A DIRECT, PLASMIN INDEPENDENT ASSAY FOR PLASMINOGEN

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We have modified the direct, I-plasminogen cleavage assay for plasminogen activator (PA) and employed it to compare urokinase (UK), tissue activator (TA), and PAs produced by cultured bovine aortic endothelial cells. The assay is based on conversion of single chain plasminogen into two chain plasmin as revealed by polyacrylamide gel electrophoresis in the presence of SOS and d-mercaptoethanol. Inclusion of Triton X-100, albumin and trasyrol in the reaction mixture reduced the adsorptive and hydrolytic loss of reactants, and increased the linearity and sensitivity of the assay. Under these conditions, plasmin formation was linear for at least 6 hrs, dose-dependent over a 20-fold range of UK concentrations, and at least 100-fold more sensitive (0.01 units/ml) than previously reported direct assays for UK. In preliminary experiments, we determined a Km value of 10μM for UK and plasminogen. Activation of plasminogen by TA was minimal in the absence of fibrin, and independent of the concentration of activator. However, in the presence of fibrin, (1) the initial rate of activation increased dramatically, (2) 100-1000 fold less TA was required, and (3) activation was proportional to the concentration of both TA and fibrin. Surprisingly, activation by UK and cellular PAs was partially inhibited by fibrin. Epsilom amino caproic acid (EACA; 0.5-100μM) stimulated activation by UK both in the presence and absence of fibrin by 30-40%. In contrast, EACA (0.1-100μM) inhibited TA activity in the presence of fibrin by over 90%. However, in the absence of fibrin, TA was inhibited by only 50%, even at high EACA concentrations (100μM). These results indicate that cleavage of 125I-plasminogen can be employed as a direct, sensitive and quantitative assay for various PAs, and offers a new approach for studying plasminogen activation and agents that stimulate or inhibit it.