The serine protease tissue-type plasminogen activator (t-PA) initiates the fibrinolytic protease cascade and plays a significant role in motor learning, memory, and neuronal cell death induced by excitotoxin and ischemia. In the fibrinolytic system, the serpin PAI-1 negatively regulates the enzymatic activity of both single-chain and two-chain t-PA (sct-PA and tct-PA). In the central nervous system, neuroserpin (NSP) is a serpin thought to regulate t-PA enzymatic activity. We report that although both sct-PA and tct-PA rapidly form acyl-enzyme complexes with NSP in vitro, the interactions are short-lived, rapidly progressing to complete cleavage of NSP and regeneration of fully active enzyme. All NSP molecules appear to transit through the detectable acyl-enzyme intermediate and progress to completion of cleavage; no subpopulation that functions as a pure substrate was detected. Likewise, all molecules were reactive, with no evidence of a latent subpopulation. The interactions between NSP and t-PA were distinct from those between plasmin and NSP, wherein the same peptide bond was cleaved but there was no evidence of a detectable plasmin-NSP acyl-enzyme complex. The interactions between t-PA and NSP contrast with the formation of long-lived, physiologically irreversible acyl enzyme complexes between t-PA and PAI-1, suggesting that the physiologic effect of t-PA-NSP interactions may be more complex than previously thought.

Tissue-type plasminogen activator (t-PA) is one of two mammalian serine proteases that activate plasminogen to plasmin. Unlike thezymogen form of most serine proteases, the single-chain form of t-PA (sct-PA) retains roughly 10–25% of the enzymatic activity of the mature two-chain form (tct-PA) (1–3). Because both sct-PA and tct-PA are active enzymes, inhibition of t-PA activity, rather than maintenance of the protease inzymogen form, is necessary to prevent undesired proteolysis. In plasma, the physiologic or cognate inhibitor of t-PA appears to be the serine protease inhibitor (serpin) plasminogen activator inhibitor type-1 (PAI-1) (4).

In addition to its role in maintaining vascular hemostasis, t-PA is a key extravascular protease in the central and peripheral nervous systems. t-PA can be detected on the axonal growth cone of developing neuroblasts (5) and is involved with motor learning and memory (6). Studies in t-PA null mice have shown that t-PA enzymatic activity mediates neuronal cell death associated with both seizure kindling and ischemia and is involved with myelinated nerve regeneration following nerve crush injury (7–9).

To date, the mechanism by which t-PA activity in the nervous system is regulated is unknown, although several studies have suggested that the recently described serpin, neuroserpin (NSP), may serve this function (10, 11). NSP is an ~45-kDa glycosylated serpin that is expressed almost exclusively in the nervous system throughout embryologic development and adulthood (12). NSP inhibits the enzymatic activity of t-PA in vitro (10), and pharmacologic administration of NSP to rats that have undergone middle cerebral artery occlusion decreases the volume of the ischemic penumbral area concomitant with inhibition of t-PA enzymatic activity (13). Also, transgenic mice that overexpress NSP show a decreased volume of neuronal cell death following middle cerebral artery occlusion compared with their wild type counterparts (14). It is presumed that NSP inhibits t-PA in a manner analogous to PAI-1 to mediate these effects.

Serpins inhibit the enzymatic activity of their cognate serine proteases via initial formation of a Michaelis complex in which the enzyme is reversibly bound and inhibited by the serpin (15). The protease initiates cleavage of the scissile peptide bond between the P1 and P1′ residues of the reactive center loop, which leads to the formation of an acyl-enzyme intermediate, with the active site serine covalently bound to the P1 residue (16, 17). However, presumably before this intermediate can be hydrolyzed to yield cleaved serpin and active protease (i.e. completion of the cleavage reaction), the protease, bound to the P1 residue of the serpin, is translocated to the opposite pole of the serpin (18). The tertiary structure of the translocated protease active site is sufficiently disrupted to prevent hydrolysis of the acyl-enzyme complex (19), making the rate of deacylation extremely slow (i.e. weeks). The complexes display new molecular determinants that allow for cellular internalization and degradation in a matter of minutes (20). Therefore, protease-serpin acyl-enzyme complex formation can usually be viewed as biologically irreversible.

