The Interaction of Streptokinase with Human, Cat, Dog, and Rabbit Plasminogens

THE FRAGMENTATION OF STREPTOKINASE IN THE EQUIMOLAR PLASMINOGEN-STREPTOKINASE COMPLEXES

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SUMMARY

Highly purified human (Lys-forms), cat, dog, and rabbit plasminogens showed significantly different sensitivities to activation by highly purified streptokinase. The most sensitive plasminogen is the human zymogen, followed by the cat, dog, and rabbit zymogens, respectively. These human, cat, dog, and rabbit zymogens reacted with streptokinase to form homogeneous equimolar plasminogen-streptokinase complexes. The electrophoretic mobilities of the human and cat complexes on cellulose acetate were different from their respective zymogens, but the electrophoretic mobilities of the dog and rabbit complexes were similar to their respective zymogens. The human, cat, and dog complexes showed different mobilities from their respective zymogens in acrylamide gel electrophoretic systems and appeared to show multiple electrophoretic forms. With these species, all of the multiple isoelectric forms of the zymogens reacted with streptokinase.

Analyses of the human (Glu- and Lys-forms), cat, dog, and rabbit equimolar plasminogen-streptokinase complexes, incubated for various time intervals, in an acrylamide gel-dodecyl sulfate-urea electrophoretic system, showed the gradual conversion, or transformation, of the plasminogen-streptokinase complexes into plasmin-streptokinase complexes. These transformations occurred at different rates in each of the mammalian plasminogen-streptokinase complexes. This acrylamide gel system also permitted a comparison between the plasmin-derived carboxymethyl heavy (A) and carboxymethyl light (B) chains produced from each of these plasminogens by urokinase activation; the human (Lys-forms) and dog carboxymethyl heavy (A and A') chains appeared to be similar in both activation systems. The carboxymethyl heavy (A* and A') chains produced by streptokinase activation of the human (Glu- and Lys-forms), cat, dog, and rabbit plasmins found in the complexes were similar but not identical with each other in molecular weight. The plasmin-derived carboxymethyl light (B') chains produced by streptokinase activation of the human (Glu- and Lys-forms), cat, dog, and rabbit plasminogens in the complex appeared to have molecular weights similar to the carboxymethyl light (B) chains derived from each of these plasminogens by urokinase activation. But, the plasmin-derived carboxymethyl light (B') chains of the four species obtained from the equimolar complexes appeared to have somewhat different molecular weights.

Streptokinase fragmentation within the complexes occurs within a few seconds after the complexes are formed, and intact streptokinase disappears after several minutes of incubation. Four major streptokinase fragments (SK1, SK2, SK3, and SK4), having molecular weights between 47,600 and 25,700, are produced in each of the equimolar human (Glu- and Lys-forms), cat, dog, and rabbit complexes. They appear to be the same fragments in each of the mammalian plasminogen-streptokinase and plasmin-streptokinase complexes; each of the complexes contains varying amounts of streptokinase fragments, SK1, SK2, SK3, and SK4. But, the rate of degradation of streptokinase into these major fragments differed with each species.

Plasmin and streptokinase moieties prepared by dissociation of an equimolar plasminogen (Lys-forms)-streptokinase complex at pH 3.0 (SUMMARIA, L., ROBBINS, K. C., AND BARLOW, G. H. (1971) J. Biol. Chem. 246, 2136-2142) were also analyzed in the acrylamide gel-dodecyl sulfate-urea electrophoretic system. The isolated plasmin moiety gave carboxymethyl heavy and carboxymethyl light chains, both of which were of lower molecular weight than the plasmin-derived carboxymethyl heavy (A) and carboxymethyl light (B) chains produced by urokinase activation. The isolated streptokinase moiety contains approximately equal amounts of streptokinase fragments SK2 and SK4, and a third smaller fragment.

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The interactions of human plasminogen and streptokinase both in the activation of the zymogen (1–4) and in the formation of bovine plasminogen activator (equimolar complexes of plasminogen and streptokinase, or plasmin and streptokinase) (5–19), have been studied in great detail. Claims have been made that a modified streptokinase can be separated from a complex of human plasminogen and streptokinase which has bovine plasminogen activator activity (20, 21). It has been reported that streptokinase is species specific in its activation of plasminogen (22). Of the mammalian species studied, only the human, monkey, and cat zymogens can be activated with low concentrations of streptokinase, whereas the dog and rabbit zymogens are activated only with high concentrations of streptokinase. Dissociation of the equimolar human plasmin-streptokinase complex gave a modified plasin moiety and fragmented streptokinase moieties (23); a streptokinase fragment was also found to be a component part of the modified plasin moiety. The fragmentation of streptokinase occurs after formation of the equimolar plasmin-streptokinase complex without apparent loss of any of the fragments.

