# Plasminogen Activation Is Stimulated by Prion Protein and Regulated in a Copper-Dependent Manner

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ABSTRACT: Prion diseases are associated with the conversion of the normal prion protein, $PrP^{C}$, to the infectious disease form $PrP^{Sc}$. Discrimination between these isoforms would significantly enhance diagnosis of these diseases, and it has recently been reported that $PrP^{Sc}$ is specifically recognized by the serine protease zymogen plasminogen (Fischer et al. (2000) *Nature 408*, 479). Here we have tested the hypothesis that PrP is a regulator of the plasminogen activation system. The effect of recombinant PrP, either containing copper (holo-PrP) or devoid of it (apo-PrP), on plasminogen activation by both uPA and tPA was determined. PrP had no effect on plasminogen activation by uPA. By contrast, the activity of tPA was stimulated by up to 280-fold. This was observed only with the apo-PrP isoforms. The copper-binding octapeptide repeat region of PrP was involved in the effects, as a mutant lacking this region failed to stimulate plasminogen activation, although a synthetic peptide corresponding to this region was unable to stimulate tPA activity. Competition experiments demonstrated that, in addition to plasminogen binding, the stimulation required a high-affinity interaction between tPA and PrP ($K_d$ < 2.5 nM). Kinetic analysis revealed a template mechanism for the stimulation, suggesting independent binding sites for tPA and plasminogen. Lack of copper-binding may be an early event in the conversion of $PrP^{C}$ to $PrP^{Sc}$, and our data therefore suggest that tPA-catalyzed plasminogen activation may provide the basis for a sensitive detection system for the early stages of prion diseases and also play a role in the pathogenesis of these diseases.

Prion diseases are fatal neurodegenerative conditions, such as Creutzfeldt−Jakob disease in humans and bovine spongiform encephalopathy and scrapie in animals. They are characterized by the accumulation in the brain of an abnormal isoform of the prion protein ($PrP^{Sc}$)[1] in amyloid deposits (*1*). The normal prion protein ($PrP^{C}$) is a glycoprotein expressed on the plasma membrane as a GPI-anchored protein and highly concentrated in synapses (*2*). $PrP^{Sc}$, which is rich in $\beta$-sheets, is generated from the largely α-helical $PrP^{C}$ by a conversion mechanism as yet unknown (*3*). Prion diseases can all be experimentally transmitted to other animals, and $PrP^{Sc}$ is inseparable from the infectious agent. The expression of $PrP^{C}$ is necessary for prion disease, as mice devoid of $PrP^{C}$ are resistant to infection (*4*). The generation of $PrP^{Sc}$ from host $PrP^{C}$ implies that the mechanism by which $PrP^{Sc}$ is generated is of fundamental importance in understanding the cause of these diseases and that understanding the difference between $PrP^{C}$ and $PrP^{Sc}$ might lead to both diagnosis and possibly treatment of prion diseases. One significant difference between these proteins that has recently been described by Fischer et al. (*5*) is that some plasma proteins, notably plasminogen, bind to $PrP^{Sc}$ but not to $PrP^{C}$.

Several studies have demonstrated that $PrP^{C}$ is a copper-binding protein (*6*−*9*). However, recent studies have indicated that $PrP^{Sc}$ does not bind copper. PrP isolated from mouse brains contains approximately 3 copper atoms per PrP molecule, and a further 2 atoms can be bound in vitro (*10*). In contrast, PrP isolated from brains of both scrapie-infected mice and humans with Creutzfeldt−Jakob disease contains less than 0.5 copper atoms per PrP molecule (*11*). Copper binding is thought to have both functional and structural effects on PrP. Copper both increases the rate of endocytosis of $PrP^{C}$ (*12*) and endows it with superoxide dismutase activity (*9*, *10*). The N-terminal half of PrP contains four tandem copies of a highly conserved octapeptide repeat which is thought to bind copper by coordination to four histidine residues (*13*). This binding of copper imparts structure in this otherwise unstructured part of the molecule (*14*). Copper can be specifically incorporated into recombinant PrP (PrP23−231), but not PrP lacking the octapeptide repeat region (PrPΔ51−90), during the refolding procedure and acquire properties resembling those of $PrP^{C}$ (*9*, *15*).

