ACTIVATION OF PLASMINOGEN BY HUMAN PLASMA KALLIKREIN*

Robert W. Colman

Departments of Medicine, Harvard Medical School and
Massachusetts General Hospital, Boston, Mass. 02114

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SUMMARY

Purified human plasma kallikrein converts human plasminogen to plasmin. When plasminogen is present in excess, the yield of plasmin is proportional to the concentration of kallikrein with a molar ratio of the plasmin to kallikrein of 1.20. Addition of increasing amounts of plasminogen results in a maximum yield of plasmin equivalent to 1.19 moles/mole kallikrein. These data suggest the formation of a stoichiometric equimolar complex of plasmin and kallikrein in the course of the activation of plasminogen by kallikrein. Hageman factor activation of fibrinolysis appears to be mediated by kallikrein-catalyzed formation of plasmin from plasminogen.

Hageman factor (factor XII) activates several plasma proteolytic enzymes with the transformation of factor XI (PTA) to its active form (MacFarlane, 1964; Davie and Ratnoff, 1964), the conversion of kallikreinogen to kallikrein (Nagasawa, 1968) and the in vitro (Niewiarowski and Prou-Wartelle, 1959) and in vivo (Holemans and Roberts, 1964) activation of plasminogen to plasmin. Although factor XII acts directly on factor XI and kallikreinogen, its effect on plasminogen is indirect. The analysis of Iatridis and Ferguson (1962) suggested that Hageman factor has a "lysokinase" activity that converts a proactivator into an activator of plasminogen but the activator has not been identified. Partially purified preparations of Hageman factor have failed to activate plasminogen (Niewiarowski, 1969).

Human plasma kallikrein, a proteolytic enzyme that hydrolyzes an lysyl-arginine bond in kininogen to liberate the nonapeptide bradykinin, has recently been purified (Colman et al, 1969). The enzyme is homogeneous by

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ultracentrifugation, disc electrophoresis, and immunoelectrophoresis and contains no detectable coagulation factors or fibrinolytic activity. This report presents evidence that purified kallikrein directly converts plasminogen to plasmin and suggests the existence of a stoichiometric complex of plasmin and kallikrein.

Materials and Methods

Kallikrein was prepared from human plasma with a specific activity of 5.25 μmoles tosyl L-arginine methyl ester (TAMe)/minute/mg protein (Colman et al, 1969). Plasminogen (Kabi Grade A) was a lyophilized preparation which when reconstituted contains 1.67 mg protein/ml with a specific activity of 15 casein units/mg. Plasmin in this preparation amounted to less than 5% of the zymogen. Plasmin (Grade A) was obtained from Kabi, acetyl L-lysine methyl ester (ALMe) and TAMe from Cyclo Chemical Corp., and Streptokinase (Varidase R) from Lederle. Caseinolytic activity was measured by a modification of the method of Alkjaersig et al (1959) in which the final assay mixture contained 1 ml and streptokinase was omitted. The buffer used for all incubation mixtures and the pH stat assay was .005M Tris-HCl pH 7.5 in 0.15M NaCl. Esterase activity using ALMe was measured by continuously recorded uptake of NaOH in the pH stat fitted with an automatic buret (Radiometer). The composition of the incubation mixture was as indicated and consisted of a total of 0.13 ml of which an aliquot of 0.10 ml was then added to 0.90 ml of 0.015M ALMe in the assay buffer. The reaction velocity at 25°C in the first three minutes was recorded. No spontaneous hydrolysis occurred during this period. Corrections were made for the esterolytic activity of kallikrein and plasmin present after the incubation of plasminogen alone. Under these conditions 1 μmole ALMe/ml/min is equivalent to 1 casein unit.

Esterase activity using TAMe was measured by a modification (Sherry et al, 1964) of the colorimetric procedure of Siegelman et al (1962), which quantifies the methanol released. Kallikrein was quantified in citrated human plasma following removal of the kallikrein inhibitor by acid treatment (Colman et al, 1969b).

Results and Discussion

When various concentrations of purified human plasma kallikrein were incubated with human plasminogen, progressive evolution of esterolytic (ALMe) activity (Fig. 1A) and proteolytic (casein) activity (Fig. 1B) were observed. The reaction followed zero order kinetics for the first five minutes and the initial rate was proportional to the kallikrein concentration. Kallikrein hydrolyzes ALMe at one-ninth the rate of a comparable concentration of plasmin (Colman et al, 1969b) and correction has been made for this small contribution. Therefore, the evolved esterase activity in Fig. 1A is due entirely to plasmin. Similarly, kallikrein does not contribute to the caseinolytic activity formed since it does not hydrolyze casein under these conditions.