The mechanism of activation of human Lys-plasminogen by streptokinase, and urokinase, involves the specific cleavage of a sensitive arginyl-valine peptide bond in the COOH-terminal portion of the zymogen to give the two-chain plasmin molecule (1, 2). The mechanism of activation of human Glu-plasminogen, and cat, dog, rabbit, and bovine plasminogens by urokinase, to plasmin, involves two specific peptide bond cleavages (24, 25). The specific cleavage of the sensitive arginyl-valine peptide bond, described for human Lys-plasminogen (1), and the specific cleavage of an X-arginyl, or X-lysyl, peptide bond (24), or an arginyl-serine and a lysyl-methionine peptide bond (25), in the NH2-terminal portion of the molecule are the two principal events which occur during activation of these zymogens. The latter event apparently results in the release of a peptide from the two-chain plasmin molecule. Additional peptide bonds are cleaved, primarily in the heavy chain portion of the molecule, during the activation of human, cat, dog, and bovine plasminogens by urokinase (24, 26). The carboxymethyl heavy (A) and carboxymethyl light (B) chain derivatives of the plasmins of all five species have been isolated, characterized, and compared (26). The chains derived from rabbit plasminogen activated by either streptokinase, or urokinase, were found to be indistinguishable in molecular weight (27).

In this paper, we describe the activation of highly purified human (Lys-forms), cat, dog, and rabbit plasminogens by highly purified streptokinase in quantitative terms. Studies will be reported on the preparation and characterization of equimolar complexes of human (Glu- and Lys-forms), cat, dog, and rabbit plasminogens with streptokinase. A comparison will be made between the plasmin-derived carboxymethyl heavy and carboxymethyl light chains produced from these mammalian zymogens by two different methods of activation, namely, equal molar ratios of streptokinase to zymogen and low molar ratios of urokinase to zymogen (1:1000). Studies on the fragmentation of streptokinase which takes place in these equimolar plasminogen-streptokinase complexes after their formation is also reported. Preliminary data on these plasminogen-streptokinase interactions has been recently reported (28).

**Materials and Methods**

**Plasminogen and Plasmins**—Human, cat, dog, rabbit, and bovine plasminogens were prepared by a modification (26, 29, 30) of the affinity chromatography method described by Deutsch and Mertz (31). Human Glu- and Lys-plasminogen forms have been prepared from plasma and plasma fractions (24, 26, 29, 30). The animal plasminogens were either Glu-, Asp-, or blocked forms (24). Plasmin was prepared by activating the plasminogens with urokinase by methods previously described (2). Proteolytic activity was determined on a casein substrate after activation of the zymogens with urokinase, or streptokinase, and directly with the enzyme (32, 33).

**Carboxymethyl Heavy (A) and Carboxymethyl Light (B) Chain Derivatives of Plasmin**—The carboxymethyl heavy (A) and carboxymethyl light (B) chain derivatives of the plasmin of all five species were prepared by methods previously described (26, 24).

**Streptokinase**—KabiKinase, Lot No. 19088, was supplied by Grant H. Barlow, Abbott Laboratories (North Chicago, Illinois). One vial containing 5,000,000 units of streptokinase was dissolved in 5.0 ml of 0.067 M phosphate buffer, pH 7.4. The solution was clarified at 4,000 rpm for 1 hour at 2°C; it contained 40 mg of soluble streptokinase protein calculated from absorbance measurements at 280 nm using an extinction coefficient of 1.0 (35).

**Cellulose Acetate Electrophoresis**—Electrophoretic analysis on cellulose acetate membranes was carried out as described previously (3) with samples at a concentration of 8 to 10 mg of protein per ml.

**Acrylamide Gel Electrophoresis**—Acrylamide gel electrophoresis was carried out in gel slabs in a Beckman Microzone acrylamide gel system (29, 30). The gel slabs contained either 0.3 M ε-amino
caproic acid or 0.3 M trans-4-aminomethylcyclohexanecarboxylic acid at pH 8.4 (5% gel) or 0.1% dodecyl sulfate-6 M urea at pH 7.0 (8.4% gel). The detailed procedures for these acrylamide gel electrophoretic analyses have been previously described (29, 30).

**Results**

**Activation of Human, Cat, Dog, Rabbit, and Bovine Plasminogens with Streptokinase**—Although human, cat, dog, rabbit, and bovine plasminogens could be completely activated using urokinase (26), these preparations showed significant differences in their sensitivities to activation by streptokinase. The degree of activation of human (Lys-forms), cat, and dog plasminogens with increasing amounts of streptokinase is shown in Fig. 1. Rabbit and bovine plasminogen (0.1 mg) could not be activated with as much as 100,000 units of streptokinase in the assay. At 25% activation, cat plasminogen required 20 times as much streptokinase and dog plasminogen required 1,600 times as much streptokinase as did human plasminogen. Dog plasminogen requires a molar ratio of streptokinase to plasminogen of 14:1 to achieve complete activation in the assay. When these plasminogens were activated by streptokinase in the absence of casein (preincubation before assay), much less streptokinase was required to produce the same degree (25%) of activation with the human, cat, and dog plasminogens; the molar ratio of streptokinase to dog plasminogen was now 0.1:1. Under these conditions, rabbit plasminogen could be activated 25% by streptokinase, but...
required very large amounts of activator; approximately 2,000 times as much streptokinase was required as was needed to activate human plasminogen (molar ratio of streptokinase to plasminogen of 3:1). Bovine plasminogen could not be activated by preincubation with streptokinase.