Pericellular proteolytic activity plays an important role in many pathological as well as physiological situations in a variety of organs, including the brain. This proteolytic activity can act to degrade components of the extracellular matrix or activate bioactive molecules such as growth factors, making it a key regulator of cellular behavior (*16*). The broad

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[1] Abbreviations used: $PrP^{Sc}$, scrapie isoform of the prion protein; $PrP^{C}$, cellular isoform of the prion protein; apo-PrP, PrP lacking bound copper; holo-PrP, PrP with bound copper; GPI, glycosyl phosphatidylinositol; uPA, urokinase-type plasminogen activator; tPA, tissue-type plasminogen activator; DiP-tPA, tPA catalytically inactivated by diisopropylfluorophosphate; ϵACA, epsilon aminocaproic acid.

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specificity serine protease plasmin is one of the principal activities involved in these processes. Plasmin is generated from the abundant zymogen plasminogen by a single proteolytic cleavage catalyzed by either of the two plasminogen activators, uPA and tPA. At the functional level, the activity of the plasminogen activation system is largely regulated by mechanisms that enhance the generation of plasmin (*17*). Thus uPA-catalyzed plasminogen activation is stimulated by the binding of uPA to its cell surface receptor uPAR (*18*) and tPA-catalyzed plasminogen activation by binding to fibrin (*19*) or cell surface binding sites (*20*). All of these situations involve the binding of plasminogen in juxtaposition to the plasminogen activator, either on the same cofactor molecule (as with fibrin) or to discrete cellular binding sites. Interactions with these molecules are mediated by "lysine binding sites" in the kringle modules of plasminogen, which can be antagonized by lysine and various aminocarboxylic acid analogues of lysine, such as $\epsilon$ACA (*21*). These kringle modules preferentially bind C-terminal lysine residues (i.e., those with a free carboxylate group), which can either be present in the native proteins or generated by the proteolytic action of plasmin.

Here we test the hypothesis that, due to its reported ability to bind plasminogen, $PrP^{Sc}$ is a regulator of plasminogen activation. We demonstrate that PrP can indeed regulate plasminogen activation but that, surprisingly, a critical determinant of this is an interaction between PrP and tPA. Furthermore, rather than being a result of the conversion of $PrP^{C}$ to $PrP^{Sc}$, the ability of PrP to interact with tPA and plasminogen, and to stimulate plasminogen activation, is related to the binding of copper to PrP. These data have implications for the diagnostic detection of the prion diseases and suggest that plasmin may have a role in prion-induced neurodegeneration.

## EXPERIMENTAL PROCEDURES

*Proteins.* Mouse recombinant prion protein was expressed in *E.coli* and purified as previously described (*22*). Immobilized metal affinity chromatography was used to purify the protein from inclusion bodies in the presence of 8 M urea. Protein was folded into its native conformation by gradual removal of the urea by the addition of water and concentration followed by a final dialysis step to remove all traces of contaminants. Copper was incorporated by addition of 1 mM $CuSO_4$ during the refolding process. The mutant form of the protein PrPΔ51−90 lacked the octapeptide repeat region and was prepared as previously described (*9*). Aggregated prion protein was produced by rapid addition of water in the presence of either 1 mM $CuSO_4$ or 500 mM NaCl. Aggregated protein was precipitated and collected by centrifugation. Samples of aggregated protein were tested for protease resistance by digesting them with 25 $\mu$g/mL proteinase K. Only resistant material was used as aggregated PrP.

