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Regular Article

Plasminogen interaction with platelets: The importance of carboxyterminal lysines

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Abstract

Introduction: Thrombin stimulation enhances plasminogen binding to platelets and promotes platelet-dependent plasmin generation. The objective of this study was to determine whether carboxyterminal lysines (C-lysines) are important for these processes, as they are in other cell types.
Materials and methods: ^{125}I-plasminogen and varying concentrations of unlabeled plasminogen were added to washed platelets that were either resting or stimulated with thrombin, thrombin receptor activating peptide, or ADP. In some experiments the platelets were digested with carboxypeptidase B to remove C-lysines. Platelet-dependent plasmin generation was also studied by adding plasminogen and tissue plasminogen activator to platelet suspensions and monitoring the conversion of a plasmin specific chromogenic substrate. The cells were either resting or stimulated with thrombin, thrombin receptor activating peptide, or ADP. The effect of the thrombin inhibitor lepirudin and the plasmin inhibitor aprotinin on plasminogen binding and the appearance of C-lysines was also investigated.
Results: Thrombin, but not thrombin receptor activating peptide or ADP, stimulated high-affinity binding of plasminogen and greatly promoted platelet-dependent plasmin generation. Digestion with carboxypeptidase B eliminated thrombin-induced high-affinity binding and reduced thrombin-induced plasmin generation by increasing the Michaelis constant. Lepirudin, but not aprotinin, inhibited thrombin-stimulated plasminogen binding to platelets.

Abbreviations: ADP, adenosine diphosphatedissociation constant; BSA, bovine serum albumin; C-lysine, carboxyterminal lysine; CpB, carboxypeptidase B; EACA, episilon aminocaproic acid; kcat, catalytic constant; Kd, dissociation constant; Km, Michaelis constant; PGE_1, prostaglandin E_1; PMSF, phenylmethyl-sulfonyl fluoride; R, binding capacity; SDS-PAGE, sodium dodecylsulfate-polyacrylamine gel electrophoresis; tPA, tissue plasminogen activator; TRAP, thrombin receptor activating peptide.

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Conclusion: C-terminal lysines are necessary for high-affinity binding of plasminogen to platelets and for platelet-supported plasmin generation. The origin of the C-lysines is not clear, but they may result from a direct effect of thrombin, rather than an intermediate enzyme such as plasmin.

Thrombi and hemostatic fibrin clots are degraded by plasmin, which is generated from plasminogen by tissue plasminogen activator (tPA) or urokinase. Binding sites for plasminogen and tPA develop on fibrin as it polymerizes, setting the stage for later fibrinolysis [1]. Initially, however, very little plasminogen is bound by the clot, as indicated by the fact that the plasminogen concentrations of serum and plasma are essentially the same [2]. But when a trace of plasmin appears, it begins cleaving fibrin to produce carboxyterminal lysines (C-lysines). These lysines provide additional binding sites for plasminogen and allow fibrinolysis to accelerate [3,4]. The acceleration is restrained, however, by carboxypeptidase B (CpB), which removes C-lysines [5]. Therefore, modulation of the C-lysine density in the clot determines its rate of lysis.

Like fibrin, platelets also influence fibrinolysis by binding plasminogen to their surface, where it becomes more easily activated by tPA [6]. Whether platelet-related plasmin generation is subject to control by CpB, however, is unknown. For other types of cells, such as monocytes and endothelial cells, plasminogen binding at sites that contain C-lysine is critically important for plasmin generation [7,8]. Miles et al. reported that plasminogen binds to fibrin that is bound to the surface of thrombin-activated platelets, but they did not address the question of whether this is partially degraded fibrin with exposed C-lysines [9]. Furthermore, Ouimet et al. observed similar binding to platelets stimulated with ADP, which in the absence of thrombin lack bound fibrin, but they also did not address the requirement for C-lysines [10].

In the current report we have shown that thrombin, but not thrombin receptor activating peptide (TRAP) or ADP, stimulates the appearance of high-affinity binding sites for plasminogen on the platelet surface and enhances platelet-dependent plasmin generation. Digestion of the platelet surface with CpB significantly reduces the affinity of plasminogen binding and increases the Km of platelet-dependent plasmin generation. Therefore, both of these processes depend upon C-terminal lysines, but the origin of the lysines is unclear.

Materials and methods

Materials

Alpha thrombin (3500 u/mg), aprotinin, and bovine serum albumin (BSA; protease-free, fraction V) were purchased from ICN (Aurora, OH). The thrombin receptor activating peptide (TRAP) SFLLRN was provided by Bachem (King of Prussia, PA) and ADP by Chrono-Log (Havertown, PA). Recombinant tPA (Activase®) was purchased from Genentech, Inc. (South San Francisco, CA), and carboxypeptidase B (porcine pancreas) (CpB) from Calbiochem (La Jolla, CA). Lysine-Sepharose® and $Na^{125}I$ were purchased from Amersham Biosciences (Piscataway, NJ). Iodo-Beads® were obtained from Pierce (Rockford, IL). Spectrozyme®PL and a murine monoclonal antibody directed against the B-chain of urokinase (Product #3689) were bought from American Diagnostica, Inc. (Greenwich, CT). Cellsep® was purchased from Larex (St. Paul, MN). Prostaglandin E_1 (PGE_1), episilon aminocaproic acid (EACA), amiloride, leupeptin, pepstatin, and phenylmethyl-sulfonyl fluoride (PMSF) were purchased from Sigma (St. Louis, MO). A monoclonal antibody against CD62 (clone AC1.2) was purchased from BD PharMingen (San Diego, CA).

Purification of Glu-plasminogen

Human plasminogen was purified from fresh 400 mL plasma samples obtained from normal donors by plasmapheresis in the Department of Transfusion Medicine at the W.G. Magnuson Clinical Center under a protocol approved by the Institutional Review Board of the National Cancer Institute. The purification scheme was a modification of previously published methods [11,12]. Briefly, 10 μg/mL aprotinin was added to the plasma, which was then centrifuged at 10,000×*g* for 10 min to remove residual cells. The plasma was diluted with an equal volume of deionized water and applied to a 70 mL column of lysine-Sepharose equilibrated in 0.05 M sodium phosphate, pH 7.5. The column was washed with the same buffer till the A_{280} of the effluent reached baseline. Then the column was washed with 10 mL 0.5 M NaCl in 0.05 M sodium