Preparation and Characterization of Equimolar Human (Glu- and Lys-Forms), Cat, Dog, and Rabbit Plasminogen-Streptokinase Complexes—Equimolar mixtures of human (Glu- and Lys-forms), cat, dog, rabbit, and bovine plasminogens with streptokinase were prepared at 0° and then incubated at 25° for 10 min. They were treated with 0.01 m DFP at 0° for 30 min. These mixtures were analyzed electrophoretically on cellulose acetate (Fig. 2, A and B), and in acrylamide gels containing either ε-aminocaproic acid (Fig. 2C) or trans-4-aminomethyl cyclohexanecarboxylic acid (Fig. 2D). The human plasminogen preparations (Glu- and Lys-forms) both had the same electrophoretic mobility on cellulose acetate. Both the human Glu- and Lys-plasminogen forms reacted with streptokinase to form homogeneous equimolar plasminogen-streptokinase complexes; the electrophoretic mobilities of these complexes were different from the electrophoretic mobilities of the zymogens. The equimolar cat plasminogen-streptokinase complex was also homogeneous with an electrophoretic mobility different from the electrophoretic mobility of either cat plasminogen or streptokinase; its mobility was similar to that of the human plasminogen-streptokinase complexes. The equimolar rabbit and dog plasminogen-streptokinase complexes migrated electrophoretically as single components, but did not have electrophoretic mobilities different from the parent rabbit and dog plasminogens. It was evident that the rabbit and dog plasminogen had reacted with streptokinase to form equimolar complexes, since no streptokinase component could be seen in these two plasminogen-streptokinase complexes. No excess streptokinase, or streptokinase fragments, could be seen in either the human, cat, dog or rabbit plasminogen-streptokinase complexes. Electrophoretic analysis of an equimolar mixture of bovine plasminogen and streptokinase indicated that no interaction occurred, since both the bovine plasminogen and the streptokinase retained their original electrophoretic mobilities.

The human (Glu- and Lys-forms), cat, dog, and rabbit plasminogen-streptokinase complexes were analyzed and compared with their respective plasminogen in acrylamide gels containing either ε-aminocaproic acid (Fig. 2C) or trans-4-aminomethyl cyclohexanecarboxylic acid (Fig. 2D). The equimolar human (Glu- and Lys-forms), cat, and dog plasminogen-streptokinase complexes had distinctly different electrophoretic mobilities from the mobilities of the multiple electrophoretic forms of their respective zymogens. The multiple electrophoretic forms of the human (Glu- and Lys-forms) and dog plasminogens are similar in both the ε-aminocaproic acid and trans-4-aminomethylcyclohexanecarboxylic acid acrylamide gel systems (Fig. 2, C and D). The electrophoretic mobilities of the human Glu-plasminogen-streptokinase and the human Lys-plasminogen-streptokinase complexes were different (Fig. 2D). There appears to be two groups of complexes in both the human Glu-plasminogen and cat plasminogen-streptokinase complexes. The human (Glu- and Lys-forms), cat, and dog equimolar complexes appear to show multiple electrophoretic forms. The apparent equimolar rabbit plasminogen-streptokinase complex contained multiple electrophoretic components with mobilities similar to the mobilities of the multiple electrophoretic forms of the parent zymogen. The rabbit plasminogen-streptokinase complex contained a large amount of a component which had an electrophoretic mobility similar to that of streptokinase. Since electrophoretic analysis of the rabbit plasminogen-streptokinase complex on cellulose acetate indicated that the streptokinase had completely reacted with the rabbit plasminogen, it is possible that the rabbit plasminogen-streptokinase complex dissociated during electrophoresis in the acrylamide gel-ε-aminocaproic acid system.

Analysis of Conversion of Plasminogen-Streptokinase Complexes to Plasmin-Streptokinase Complexes—Equimolar human (Glu- and Lys-forms), cat, dog, and rabbit plasminogen-streptokinase complexes were prepared at 0°. These complexes were incubated at 25° for various time intervals up to 40 min. The time required for mixing the components was approximately 0.25 min and this sample is the first one analyzed in the following experiments. The reaction was stopped by treating each plasminogen-streptokinase complex with 0.01 m DFP at 0° for 30 min and the preparations were stored at -25°. Each DFP-treated complex was reduced in 8 m urea with 0.1 m 2-mercaptoethanol and alkylated with 0.1 m iodoacetate (26, 34). The mixtures were adjusted to 1% dodecyl sulfate and then analyzed in the acrylamide gel-dodecyl sulfate-urea electrophoretic system.

The equimolar complexes of streptokinase with human (Lys-forms), cat, dog, and rabbit plasminogen, incubated for 10 min, were first compared (Fig. 3A). For purposes of comparison and identification, samples of carboxyethyl plasminogen, carboxymethyl heavy (A) and carboxymethyl light (B) chains of the plasmin (urokinase-activated) (26) and streptokinase were used as controls. In this experiment, the residual plasminogen components of the four species which were compared had slightly different molecular weights; rabbit plasminogen appeared to be the largest of the zymogens. Also, the four pairs of plasmin-derived carboxymethyl heavy and carboxymethyl light chains differed in their molecular weights. The components seen between the carboxymethyl heavy and carboxymethyl light chains are large streptokinase fragments; very little intact streptokinase remained. The identification of the streptokinase fragments is discussed in the following experiments. Peptides smaller in size than the carboxymethyl light chain were found in each preparation.