Single-chain tPA was obtained from Biopool AB (Umeå, Sweden) and catalytically inactivated as previously described (*20*). uPA was obtained from Serono S. A. (Geneva, Switzerland). Lys−plasminogen (i.e., with $Lys^{77}$ as the N-terminus) was obtained from Enzyme Research Laboratories (Swansea, U.K.).

*Effect of PrP on Plasminogen Activation.* To determine the effect of the various PrP isoforms on plasminogen activation, we incubated varying concentrations of PrP with either uPA (0.25 nM) or tPA (0.25 nM) and Lys−plasminogen (25 nM) in 0.05 M Tris−HCl, pH 7.4, 0.1 M NaCl, and 0.01% Tween 80 in the presence 0.2 mM of the plasmin specific fluorogenic substrate H−d-Val−Leu−Lys−7-amido-4-methylcoumarin (Bachem AG, Bubendorf, Switzerland) at 37 °C. Substrate hydrolysis was measured by continuous recording of fluorescence intensity in a SPECTRAmax Gemini fluorescence microplate reader (Molecular Devices, Sunnyvale, CA) using excitation and emission wavelengths of 360 and 440 nm, respectively. Plasmin generation was determined as $\delta F/\delta t$ and either expressed as fold-increase over plasmin generation in the absence of PrP or in some cases converted to molar rates of plasmin generation by comparison to standard curves constructed using active-site-titrated plasmin (*18*).

*Competition Experiments.* The contribution of tPA and plasminogen binding to apo-PrP were determined in competition experiments performed in a manner similar to those described above, but including increasing concentrations of either $\epsilon$ACA or DiP-tPA. $IC_{50}$ values for the competitive effects of these ligands were determined by nonlinear regression analysis of the binding isotherms obtained.

*Analysis of Template Mechanism.* The mechanism of stimulation of tPA-catalyzed plasminogen activation was analyzed over a wide range of apo-PrP concentrations up to 1 mg/mL. These data were plotted in a log-normal manner and shown to conform to a classical template model (*23*).

## RESULTS

From the observation of Fischer et al. (*5*) that $PrP^{Sc}$ can bind plasminogen, we have hypothesized that this binding has a functional effect and that PrP can act as a regulator of plasminogen activation. To test this hypothesis, we have studied both uPA- and tPA-catalyzed plasminogen activation in the presence recombinant murine PrP23−231 in its holo- and apo-isoforms, i.e., copper-containing and copper-free, resembling $PrP^{C}$ and $PrP^{Sc}$, respectively. Neither form of PrP was found to have an effect on uPA-catalyzed plasminogen activation (data not shown), but in sharp contrast, plasminogen activation catalyzed by tPA was greatly enhanced by apo-PrP (Figure 1). This stimulation was concentration-dependent and rapid, with no lag-phase in plasmin generation. Both the soluble and aggregated forms of apo-PrP had a similar effect, the latter stimulating plasminogen activation by up to 280-fold, but neither form of copper-containing holo-PrP had any stimulatory effect (Figure 2A).

The observation that tPA-catalyzed plasminogen activation is specifically stimulated by apo-PrP suggests that the copper-binding N-terminal octapeptide repeat region of PrP has a role in this effect. This was confirmed by using the mutant PrPΔ51−90, lacking the octapeptide repeat region, which was much less effective as a stimulator of plasminogen activation under all conditions (Figure 2B). To determine if the octapeptide repeat region was directly responsible for the effects on plasminogen activation, we used a synthetic peptide corresponding to this region, $(PHGGGWGQ)_4$ in similar experiments. This peptide was unable to stimulate tPA-catalyzed plasminogen activation at concentrations up to 1 mM. Therefore, only PrP containing the copper-binding octapeptide repeat region, but lacking bound copper, was able to stimulate plasminogen activation.

