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 plasmins. Two major forms of each heavy chain can be eluted, after adsorption to Sepharose/lysine, utilizing a gradient of ε-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 ε-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, generates 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, yield a large conformational alteration in this polypeptide which has lost the activation peptide. Due to this inconsistency, we have attempted to isolate the heavy chain containing the activation peptide intact (Glu-H) in order to compare its ε-ACA binding properties with those of Lys-H. In addition, we felt that much could be learned about the activation of human plasminogen from a study of the isolated plasmin heavy chains. This report summarizes our findings in these areas.

**EXPERIMENTAL PROCEDURES**

Since the materials and methods employed represent only minor modifications of those previously described by ourselves and others, this section has been placed in the adjacent miniprint.

**RESULTS AND DISCUSSION**

In this report, we have isolated the heavy chains of human Glu-plasmin and human Lys-plasmin, in native solvents, utilizing a modification of the procedure described by Rickli and Otavsky (10), after partial reduction of the plasmins. Establishment of the reduction conditions for cleavage of the interchain disulfide for production of Glu-H with minimal intrachain reduction is shown in Fig. 1, supplement. Upon adsorption of partially reduced and carboxymethylated Lys-plasmin

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or Glu-plasmin to a Sepharose/lysin affinity matrix and elution with a gradient of e-ACA. Two major forms of the Lys-plasmin heavy chain (Lys-H) and the Glu-plasmin heavy chain (Glu-H) are obtained (Figs. 2 and A, B, supplement). Molecular weights, obtained by high speed sedimentation equilibrium centrifugation, and quantitative NH-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-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 (des-Lys) in quantities of 0.6 mol 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 e-ACA on the $s_{20w}$ of Lys-H and Glu-H has been studied. The native $s_{20w}$ value of 3.9 S for Lys-H is only slightly altered to 3.8 S upon complete binding of e-ACA. This is in contrast to the results reported by Rickli and Otavsky (10), who found a much larger decrease in the $s_{20w}$ of this heavy chain upon e-ACA binding. However, they also found a parallel increase in the $D_{20w}$, indicating that a molecular weight change occurred in their experiment. Our studies on the isolated Lys-H fortify our previous observations (11) that e-ACA binding to Lys-Pg and Lys-Pm is accompanied by only minor alterations in the conformation of plasminogen or plasmin which lack the activation peptide. On the other hand, the results of Fig. 6 show that urokinase, when present in quantities of 2,400 Plough units/ml, was completely incapable of catalyzing the Glu-H to Lys-H conversion up to at least 45 min. At these concentrations of urokinase and under the temperature and buffer conditions employed, equivalent amounts of Glu-Pg would be converted to Lys-Pm at times of less than 10 min. Thus, we conclude that the activator, urokinase, is incapable of removing the activation peptide from the Glu-Pm heavy chain at rates of importance to its mechanism of action. Similar conclusions have previously been forwarded (5) concerning the action of urokinase on Glu-Pg.

Finally, we wished to determine whether the streptokinase binding site on human plasminogen existed on the isolated human plasmin heavy chain. Sucrose density centrifugation with streptokinase and Lys-H did not yield evidence for an interaction between the components, as seen from Fig. 7, supplement. Thus, the tight streptokinase binding site on plasminogen and plasmin is not an exclusive property of the latent or real plasmin heavy chain.

REFERENCES


Fig. 5 (left). The conversion of Glu-H to Lys-H by plasmin. A level of plasmin at 5% of the weight of Glu-H was incubated with Glu-H. Samples were removed at times of 0 min (Gel 1), 2 min (Gel 2), 5 min (Gel 3), 10 min (Gel 4), and 45 min (Gel 5) for reduced SDS-gel electrophoretic analysis of the Glu-H to Lys-H conversion. All gels contained 6 M urea and 5% acrylamide.

Fig. 6 (right). The conversion of Glu-H to Lys-H by urokinase. A level of urokinase of 2,400 Plough units/ml was added to Glu-H. Samples were removed at times of 2 min (Gel 1), 5 min (Gel 2), 10 min (Gel 3), and 45 min (Gel 4). All conditions are as in Fig. 5.
Plasmin Heavy Chains 2177

Supplementary Material

Plasmin and Some Properties of the C2a and C2b Fragments

Arctic Oceanic Environments, Alaska

Michael J. Carthew

The results of this investigation, as well as the data and observations, are shown in the present paper. The sequence of events and results yield a detailed and usable model for the determination of the concentration of heavy and light subunits used together with a more precise procedure for isolation of the subunits.

Methods

The heavy chains of human plasmin were purified by the method of Sokolov and coworkers (11). The plasminogen used was a gift from Dr. J. R. Wessling, New York, N.Y.

Preparation of plasminogen. Plasminogen was prepared by Dr. J. R. Wessling and associates (12) using a modification of the method of Hamburger (13). The plasminogen was isolated by the method of Sokolov and coworkers (11). The plasminogen was isolated by the method of Sokolov and coworkers (11). The plasminogen was isolated by the method of Sokolov and coworkers (11). The plasminogen was isolated by the method of Sokolov and coworkers (11). The plasminogen was isolated by the method of Sokolov and coworkers (11).

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Purification and some properties of the Glu- and Lys-human plasmin heavy chains.
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