Isolation, Characterization, and Comparison of the S-Carboxymethyl Heavy (A) and Light (B) Chain Derivatives of Cat, Dog, Rabbit, and Bovine Plasmins*

Louis Summardia, Leonida Arzadon, Priscilla Bernare, and Kenneth C. Robbins

From the Biochemistry Section, Blood Center, Department of Medicine, Michael Reese Hospital and Medical Center, Chicago, Illinois 60616 and The Department of Medicine, The Pritzker School of Medicine, The University of Chicago, Chicago, Illinois 60637

SUMMARY

Highly purified preparations of cat, dog, rabbit, and bovine plasminogens, isolated by affinity chromatography on lysine-substituted Sepharose columns, were found to be similar in molecular weight to human plasminogen in acrylamide gel-dodecyl sulfate electrophoresis. These plasminogens showed multiple electrophoretic forms in acrylamide gel electrophoresis in 0.3 M L-aminocaproic acid at pH 8.4 and in acrylamide gel-isoelectric focusing electrophoresis. The isoelectric points of the multiple forms of rabbit and bovine plasminogens were similar to the isoelectric points of the multiple isoelectric forms of human Lys-plasminogen; the cat and dog plasminogen isoelectric forms were similar to one another and more electronegative than the human Lys-plasminogen isoelectric forms.

Cat, dog, rabbit, and bovine plasmins (urokinase-activated plasminogens) reacted stoichiometrically with L-1-chloro-3-tosylamido-7-amino-2-heptanone and were completely inhibited. Cm-heavy (A) and Cm-light (B) chain preparations were isolated from reduced and alkylated plasmin preparations from each species by dialysis against 0.002 M ammonium bicarbonate. The incorporation studies with L-1-chloro-3-tosylamido-7-amino-2-heptanone indicated a single "active site" histidine, for each species of plasmin, located in the Cm-light (B) chain.

The Cm-light (B) chain derivatives of the cat, dog, rabbit, and bovine plasmins were essentially homogeneous and similar in molecular weight to the human Cm-light (B) chain derivative in acrylamide gel-urea-dodecyl sulfate electrophoresis. These derivatives showed multiple isoelectric forms in acrylamide gel-urea-isoelectric focusing electrophoresis. The isoelectric points of cat and dog Cm-light (B) chain forms were similar to the isoelectric points of the human Cm-light (B) chain forms; the rabbit Cm-light (B) chain forms were more electronegative and the bovine Cm-light (B) chain forms were more electropositive than the human Cm-light (B) chain forms.

The Cm-heavy (A) chain preparations of cat, dog, rabbit, and bovine plasminogens all contained both major and minor chains in acrylamide gel-urea-dodecyl sulfate electrophoresis. The cat, dog, and rabbit preparations contain a 42,000 and minor chain that had the same molecular weight as the major human Cm-heavy (A) chain. The major cat Cm-heavy (A1) chain was similar in molecular weight to the minor human Cm-heavy (A1) chain. The major dog Cm-heavy (A2) chain had a lower molecular weight than both the major and minor human Cm-heavy (A and A1) chains. The bovine Cm-heavy (A3) chain was of lower molecular weight than the dog, Cm-heavy (A2) chain. The Cm-heavy (A) chain derivatives of all four animal species showed multiple isoelectric forms in acrylamide gel-urea-isoelectric focusing electrophoresis with isoelectric points slightly more electronegative than the isoelectric points of the multiple human Cm-heavy (A) chain forms.

Plasminogens have been isolated from several animal plasmas in a highly purified state by various types of chromatographic methods (1-6). The molecular weights of bovine and rabbit plasminogens were determined to be between 80,000 and 90,000 (1, 2, 4, 5). Dog (3) and rabbit (5) plasminogens showed multiple electrophoretic forms in starch gel and acrylamide gel electrophoresis, respectively. Bovine plasminogen activated by urokinase (7) and rabbit plasminogen activated by streptokinase (5) give plasmin which, after reduction and alkylation, show two chains for each species. The Cm-heavy (A) and Cm-light (B) chain derivatives of bovine plasmin have been isolated (7). The molecular weight of the Cm-heavy (A) chain was reported to be 35,000 and the molecular weight of the Cm-light (B) chain was reported to be 23,500. In this report, cat, dog, rabbit, and bovine plasminogens were isolated by an affinity chromatographic method.
graphic method using L-lysine-substituted Sepharose (8). These plasminogens were characterized and compared in several acrylamide gel electrophoretic systems. These zymogens were activated with urokinase and the plasin-derived Cm-heavy (A) and Cm-light (B) chains were isolated, characterized, and compared with each other and with similar human preparations.