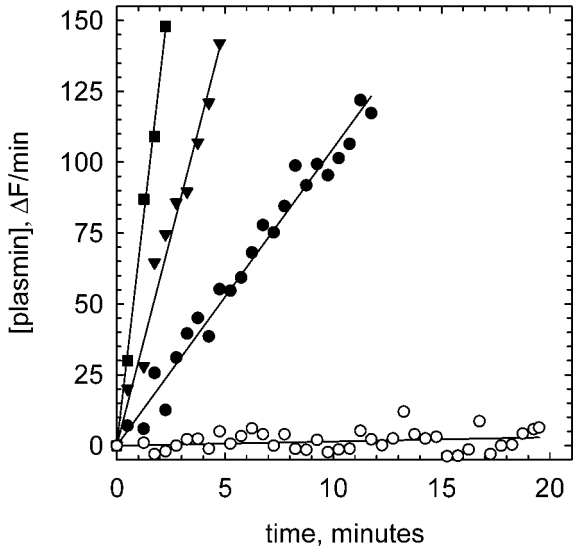


FIGURE 1: Stimulation of tPA-catalyzed plasminogen activation by apo-PrP. tPA (0.25 nM) and plasminogen (25 nM) were incubated with increasing concentrations of apo-PrP; 0 $\mu$g/mL (○), 3 $\mu$g/mL (●), 10 $\mu$g/mL (▼), 30 $\mu$g/mL (■). This highest concentration is equivalent to 1.3 $\mu$M PrP. Plasmin generation was determined by hydrolysis of the plasmin-specific fluorogenic substrate H−d-Val−Leu−Lys−7-amido-4-methylcoumarin and expressed as $\delta F/\delta t$. The initial rates of plasmin generation were calculated from these linear slopes.

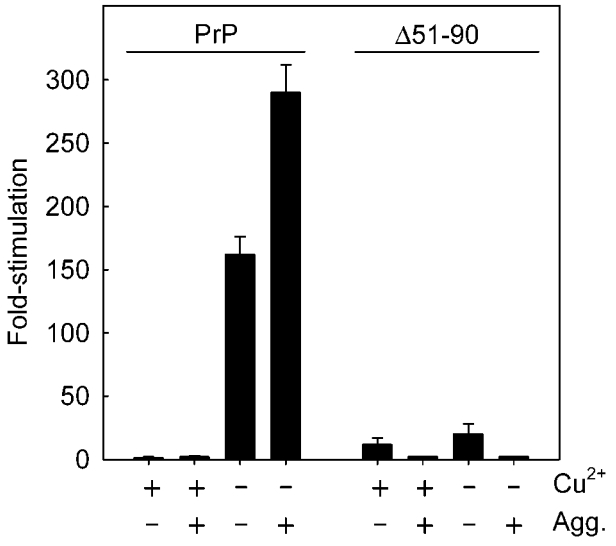


FIGURE 2: Comparison of the effects of various forms PrP on tPA-catalyzed plasminogen activation. Data such as those shown in Figure 1, obtained with the various PrP preparations at a fixed concentration of 25 $\mu$g/mL, were expressed as fold-stimulation over plasmin generation in the absence of PrP. Data are shown for full-length PrP (PrP23−231) and PrPΔ51−90, refolded in the presence and absence of $Cu^{2+}$ and in soluble and aggregated forms.

The stimulation of plasminogen activation by apo-PrP is consistent with the binding of plasminogen to PrP, although different effects were observed with the two plasminogen activators. Therefore, to confirm that an interaction with plasminogen was involved in the stimulatory mechanism, $\epsilon$ACA, a ligand for plasminogen's lysine binding sites, was used as an antagonist of plasminogen binding. The stimulation of tPA-catalyzed plasminogen activation by apo-PrP was completely abolished by $\epsilon$ACA (Figure 3A). This effect had an $IC_{50}$ of 12 $\mu$M, consistent with occupation of the high-affinity lysine binding sites present in kringles 1 and 4 of

