6,000 to 8,000 peptide from the NH₂ terminus of the native plasminogen molecule. The remaining plasminogen is an altered, lower molecular weight form of the native molecule. We have previously shown that an analogous reaction takes place in the sheep system (14). Treatment of native sheep plasminogen (SPg-a) with sheep plasmin (SPm) results in rapid loss of a small peptide of Mₜ = 6,000 to 8,000, yielding sheep plasminogen b (SPg-b). In addition, however, protracted treatment of SPg-b with SPm results in the loss of a second, and much larger peptide (P) of Mₜ of approximately 30,000 to 32,000. The portion of the molecule that remains is a fully activatable plasminogen (SPg-c) of Mₜ = 50,000 to 52,000.

Sheep plasminogen is pertinent to our studies on the mechanism of activation of plasminogen, since it is apparently insensitive to action of streptokinase alone. Further, the plasminolysis reaction of sheep plasminogen provides convenient natural cleavage products with which the structure-function relationships of the molecule can be examined. We have previously described the purification and physical characterization of the native and altered, lower molecular weight forms of sheep plasminogen, as well as the large peptide released from SPg-b by SPm. In this paper, we extend our studies to comparison of the urokinase and streptokinase sensitivities of the various forms of the sheep plasminogen molecule.

**EXPERIMENTAL PROCEDURES**

Proteins—SPg-a, SPg-b, SPg-c, and P were prepared as previously described (14). The only exception was that the SPg-c and P used in these studies was prepared by incubating SPg-a with urokinase-free SPm for 2.5 h, rather than 4 h. Only slightly lower yields of SPg-c and P were obtained with this modification. Urokinase was obtained from G. H. Barlow of Abbott Laboratories, and further purified as previously described (19). The starting material used for purification of

¹ The abbreviations used are: 6-Ahx, 6-aminohexanoic acid; Tos-ArgOMe, N-o-tosyl-L-arginine methyl ester; DdSO₄, sodium dodecyl sulfate; PFP, phenylphosphorofluoridate; SPg-a, native sheep plasminogen; SPm, sheep plasmin; SPg-b, sheep plasminogen b; P, peptide; SPg-c, sheep plasminogen c; CTA, Committee on Thrombolytic Agents.

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S1P-g was Kabikinase, obtained from AB Kabio. The material had as its major impurity plasma albumin, added by the manufacturers as a stabilizer. The albumin was removed as previously published (23). Urokinase-free S1Pm was prepared by treatment of S1P-g with Sepharose-urokinase (14, 21).

Quantitative NH\textsubscript{2}-terminal Amino Acid Analysis—The method used was essentially that of Stark (24). The concentration of protein taken was determined by a modification of the method of Lowry et al. (25) as described above. The NH\textsubscript{2}-terminal amino acid analyses were performed on a Beckman model 121 amino acid analyzer, using S1P-c and P samples, the phenylthiohydantoins were identified with the aid of a Beckman GC 45 gas chromatograph on a glass column (2 mm x 4 feet) packed with SP-400 (10% on Supelcoport. 100/200 mesh Supelco, Inc.).Silica thin layer chromatography, employing Solvent XM (26), was used for discriminations between Thr and Gyl, and between Thr and Pro derivatives.

S1P-g was found to strongly bind Quartus, thus making gas chromatographic analysis of the phenylthiohydantoins difficult to accomplish. Instead, the samples obtained after each cycle were hydrolyzed and the samples derived from the mixture obtained at the cyclization step.

Reduced and nonreduced DodSO\textsubscript{4}-gel electrophrograms of the time course of urokinase activation of S1P-g are shown in the text. This activation was performed at 30°C in 0.05 M Tris-HCL, 0.1 M L-lysine, pH 8.0. The final concentration of S1P-g was 3.07 mg/ml, and the final concentration of urokinase was 649 CTA units/ml. At the times indicated, 0.01 ml of the activation mixture was added to 0.05 ml of 0.1% DodSO\textsubscript{4}, 0.01 M phosphate, 6 M urea, pH 7.0, for nonreduced samples, or 0.05 M mercaptoethanol for reduced samples. The samples were analyzed by DodSO\textsubscript{4}-gel electrophoresis, 5% in polyacrylamide and 6 M in urea.