The work presented here demonstrates that the interaction of NSP with its putative cognate protease, t-PA, differs from the currently held paradigm in that the acyl-enzyme intermediates between t-PA and NSP are much less stable than other...
cognate protease-serpin complexes. Indeed, t-PA seems to handle NSP more like a substrate than a suicide inhibitor, with the acyl-enzyme complex being a detectable intermediate. These findings may provide insights into the physiologic interaction between NSP and t-PA, which may differ from certain functions inferred from studies with pharmacological concentrations of NSP in animal models.

MATERIALS AND METHODS

Proteins and Reagents—Sct-PA (greater than 95% single-chain t-PA) isolated from Bowes melanoma cells was purchased from Biopool and from Calbiochem. Tct-PA was generated by treating sct-PA with plasmin linked to Sepharose beads (plasmin-Sepharose) at 37 °C for times determined in preliminary experiments, to yield complete cleavage of sct-PA by that batch of plasmin-Sepharose as determined by silver staining of SDS-polyacrylamide gels run under reducing conditions. Human scu-PA was a generous gift from Dr. Jack Henkin of Abbott Laboratories. Human tcu-PA (Winkinase) was obtained from Dr. Gene Murano, Monsanto, St. Louis, MO. The cDNA for human NSP was obtained from Human Genome Sciences, Rockville, MD. Recombinant human NSP was expressed and purified from a baculovirus-based system. PAI-1 (14-1b mutant) was expressed and purified from Escherichia coli as described previously (10, 21) and was ~50% active as measured by stable inhibition of t-PA. Polyclonal antibodies against NSP were generated in rabbits (10). Plasminogen was radiiodinated as described previously (22, 23). Plasmin, aprotinin, and spectrozyme t-PA were purchased from Amer- icon Diagnostica, Greenwich, CT. Bovine serum albumin, obtained from Sigma, was treated with 100 μM diisopropyl fluorophosphate to inhibit potentially contaminating serine proteases, followed by exhaustive dia- lysis. Horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody was purchased from Pierce. Kodak 1-D Image software was used to digitize and quantify bands on autoradiograms and Western blots. All electrophoresis and protein transfers were carried out in the Bio-Rad Mini-protein 3 system. Electrophoresis reagents and pre- parations were purchased from Bio-Rad.

SDS-Polyacrylamide Electrophoresis and Western Blotting—10% polyacrylamide gels with a 3% stacking gel or precast 8–16% gels with a 4% stacking gel were used as noted in each figure legend. The gradient gel system was selected for optimal separation of 40- and 45-kDa NSP species but does not allow for optimal detection of NSP-PA acyl-enzyme complexes. A 10% separating gel was used for experiments in which optimal detection of NSP-t-PA acyl-enzyme complexes was necessary. NSP was detected immunologically after electrophoresis by electroblotting proteins from the gel to polyvinylidene difluoride membrane for 1 h at 100 V (24, 25). Membranes were then blocked with Tris-buffered saline, 0.01 M Tris, 0.15 M NaCl, pH 7.2, containing 0.05% Tween and 0.25% gelatin and then blotted with a 1:5000 dilution of horseradish peroxidase-conjugated goat anti- body against rabbit IgG in the blocking buffer. Membranes were then probed with a 1:5000 dilution of anti-NSP antibody followed by blotting with a 1:5000 dilution of horseradish peroxidase-conjugated goat antibody against rabbit IgG in the blocking buffer. Membranes were then rinsed several times in Tris-buffered saline with 0.05% Tween and subsequently treated with Pierce West Super Pico Enhanced Chemilu- minescence Reagent, then exposed to film.