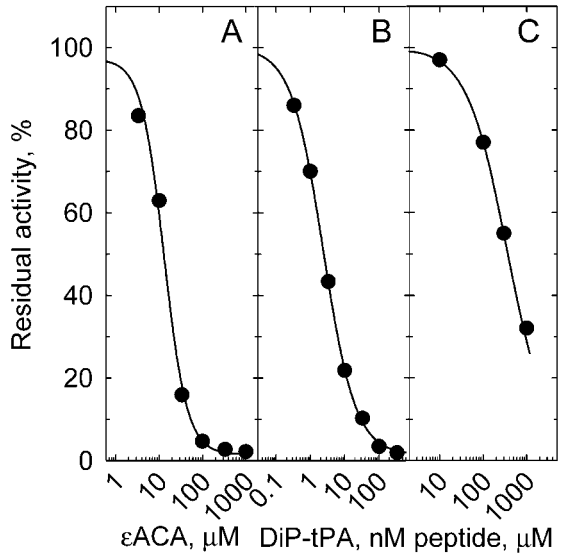


FIGURE 3: Effect of competitors of plasminogen and tPA binding on apo-PrP-stimulated plasminogen activation. tPA-catalyzed plasminogen activation stimulated by apo-PrP (25 $\mu$g/ml) was performed in the presence of increasing concentrations of $\epsilon$ACA as a competitor of plasminogen binding (panel A), of DiP−tPA as a competitor of tPA binding (panel B), or of the $(PHGGGWGQ)_4$ peptide (panel C). The $IC_{50}$ values, calculated by nonlinear regression analysis using a single-site binding model, were found to be 12 $\mu$M, 2.5 nM, and 400 $\mu$M, respectively. The last $IC_{50}$ fitted well despite being close to the maximum peptide concentration used (1 mM). The concentrations of competitors used were shown to have no effect on the unstimulated reactions in control experiments.

plasminogen (*21*) and demonstrating the specificity of the interaction with plasminogen.

Having established that the interaction of plasminogen with apo-PrP was necessary for the stimulation of plasminogen activation, we addressed the question of why only its activation by tPA was affected. This observation suggested that apo-PrP may have an additional interaction with tPA but not with uPA. To determine whether this was the case, we used a catalytically inactivated form of tPA (DiP-tPA) to attempt to compete this interaction. Consistent with a functionally important interaction between tPA and apo-PrP, DiP-tPA abolished the stimulation completely and with remarkably high efficiency (Figure 3B). The $IC_{50}$ value was calculated to be 2.5 nM, which under these experimental conditions is an upper estimate of the $K_d$ for the interaction of tPA with apo-PrP.

Because competition for the binding of either tPA or plasminogen to apo-PrP abolished the stimulation of plasminogen activation, we used the synthetic octarepeat peptide in similar experiments to determine whether this region of the protein could also act as a competitor despite being unable to directly stimulate plasminogen activation. Figure 3C shows that this peptide appeared to behave as a competitor in the same way as $\epsilon$ACA and DiP-tPA, suggesting that either tPA or plasminogen bind to this region of PrP. However, it should be noted that the $IC_{50}$ for the peptide (400 $\mu$M) is far above that for DiP-tPA (2.5 nM), although these are not directly comparable as the basis of the competition is different in either case.

Plasminogen activation by tPA (0.25 nM) was measured in the absence and presence of apo-PrP (25 $\mu$g/mL) at plasminogen concentrations varying from 2.5 nM to 2.5 $\mu$M.

Table 1: Effect of PrP on the Kinetic Constants for tPA-Catalyzed Plasminogen Activation

| | $K_m$, M | $k_{cat}$, $s^{-1}$ | $k_{cat}/K_m$, $M^{-1}$ $s^{-1}$ |
|---|---|---|---|
| minus PrP | $1.6 \times 10^{-5}$ | 3.6 | $2.2 \times 10^5$ |
| apo-PrP | $4.5 \times 10^{-8}$ | 2.7 | $6.0 \times 10^7$ |

Kinetic constants were calculated by fitting the primary data to the Michaelis−Menten equation by nonlinear regression. Data obtained in the presence of holo-PrP (25 $\mu$g/mL) were similar to those in the absence of PrP.