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The molar ratio of human plasminogen-streptokinase was equal to 1:4:1, and the final lysine concentration was 0.08 M. The mixture was incubated at 22°C for 5 min, after which 0.01 ml was withdrawn and added to 0.538 mg of SPg-a (affinity chromatography form 2) dissolved in 0.14 ml of 0.05 M Tris-HCl, 0.1 M L-lysine, pH 8.0. The activation was performed at 30°C in 0.05 M Tris-HCl, 0.1 M L-lysine, pH 8.0. The final SPg-a concentration was equal to 41.3 nmol/ml (3.59 mg/ml), and the molar ratio of SPg-a:human activator was equal to 17.4:1. The time course of the activation was monitored by potentiometric techniques using the synthetic substrate TosArgOMe. Controls for the experiment included a human activator blank, to determine the amount of plasmin activity contributed by the activator, and individual mixtures of human plasminogen with SPg-a, and streptokinase with SPg-a.

The human activator activation of SPg-c (affinity chromatography form 2) was performed as described above, except that the human plasminogen and streptokinase mixture was incubated at 22°C for 10 min, and the final concentration of SPg-c in the activation mixture was 50.7 nmol/ml (2.59 mg/ml).

Results

The urokinase activation of the two major affinity chromatography forms of SPg-a is shown in Fig. 1. Both form 1 and form 2 SPg-a are activated at the same rate, and to the same extent, by urokinase. The time course of the reaction was followed by DodSO₄-gel electrophoresis in the presence and absence of reducing agent (Fig. 2, A and B, respectively). The results shown for affinity form 2 SPg-a indicate that the final plasmin formed has a much lower molecular weight than that reported for human and rabbit plasmin. The heavy and light chains of human and rabbit plasmin have molecular weights of approximately 60,000 and 24,000, respectively. The molecular weights of the heavy and light chains of SPm have been determined by calibrated DodSO₄-gel electrophoresis. The SPm heavy chain has a molecular weight of 29,000 to 32,000 and the light chain has a molecular weight of 23,000 to 26,000. The molecular weights of the SPm-component chains are similar to those previously reported for bovine plasmin (heavy chain, Mₗ = 35,000; light chain, Mᵢ = 23,500 (30)).

SPg-a affinity chromatography form 2 was incubated with various amounts of streptokinase and the results are shown in Fig. 3. Little or no plasmin activity was detected when SPg-a was incubated with streptokinase at molar ratios of SPg-a:streptokinase equal to 8:6:1, 5:1, 2:6:1, or 1:1. However, plasmin activity was rapidly generated when SPg-a was incubated with a human plasminogen-streptokinase activator complex at a molar ratio of SPg-a to activator equal to 17:4:1.

SPg-a and streptokinase were mixed in equal molar quantities and the time course of the reaction was analyzed under reducing conditions by DodSO₄-gel electrophoresis (Fig. 4). The gels show that the streptokinase in the activation mixture was rapidly degraded. No change in the migration of the SPg-a or streptokinase bands were observed when the active site titrant p-nitrophenyl-p'-guanidinobenzoate was present in the incubation mixture (data not shown). Examination of the 120-min sample of Fig. 4 reveals significant amounts of SPg-c and P formed during the incubation, at times beyond the stability limit of streptokinase, or streptokinase fragments, as evidenced by their disappearance from the gel profiles. This indicates that a small amount of plasmin was likely produced by the reaction, subsequently degrading SPg-a to these peptides (14).