Inhibition of t-PA by NSP—Assays to monitor the cleavage of 125I-plasminogen were performed as described previously (26). Plasminogen activation was quantified by measuring the plasmin light chain band (26). The percentage of t-PA activity remaining for each concentration of inhibitor was determined by the formula,

\[
\text{% t-PA activity} = \frac{100 \times (\text{density of plasmin light chain in reaction with inhibitor})}{\text{density of plasmin light chain in reaction with uninhibited t-PA)}
\]  

(Eq. 1)

Assays of t-PA activity usingchromogenic substrates were performed in Tris-imidazole buffer, ionic strength 0.3, pH 8.4, at room tempera- ture. Bovine serum albumin (145–150 nmol each) was incubated for the indi- cated times followed by the addition of spectrozyme t-PA at a final concentration of 500 μM. The kinetics program on a Beckman DU-640 spectrophotometer was used at 1 reading/s at 440 nm for 1 min. Measurements were normalized to exclude substrate autohydrolysis (meas-
characteristic of acyl-enzyme complex formation (high $M_r$ band). However, at subsequent time points the intensity of the acyl-enzyme band decreased, and coincident with this an $\sim$40-kDa band was observed, suggesting that NSP in complex with t-PA was undergoing completion of the cleavage reaction. Both the appearance of acyl-enzyme complexes and their presumed hydrolysis occurred much more rapidly with two-chain than with single-chain t-PA, consistent with the greater catalytic efficiency of tct-PA in solution. These results suggest that, similar to PAI-1, NSP inhibits t-PA via acyl-enzyme complex formation (inhibition detected in assay over short time). However, in contrast to t-PA/PAI-1, t-PA/NSP acyl-enzyme complexes are relatively unstable, undergoing deacylation in a matter of minutes (assays requiring longer time courses do not detect this transient inhibition). These data suggest that the acyl-enzyme complex between either form of t-PA and NSP might be characterized as a "stutter-step," e.g., a detectable intermediate in the process of peptide bond cleavage by the protease. An interesting structural implication is that the deformation of the active site that accompanies rearrangement of protease-serpin pairs may not be as extensive for t-PA/NSP as for other protease-serpin pairs.

To determine whether such transient acyl-enzyme complexes are a property of NSP interactions with any protease, reactions between plasmin and NSP and between u-PA and NSP were examined. Fig. 3 demonstrates that plasmin efficiently cleaves NSP with no detection of plasmin-NSP complexes. Fig. 4 demonstrates that scu-PA yields neither detectable complex formation with nor cleavage of NSP; whereas tctu-PA cleaves NSP with a trace of transient acyl-enzyme complexes detected on overexposed autoradiograms (not shown). Hence, the existence of a quantitatively significant acyl-enzyme intermediate seems to be a unique property of the NSP-t-PA interaction.

Examination of the amino acid sequence of the reactive center loop of NSP suggests three potential sites for cleavage by trypsin-like proteases Arg362, Arg384, and Arg393. Thus, it was conceivable that different proteases hydrolyze different peptide bonds in NSP, with the one targeted by t-PA yielding a loop of the appropriate length to allow for translocation of the protease in a transiently stable arrangement and the site targeted by...
plasmin yielding a loop that was too long for stabilization of the intermediate (28). However, for both enzymes, the target P1-
P1’ bond was determined to be Arg362-Met363 by N-terminal sequencing of the C-terminal cleavage peptide (Table II).
Hence, plasmin and t-PA both cleave the same P1-P1’ bond, but the outcome of the interaction of t-PA with NSP is a detectable albeit transient acyl-enzyme, whereas plasmin treats NSP solely as a substrate without evidence of the covalent serpin-protease complex. It is not clear whether this reflects a more rapid rate of deacylation by plasmin such that there is insufficient time for translocation (18) or whether the different surface topographies of the proteases result in a molecular alignment between NSP and plasmin that is suboptimal for the active site deformation required for a detectable acyl-enzyme complex.