These data suggest that apo-PrP, but not copper-containing holo-PrP, simultaneously binds both plasminogen and tPA and thereby promotes catalysis. If the effect of apo-PrP on plasminogen activation is principally due to such a "template" mechanism, two consequences would be predicted. First, the kinetic mechanism for the observed stimulation should be due to a reduction in the apparent $K_m$ for plasminogen activation, i.e., an increase in local substrate concentration. Second, higher concentrations of PrP should lead to a reduction in the stimulatory effect, as tPA and plasminogen become increasingly less likely to simultaneously interact with the same molecule of PrP and the formation of PrP-tPA and PrP-plasminogen bimolecular complexes becomes favored over the formation of functional trimolecular complexes. To test the first prediction, we determined the $K_m$ for plasminogen activation in the absence and presence of both apo- and holo-PrP. As shown in Table 1, holo-PrP had no effect on the $K_m$ compared to the absence of PrP, whereas the $K_m$ in the presence of apo-PrP was reduced by greater than 350-fold. By comparison, no increase in the $V_{max}$ for the reaction was observed. The second prediction was also confirmed, as concentrations of apo-PrP above approximately 100 $\mu$g/mL led to a reduction in the stimulation, which was reduced almost to unstimulated levels at the highest concentrations tested (Figure 4). The experimental data fitted well to a log-normal distribution consistent with a template model of catalysis.

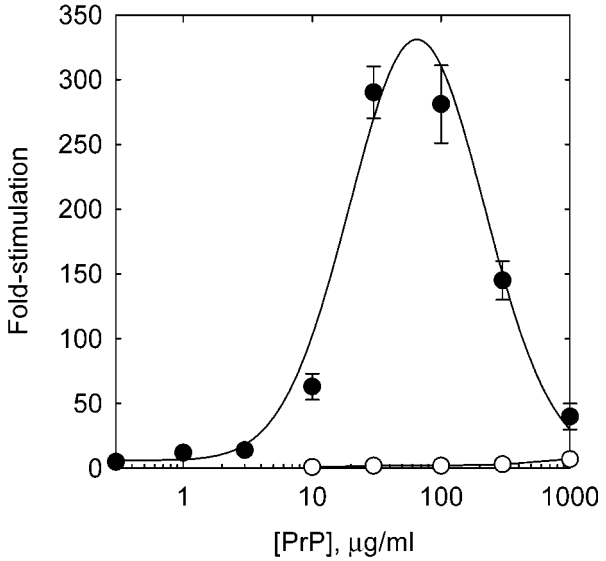


FIGURE 4: Demonstration of template mechanism of stimulation of plasminogen activation by apo-PrP. Experiments such as those shown in Figure 1 were repeated at concentrations of PrP far in excess of those giving maximal stimulation of plasmin generation by apo-PrP. Data are shown for apo-PrP (●) and holo-PrP (○). The data obtained with apo-PrP fitted a log-normal peak model, indicative of a template mechanism.

## DISCUSSION

We demonstrate here that certain isoforms of PrP, the protein infectious agent responsible for the prion diseases, can stimulate the generation of the powerful serine proteinase plasmin. This effect was specific to apo-PrP, i.e., PrP23−231 devoid of bound copper, and involved both the known interaction of PrP with plasminogen (*5*) and a newly identified high-affinity interaction with the plasminogen activator tPA. The observations that apo-PrP interacts with both tPA and plasminogen and that these interactions have functional consequences for the generation of plasmin potentially have wide-ranging implications for our understanding of the fundamental nature of $PrP^{Sc}$ and the pathogenesis of the prion diseases.