The time course of stability of streptokinase in the presence of SPm was next determined. Streptokinase was incubated with urokinase-free SPm in a molar ratio of streptokinase:plasmin equal to 25:1 (Fig. 5). The streptokinase was rapidly degraded. No change in the migration of the SPg-a or streptokinase bands were observed when the active site titrant p-nitrophenyl-p'-guanidinobenzoate was present in the incubation mixture (data not shown). Examination of the 120-min sample of Fig. 4 reveals significant amounts of SPg-c and P formed during the incubation, at times beyond the stability limit of streptokinase, or streptokinase fragments, as evidenced by their disappearance from the gel profiles. This indicates that a small amount of plasmin was likely produced by the reaction, subsequently degrading SPg-a to these peptides (14).

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The nature of the streptokinase fragmentation is qualitatively similar to those produced during the incubation of SPg-a with streptokinase. The nature of the streptokinase fragmentation is qualitatively consistent with previous studies employing human and rabbit plasmin (18, 31, 32) for this purpose, although the stability of the various fragments is different in all three systems.

The NH₂-terminal amino acid sequences of SPg-a, SPg-b, SPg-c, and P derived from affinity chromatography form 2 are shown in Table I. SPg-b was found to be resolved by routine gas chromatographic analysis. Instead, the samples were hydrolyzed as described under "Experimental Procedures," and the residues were identified by amino acid analysis on a Beckman model 121 amino acid analyzer. Clearly, the NH₂ terminus of the native sheep plasminogen molecule is lost during the formation of SPg-b. Furthermore, the same NH₂-terminal amino acid sequence found on SPg-b is also present on the large peptide released during extended treatment of SPg-a with plasmin. This indicates that P originates as the NH₂ terminus of SPg-b.

Quantitative NH₂-terminal analysis (mol/mol sample)

| Sequence no. | SPg-a | SPg-b | P | SPg-c |
|--------------|-------|-------|---|-------|
| 1            | Asp   | Ser   | Met |
| 2            | Leu   | Ile   | Asp |
| 3            | Leu   | Tyr   | Tyr |
| 4            | Asp   | Leu   | Ser |
| 5            | Asp   | Ser   | Val |
| 6            | Tyr   | Glu   | Gly |
| 7            | Val   | Ser   | Ala |
| 8            | Asn   | Lys   | Gly |
| 9            | Gly   | Ile   | Thr |
| 10           | Gly   | Gly   | Pro |

Quantitative NH₂-terminal analysis (mol/mol sample)

|        |        | Aasp 0.80 | n.d.  | n.d. | Met 0.79 |

* n.d., not determined.
Fig. 6. A, relative rate of activation of affinity form 1 SPg-a and SPg-b to plasmin by urokinase. Affinity chromatography form 1 SPg-a \( \bullet \bullet \bullet \) and SPg-b \( \bigcirc \bigcirc \) plus urokinase, as described in the text. Activity is expressed as micromoles of TosArgOMe cleaved per min per nmol of protein. B, relative rate of activation of affinity form 2 SPg-a and SPg-b to plasmin by urokinase. As described in A, except that affinity chromatography form 2 plasminogens were used.

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Fig. 7. Relative rate of activation of SPg-c, P, and SPg-c + P to plasmin by urokinase. Shown are the urokinase-mediated activation rates of affinity chromatography form 2 SPg-c \( \bigcirc \bigcirc \), P \( \bigtriangleup \), and SPg-c + P \( \bullet \). Activities are expressed as micromoles of TosArgOMe cleaved per min per nmol of protein added to the pH 8.5 Tris buffer. For comparison, the activation rate of SPg-a, under similar conditions \( \bullet \bullet \bullet \), is also indicated.

large peptide. SPg-c was activated with urokinase until maximum plasmin activity was reached. The plasmin produced was then separately mixed with a 3-fold molar excess of P and buffer alone. The results showed that little or no change in plasmin activity occurred.

DodSO4 gel electrophoresis under reducing conditions of the plasmin produced by partial activations of SPg-a, SPg-b, and SPg-c showed electrophoretically similar plasmin upon activation by urokinase, while urokinase does not affect the mobility of P.

While SPg-c is insensitive to activation by streptokinase alone, it is rapidly activated by small amounts of a mixture of human plasmin and streptokinase (Fig. 9). In this regard, SPg-c closely resembles SPg-a (Fig. 3).