An analysis of the equimolar human Lys-plasminogen and Glu-plasminogen-streptokinase complexes, incubated for 0.25, 0.50, 1, 2, 5, and 10 min, is shown in Fig. 3, B and C. Both of these complexes show a gradual decrease in the plasmin component as the preparations were incubated and a gradual increase in the plasin component. The plasmin carboxymethyl heavy chain produced in the Lys-complexes (Fig. 3B) appeared to be identical in molecular weight with the carboxymethyl heavy (A) chain produced by urokinase activation. The plasmin carboxymethyl heavy chain produced in the Glu-complexes (Fig. 3C) appeared to be larger than the plasmin carboxymethyl heavy (A) chain. The plasmin carboxymethyl light chains formed in all of the plasmin-streptokinase complexes appeared to be nearly identical in molecular weight with the plasmin carboxymethyl light (B) chains produced by urokinase activation. Very little intact streptokinase remains after 0.50 min. The streptokinase fragments appearing in the Lys-complexes appeared to be the same fragments as were produced in the Glu-complexes. The rate at which each streptokinase fragment developed was similar for both types of complexes.

In order to obtain a better comparison of the components in
Fig. 2. A, cellulose acetate electrophoretogram: 1, human Glu-plasminogen; 2, human 10-min Glu-complex; 3, human 10-min Lys-complex; 4, streptokinase; 5, cat plasminogen; 6, cat 10-min complex (nonincubated); 7, cat 10-min complex; 8, streptokinase (1:2). B, cellulose acetate electrophoretogram: 1, human Lys-plasminogen; 2, human 10-min Lys-complex; 3, rabbit plasminogen; 4, rabbit 10-min complex; 6, dog plasminogen; 7, dog 10-min complex; 8, streptokinase; 9, streptokinase (1:2). C, acrylamide gel-ε-aminocaproic acid electrophoretogram: 1, human Lys-plasminogen; 2, human 10-min Lys-complex; 3, cat plasminogen; 4, cat 10-min complex; 5, dog plasminogen; 6, dog 10-min complex; 7, rabbit plasminogen; 8, rabbit 10-min complex. D, acrylamide gel-trans-4-aminomethylcyclohexane carboxylic acid electrophoretogram: 1, dog plasminogen; 2, dog 10-min complex; 3, human Lys-plasminogen; 4, human 10-min Lys-complex; 5, human Glu-plasminogen; 6, human 10-min Glu-complex; 7, streptokinase.
the incubated Lys- and Glu-complexes, samples of these complexes, incubated for 0.25, 2, and 10 min, were subjected to electrophoresis for 16 hours, instead of 5 hours (Fig. 3D). In this experiment, the plasminogen component seen in the Glu-complexes had an apparent higher molecular weight than the plasminogen component seen in the Lys-complexes. On further incubation the plasminogen component in the Glu-complexes resolved into two distinct components, one apparently similar in molecular weight to the Lys-plasminogen component. The plasmin carboxymethyl heavy (A*) chain seen in the Glu-complexes also had a higher molecular weight than the plasmin carboxymethyl heavy (A') chain found in the Lys-complexes.
Fig. 3. A to I. Acrylamide-gel-urea-dodecyl sulfate electrophoreograms: (A) 1, human carboxymethyl Lys-plasminogen; 2, human Lys-carboxymethyl heavy (A) chain; 3, human 10-min carboxymethyl Lys-complex; 4, cat 10-min carboxymethyl complex; 5, dog 10-min carboxymethyl complex; 6, rabbit 10-min carboxymethyl complex; 7, human Lys-carboxymethyl light (B) chain; 8, streptokinase. (B) 1, human Lys-carboxymethyl heavy (A) and carboxymethyl light (B) chains; 2, human 0.25-min carboxymethyl Lys-complex; 3, human 0.5-min carboxymethyl Lys-complex; 4, human 1-min carboxymethyl Lys-complex; 5, human 2-min carboxymethyl Lys-complex; 6, human 5-min carboxymethyl Lys-complex; 7, human 10-min carboxymethyl Lys-complex; 8, streptokinase. (C) 1, human Lys-carboxymethyl heavy (A) and carboxymethyl light (B) chains; 2, human 0.25-min carboxymethyl Glu-complex; 3, human 0.5-min carboxymethyl Glu-complex; 4, human 1-min carboxymethyl Glu-complex; 5, human 2-min carboxymethyl Glu-complex; 6, human 5-min carboxymethyl Glu-complex; 7, human 10-min carboxymethyl Glu-complex; 8, streptokinase. (D) 1, human Lys-carboxymethyl heavy (A) and carboxymethyl light (B) chains; 2, human 0.25-min carboxymethyl Glu-complex; 3, human 0.25-min carboxymethyl Lys-complex; 4, human 1-min carboxymethyl Lys-complex; 5, human 2-min carboxymethyl Lys-complex; 6, human 10-min carboxymethyl Lys-complex; 7, human 10-min carboxymethyl Lys-complex. (E) 1, human Lys-carboxymethyl heavy (A) and carboxymethyl light (B) chains; 2, human carboxymethyl plasmin moiety from carboxymethyl Lys-complex; 3, streptokinase; 4, streptokinase moiety from carboxymethyl Lys-complex; 5, streptokinase fragment (Peak 1, Fig. 4D of Ref. 23); 6, human 2-min carboxymethyl Lys-complex. (F) 1, cat carboxymethyl plasminogen; 2, cat carboxymethyl heavy (A'1) chain; 3, cat 0.25-min carboxymethyl complex; 4, cat 2-min carboxymethyl complex; 5, cat 10-min carboxymethyl complex; 6, cat carboxymethyl light (B) chain; 7, streptokinase; 8, human carboxymethyl heavy (A) chain. (G) 1, dog carboxymethyl plasminogen; 2, streptokinase; 3, dog 0.25-min carboxymethyl complex; 4, dog 2-min carboxymethyl complex; 5, dog 10-min carboxymethyl complex; 6, dog 20-min carboxymethyl complex; 7, dog carboxymethyl light (B) chain; 8, dog carboxymethyl heavy (A and A2) chain. (H) 1, rabbit carboxymethyl plasminogen; 2, rabbit carboxymethyl heavy (A) chain; 3, rabbit 2-min carboxymethyl complex; 4, rabbit 10-min carboxymethyl complex; 5, rabbit 20-min carboxymethyl complex; 6, rabbit 40-min carboxymethyl complex; 7, rabbit carboxymethyl light (B) chain; 8, streptokinase. (I) 1, rabbit carboxymethyl heavy (A) and carboxymethyl light (B) chains; 2, rabbit 10-min carboxymethyl complex (1:1); 3, rabbit 10-min carboxymethyl complex (2:1); 4, rabbit 20-min carboxymethyl complex (3:1); 5, rabbit 20-min carboxymethyl complex (1:1); 6, rabbit 20-min carboxymethyl complex (2:1); 7, rabbit 20-min carboxymethyl complex (3:1); 8, streptokinase. SK, streptokinase.