**MATERIALS AND METHODS**

**Animal Plasmas**—Pooled citrated dog plasma was obtained from Abbott Laboratories, North Chicago, Ill., and Pel-Freez Inc., Rogers, Ark. Pooled citrated cat and rabbit plasmas were obtained from Pel-Freez, Inc. All plasma was stored frozen for a period of from 3 to 6 months at -20°C. Prior to use, each plasma sample was thawed at 20°C and the cryoprecipitate was removed by centrifugation at 4000 rpm for 1 hour.

**Plasminogens and Plasmins**—Cat, dog, rabbit, and bovine plasminogens were prepared from plasma by a modification of the method described by Deutsch and Mertz using an affinity chromatography method with L-lysine-substituted Sepharose at 2°C (8). Human plasminogen was prepared from plasma Fractions III and III2,3 (9–11). Plasma Fraction III contains Glu- and Lys-plasminogens whereas plasma Fraction III2,3 contains Lys-plasminogen only. The eluted plasminogen was precipitated by adding 3.1 g of ammonium sulfate per 10 ml of solution. After 18 hours at 2°C, the suspension was centrifuged at 2°C at 4000 rpm (International Centrifuge PR II, Head No. 296, multispeed attachment) at 2°C. After isolation, the Cm-heavy (A) and Cm-light (B) chain derivatives of all species were dialyzed at 2°C against 1 mM acetic acid and lyophilized.

**Acrylamide Gel Electrophoresis**—Acrylamide gel electrophoresis was carried out in gel slabs in a Beckman Mierozone acrylamide gel system at pH 8.4 and 7.0 (9, 10). The gel slabs (5 and 7.5%) were prepared in 0.037 M Tris-0.2 M glycine buffer, pH 8.4, containing 0.3 M e-aminoacetic acid. Gel slabs (5% for plasminogen and 7.5% for Cm-heavy (A) and Cm-light (B) chains) were also prepared in 0.1 M phosphate buffer, pH 7.0, 0.1% dodecyl sulfate, with 6 M urea. Isoelectric focusing in acrylamide gel (5% for plasminogens and 7.5% for Cm-heavy (A) and Cm-light (B) chains), with 8 M urea was carried out in gel slabs using a modification of the method described by Vesterberg (14). The detailed procedures for all of the acrylamide gel electrophoretic analyses have been previously described (9, 10). Qualitative determinations for carbohydrate were made in certain gels by staining for 3 hours with Schiff’s stain (15). The gels were washed and stored in 7% acetic acid.

**RESULTS**

**Preparation of Cat, Dog, Rabbit, and Bovine Plasminogens**—The recoveries and activities of cat, dog, rabbit, and bovine plasminogens, prepared by the affinity chromatography method using a lysine-substituted Sepharose column (2.5 X 10 cm) are summarized in Table I. Volumes of approximately 500 ml of the various plasmas were added to the column. A single symmetrical peak was obtained containing 0.1 to 0.2 mg of protein per ml of plasma applied. The specific activities for all four species of plasminogen were between 20 and 23 casein units per mg of protein. These specific activities are similar to the activities of human plasminogen prepared from Fractions III and III2,3 by this method (10). Nearly complete recovery of plasminogen activity was found with the cat, dog, and rabbit plasma, but not with bovine plasma.

**Analysis of Plasminogen by Acrylamide Gel Electrophoresis**—Cat, dog, rabbit, and bovine plasminogens were compared with the same manner as that described for the reduction and alkylation of human plasmin (13). The Cm-heavy (A) and Cm-light (B) chain derivatives of the [3H]TLCK-plasminogens of all species were separated and isolated by dialysis against 0.002 M ammonium bicarbonate at 4°C (13). The insoluble Cm-light (B) chain derivatives were recovered by centrifugation at 16,000 rpm for 2 hours (International Centrifuge, PR II, Head No. 296, multispeed attachment) at 2°C. After isolation, the Cm-heavy (A) and Cm-light (B) chain derivatives of all species were dialyzed at 2°C against 1 mM acetic acid and lyophilized.