DISCUSSION

Sheep plasminogen has previously been reported to be insensitive to activation by the bacterial protein streptokinase. Wulf and Mertz (16), using crude sheep plasminogen preparations, reported that this zymogen could not be activated by crude streptokinase at any level of streptokinase tested. The streptokinase sensitivity of sheep plasminogen was also examined in this present study utilizing highly purified sheep plasminogen and streptokinase preparations. Streptokinase was incubated with SPg-a, at molar ratios of streptokinase: SPg-a up to and including 1:1 (Fig. 3). In each case, little or no plasmin activity could be detected in the activation mixtures. Similar results have been obtained for 1:1 mixtures of SPg-b, and SPg-c with streptokinase. DodSO4 electrophoretic analysis of this process reveals that in 1:1 mixtures of SPg-a, SPg-b, and SPg-c with streptokinase, the streptokinase is rapidly degraded to fragments (SK-3 and SK-4) which are inactive, when formed in a complex with human plasmin, in sheep plasminogen activation (32). It is seen, however, in Fig. 4, upon examination of the longer incubation time, that significant amounts of SPg-c and P are formed long after the streptokinase has disappeared from the gels. Since SPg-c and P are readily produced from SPm, by small amounts of SPm, these results indicate that some small amount of SPm is likely produced during the reaction. Fig. 5 shows the effect of urokinase-free SPm on streptokinase. Here, low levels of SPm rapidly degrade the streptokinase to fragments electrophoretically similar to those produced upon incubation of SPg-a, SPg-b, and SPg-c with streptokinase. Thus, the inability of streptokinase to activate sheep plasminogen is contributed to by the extreme instability of streptokinase in the presence of initial small quantities of SPm formed. Concomitant with the small amounts of SPm formed, rapid degradation of the streptokinase to lower molecular weight fragments (SK-3 and SK-4), proceeds further SPm formation (32). The instability of streptokinase in the presence of SPm is apparently not pronounced when formed in a complex with human plasmin. The results given in Fig. 3 show that low levels of a complex of streptokinase and human plasmin can rapidly activate sheep plasminogen.

A significant observation in the sheep plasminogen system deals with the existence of significantly smaller plasminogen intermediates, produced by plasminolysis of SPg-a. Sheep plasmin rapidly cleaves a small peptide, \( M_r = 6,000 \) to \( 8,000 \), from SPg-a, forming an altered and somewhat lower molecular weight form of the molecule, SPg-b. Continued exposure of SPg-b to SPm results in the loss of a second and much larger peptide (P), \( M_r = 30,000 \), from SPg-b. The portion of the molecule that remains, SPg-c, possesses an approximate \( M_r = 51,000 \). In the conversion from SPg-a to SPg-c, more than 40% of the molecular weight of the SPg-a molecule is lost.

Urokinase activations of the various forms of the sheep plasminogen molecule were performed to assess what affect the loss of a large portion of the NH2 terminus had on the urokinase-mediated activation of sheep plasminogen. While SPg-c possesses a substantially smaller molecular weight than SPg-a, it remains a fully activatable form of the sheep plasminogen molecule. The large \( M_r = 50,000 \) peptide P, cleaved during the formation of SPg-c, appears to be significant in describing the mechanism of activation of sheep plasminogen by urokinase. SPg-c activates significantly faster than SPg-a or SPg-b. The addition of P to the SPg-c activation mixture, however, restores the SPg-a and SPg-b activation rate. The exact process by which the large peptide modifies the activation rate of SPg-c is uncertain. One possible mechanism would be through a noncovalent interaction with SPg-c, which may restore the SPg-a and SPg-b conformation.

It should be pointed out that the urokinase activations of the native and lower molecular weight forms of sheep plasminogen were performed in the presence of 0.1 M L-lysine.
This was necessary since the SPm formed was extremely insoluble in all solvents which did not contain L-lysine or 6-AHx. Lysine and 6-AHx are known to cause a gross conformational change in SPg-a, a process which greatly facilitates its activation by urokinase (12, 15, 33, 34). In comparison, SPg-b and SPg-c do not exhibit the dramatic decrease in SPm, in the presence of 6-AHx which is characteristic of the gross conformational alteration of SPg-a in the presence of this agent (14). While the studies described above represent a necessary comparison of the activation rates of the various forms of sheep plasminogen in 0.1 M L-lysine, similar results may not be obtained in the absence of this amino acid.

