Isolation and Functional Properties of the Heavy and Light Chains of Human Plasma Kallikrein*

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Human plasma kallikrein was prepared by proteolytic activation of prekallikrein with β-Factor XIIa (M, = 28,000). Two forms of kallikrein were generated that were each composed of two disulfide-linked polypeptide chains: a heavy chain of apparent M, = 43,000 and a light chain of apparent M, = either 36,000 or 33,000. Following reduction and alkylation, the heavy and light chains of kallikrein were isolated by affinity chromatography using insolubilized high molecular weight kininogen. The alkylated light chain of kallikrein did not bind to high molecular weight kininogen-Sepharose while the heavy chain did bind with high affinity and was subsequently eluted. The light chain retained the specific amidolytic activity of native kallikrein. The K, and kcat values for the hydrolysis of H-D-Pro-Phe-Arg-p-nitroanilide by kallikrein or its light chain were identical. Activation of Factor XII in solution was equally well catalyzed by kallikrein and its light chain. However, in kaolin-dependent coagulation, kallikrein was 180 times more effective than the light chain in correcting the clotting defect of prekallikrein-deficient plasma. Furthermore, the light chain was 3.5 times less potent than kallikrein in cleaving high molecular weight kininogen in solution. These observations indicate that the light chain region contains the enzymatic active site and adequately accounts for the enzymatic properties of kallikrein in solution, on the protein substrate, Factor XII, and on oligopeptide substrates. However, the heavy chain region of kallikrein is required for binding to high molecular weight kininogen, for surface-dependent activation of coagulation, and for optimal cleavage of high molecular weight kininogen.

Human plasma prekallikrein is a γ-globulin glycoprotein that participates in the early phase of intrinsic blood coagulation, kinin formation, and fibrinolysis. These contact activation reactions are initiated upon exposure of plasma to negatively charged surfaces (1–3). The mechanism of surface-dependent activation probably involves the binding of blood coagulation Factor XII to the surface, followed by the reciprocal proteolytic activation of Factor XII and prekallikrein.

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§ Supported in part by a grant from the Medical Research Council, The Netherlands.
gen had applied at paralton Conductivity at 4°C then brother purified high buffer containing was applied at Seven 31°C. 8.3. 8-Factor XIIa Aetlvatlon k allowed observed to wash the column The bound protein to elute was determined by SDS-PAGE gels with acetic acid, 0.15

Electrophoresis. Polyacrylamide gel
determlned by
the isolated alkylated heavy chain was analyzed by SDS-PAGE of human plasma
Kininogen-Sepharose. The fractions containing protein were analyzed for activity by adding 0.08 mm dithiothreitol, 5 mM benzamidine for 1 h under nitrogen. Under these conditions, reduction of kalilkrine was greater than 95% as judged by the separation of polypeptides on nonreduced SDS gels (Fig. 2). The reduced kalilkrine was alkylated with iodoacetamide. After dialysis, the specific amilolytic activity of the reaction mixture was 95% of the original kalilkrine. The mixture of the alkylated heavy and light chains was applied to a column containing high molecular weight kininogen-Sepharose. The fractions containing protein that did not adhere to the resin were found to have amilolytic activity toward S-2302. Analysis of these fractions by SDS-polyacrylamide gel electrophoresis showed two bands with apparent M, 36,000 and 33,000, corresponding to the molecular weight values of the light chains of kalilkrine (Fig. 2). Protein that bound to the resin was eluted with 3 M NaSCN and gave one band with apparent M, 43,000 on 10% SDS-polyacrylamide gels, corresponding to that of the heavy chain of kalilkrine (Fig. 2). Stability of the Light Chain—To test whether the enzymatic activity of the alkylated light chain remains stable upon dilution at different pH, a stock solution of the light chain was diluted a hundredfold in three different buffers: 5 mM sodium

RESULTS

Preparation and Purification of the Heavy and Light Chains of Kalilkrine—Prekalilkrine was converted to kalilkrine by incubation of 4.1 mg of prekalilkrine with 14 mg of β-Factor XIIa as described under "Materials and Methods." Analysis of kalilkrine on 7.5% SDS-polyacrylamide gels in the presence of β-mercaptoethanol showed three fragments with apparent molecular weights of 43,000, 36,000, and 33,000 (Fig. 1). In the absence of reducing agent, kalilkrine gave two protein bands with apparent M, 80,000 and 82,000 similar to those seen for prekalilkrine. This indicates that prekalilkrine was proteolytically cleaved by β-Factor XIIa and that the resulting polypeptide chains designated heavy and light chains are held together by one or more disulfide bonds, as previously established (11, 12).

