The heavy polypeptide chains of human Glu-plasmin and human Lys-plasmin have been isolated in native solvents, after partial reduction and carboxymethylation of the corresponding plasminogens. Two major forms of each heavy chain can be eluted, after adsorption to Sepharose/lysine, utilizing a gradient of e-aminocaproic acid as the eluant. The elution profile of these heavy chains is practically identical to the elution behavior previously observed for human Glu- and Lys-plasminogen, and human Glu- and Lys-plasmin, adsorbed to these columns. Sedimentation velocity analysis of the heavy chain of human Glu-plasmin, in the presence of e-aminocaproic acid, demonstrated that a gross conformational alteration occurs in this peptide accompanying binding of this amino acid. A much smaller conformational alteration occurs under similar circumstances with the human Lys-plasmin heavy chain. We find that the NH₂-terminal peptide released in the Glu plasminogen to Lys plasminogen and Glu-plasmin to Lys-plasmin conversions is also released in the Glu-plasmin heavy chain to Lys-plasmin heavy chain conversion. This reaction is catalyzed at a significant rate only by plasmin and not by urokinase. Finally, no strong interaction between streptokinase and the isolated plasmin heavy chains is observed.

It is now well established that at least two peptide bonds are cleaved in conversion of single chain human plasminogen to a two-chain plasmin molecule. Cleavage of an arginyl-valine bond, in the interior of the plasminogen molecule, yields a two-chain plasmin, stabilized by a single disulfide bond (1). Another cleavage, at the NH₂-terminus of the original plasminogen molecule, liberates a small activation peptide of molecular weight 6,000 to 8,000 (2-5). Our recent studies on two-chain plasmin, stabilized by a single disulfide bond, in the interior of the plasminogen molecule, yields a significant rate only by plasmin and not by urokinase. Finally, no strong interaction between streptokinase and the isolated plasmin heavy chains is observed.

1 The abbreviations used are: e-ACA, e-aminocaproic acid; SDS, sodium dodecyl sulfate; Glu-P₉ and Lys-P₉, human plasminogen containing NH₂-terminal glutamic acid and lysine, respectively; Glu-P₉ and Lys-P₉, human plasmin containing heavy chains with NH₂-terminal glutamic acid and lysine, respectively; Glu-H and Lys-H, the isolated heavy chains of Glu-P₉ and Lys-P₉, respectively. These heavy chains contain NH₂-terminal glutamic acid and lysine, respectively.

2 Some sections of this manuscript, as well as part of the data obtained, are presented as a miniprint supplement immediately following this paper. For the convenience of those who prefer to receive the supplementary material in the form of 10 pages of full size photocopies, it is available as JBC Document Number 76M-1134. Orders should specify the title, authors, and reference to this paper, the JBC Document Number, and the number of copies desired. Orders should be addressed to the Journal of Biological Chemistry, 8650 Rockville Pike, Bethesda, Md. 20014 and must be accompanied by a remittance to the order of the Journal in the amount of $1.50.
or Glu-plasmin to a Sepharose/lysin affinity matrix and elution with a gradient of \( \epsilon \)-ACA. Two major forms of the Lys-plasmin heavy chain (Lys-H) and the Glu-plasmin heavy chain (Glu-H) are obtained (Fig. 2, A and B, supplement). Molecular weights, obtained by high speed sedimentation equilibrium centrifugation, and quantitative NH\(_2\)-terminal amino acid analysis, were determined for affinity chromatography form 2 of Lys-H and Glu-H. From the plots shown in Fig. 3, A and B, supplement, a molecular weight of 61,500 ± 2,500 was obtained for Lys-H, and 69,000 ± 2,500 was obtained for Glu-H in native solvents. No evidence for aggregation of the peptides over the concentration range indicated was noted. Similar molecular weights were obtained for these heavy chains in calibrated SDS gels. The unique NH\(_2\)-terminal amino acid found on Glu-H was glutamic acid in quantities of 0.9 mol/mol of peptide; whereas, Lys-H contained lysine and valine (determined in quantities of 0 and 0.2 mol/mol of peptide, respectively. Qualitative immunodiffusion analysis, shown in Fig. 4, supplement, indicates that Glu-H, Glu-Pg, Lys-H, and Lys-Pg all cross-react with rabbit antibodies against human Lys-H.

The effect of \( \epsilon \)-ACA on the \( \Delta_{\text{N-20}} \) of Lys-H and Glu-H has been studied. The native \( \Delta_{\text{N-20}} \) value of 3.9 S for Lys-H is only slightly altered to 3.8 S upon complete binding of \( \epsilon \)-ACA. This is in contrast to the results reported by Rickli and Otavsky (10), who found a much larger decrease in the \( \Delta_{\text{N-20}} \) of this heavy chain upon \( \epsilon \)-ACA binding. However, they also found a parallel increase in the \( D_{\text{N-20}} \), indicating that a molecular weight change occurred in their experiment. Our studies on the isolated Lys-H fortify our previous observations (11) that \( \epsilon \)-ACA binding to Lys-Pg and Lys-Pm is accompanied by only minor

REFERENCES

Purification and some properties of the Glu- and Lys-human plasmin heavy chains.
M Gonzalez-Gronow, B N Violand and F J Castellino


Access the most updated version of this article at http://www.jbc.org/content/252/7/2175

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/252/7/2175.full.html#ref-list-1