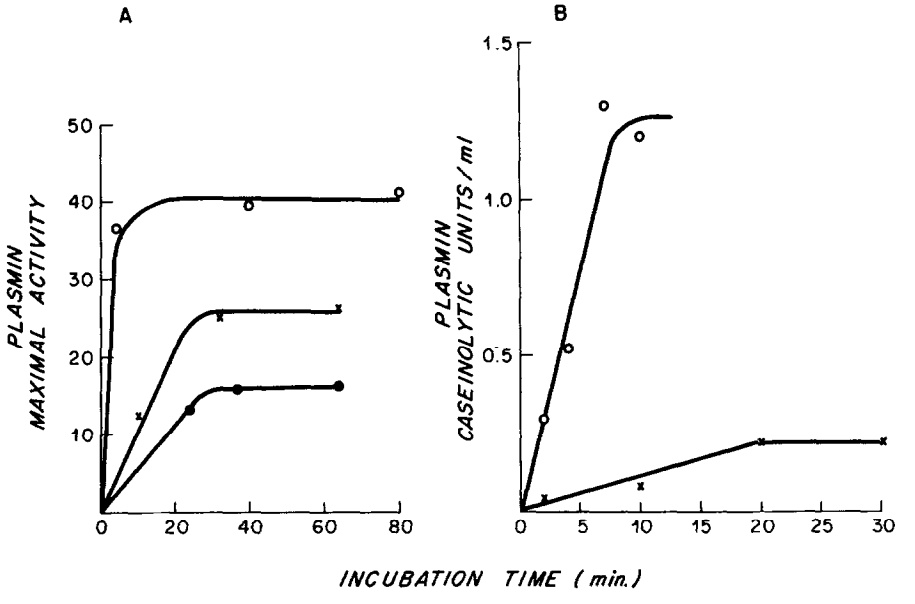

Figure 1. Activation of plasminogen by kallikrein. Plasminogen (830µg/ml) and kallikrein incubated at 25°. Aliquots 100 ul (A) or 50 ul (B) removed at various times.
A. Assay for ALMe esterolytic activity as in methods expressed in % of maximal plasmin activity activated by streptokinase 150 units/ml.
Kallikrein µg/ml .——. 130 x——x 260 o——o 390
B. Assay for caseinolytic activity as in methods.
Kallikrein µg/ml x——x 100 o——o 650

Evidence that plasmin is formed from plasminogen by the enzymatic activity of kallikrein is shown in Table I. The interaction of plasminogen and kallikrein results in considerable increase of TAMe esterolytic activity over that predicted if summation occurred. Inclusion of a competitive substrate of kallikrein, TAMe (Colman et al, 1969) in the incubation mixture inhibits the activation of plasminogen by 80%. Kallikrein resembles trypsin (Alkjaersig et al, 1958) and thrombin (Engel et al, 1966), known activators of plasminogen in that it hydrolyzes substituted basic amino acid esters and is inhibited by DFP. Recent studies in this laboratory have demonstrated that kallikrein is capable of converting chymotrypsinogen to chymotrypsin, a reaction also catalyzed by trypsin and thrombin (Engel and Alexander, 1966). Unlike trypsin and thrombin, kallikrein has no effect on factor V (Colman, 1969).

TABLE I: INHIBITION BY TAMe OF ACTIVATION OF PLASMINOGEN BY KALLIKREIN

Incubation Mixture[a]	Esterase (μmoles TAMe/ml/min) Observed	Predicted for No Activation[b]	Net Plasmin Formed[c]
Kallikrein	2.80		
Plasminogen	0.59		
Kallikrein + Plasminogen	6.05	3.39	2.66
Kallikrein + Plasminogen + TAMe	3.90	3.39	0.51

a. The incubation mixture contained a total of .08 ml containing where indicated kallikrein (870 μg/ml), plasminogen (830 μg/ml) and TAMe 1.6 μmoles/ml in .005M Tris-HCl pH 7.5, 0.15M NaCl. After 30 minutes at 25°, 0.80 ml of .05M TAMe in 0.1M Na phosphate buffer pH 7.6, 0.15M NaCl was added and esterase activity by the colorimetric procedure (Methods) was measured. The contribution of TAMe in the incubation mixture to the assay mixture was less than 3%.

b. Predicted value is the sum of kallikrein esterolytic activity and that of plasminogen preparation incubated separately under the same conditions for their joint incubation.

c. Net plasmin formed is difference of observed and predicted.