**Table I**

| Plasma source | Plasminogen preparation | Recovery of protein | Specific activity |
|---------------|-------------------------|--------------------|------------------|
| Cat           | 3.2 | 98 | 0.18 | 21.0 |
| Dog           | 2.4 | 93 | 0.14 | 21.9 |
| Rabbit        | 3.2 | 96 | 0.14 | 23.2 |
| Bovine        | 1.6 | 80 | 0.10 | 20.2 |

*The extinction coefficients E1%cm for cat, dog, rabbit, and bovine plasminogens, uncorked for moisture and ash, were 15.0, 16.2, 18.1, and 18.3, respectively. These values were used to calculate protein concentrations.
each other and with human Lys-plasminogen in acrylamide gel-urea-dodecyl sulfate electrophoresis (Fig. 1a). All of the plasminogens appeared to have similar, but not identical molecular weights.

Cat, dog, rabbit, and bovine plasminogens were compared with each other and to human Glu- and Lys-plasminogens in acrylamide gel-ε-aminocaproic acid electrophoresis (Fig. 1b). Multiple

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FIG. 1. See figure legend on page 6525.
electrophoretic forms were observed in each plasminogen. The multiple electrophoretic bovine and rabbit plasminogen forms were similar to the human Lys-plasminogen forms. The multiple electrophoretic bovine and rabbit plasminogen forms and the plasminogen molecular weights in acrylamide gel-dodecyl sulfate electrophoresis appear to be similar, these data probably indicate that one “active site” histidine is present in each of the plasmin species.

Characterization and Comparison of Isolated Cm-Heavy (A) and Cm-Light (B) Chain Derivatives—The distribution of protein and radioactivity in the Cm-heavy (A) and Cm-light (B) chain preparations derived from dog, cat, rabbit, bovine, and Lys-human plasmins are shown in Table II. The relative quantities of Cm-heavy (A) and Cm-light (B) chains obtained from dog, cat, rabbit, and bovine plasmins were similar to those obtained from human Lys-plasmin. The distribution of radioactivity in the Cm-heavy (A) and Cm-light (B) chains of the animal plasmins contains the “active site” histidine, as previously reported for human Lys-plasmin (15). The reduced incorporation of [3H]TLCK per pmole of Cm-light (B) chain for the animal species could be due to the sensitivity of the radiolabelling to reduction and alkylation, or to handling (17).

Each Cm heavy (A) chain preparation was contaminated with small amounts of Cm-light (B) chain derivative.