The stimulation of plasminogen activation by apo-PrP was specific to tPA, was due to a 350-fold reduction in $K_m$ for plasminogen, and required the interaction of both plasminogen and tPA with apo-PrP. This suggests that the mechanism underlying the stimulation is coincident binding of tPA and plasminogen to the same molecule of apo-PrP. Consistent with this, the stimulation was shown to display a bell-shaped curve with increasing apo-PrP concentration indicative of such a template mechanism. Therefore, tPA and plasminogen must interact with independent binding sites on apo-PrP. The lack of stimulation observed with the PrPΔ51−90 mutant appears to implicate the octapeptide repeat region directly in this binding, with copper binding to this region excluding tPA/plasminogen binding. A synthetic peptide corresponding to this region was unable to stimulate plasminogen activation, demonstrating that this region in isolation is not responsible for the effects on plasminogen activation, but the peptide did appear to compete for the binding of tPA to apo-PrP, consistent with the octarepeat region containing the putative tPA binding site. However, as there is a large discrepancy between the $IC_{50}$'s for the octarepeat peptide and DiP−tPA, it is not possible to draw firm conclusions as to whether this region constitutes the binding site for tPA. It is also possible that the effects of this region are indirect and that the known conformational changes on copper binding to the octapeptide repeat (*14*) lead to a structural rearrangement that masks binding sites on other parts of the PrP molecule.

Fibrillar structures in addition to fibrin have been shown to stimulate plasminogen activation, such as the fibrillar form of amyloid $\beta$-protein (*24*), suggesting that aggregation of PrP could have an important role in the effects observed here. However, this does not appear to be the case. Although the aggregated form of apo-PrP did give a greater stimulation than the nonaggregated form, this difference was small (less than 2-fold) compared to the overall 300-fold increase in activity. Furthermore, PrPΔ51−90 is also able to form aggregates, but these had no effect on plasminogen activation.

The interaction of tPA and plasminogen with apo-PrP is remarkable in two respects, reinforcing the notion that these interactions are highly specific and of biological relevance. First, tPA appears to bind with very high affinity, with a $K_d$ below 2.5 nM. By comparison, tPA binds to a number of sites on its classical cofactor fibrin with affinities between 0.1 and 0.6 $\mu$M (*25*, *26*). Second, plasminogen and to some extent tPA bind to proteins having a lysine residue at the

C-terminus, aminocarboxylic acids being the preferential ligands for lysine-binding kringle modules in both plasminogen and tPA. The recombinant PrP used here does not have a C-terminal lysine residue, but these can also be generated from internal sequence by the proteolytic action of plasmin (which cleaves C-terminally of lysine and arginine residues), and in some cases, such as with fibrin, this constitutes an amplification mechanism for plasmin generation. However, this does not appear to be involved in the stimulation by apo-PrP, as plasmin generation was linear with time and displayed no evidence of the lag-phase that would be expected with this mechanism. It is possible that appropriately spaced internal lysine residues can interact with more than one kringle domain to provide sufficient binding energy. PrP contains three clusters of lysine residues that could potentially fulfill this function, two of which ($Lys^{23,24,27}$ and $Lys^{100,103,105,109}$) flank the octapeptide repeat region (residues 51−90).