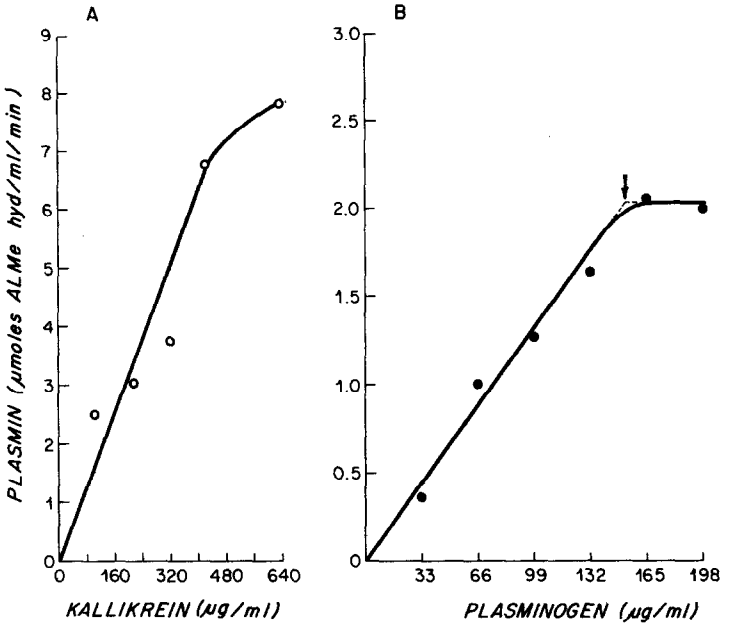

Figure 2. Stoichiometric relationship of kallikrein in plasminogen activation. Plasminogen and kallikrein were incubated at 25° for 30 min and aliquot removed for assay of ALMe esterolytic activity as in methods.
A. Plasminogen - 830μg/ml and various amounts of kallikrein.
B. Kallikrein - 17.4μg/ml and various amounts of plasminogen.
Arrow indicates equivalence of plasminogen to 17.4μg/ml kallikrein.

Moreover, kallikrein fails to convert prothrombin to thrombin (unpublished results).

The reaction of kallikrein with plasminogen exhibits a dependence of the plasmin yield on the amount of kallikrein present (Fig. 1A). Preliminary experiments demonstrated that at the concentrations of plasminogen and kallikrein investigated the reaction was complete in 30 minutes. No destruction of kallikrein by plasmin was observed during this interval nor was the activity of preformed plasmin affected by kallikrein. Figure 2A shows that the maximum amount of plasmin formed was directly proportional to the amount of kallikrein present when plasminogen concentration was constant; thus a stoichiometric relationship between kallikrein and plasmin is suggested.

The molar concentrations of kallikrein and plasmin were calculated from the specific activities of the preparations used (see Methods), the maximum specific activities for plasminogen (24 casein units/mg, Robbins et al, 1965) and kallikrein (9.3 μmoles TAMe/mg protein, Colman et al, 1969a), and the molecular weights of plasmin (89,000) and kallikrein (99,500). The ratio of plasmin to kallikrein at points along the linear portion of Fig. 2A was 1.20 suggesting an equimolar complex of plasmin and kallikrein. If this hypothesis is true, then addition of increasing amounts of plasminogen to constant kallikrein should eventually result in a constant maximal yield of plasmin whereas in a purely enzymatic reaction raising the substrate concentration increases the yield of product. Figure 2B indicates the results of such an experiment which shows a maximum consistent with a stoichiometric reaction. At the equivalence point, the molar ratio of plasmin to kallikrein is 1.18, again suggesting an equimolar complex. Similar kinetics have been observed for streptokinase activation of plasminogen (Ling et al, 1965) where physical evidence for a plasmin-streptokinase complex exists (DeRenzo et al, 1967).

To attempt to assess the contribution of kallikrein in converting plasminogen to plasmin in plasma, kallikrein was maximally activated by exposing plasma (which was artificially depleted of plasmin and kallikrein inhibitors) to kaolin (see Methods). Caseinolytic activity, which in plasma reflects only plasmin function, was measured simultaneously. Under the conditions of the experiment, kallikrein activity was 3.48 umoles TAMe/ml plasma/min compared with plasmin 0.375 casein units. The magnitude of the fibrinolytic response is similar to that observed after maximal activation of Hageman factor (Eisen, 1963).

The observations of this study indicate that kallikrein may be included with the two other plasma proteases thrombin and plasmin known to activate plasminogen. The influence of Hageman factor on fibrinolysis appears to be mediated through its activation of plasma kallikrein.

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