The cat, dog, rabbit, and bovine plasmin-derived Cm-heavy (A) and Cm-light (B) chains were compared with each other and with the human Lys-plasmin-derived chains in acrylamide gel-urea-dodecyl sulfate electrophoresis (Fig. 1, d and e). The Cm-light (B) chain derivatives of these species were essentially homogeneous, except for the cat Cm-light (B) chain preparation which contained two additional minor components (Fig 1d, position 8). All of the Cm-light (B) chain derivatives had similar if not identical electrophoretic mobilities, indicating that the Cm-light (B) chains derived from cat, dog, rabbit, and bovine plasmins probably have molecular weights very similar to that of the human plasmin Cm-light (B) chain derivative, which is 25,700 (18). Each of the Cm-heavy (A) chain preparations (Fig 1e) contained more than one Cm heavy (A) chain derivative, in different amounts, as well as a trace Cm-light (B) chain contaminant. The major rabbit Cm-heavy (A) chain and the plasmin (Cm-heavy (A) chain derivative), c, acrylamide gel-dodecyl sulfate electrophoretogram, pH 7.0: 1, human Lys-plasmin (Fraction II12,1); 2, dog plasmin; 3, bovine plasmin; 4, rabbit plasmin; 5, bovine plasmin; 6, acrylamide gel-dodecyl sulfate electrophoretogram, pH 5.4: 1, human Lys-plasmin (Fraction III11); 2, human Lys-plasminogen (Fraction II111,1); 3, human Lys-plasminogen (Fraction II111,1); 4, human Lys-plasminogen (Fraction II111,1); 5, human Lys-plasminogen (Fraction II111,1); 6, human Lys-plasminogen (Fraction II111,1); 7, rabbit plasmin; 8, human Lys-plasminogen, pH 3 to 10, electrophoretogram: 1, human Lys-plasmin (Fraction III11); 2, human Lys-plasminogen (Fraction III111,1); 3, human Lys-plasminogen (Fraction III111,1); 4, human Lys-plasminogen (Fraction III111,1); 5, human Lys-plasminogen (Fraction III111,1); 6, human Lys-plasminogen (Fraction III111,1); 7, rabbit plasmin; 8, human Lys-plasminogen, acrylamide gel-dodecyl sulfate electrophoretogram, pH 7.0: 1, human Lys-plasmin (Cm-light (B) chain derivative); 2, dog plasmin (Cm-light (B) chain derivative); 3, cat plasmin (Cm-light (B) chain derivative); 4, rabbit plasmin (Cm-light (B) chain derivative); 5, bovine plasmin (Cm-light (B) chain derivative); 6, rabbit plasmin (Cm-light (B) chain derivative); 7, bovine plasmin (Cm-heavy (A) chain derivative); 8, rabbit plasmin (Cm-heavy (A) chain derivative); 9, acrylamide gel-urea-Ampholine, pH 4 to 8, electrophoretogram: 1, human Lys-plasmin (Cm-heavy (A) chain derivative); 2, dog plasmin (Cm-heavy (A) chain derivative); 3, cat plasmin (Cm-heavy (A) chain derivative); 4, rabbit plasmin (Cm-heavy (A) chain derivative); 5, bovine plasmin (Cm-heavy (A) chain derivative); 6, rabbit plasmin (Cm-heavy (A) chain derivative); 7, bovine plasmin (Cm-heavy (A) chain derivative); 8, rabbit plasmin (Cm-heavy (A) chain derivative).
minor dog Cm-heavy (A) chain derivatives were of the same molecular weight as the major Cm-heavy (A) chain derivative of human plasmin. The major cat Cm-heavy (A1) chain derivative was smaller than the major rabbit and human Cm-heavy (A) chain derivatives but corresponded in molecular weight to the minor human Cm-heavy (A1) chain derivative. The bovine Cm-heavy (A) chain preparation contained no Cm-heavy (A) chain derivative that corresponded to either the major or minor, human Cm-heavy (A) chain derivatives. The bovine preparation contained a Cm-heavy (A3) chain derivative which was smaller than the human Cm-heavy (A) chain derivative. A bovine Cm-heavy (A) chain derivative with a molecular weight of 35,000 has been reported (7). The dog Cm-heavy (A) chain preparation contained a major Cm-heavy (A2) chain derivative that was larger than the bovine Cm-heavy (A3) chain derivative. Trace amounts of this Cm-heavy (A2) chain derivative could also be seen in the cat and rabbit Cm-heavy (A) chain preparations. When these Cm-heavy (A) and Cm-light (B) chain preparations were subjected to electrophoresis in acrylamide gel-urea-dodecyl sulfate and stained with Schiff's stain, all of the Cm-heavy (A) and Cm-light (B) chain derivatives showed a positive reaction, indicating the presence of carbohydrate on both the Cm-heavy (A) and Cm-light (B) chain derivatives of all species.

In acrylamide gel-urea-isoelectric focusing, pH range of 4 to 8 (Fig. 1c), all of the Cm-light (B) chain derivatives contained multiple forms. The dog and cat Cm-light (B) chain forms had isoelectric points similar to those of the human Cm-light (B) chain forms. We had previously determined in column isoelectric focusing that the human Cm-light (B) chain forms had isoelectric points of pH 5.3, 5.8, 5.9, 6.0, and 6.2 (9). The bovine Cm-light (B) chain forms were more electropositive than the human Cm-light (B) chain forms, while the rabbit Cm-light (B) chain forms were more electronegative than the human Cm-light (B) chain forms. Similar results were obtained in acrylamide gel-urea electrophoresis. In acrylamide gel-urea-isoelectric focusing, pH range of 4 to 8 (Fig. 1g), the Cm-heavy (A) chain preparations of all species showed multiple isoelectric forms. We had reported multiple isoelectric forms of the human Cm-heavy (A) chain derivative (10). Many closely migrating bands differing by probably 0.01 pH unit could be seen in every Cm-heavy (A) chain preparation (Fig. 1g). The isoelectric points of the rabbit multiple Cm-heavy (A) chain forms appeared to be the same as the isoelectric points of the human Cm-heavy (A) chain forms, which are approximately pH 4.9 (9). The isoelectric points of the dog, cat, and bovine Cm-heavy (A) chain forms were all slightly more electronegative than pH 4.9.

