JCI The Journal of Clinical Investigation

Carboxypeptidases: new regulators of plasminogen activation in vivo?

D J Loskutoff

J Clin Invest. 1995;96(5):2104-2105. https://doi.org/10.1172/JCI118262.

Research Article

It is now well established that both tissue-type plasminogen activator (t-PA) and plasminogen bind to fibrin to form a ternary complex which efficiently generates plasmin (reviewed in reference 1). Plasminogen bound to fibrin is a much better substrate for t-PA than free, circulating plasminogen, and the plasmin that remains bound to the fibrin surface is protected from rapid inactivation by its primary endogenous inhibitor, α_2-antiplasmin. Thus, physiological fibrinolysis is regulated and contained, in large part by the fibrin surface itself. Treatment of fibrin with pancreatic carboxypeptidase B greatly reduces the extent of plasminogen binding, suggesting that carboxy-terminal lysines on fibrin are involved. This hypothesis is supported by the observations that these interactions are not only inhibited by lysine analogs, but also are dependent upon lysine binding sites in t-PA and plasminogen. Interestingly, t-PA and plasminogen also bind to cell surfaces, and this binding is again mediated, in part, by carboxy-terminal lysines (reviewed in references 2 and 3). In fact, there is an intriguing similarity between the way fibrin and cells can assemble, localize, and restrict plasminogen activation to specific surfaces and thereby minimize the escape of this potentially destructive enzyme system. The binding of plasminogen to fibrin and to cell surfaces is of relatively low affinity ($\sim 1\ \mu M$), with the number of receptors ranging from $< 10^5$ to $> 10^7$ per cell. Since plasminogen circulates at 2 μM, it is expected that these binding sites will be half occupied under normal conditions. A variety of mechanisms may exist to increase the number of plasminogen binding sites on surfaces. For example, as fibrin is degraded by plasmin, additional carboxy-terminal lysine residues are generated, and occupancy of these new sites may accelerate fibrinolysis significantly (1). Similarly, removing peripheral blood monocytes from blood and culturing them in vitro, increases the number of plasminogen binding sites by more than one order of magnitude (2). This latter observation suggests that plasma may contain molecules that suppress plasminogen binding to circulating cells. In this regard, plasma contains at least two carboxypeptidases, and one of them, carboxypeptidase N, is constitutively active (reviewed in reference 4). By continuously removing exposed carboxy-terminal lysines, the plasma carboxypeptidases could suppress plasminogen binding to and activation on surfaces. The effects of plasma carboxypeptidases on the plasminogen system have not been reported. In this issue of *The Journal* Redlitz et al. examine this question (5). These investigators show that whole plasma is, in fact, a potent inhibitor of plasminogen binding to cells and to fibrin, and they use specific inhibitors and monoclonal antibodies to provide convincing evidence that these effects are mediated by circulating carboxypeptidases. Perhaps more interestingly, their data suggest that plasma carboxypeptidase B is the primary enzyme involved in this process. Since this enzyme can be activated by thrombin (6), the authors speculate that it may function to stabilize nascent blood clots by dampening fibrinolysis. This report is an important extension of earlier work by this group and suggests that basic carboxypeptidases in plasma may play a previously unsuspected role in the regulation of plasminogen activation in vivo. These observations are certain to spawn a number of subsequent studies to characterize this system in greater detail, and to evaluate the effect of manipulating this system in the clinical setting. At the same time, these observations raise a number of questions that once resolved, should provide additional insights into the physiological role of carboxy-terminal lysines in the control of fibrinolysis and cell-mediated proteolysis. Foremost among these is the question of the number of plasminogen binding sites on cells in vivo. Although this plasminogen binding system is generally considered to be of high capacity, this conclusion is based primarily on cell culture studies. The presence of carboxypeptidase activity in plasma suggests that the number of plasminogen binding sites on circulating cells in vivo may be considerably lower. Whether the number of sites can actually be up-regulated in vivo, especially in the presence of the constitutively active carboxypeptidase N, also remains to be determined. In this regard, the increase in plasminogen binding to cultured monocytes may result simply from the removal of the cells from plasma (i.e., removal from active carboxypeptidase activity). Consequently, it remains unclear whether the observed increase in plasminogen binding capacity is physiologically relevent. Alternatively, it is possible that the number of carboxy-terminal lysines on cells in plasma can be up-regulated much like it is on fibrin in plasma. That is, the initial formation of plasmin on cells may generate additional binding sites, again amplifying this system locally. The observation that carboxypeptidase treatment only reduced plasminogen binding by 50% implies the existence of other plasminogen binding molecules on cell surfaces. In fact, gangliosides on cells also bind plasminogen, and this interaction is again inhibited by lysine analogs (7). Thus, another unresolved question concerns the relative functional importance of carboxy-terminal lysines, gangliosides, and perhaps other molecules, for plasminogen binding and activation on cell surfaces in vivo. Finally, there are sure to be questions about the regulation of carboxypeptidase activity itself. Redlitz et al. showed that carboxypeptidase inhibitors like guanidinoethylmercaptosuccinic acid and potato carboxypeptidase inhibitor stimulated plasminogen binding to fibrin and cells. Whether naturally occurring inhibitors in cells and/or plasma also regulate plasminogen activation is an important, unresolved question. Such inhibitors could prove to be useful thrombolytic agents when used in conjunction with t-PA.

David J. Loskutoff
Department of Vascular Biology
The Scripps Research Institute

J. Clin. Invest.

0021-9738/95/11/2104/02 $2.00
Volume 96, November 1995, 2104–2105

References

1. Collen, D., and H. R. Lijnen. 1992. Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood.* 78:3114–3124.

2. Plow, E. F., J. Felez and L. A. Miles. 1991. Cellular regulation of fibrinolysis. *Thromb. Haemostasis.* 66:32–36.

3. Gonzalez-Gronow, M., S. Stack, and S. V. Pizzo. 1991. Plasmin binding to the plasminogen receptor enhances catalytic efficiency and activates the receptor for subsequent ligand binding. *Arch. Biochem. Biophys.* 286:625–628.

4. Skidgel, R. A. 1988. Basic carboxypeptidases: regulators of peptide hormone activity. *Trends Pharmacol. Sci.* 9:299–304.

5. Redlitz, A., A. K. Tan, D. L. Eaton, and E. F. Plow. 1995. Plasma carboxypeptidases as regulators of the plasminogen system. *J. Clin. Invest.* 96:2534–2538.

6. Tan, A. K., and D. L. Eaton. 1995. Activation and characterization of procarboxypeptidase B from human plasma. *Biochemistry.* 34:5811–5816.

7. Miles, L. A., C. M. Dahlberg, E. G. Levin, and E. F. Plow. 1989. Gangliosides interact directly with plasminogen and urokinase and may mediate binding of these components to cells. *Biochemistry.* 280:9337–9343.