Many studies have shown that copper is involved in the biology of PrP. Our observation, that the ability of PrP to interact with tPA and plasminogen and to stimulate plasminogen activation is also influenced by copper binding, adds to the evidence that copper regulates PrP function. Copper-containing holo-PrP had no effect on plasminogen activation, whereas copper-free apo-PrP led to a dramatic stimulation of plasminogen activation. Our data support the conclusion of Fischer et al. (*5*) that plasminogen can bind to PrP and furthermore rule out the involvement of other molecules in mediating this binding, a possibility not excluded in the previous study. However, our data differ in one fundamental respect. Fischer et al. showed that $PrP^{Sc}$ from scrapie-infected mouse brain, but not $PrP^{C}$ from normal brain, bound to immobilized plasminogen and could subsequently be detected by Western blotting, and they concluded that $PrP^{Sc}$ uniquely had the ability to bind plasminogen. Our data, using enzymological techniques, show that plasminogen binding is clearly not specific for $PrP^{Sc}$ and suggest that the essential difference governing the interaction of PrP isoforms with plasminogen is whether they have bound copper. Nevertheless, our respective studies are consistent as $PrP^{Sc}$ can be considered to be a form of apo-PrP. It has been shown in both mouse and human brains that wild-type $PrP^{C}$ has substantial amounts of bound copper, whereas $PrP^{Sc}$ has less than stoichiometric amounts bound (*10*, *11*). This implies that two of the known differences between $PrP^{C}$ and $PrP^{Sc}$, the ability to bind copper and the ability to stimulate plasminogen activation, are inversely related. Other divalent metal cations, in particular manganese, can also interact with PrP. This can substitute for copper causing the protein to slowly decay in vitro into a form resembling $PrP^{Sc}$ (*22*), and $PrP^{Sc}$ in vivo has elevated levels of bound manganese and, to a lesser extent, zinc (*11*). Determining the effect of these metals on tPA binding and plasminogen activation may help to further elucidate the changes in PrP mediating these effects.

The demonstration here that PrP can interact with both tPA and plasminogen and thereby stimulate plasmin generation adds to the spectrum of mechanisms regulating the activity of this powerful proteolytic system and suggests a possible biological function. tPA and plasminogen are both expressed by neurons in the brain (*27*−*29*) and are known to have both physiological and pathological roles there. Studies in gene-targeted mice have implicated tPA in various aspects normal brain functions and neural plasticity, including long-term potentiation (*30*) and neuronal cell migration (*31*). These mice are also protected from excitotoxin-induced neurodegeneration and seizure (*32*), a process that involves plasmin-mediated degradation of the extracellular matrix protein laminin (*33*) and, potentially, proteolytic cleavage of the glutamatergic NMDA receptor (*34*). In contrast to this role in neurodegeneration, tPA may be neuroprotective in other situations, as it is implicated in the activation of certain growth factors with major neuroprotective roles. Plasmin has recently been shown to proteolytically activate pro-forms of the nerve growth factor family members, brain-derived neurotrophic factor (BDNF) and $\beta$-NGF (*35*), converting them to neuronal cell-survival factors, and it has a well-established role in regulating the bioavailability of FGF-2 (*36*), which also promotes neuronal survival (*37*). tPA has also been shown to attenuate zinc neurotoxicity in a nonproteolytic manner (*38*). Therefore, if PrP is a regulator of tPA activity and plasmin generation in the brain, it could be hypothesized that changes in copper-binding associated with the formation of $PrP^{Sc}$ will lead to an increase in plasmin generation which will influence the pathogenesis of prion disease. Whether this influence is to contribute to the ongoing neurodegeneration or whether it is neuroprotective must await infection experiments in tPA-deficient mice.

In conclusion, we have shown that PrP lacking bound copper interacts with both tPA and plasminogen, leading to a stimulation of plasminogen activation. The changes in copper binding accompanying the conversion of $PrP^{C}$ to $PrP^{Sc}$, together with the known neurodegenerative and neuroprotective roles of tPA, suggest that apo-PrP-mediated plasmin generation may have relevance to the pathogenesis of prion disease. Levels of $PrP^{Sc}$ have been shown to vary quite dramatically in prion disease, with reports of undetectable levels of $PrP^{Sc}$ associated with scrapie infectivity (*39*). If changes in copper binding precede conversion to $PrP^{Sc}$, tPA binding and plasminogen activation may prove to be useful markers of early infection before the onset of $PrP^{Sc}$ accumulation.

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