**DISCUSSION**

Affinity chromatography has proven to be a simple and effective method for isolating nearly homogeneous preparations of plasminogen from a number of mammalian species (5, 6, 8-10). In this study, a comparison of the molecular weights of cat, dog, rabbit, and bovine plasminogens was made in acrylamide gel-urea-dodecyl sulfate electrophoresis; they were found to be similar to each other and to human plasminogen (10, 16). Molecular weights determined by ultracentrifugal methods showed the same results (1-5). Multiple isoelectric forms for dog (3) and rabbit (5) plasminogens have been previously reported. It was previously reported that bovine plasminogen had a single isoelectric form (4). In this study, we have shown that cat and bovine plasminogens also show multiple isoelectric forms. The multiple electrophoretic forms of the rabbit and bovine plasminogens were similar in electrophoretic mobility to human Lys-plasminogen; the multiple electrophoretic forms of the cat and dog plasminogens were more electronegative than the forms found in human Lys-plasminogen. Isoelectric focusing in acrylamide gel was found to be an excellent method for demonstrating the isoelectric forms of plasminogen.

After activation of cat, dog, rabbit, and bovine plasminogens with urokinase, we found that the plasmins of each species could be inhibited with TLCK in a manner analogous to the inhibition of human plasmin. Radioactivity incorporation studies with [3H]TLCK showed that each species of plasmin contained one "active site," histidine assuming similar molecular weights, which was located in the Cm-light (B) chain. Approximately 0.8 to 0.9 pmole of [3H]TLCK was incorporated per pmole of plasmin.

The analysis method developed in our laboratory for separating the Cm-heavy (A) and Cm-light (B) chain derivatives of human plasmin (13) was used in this study to prepare Cm-heavy (A) and Cm-light (B) chain preparations from the cat, dog, rabbit, and bovine, reduced and alkylated, [3H]TLCK-plasminogens. These Cm-heavy (A) and Cm-light (B) chain preparations were analyzed and compared in several different acrylamide gel electrophoretic systems. We found that the Cm-light (B) chain derivatives were essentially homogeneous and similar in molecular weight to the human plasmin Cm-light (B) derivative. The Cm-light (B) chain derivatives also consisted of different multiple isoelectric forms. The parameter (or parameters) responsible for the differences between the multiple isoelectric forms of these Cm-light (B) chain derivatives has not been determined. The multiple components found in the Cm-heavy (A) chain preparations are probably the result of specific secondary peptide bond cleavages that occur during activation. Evidence that the rabbit plasmin heavy chain but not the rabbit plasmin light chain undergoes proteolytic degradation when activation is prolonged has been reported (5).

This comparative study of cat, dog, rabbit, and bovine plasminogens, and plasmins, with human plasminogen and plasmin has revealed basic similarities that exist in these different species of molecules. These similarities are: (a) molecular weights; (b) multiple isoelectric forms; (c) activatable to plasmin by urokinase, resulting in disulfide-linked heavy (A) and light (B) chains; (d) a TLCK-reactive site on the light (B) chain portion of the plasmin molecule; (e) the Cm-light (B) chain derivatives are similar in molecular weight; (f) each of the Cm-heavy (A) chain preparations, except the bovine, contained a Cm-heavy (A) derivative of similar molecular weight; and (g) the Cm-heavy (A) and Cm-light (B) chain derivatives exist in multiple isoelectric forms.

Studies on the activation of mammalian plasminogens by both streptokinase and urokinase, two well characterized activators, should permit us to develop a common mechanism by which the fibrinolytic system is activated and to define the molecular events which occur during activation. We have selected the non-human mammalian species reported in this study because of their varied reactivity to streptokinase (19). Subsequent reports will describe the molecular events which take place during the activation of these plasminogens by urokinase and the molecular interactions between these plasminogens and streptokinase.

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