DodSO₄-gel electrophoretic analysis of the plasmins produced by urokinase activation of SPg-a, SPg-b, and SPg-c (Fig. 8) reveals that all of the plasmins are electrophoretically similar in nature. We have previously shown that the plasmin derived from SPg-c possesses fibrinolytic activity (35). Thus, it appears that a large portion of the NH₂ terminus of SPg-a is not necessary for the cleavage of fibrin.

The streptokinase sensitivities of SPg-b and SPg-c were also tested by separately incubating each altered form of the plasminogen molecule with an equimolar amount of streptokinase. In each case, the streptokinase was rapidly cleaved to plasminogen molecule with an equimolar amount of streptokinase and human plasminogen. Here, SPg-c was incubated with a 1:1 m/m complex (preformed) of streptokinase and human plasmin. The molar ratio of SPg-c to the complex was 17.4:1. The time course of the formation of SPm activity was monitored by TosArgOMe hydrolysis, as described in the text. Activities are expressed as micromoles of TosArgOMe cleaved per min per nmol of SPg-c originally present.

is totally speculative, it is not without precedent for peptides released in other humoral systems. Peptides released during the activation of the third and fifth components of the complement system have dramatic biological activities. Each causes contraction of smooth muscle; release of histamine from mast cells; and directed, chemotactic migration of polymorphonuclear leukocytes (36).

Finally, the comparison of the activation properties of the native and lower molecular weight forms of sheep plasminogen may be particularly valuable in light of recent preliminary observations by Yecies and Kaplan (37), suggesting that lower molecular weight forms of the plasminogen molecule exist in human plasma.

REFERENCES

1. Deutsch, D., and Mertz, E. T. (1970) Science 170, 1095-1096
2. Brockway, W. J., and Castellino, F. J. (1972) Arch. Biochem. Biophys. 151, 194-199
3. Sodetz, J. M., Brockway, W. J., and Castellino, F. J. (1972) Biochemistry 11, 4451-4457
4. Castellino, F. J., Sodetz, J. M., Jr., Sodetz, J. M., and Bretthauer, R. K. (1973) Biochem. Biophys. Res. Commun. 53, 845-851
5. Siefring, E. J., and Castellino, F. J. (1974) J. Biol. Chem. 249, 1434-1438
6. Siefring, E. J., Jr., and Castellino, F. J. (1974) J. Biol. Chem. 249, 7742-7746
7. Castellino, F. J., Sodetz, J. M., and Siefring, E. J., Jr. (1975) in Enzymes I. Molecular Structure (Market, C. L., ed) pp. 245-258, Academic Press, New York
8. Castellino, F. J., Brockway, W. J., Thomas, J. K., Liao, H. T., and Rawitch, A. B. (1973) Biochemistry 12, 2787-2791
9. Summaria, L., Arzadon, L., Bernabe, P., and Robbins, K. C. (1972) J. Biol. Chem. 247, 4691-4696
10. Robbins, K. C., Bernabe, P., Arzadon, L., and Summaria, L. (1972) J. Biol. Chem. 247, 4757-4762
11. Summaria, L., Arzadon, L., Bernabe, P., Robbins, K. C., and Barlow, G. H. (1973) J. Biol. Chem. 248, 2984-2991
12. Sjoholm, I., Wiman, B., and Wallen, P. (1973) Eur. J. Biochem. 39, 471-478
13. Summaria, L., Spitz, F., Arzadon, L., Borezha, I., and Robbins, K. C. (1975) J. Biol. Chem. 251, 3693-3699
14. Paoni, N. F., Violand, B. N., and Castellino, F. J. (1975) J. Biol. Chem. 252, 7725-7732
15. Violand, B. N., Sodetz, J. M., and Castellino, F. J. (1975) Arch. Biochem. Biophys. 170, 390-395
16. Wulf, H. J., and Mertz, E. T. (1969) Can. J. Biochem. 47, 927-933
17. Schick, L. A., and Castellino, F. J. (1973) Biochemistry 12, 4315-4321