(see Table I for description of chain nomenclature). Upon further incubation, a second, smaller plasmin carboxymethyl heavy (A') chain appeared to develop in the Glu-complexes, perhaps similar in size to the Lys-plasmin-derived carboxymethyl heavy (A') chain. No resolution into two plasminogen, or two plasmin carboxymethyl heavy (A') chain components occurred in the Lys-complexes. The plasmin carboxymethyl light (B') chain formed in both the Glu- and Lys-complexes appeared to be
identical in molecular weight with each other, and with the plasmin carboxymethyl light (B) chain produced by urokinase activation. The resolution of the streptokinase fragments was improved in this experiment; three additional minor streptokinase fragments could be identified in the two 0.25-min mixtures. Some intact streptokinase was present but most of the streptokinase was already converted to the first and second major streptokinase fragments (SK1 and SK2) after only 0.25 min. After 2 min, streptokinase and streptokinase fragment SK1, had nearly disappeared, and streptokinase fragment SK2 is now the major streptokinase component, with small amounts of streptokinase fragment SK3 appearing. After 10 min, streptokinase fragment SK4 appears as streptokinase fragment SK3 disappears; however, streptokinase fragment SK2 persists throughout the incubation and is the major streptokinase fragment in the 10-min incubation mixtures. Streptokinase fragment SK2 appears to be the major fragment in the human complexes.

In a previous study, we had reported on the dissociation of an equinuline human Lys-plasminogen-streptokinase complex, which had been incubated for 10 min at pH 3.0 (23). The plasmin and streptokinase moieties were separated from one another on Sephadex G-150 at pH 3.0 (see Fig. 3 of Ref. 23). The streptokinase moiety was dissociated into its component fragments in Sephadex G-200 column in the same buffer system (see Fig. 4 of Ref. 23). The streptokinase moiety (Peak 1 of Fig. 3) and the largest of the streptokinase fragments (Peak 1 in Fig. 4D of Ref. 23) were analyzed in the acrylamide gel system (Fig. 3f). The streptokinase moiety contains approximately equal amounts of streptokinase fragments SK2, SK3, and a smaller fragment. Peak 1 in Fig. 4D of Ref. 23 contains primarily streptokinase fragment SK2 and small amounts of streptokinase fragments SK3 and SK4. The sedimentation coefficient, $s_{20,w}$, of this fraction was determined to be approximately 2 S which is probably the $s_{20,w}$ value for streptokinase fragment SK2. The plasmin moiety (Peak 2 of Fig. 3) showed one carboxymethyl heavy chain and one carboxymethyl light chain, both of which were lower in molecular weight than the plasmin-derived carboxymethyl heavy (A) and carboxymethyl light (B) chains produced by urokinase activation. It appears that the plasmin moiety of this complex was partially degraded in both plasmin chains.