For the preparation and purification of the heavy and light chains, 3.5 mg of kalilkrine were reduced in the presence of 0.08 mm dithiothreitol, 5 mM benzamidine for 1 h under nitrogen. Under these conditions, reduction of kalilkrine was greater than 95% as judged by the separation of polypeptides on nonreduced SDS gels (Fig. 2). The reduced kalilkrine was alkylated with iodoacetamide. After dialysis, the specific amilolytic activity of the reaction mixture was 95% of the original kalilkrine. The mixture of the alkylated heavy and light chains was applied to a column containing high molecular weight kininogen-Sepharose. The fractions containing protein that did not adhere to the resin were found to have amilolytic activity toward S-2302. Analysis of these fractions by SDS-polyacrylamide gel electrophoresis showed two bands with apparent M, 36,000 and 33,000, corresponding to the molecular weight values of the light chains of kalilkrine (Fig. 2). Protein that bound to the resin was eluted with 3 M NaSCN and gave one band with apparent M, 43,000 on 10% SDS-polyacrylamide gels, corresponding to that of the heavy chain of kalilkrine (Fig. 2). Stability of the Light Chain—To test whether the enzymatic activity of the alkylated light chain remains stable upon dilution at different pH, a stock solution of the light chain was diluted a hundredfold in three different buffers: 5 mM sodium

The abbreviation used is: SDS, sodium dodecyl sulfate.
To the amount of enzyme added (data not shown). As seen in Fig. 3, the initial rate of activation of Factor XII in solution was catalyzed equally well by kallikrein as by the light chain. No activation of Factor XII occurred in the absence of kallikrein or light chain. These data suggest that the light chain of kallikrein is participating in the activation of Factor XII as an enzyme and that its specific activity is essentially identical with that of kallikrein in this reaction.

**Procoagulant Activity of Kallikrein and the Light Chain**—The ability of kallikrein and of the light chain to correct the

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\text{TABLE I}
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Specific amidolytic activities of human kallikrein and its light chain on the oligopeptide substrate S-2302

<table>
<thead>
<tr>
<th>Substance</th>
<th>Native kallikrein</th>
<th>Light chain</th>
</tr>
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<tbody>
<tr>
<td>μmol pNA formed/min/mg protein</td>
<td>52.4</td>
<td>109.1</td>
</tr>
<tr>
<td>μmol pNA formed/min/μmol</td>
<td>4192.0</td>
<td>3820.0</td>
</tr>
</tbody>
</table>

**TABLE II**

Kinetic constants for the hydrolysis of S-2302 by kallikrein and the light chain

<table>
<thead>
<tr>
<th>Substrate</th>
<th>K_m (mm)</th>
<th>k_cat (s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kallikrein</td>
<td>0.32</td>
<td>275.0</td>
</tr>
<tr>
<td>Light chain</td>
<td>0.33</td>
<td>271.0</td>
</tr>
</tbody>
</table>
coagulation defect of prekallikrein-deficient (Fletcher trait) plasma was determined. Sulfatides as well as kaolin were used in coagulation assays because they are very effective in the surface-dependent activation of the intrinsic coagulation pathway (25, 27). Both kallikrein and light chain shortened the clotting time of prekallikrein-deficient plasma (Table III). However, on a molar basis, kallikrein was 180 times more active than the light chain when kaolin provided the surface. With sulfatides, kallikrein proved to be 145 times more active than the light chain.

**Cleavage of High Molecular Weight Kininogen by Kallikrein or the Light Chain**—The rate of cleavage of high molecular weight kininogen by the light chain of kallikrein was studied and compared to that by kallikrein. High molecular weight kininogen was mixed with the light chain or kallikrein in an enzyme to substrate ratio of 1:220. Aliquots of the reaction mixture were analyzed at various times for cleavage of high molecular weight kininogen on stained reduced SDS gels. Cleavage of high molecular weight kininogen was determined from absorbance profiles of scanned stained SDS gels. Incubation of high molecular weight kininogen with the light chain led to the disappearance of the $M_r = 110,000$ band with the simultaneous appearance of a protein band at $M_r = 65,000$. Prolonged incubation led to the generation of a third band at $M_r = 52,000$ (Fig. 4). The same pattern of cleavage was observed with kallikrein, and fragments with similar molecular weights were obtained. The initial rate of cleavage of high molecular weight kininogen by the light chain was about 3.5 times slower than that by kallikrein (Fig. 5). Thus, although a similar mechanism of cleavage of high molecular weight kininogen occurs, kallikrein is significantly more effective than the light chain.

**Immunological Studies of the Heavy and Light Chains of Kallikrein**—Immunodiffusion experiments using an unabsorbed anti-prekallikrein antiserum revealed a single precipitation line against kallikrein, and this line showed a reaction of partial identity with the single line obtained against either

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**Table III**

**Procoagulant activities of kallikrein and its light chain**

Assay conditions are described under "Material and Methods." Serial dilutions of kallikrein and light chain were used and converted to clotting units by comparison with the clotting time of dilutions of a pool of normal human plasma. A linear relationship was observed between the clotting time and the log of the concentration of normal plasma, kallikrein, or light chain at the concentrations tested. The lines for each procoagulant were parallel. Kaolin was used in a final concentration of 2 mg/ml and sulfatides at 20 μM.