To ensure that the observed transient stability of t-PA-NSP acyl-enzyme complexes was a true reflection of the protein properties and not a function of an SDS-PAGE-based assay (29, 30), a chromogenic substrate-based assay of t-PA enzymatic activity was used. Equimolar amounts of NSP and t-PA were incubated for the time indicated at 37 °C. In B, second lane, 14 nM tct-PA and 14 nM NSP were incubated for the indicated time at 37 °C for a comparison of the extent of NSP cleaved by tct-PA and by tcu-PA within the same time frame and under the same conditions. Reactions were analyzed by SDS-PAGE and immunoblotting as described under “Materials and Methods.”

Fig. 4. u-PA does not form acyl-enzyme complexes with NSP. NSP (14 nM) alone (first lane of each gel) or with scu-PA (14 nM; A) or tcu-PA (14 nM; B) was incubated for the times indicated at 37 °C. In B, second lane, 14 nM tct-PA and 14 nM NSP were incubated for the indicated time at 37 °C for a comparison of the extent of NSP cleaved by tct-PA and by tcu-PA within the same time frame and under the same conditions. Reactions were analyzed by SDS-PAGE and immunoblotting as described under “Materials and Methods.”

The data presented thus far suggest that the interactions between NSP and t-PA are more similar to cleavage of a substrate with a protracted intermediate than the biologically irreversible inhibition usually associated with serpins. If this is the case, inclusion of NSP in an assay that measures t-PA enzymatic activity over a time period that allows deacylation to occur should yield a pattern more similar to that of a substrate than to the pattern seen with PAI-1. To test this hypothesis, the inhibition profiles of scu-PA and tct-PA-mediated 125I-plasminogen cleavage by PAI-1, NSP, and a physiologic substrate, unlabeled plasminogen, were compared. The NSP inhibition profiles of both forms of t-PA are very similar to the profiles of inhibition by a competitive substrate, unlabeled plasminogen, and differ from the inhibition profiles of PAI-1 (Fig. 7). This difference is unlikely to be due solely to differences in affinities of t-PA for PAI-1 versus NSP, as the time to onset and extent of maximal protease inhibition by PAI-1 and NSP appeared to be similar in the chromogenic substrate-based assays detailed in Fig. 5.

It seems that NSP is neither a pure inhibitor nor a pure substrate of t-PA, as the rate of NSP-t-PA acyl-enzyme complex deacylation in vitro is rapid enough to make inhibition transient, at best, but slow enough to constitute a significant kinetic stutter step in the substrate behavior of NSP and therefore to interrupt proteolysis of an alternative substrate by t-PA. Such an interpretation is consistent with the protective effects seen with transgene-driven overexpression of NSP or with the administration of pharmacological concentrations of NSP into the central nervous system of mice with middle cerebral artery occlusion (13, 16).

**TABLE II**

| Residue | Plasmin digest of NSP | Tct-PA digest of NSP | NSP sequence from residue 263–273 (33) |
|---------|---------------------|---------------------|-------------------------------------|
| P1'     | Met                 | Met                 | Met                                 |
| P2'     | Ala                 | h, a                | Ala                                 |
| P3'     | Val                 | Val                 | Val                                 |
| P4'     | Leu                 | Leu                 | Leu                                 |
| P5'     | Tyr                 | Tyr                 | Tyr                                 |
| P6'     | Pro                 | Pro                 | Pro                                 |
| P7'     | Gln                 | Ser                 | Gln                                 |
| P8'     | Val                 | Val                 | Val                                 |
| P9'     | Ile                 | Ile                 | Ile                                 |
| P10'    | Val                 | Val                 | Val                                 |

**Neuroserpin-t-PA Acyl-Enzyme Complexes**

Plasmin and t-PA both cleave NSP at the same residue of the reactive center loop. NSP was incubated with either plasmin or tct-PA. The reaction products were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane, and the C-terminal peptide of NSP generated by proteolytic cleavage was subjected to Edman degradation analysis.