An analysis of the cat plasminogen-streptokinase complex incubated for 0.25, 2, and 10 min is shown in Fig. 3E. The plasmin carboxymethyl heavy (A') chain that is formed appears to be larger than the carboxymethyl heavy (A1) chain produced by urokinase activation and is similar to the human carboxymethyl heavy (A) chain in molecular weight. No plasmin carboxymethyl heavy (A1) chain is formed even after the cat complex is incubated for 10 min. The plasmin carboxymethyl light (B') chain that is produced during this conversion appears to be slightly lower in molecular weight than the plasmin carboxymethyl light (B) chain produced by urokinase activation. It appears that there is nearly complete conversion of the cat plasminogen-streptokinase complex to a cat plasmin-streptokinase complex after 10 min, but only partial conversion after 2 min. The 0.25-min cat complex contains intact streptokinase. After 2 min, very little intact streptokinase remains and three of the major streptokinase fragments (SK1, SK2, and SK3) can be seen. After 10 min, no intact streptokinase remains, the largest streptokinase fragment (SK1) has also disappeared, and a fourth streptokinase fragment (SK4) slightly larger than the carboxymethyl light (B) chain appears. These four major streptokinase fragments appear to be the same as those formed in the human Glu- and Lys-complexes (see Fig. 3, B and C).

An analysis of the dog plasminogen-streptokinase complex incubated for 0.25, 2, 10, and 20 min is shown in Fig. 3G. A major plasmin carboxymethyl heavy (A') chain is formed which appears to be identical with the plasmin carboxymethyl heavy (A) chain produced by urokinase activation; there is no plasmin carboxymethyl heavy (A2) chain formed even after 20 min. The plasmin carboxymethyl light (B') chain that is formed appears to be slightly lower in molecular weight than the plasmin carboxymethyl light (B) chain produced by urokinase-activation. The dog complex incubated for 0.5 min contains some intact streptokinase and four major streptokinase fragments (SK1, SK2, SK3, and SK4). Intact streptokinase cannot be found after 2 min, and two major streptokinase fragments (SK2 and SK3) appear. After 10 min, the SK2 and SK3 streptokinase fragments disappear and a new streptokinase fragment (SK4) appears.

Two types of rabbit plasminogen preparations were obtained from different batches of rabbit plasma. One could be activated to plasmin by streptokinase and the other could not be activated to plasmin. An analysis of the rabbit plasminogen-streptokinase complex prepared with the rabbit plasminogen that could be activated by streptokinase, incubated for 2, 10, 20, and 40 min, is shown in Fig. 3H. Even after 40 min, only a part of the plasminogen was converted to plasmin. Two plasmin carboxymethyl (A* and A') chains were produced; equal amounts were observed in the 20-min complex. The 40-min complex contained the plasmin carboxymethyl heavy (A') chain only. The 10-min complex contained a single plasmin carboxymethyl heavy (A*) chain which was of higher molecular weight than any of the rabbit plasmin carboxymethyl heavy (A) chain derivatives previously reported (26). This type of carboxymethyl heavy chain transformation was not observed in either of the incubated cat or dog plasminogen-streptokinase complexes. The plasmin carboxymethyl light (B') chain appeared to be identical in molecular weight with the plasmin carboxymethyl light (B) chain produced by urokinase activation. A large amount of intact streptokinase was observed in the rabbit plasminogen-streptokinase complex incubated for 2 min; this complex also contains one major streptokinase fragment (SK1) and trace amounts of two other streptokinase fragments (SK2 and SK3). After 10 min, both the intact streptokinase and the largest streptokinase fragment (SK1) had disappeared with the appearance of two major smaller streptokinase fragments (SK3 and SK4). Both of the SK3 and SK4 fragments gradually disappeared on further incubation (20 and 40 min).

The second type of rabbit plasminogen, which could not be activated by streptokinase, was incubated with streptokinase in molar ratios of 1:1, 2:1, and 3:1 (ratio of streptokinase to plasminogen) for 10 and 20 min. An analysis of these mixtures is shown in Fig. 3I. Samples of a mixture of rabbit plasmin carboxymethyl heavy (A) and carboxymethyl light (B) chain derivatives of plasmin (urokinase-activated), and streptokinase, were used as controls. None of these incubation mixtures contained a component that resembled the rabbit plasmin carboxymethyl light (B) chain. A trace component similar in size to the rabbit plasmin carboxymethyl heavy (A) chain was present in nearly all of these incubated mixtures. Of particular interest in these analyses is that although apparently little, or no, rabbit plasmin was formed, no intact streptokinase could be found. The three major streptokinase fragments (SK2, SK3, and SK4) formed at 10 min appeared to be the same three fragments pro-
duced in the rabbit plasminogen-streptokinase complex prepared with the rabbit plasminogen preparation that could be activated by streptokinase (Fig. 3H). After 20 min, the SK2 and SK3 streptokinase fragments disappeared and the SK4 streptokinase fragment increased in amount; the rate of degradation of streptokinase was similar with both types of rabbit plasminogen preparations (Fig. 3, H and I). Two major streptokinase peptides were present and both appeared to increase in amount with increasing streptokinase ratios.