Fig. 8. DodSO₄-gel electrophoretograms produced under reducing conditions of the plasmins produced by the urokinase-mediated activation of SPg-a, SPg-b, and SPg-c. Gel 1, SPg-a; Gel 2, partial activation of SPg-a; Gel 3, SPg-b; Gel 4, partial activation of SPg-b; Gel 5, SPg-c; Gel 6, partial activation of SPg-c. Also shown are P (Gel 7), and P + urokinase (Gel 8). H and L refer to the heavy and light chains of SPm, respectively. Other abbreviations are as used previously.

Fig. 9. Activation of SPg-c by a mixture of streptokinase and human plasmin. Here, SPg-c was incubated with a 1:1 m/m complex (preformed) of streptokinase and human plasmin. The molar ratio of SPg-c to the complex was 17.4:1. The time course of the formation of SPm activity was monitored by TosArgOMe hydrolysis, as described in the text. Activities are expressed as micromoles of TosArgOMe cleaved per min per nmol of SPg-c originally present.
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18. Summaria, L., Arzadon, L., Banabe, P., and Robbins, K. C. (1974) J. Biol. Chem. 249, 4760–4769
19. Violand, B. N., and Castellino, F. J. (1976) J. Biol. Chem. 251, 3906–3912
20. Sodetz, J. M., Brockway, W. J., Mann, K. G., and Castellino, F. J. (1974) Biochem. Biophys. Res. Commun. 60, 729–736
21. Sodetz, J. M., and Castellino, F. J. (1975) J. Biol. Chem. 250, 3041–3049
22. Sodetz, J. M., and Castellino, F. J. (1975) in Cold Spring Harbor Conferences on Cell Proliferation (Reich, E., Rifkin, D. B., and Shaw, E., eds) Vol. 2, pp. 311–324, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.
23. Castellino, F. J., Sodetz, J. M., Brockway, W. J., and Siefring, G. E. (1975) Methods Enzymol. 45, 244–257
24. Stark, G. L. (1967) Methods Enzymol. 11, 125–138
25. Hermodson, M. A., Ericsson, L. H., Titani, K., Neurath, H., and Walsh, K. A. (1972) Biochemistry 11, 4493–4502
26. Inagami, T., and Murakami, K. (1972) Anal. Biochem. 47, 501–504
27. Weber, K., and Osborn, M. (1969) J. Biol. Chem. 244, 4405–4412
28. Robbins, K. C., Summaria, L., Elwyn, D., and Barlow, G. H. (1965) J. Biol. Chem. 240, 541–550
29. Taylor, F. B., and Tumor, R. H. (1970) Methods Enzymol. 19, 807–821
30. Summaria, L., Arzadon, L., Bernabe, P., and Robbins, K. C. (1973) J. Biol. Chem. 248, 6022–6027
31. Brockway, W. J., and Castellino, F. J. (1974) Biochemistry 13, 2063–2070
32. Siefring, G. E., and Castellino, F. J. (1975) J. Biol. Chem. 250, 3913–3920
33. Brockway, W. J., and Castellino, F. J. (1971) J. Biol. Chem. 246, 4641–4647
34. Claey, H., and Vermill, J. (1974) Biochim. Biophys. Acta 342, 351–359
35. Paoni, N. F., and Castellino, F. J. (1975) Biochem. Biophys. Res. Commun. 65, 757–764
36. Muller-Eberhard, H. J. (1975) Annu. Rev. Biochem. 44, 697–727
37. Yecies, L. D., and Kaplan, A. P. (1975) Fed. Proc. 34, 874
A comparison of the urokinase and streptokinase activation properties of the native and lower molecular weight forms of sheep plasminogen.
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