Figs. 2 and 5 also demonstrate that NSP cleavage by either form of t-PA proceeds via the transient acyl-enzyme intermediate, with little evidence for a NSP population that acts as a pure t-PA substrate. Slowing the reaction by carrying it out at 4 °C revealed that cleaved NSP was preceded by the appearance of acyl-enzyme complexes (Fig. 6). Hence, even under conditions that favor substrate behavior by serpins (lowering the reaction temperature fosters the substrate behavior of serpins due to slowing of protease translocation, a temperature-sensitive process, with little influence on deacylation) (31), all NSP proceeds to cleavage via the acyl-enzyme intermediate. In addition, all of the NSP is eventually cleaved, without evidence of a latent form of the serpin (Fig. 2 for tct-PA, not shown for scu-PA). Therefore, NSP appears to act as a single population of molecules that is cleaved by t-PA via transition through a detectable acyl-enzyme intermediate.

Determination of the proteolytic cleavage site in neuroserpin

Plasmin and t-PA both cleave NSP at the same residue of the reactive center loop. NSP was incubated with either plasmin or tct-PA. The reaction products were separated by SDS-PAGE and transferred to a polyvinylidene difluoride membrane, and the C-terminal peptide of NSP generated by proteolytic cleavage was subjected to Edman degradation analysis.

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Another aspect to be considered is the effect of plasmin on NSP-sensitive processes. If t-PA effect in the central nervous system is plasminogen-dependent, as much data suggest (32), the rapid and efficient cleavage of NSP by plasmin will have to be considered and balanced against the blunting of plasmin generation by NSP. Likewise, if NSP is ultimately cleaved by both t-PA and plasmin, a potential but heretofore unknown function for cleaved NSP may be part of the process.

According to its predicted structure, NSP bears a series of negatively charged residues in an alignment similar to the positively charged residues on the face of the D-helix in certain serpins that bind heparin with resultant augmented inhibitory activity (33). As such, there is the possibility that a positively charged cofactor binds NSP and augments or stabilizes its inhibitory activity. Such a cofactor was sought by combining NSP with homogenates of mouse hippocampus to allow for interaction between NSP and a potential cofactor, followed by the addition of t-PA. There was no evidence of improved effi-

**Fig. 5.** t-PA enzymatic activity is recovered with deacylation of NSP-t-PA acyl-enzyme complexes. Sct-PA (A) or tct-PA (B) and NSP or PAI-1 (all reactants were at 144 nM) were incubated at room temperature for the times noted. An aliquot of each reaction was added to the chromogenic substrate Spec-t-PA (500 μM), and the % active t-PA was determined as described under “Materials and Methods.” The fate of the NSP in reactions A and B was determined by subjecting an aliquot of each reaction to electrophoresis on an 8–16% gradient gel followed by detection of NSP antigen as described under “Materials and Methods” (C and D, respectively). Note that the 8–16% gradient gel system was used expressly to detect the 40-kDa cleaved NSP species as evidence of acyl-enzyme deacylation.

**Fig. 6.** NSP cleavage proceeds via the detectable acyl-enzyme intermediate without evidence for a population of NSP molecules that behave as a pure substrate. 14 nM NSP was incubated with 14 nM act-PA (A) or with 14 nM tct-PA (B) for the indicated times at 4 °C to slow the reaction. Samples were quenched by the addition of noreducing SDS sample buffer. Reactions were analyzed by SDS-PAGE and immunoblotting as described under “Materials and Methods.”

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**Fig. 7.** NSP interacts with t-PA with a profile more consistent with a competitive substrate than an irreversible serpin. Sct-PA and tct-PA (1.7 nM) were incubated with 42 nM 125I-plasminogen at 37 °C in the presence of the indicated concentrations of either NSP, PAI-1, or unlabeled plasminogen. The reactions were analyzed by SDS-PAGE under reducing conditions followed by autoradiography. The percentage inhibition of t-PA was determined by comparing the intensity of the 125I-plasmin light chain band to the band obtained in the reaction lacking any inhibitor as described under “Materials and Methods.”
ciency of t-PA inhibition or of stabilization of t-PA-NSP complexes (data not shown).