**DISCUSSION**

In this study, we have re-evaluated the sensitivities of human, cat, dog, and rabbit plasminogens to streptokinase using highly purified zymogen preparations and streptokinase. We have shown in these studies that the human, cat, and dog plasminogens, but not rabbit plasminogen, can be completely activated by streptokinase in a standard casein assay (Fig. 1). Rabbit plasminogen can be partially activated only if preincubated with large amounts of streptokinase (molar ratio of streptokinase to plasminogen is 3:1) before addition of the casein substrate. The most sensitive plasminogen is the human zymogen, followed by the cat, dog, and rabbit zymogens, respectively. Wulf and Mertz (22) working with crude preparations of both zymogens and activator reported that in addition to human plasminogen, only cat, monkey, and rabbit plasminogens, but not dog plasminogen, could be activated in a casein assay. Schick and Castellino (19) have recently reported, using highly purified reagents, that the rabbit zymogen can be completely activated when streptokinase-plasminogen ratios of greater than 10:1 are used. We have found a type of rabbit zymogen which could not be activated at a streptokinase-plasminogen ratio of 16:1, under the conditions of our experiment.

Equimolar complexes of human (Glu- and Lys-forms), cat, dog, and rabbit plasminogens with streptokinase have been prepared and shown to be homogeneous by electrophoresis on cellulose acetate (Fig. 2, A and B). Electrophoretic analyses of the complexes in acrylamide gels containing either e-aminocaproic acid, or trans-4-aminomethylcyclohexanecarboxylic acid, showed that the equimolar human (Glu- and Lys-forms) cat, and dog plasminogen-streptokinase complexes had different mobilities from their respective zymogens, and showed multiple isoelectric forms. In these species, all of the zymogen isoelectric forms had reacted with streptokinase to form the equimolar complexes.

Analyses of equimolar plasminogen-streptokinase complexes in acrylamide gel-dodecyl sulfate-urea electrophoretic systems allowed us to study the plasminogen, plasmin, and streptokinase components of the complexes (Fig. 3, A to I). First, it permitted an evaluation of the relative amounts of plasminogen and plasmin in any plasminogen-streptokinase mixture at any time, since the quantity of plasmin could be estimated by the appearance of carboxymethyl heavy and carboxymethyl light chains. It was possible to compare the relative rates of transformation of each mammalian plasminogen-streptokinase complex into a plasmin-streptokinase complex. It appeared that this transformation occurred at a different rate with each mammalian plasminogen-streptokinase complex. An active site in each component of the complexes (Fig. 3, A to I). Second, this system permitted a comparison between the carboxymethyl heavy (A) and carboxymethyl light (B) chains produced from the human, cat, dog, and rabbit plasminogen after urokinase activation (molar ratio of urokinase to plasminogen is 1:1000) with the carboxymethyl heavy and carboxymethyl light chains produced from these same plasminogens activated with equal molar ratios of streptokinase.

It appeared that in each species the plasmin-derived carboxymethyl light (B) chain produced by urokinase activation had approximately the same molecular weight as the carboxymethyl light (B') chain produced by streptokinase activation in the equimolar complex. But, the carboxymethyl light (B') chains of all four species appeared to have somewhat different molecular weights. In some of the species, the carboxymethyl heavy (A* and A') chains produced by streptokinase activation in the equimolar complex had different molecular weights than the carboxymethyl heavy (A, A1, and A2) chains derived from these plasminogens by urokinase activation. The carboxymethyl heavy (A*) chain produced by streptokinase activation of human Glu-, cat, and rabbit plasminogens appeared to have higher molecular weights than the carboxymethyl heavy (A and A1) chains seen in these two plasmines. Only one carboxymethyl heavy (A') chain was produced during streptokinase activation of dog and human Lys-plasminogens. With both of these plasmins, the carboxymethyl heavy (A') chain had a molecular weight similar to that found for the urokinase-produced carboxymethyl heavy (A) chain. The smaller dog carboxymethyl heavy (A2) chain was not produced during streptokinase activation. The appearance of a carboxymethyl heavy (A*) chain produced during streptokinase activation of the rabbit and human Glu-plasminogens that is larger than the carboxymethyl heavy (A) chain produced from these two plasminogens by urokinase activation indicates the possibility that a Glu-plasmin is initially formed in these two species. It is possible that the initial bond cleaved during streptokinase activation of plasminogen in the complex is the arginyl-valine bond, yielding a plasmin consisting of a Glu-carboxymethyl heavy (A*) chain and a carboxymethyl light (B') chain.

We have summarized in Table I the different types of carboxymethyl heavy and carboxymethyl light chains produced by activation of the mammalian plasminogens with both high molar ratios of zymogen to urokinase (1000:1) and equal molar ratios of zymogen to streptokinase in the complex. These two activation systems apparently produce different plasmin-derived heavy chains.