<table>
<thead>
<tr>
<th></th>
<th>Native kallikrein</th>
<th>Light chain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kaolin Sulfatides</td>
<td>Kaolin Sulfatides</td>
</tr>
<tr>
<td>Clotting units/mg</td>
<td>471.0 287.0</td>
<td>6 4.4</td>
</tr>
<tr>
<td>Clotting units/nmol</td>
<td>37.7 22.2</td>
<td>0.21 0.154</td>
</tr>
</tbody>
</table>
the isolated heavy or light chain (Fig. 6). This indicates that the anti-prekallikrein antiserum contains antibodies directed against antigenic determinants present on the heavy chain and on the light chain of kallikrein. The precipitin lines obtained against the heavy chain and the light chain showed a reaction of nonidentity, indicating that the heavy and light chains have different antigenic determinants.

**DISCUSSION**

This study was undertaken to explore structure-function relationships for human plasma kallikrein. Activation of human prekallikrein by β-Factor XIIa is associated with limited proteolysis (12). Two slightly different forms of kallikrein are obtained that reflect the presence of two slightly different forms of prekallikrein as visualized on SDS gels in the absence of reducing agents. Kallikrein molecules are composed of two polypeptide chains linked by disulfide bonds, a heavy chain of 43,000 daltons and a light chain of either 36,000 or 33,000 daltons. As described here, under appropriate conditions, reduction and alkylation allow the formation of heavy and light chains that can be isolated using affinity chromatography on high molecular weight kininogen-Sepharose. The use of this affinity column was based on success in isolation of prekallikrein (28) and Factor XI (29). The heavy chain of kallikrein binds tightly to high molecular weight kininogen-Sepharose, whereas the light chain passes directly through the column with no apparent affinity for high molecular weight kininogen. These results demonstrate that the heavy chain of kallikrein possesses a high affinity binding site for high molecular weight kininogen and provide a rapid and convenient technique for separating the alkylated polypeptide chains of kallikrein. Previous studies had indicated that prekallikrein and kallikrein form equimolar complexes with the $M_r = 44,000$ light chain of high molecular weight kininogen (21, 30). Consequently, high affinity interactions between these molecules involve binding sites localized in the heavy chain region of kallikrein and the light chain region of high molecular weight kininogen.

The isolated alkylated light chains exhibit the full specific amidolytic activity of native kallikrein. The kinetic parameters, $K_a$ and $k_{cat}$, for the hydrolysis of H-D-Pro-Phe-Arg-p-nitroanilide by kallikrein or its light chain were identical. Moreover, the activation of Factor XII in solution was catalyzed equally well by kallikrein and its light chain. This demonstrates that the enzymatic active site of kallikrein is located in the light chain of the molecule, as previously suggested based on the observation that the molecule with diisopropyl fluorophosphate (11). The fact that the light chain is as effective as native kallikrein in cleaving oligopeptide chromogenic substrates and Factor XII suggests that the heavy chain region of kallikrein plays no significant role in forming the enzyme active site. However, a major role for the heavy chain region of kallikrein may be inferred from the observation that the light chain is much less procoagulant than native kallikrein. For example, in surface-dependent coagulation, the light chain was 180 times less effective than kallikrein in the presence of kaolin and 145 times less effective in the presence of sulfatides. These coagulation assays involved the addition of purified enzymes to plasma, and the specific reactions that are affected by the absence of the heavy chain region of kallikrein cannot be definitively specified. Nonetheless, it is tempting to speculate that the light chain fails to cleave surface-bound Factor XII as effectively as native kallikrein does. This is based on the facts that high molecular weight kininogen is required for normal binding of prekallikrein to negatively charged surfaces (20) and that surface-bound Factor XII in the presence of high molecular weight kininogen is 500 times more susceptible to proteolytic activation by kallikrein in comparison to Factor XII in solution (15).

Immunologic characterization of the heavy and light chains of kallikrein indicates that the two chains have different antigenic determinants as recognized by anti-prekallikrein antibodies. In double immunodiffusion studies, a reaction of nonidentity between the heavy and light chains was observed. Each polypeptide chain showed a reaction of partial identity with kallikrein, indicating that antigenic determinants of both polypeptide chains are exposed in the native molecule.

Previous workers (31) suggested that the high affinity binding of kallikrein to high molecular weight kininogen is mediated by the light chain region. This suggestion is not supported by the observation that the light chain of kallikrein does not adhere to high molecular weight kininogen-Sepharose, whereas the heavy chain region binds with high affinity. The previous work was based on kinetic studies of the rate of inactivation of kallikrein by C1 inhibitor (31). In preliminary studies, we have not been able to demonstrate a significant decrease in the rate of inactivation of kallikrein by C1 inhibitor in the presence of high molecular weight kininogen. Perhaps further studies may show effects of high molecular weight kininogen on this reaction as due to substrate or product inhibition rather than due to the specific high affinity binding that occurs between sites on the heavy chain region of kallikrein and the light chain region of high molecular weight kininogen.

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**REFERENCES**

Function of Heavy and Light Chains of Plasma Kallikrein