The data presented above raise intriguing questions about the nature of the physiologic interaction between t-PA and NSP. In processes involving other protease-serpin pairs, the acyl-enzyme complex presents molecular determinants not present on the individual reactants that allow cellular internalization of the complex, classically via the LDL receptor-related protein molecule (17). The time required for complex internalization in \textit{in vitro} experiments (33) suggests that this process may not be rapid enough to clear t-PA-NSP complexes before deacylation at 37°C. Hence, it will be of interest to determine whether t-PA-NSP acyl-enzyme complexes have a different fate than do other protease-serpin pairs. Alternatively, given that sct-PA-NSP complexes are longer lived than tct-PA-NSP complexes, NSP may serve to selectively inactivate or mediate clearance of sct-PA. At present, however, these remain speculations to be tested experimentally.

Given that stable acyl-enzyme complexes are characteristic of cognate protease-serpin pairs, with relatively unstable complexes being observed in some noncognate pairs (35), it seems reasonable to examine whether NSP is indeed a physiologic inhibitor of t-PA. Relating the data in statement (i) that families with a point mutation of NSP, resulting in \textit{in situ} polymor- 

REFERENCES

1. Boose, J. A., Kuismanen, E., Gerard, R., Sambrook, J., and Gething, M. J. Biochemistry 28, 635–643, 1987
2. Ranby, M., Bergsdorf, N., and Nilsson, T. (1982) Thromb. Res. 27, (suppl.) 175–183
3. Tachias, K., and Madison, R. L. (1997) J. Biol. Chem. 272, 28–31
4. Kruthof, E. K., Tran-Thang, C., Ransang, A., and Bachmann, F. (1984) Blood 64, 907–913
5. Pittman, R. N., Ivins, J. K., and Buettner, H. M. (1989) J. Neurosci. 9, 4269–4289
6. Calabresi, P., Napolitano, M., Centonze, D., Marfia, G. A., Gubellini, P., Teule, M. A., Berretta, N., Bernardi, G., Prati, L., Tol, M., and Galino, A. (2000) Eur. J. Neurosci. 12, 1002–1012
7. Tsirka, S. E., Gualandris, A., Amaral, D. G., and Strickland, S. (1995) Nature 377, 340–344
8. Tsirka, S. E., Hogwe, A. D., and Strickland, S. (1996) Nature 384, 123–124
9. Akkasoglou, K., Kombrinck, R. W., Degen, J. L., and Strickland, S. (2000) J. Cell Biol. 150, 1157–1166
10. Hastings, G. A., Coleman, T. A., Handenbush, C. C., Stefansson, S., Smith, E. P., Barthlow, R., Cherry, S., Sandkvist, M., and Lawrence, D. A. (1997) J. Biol. Chem. 272, 33062–33067
11. Osterwalder, T., Cinelli, P., Baicica, A., Pennella, A., Krueger, S. R., Schrimpf, S. P., Meins, M., and Sonderegger, P. (1998) J. Biol. Chem. 273, 2312–2321
12. Krueger, S. R., Ghisi, G. P., Cinelli, P., Gschwend, T., P., Osterwalder, T., Wolfer, D., P., and Sonderegger, P. (1997) J. Neurosci. 17, 8884–8896
13. Yepes, M., Sandkvist, M., Wong, M. K., Coleman, T. A., Smith, E., Cohen, S. L., and Lawrence, D. A. (2000) Blood 96, 569–576