<table>
<thead>
<tr>
<th>Plasminogen species</th>
<th>Plasminogen to urokinase ratio of 1000:1 used to prepare chains</th>
<th>Plasminogen to streptokinase ratio of 1:1 used to prepare chains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu-forms</td>
<td>A, A1</td>
<td>A*, A'</td>
</tr>
<tr>
<td>Lys-forms</td>
<td>A, A1</td>
<td>A'</td>
</tr>
<tr>
<td>Cat</td>
<td>A, A1</td>
<td>A'</td>
</tr>
<tr>
<td>Dog</td>
<td>A, A2</td>
<td>A'</td>
</tr>
<tr>
<td>Rabbit</td>
<td>A</td>
<td>A*, A'</td>
</tr>
</tbody>
</table>

*Major A chains are italicized; A' chains are A chains not found in urokinase activation systems; A* and B' chains found in streptokinase activation (complex) systems may, or may not, be identical with the A and B chains found in urokinase activation systems.
and light chains. In the activation of human (Glu-forms), cat, dog, rabbit, and bovine plasminogens by low molar ratios of urokinase to plasminogen (1:1000), the specific cleavage of the sensitive arginyl-valine peptide bond in the COOH-terminal portion of the molecule and an X-arginyl, or X-lysyl, peptide bond in the NH2-terminal portion of the molecule are the two principal events which occur during activation of the zymogens (24). Other investigators have reported other specific cleavages of peptide bonds by urokinase activation at the NH2-terminal portion of the molecule (25, 26). During the conversion of plasminogen to plasmin in the equimolar human (Glu-forms) and rabbit plasminogen-streptokinase complexes, we have found larger carboxymethyl heavy (A*) chains than obtained by urokinase activation indicating the possibility that a specific peptide bond cleavage did not occur at the NH2-terminal portion of the molecule.

Finally, the acrylamide gel-dodecyl sulfate-urea electrophoretic analyses demonstrated the systematic degradation of streptokinase in the intact complex into lower molecular weight fragments. Although, the rates at which streptokinase was fragmented appeared to be different in each mammalian plasminogen-streptokinase complex, the specific fragments produced appeared to be the same for each species (Fig. 3, A to I). Four major streptokinase fragments (SK1, SK2, SK3, and SK4) were produced in each of the equimolar human (Glu- and Lys-forms), cat, dog, and rabbit plasminogen-streptokinase complexes having molecular weights between approximately 47,600 (molecular weight of streptokinase) and approximately 25,700 (molecular weight of human carboxymethyl light (B) chain). Some smaller streptokinase peptide fragments are also produced in all of the mammalian species which are probably attached to the plasminogen, or plasmins, in the complexes. Analyses of these complexes by cellulose acetate and acrylamide gel electrophoresis indicated that all of the large streptokinase fragments remained attached to the plasminogen or plasmin moiety. This confirmed, as we had previously reported (23), that multiple bindings sites for each other exist on both the plasminogen and streptokinase molecules. Each complex contains varying amounts of each of the large major streptokinase fragments, SK1, SK2, SK3, and SK4.

The fragmentation of streptokinase in the complexes occurs during the preparation and incubation of each complex. No intact streptokinase remained in any complex after 10 min of incubation. The rate at which streptokinase fragmentation occurs appears to parallel the formation of plasmin in the human, cat, and dog complexes, but not in the rabbit complex. With one rabbit complex, the streptokinase was fragmented when only a trace amount of plasmin was found in the preparation (Fig. 3F). The small peptide fragments seen in this experiment are obviously derived from streptokinase and not plasminogen. Streptokinase in the rabbit plasminogen-streptokinase complex must be degraded by this complex. Schick and Castellino (19) have recently reported that the activator for rabbit plasminogen is the rabbit plasminogen-streptokinase complex.

Because we have used several mammalian species of plasminogen in these studies, we should now be able to prepare and isolate any one of the large specific streptokinase fragments (SK1, SK2, SK3, and SK4) by first selecting the appropriate zymogen and then by stopping the reaction in the equimolar complex at an appropriate time. We had previously reported on the dissociation of an equimolar human Lys-plasminogen-streptokinase complex which had been incubated for 10 min at pH 3.0 (23). The plasmin and streptokinase moieties were separated and the streptokinase moiety was analyzed in the acrylamide gel-dodecyl sulfate-urea electrophoretic system and found to contain approximately equal amounts of streptokinase fragments SK2 and SK4 and a smaller fragment (Fig. 3E). Streptokinase fragment SK2 was isolated by gel filtration.

These studies have also shown that those species of plasminogen (human, cat, dog, and rabbit) that can be activated by streptokinase to form plasmin can also form equimolar plasminogen-streptokinase complexes. The activation of these plasminogens is probably by the intermediary plasminogen-streptokinase (15, 16, 19) and plasmin-streptokinase complexes (6, 7, 12-14). The human, cat, and dog equimolar plasminogen-streptokinase complexes, but not the rabbit equimolar complex, are all activators of bovine plasminogen, but with different specific activities.2 The human complex has the highest specific activity and the dog complex has the lowest.

REFERENCES


The Interaction of Streptokinase with Human, Cat, Dog, and Rabbit Plasminogens: THE FRAGMENTATION OF STREPTOKINASE IN THE EQUIMOLAR PLASMINOGEN-STREPTOKINASE COMPLEXES
Louis Summaria, Leonida Arzadon, Priscilla Bernabe and Kenneth C. Robbins


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