14. Cinelli, P., Madani, R., Tsuchiya, N., Velt, P., Arras, M., Zhao, C. N., Osterwalder, T., Balickie, T., and Sonderegger, P. (2001) Mol. Cell. Neurosci. 18, 443–457
15. Ye, S., Ceha, A. L., Belmares, R., Bergstrom, R. C., Tong, Y., Corey, D. R., Kanouet, M. R., and Goldsmith, E. J. (2001) Nat. Struct. Biol. 8, 979–983
16. Lawrence, D. A., Ginsburg, D., Day, D. E., Benkerpas, M. B., Verhamme, I. M., Vassmann, J. O., and Shore, J. D. (1995) J. Biol. Chem. 270, 25309–25312
17. Higazi, A. A., Saleh, R. H., Cohen, R. L., Manopoulos, J., Bognacki, J., Henkin, J., McCrosk, K. R., Kounnas, M. Z., Strickland, D. K., Preissner, K. T., Lawler, J., and Cines, D. B. (1996) Blood 88, 542–551
18. Petemp, J., Korous, E., and Travin, J. (1994) J. Biol. Chem. 269, 13587–13586
19. Huntington, J. A., Read, R. J., and Carrell, R. W. (2000) Nature 407, 923–926
20. Strickland, D. K., Kounnas, M. Z., and Argraves, W. S. (1995) FASEB J. 9, 890–898
21. Lawrence, D., Strandberg, L., Grunstrom, T., and Ny, T. (1989) Eur. J. Biochem. 186, 523–533
22. Deutsch, D. G., and Mertz, E. T. (1970) Science 170, 1095–1096
23. Schwartz, B. S., and Espana, F. (1999) J. Biol. Chem. 274, 15278–15283
24. Lasnamb, U. K. (1970) Nature 227, 680–685
25. Gallagh, S., Winston, S. E., Fuller, S. A., and Hurrell, J. G. R. (1997) in Current Protocols in Molecular Biology, Vol. 2, pp. 10.8.1–10.8.21, John Wiley & Sons, Etobicoke, Ontario, Canada
26. Mussoni, L., Lawrence, D., and Laskooff, D. L. (1984) Thromb. Res. 34, (suppl.) 241–254
27. Schagger, H., and Jagow, G. (1986) Anal. Biochem. 173, 201–205
28. Zhou, A., Carrell, R. W., and Huntington, J. A. (2001) J. Biol. Chem. 276, 27541–27547
29. Gausse, P., Gralibe, P., and Alegre-Anco, E. (1993) J. Biol. Chem. 268, 12150–12155
30. Gils, A., and Declerck, P. J. (1998) Thromb. Haemostasis 80, 286–291
31. Kollner, L., Marsten, P. M., Sottrup-Jensen, L., Justesen, J., Rodenburg, K. W., and Andrews, P. A. (1996) EMBO J. 15, 38–46
32. Chen, Z. L., and Strickland, S. (1997) Cell 91, 917–925
33. Osterwalder, T., Contartese, J., Stoockel, E. T., Kuhn, T. B., and Sonderegger, P. (1996) EMBO J. 15, 2944–2950
34. Zhang, J.-C., Sakthivel, R., Kniss, D., Graham, C. H., Strickland, D. K., and McCrae, K. R. (1998) J. Biol. Chem. 273, 32273–32280
35. Plotnik, M. I., Sandkur, M., Wang, Z. M., Liu, X., Rubin, H., Schechter, N. M., and Selwood, T. (2002) Biochemistry 41, 334–342
36. Takao, M., Mison, M., Murrell, J., Yaraki, M. Piccardo, P., Unverzagt, F., Davie, R., Holohan, P., Laurens, D., Richardson, R., Fralow, M., and Tett, B. (2000) J. Neurochem. Exp. Neurol. 59, 1070–1086
37. Yepes, M., Sandkur, M., Coleman, T. A., Moore, E., Wu, J. Y., Mitola, D., Bugge, T. H., and Lawrence, D. A. (2002) J. Clin. Invest. 109, 1571–1578
Acyl-Enzyme Complexes between Tissue-type Plasminogen Activator and Neuroserpin are Short-lived \textit{in Vitro}
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