Differential interactions of human kallikrein-binding protein and α_1 -antitrypsin with human tissue kallikrein

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The characteristics of a new kallikrein-binding protein in human serum and its activities were studied. Both the kallikrein-binding protein and α_1 -antitrypsin form 92 kDa SDS-stable and heat-stable complexes with human tissue kallikrein. In non-SDS/PAGE, the mobility of these complexes differ. Complex-formation between kallikrein and the binding protein is inhibited by heparin, whereas that between kallikrein and α_1 -antitrypsin is heparin-resistant. In normal or α_1 -antitrypsin-deficient-serum, the amount of 92 kDa SDS-stable complex formed upon addition of kallikrein is not related to serum α_1 -antitrypsin levels. The rate of complex-formation between kallikrein and the binding protein is 12 times higher than that between kallikrein and α_1 -antitrypsin. Purified α_1 -antitrypsin, which exhibits normal elastase binding, has a kallikrein-binding activity less than 5% of that of serum. Binding of tissue kallikrein in serum is not inhibited by increasing elastase concentrations, and elastase binding in serum is not inhibited by excess tissue kallikrein. A specific monoclonal antibody to human α_1 -antitrypsin does not bind to either 92 kDa endogenous or exogenous kallikrein complexes isolated from human serum. The studies demonstrate a new tissue kallikrein-binding protein, distinct from α_1 -antitrypsin, is present in human serum.

INTRODUCTION

Tissue kallikreins (EC 3.4.21.35) are capable of liberating kinins from kininogen by limited proteolysis, which in turn cause local vasodilation and alterations in ion transport in the kidney and exocrine glands (Schacter, 1980). Tissue kallikreins have been identified and isolated from several exocrine and endocrine tissues, including kidney, salivary gland, pancreas, colon, spleen, brain, pituitary gland, vascular wall and heart (Simson *et al.*, 1979; Schacter, 1980; Chao *et al.*, 1983, 1984, 1987; Powers & Nasjletti, 1983; Nolly *et al.*, 1985). Active kallikreins are able to form high-molecular-mass entities with proteins in serum or tissues (Chao *et al.*, 1986a; Chao & Chao, 1988), and endogenous complexes of kallikrein and a binding protein have been isolated from serum, urine and kidney (Chao *et al.*, 1986a,b). However, specific tissue kallikrein-binding proteins or inhibitors that are capable of regulating kallikrein activity or degradation have not been identified.

It has been known for some time that tissue kallikrein may be released from its site of synthesis and appear in the circulation (Nustad *et al.*, 1978; Rabito *et al.*, 1980; Lawton *et al.*, 1981; Shimamoto *et al.*, 1984). The enzymic activity of circulating tissue kallikrein appears to be very low, and the presence of high-molecular-mass complexes of kallikrein in sera suggests that circulating kallikrein may be bound to inhibitors, binding proteins or circulating autoantibodies to tissue kallikrein (Shimamoto *et al.*, 1984; Chao *et al.*, 1988). Previous studies have shown that α_1 -antitrypsin is a slow progressive inhibitor of human urinary kallikrein (Geiger *et al.*, 1985). In search of kallikrein modulators we have recently identified kallikrein-binding protein(s) in serum, tissues and secreted into the media by several cell culture lines (Chao *et al.*, 1986a; Chao & Chao, 1988). Our results show a differential interaction of human tissue kallikrein with α_1 -antitrypsin and kallikrein-binding protein in serum and suggest that the newly identified kallikrein-binding protein may play a more important role in binding to circulating tissue kallikrein than α_1 -antitrypsin.

EXPERIMENTAL

Materials

Human sera containing different phenotypes of α_1 -antitrypsin were a gift from Dr. Robert Allen of the Department of Pathology of this institution. Concentrations of α_1 -antitrypsin in each serum were determined by the Diagnostic Immunology Department at this institution.

Commercially purified α_1 -antitrypsin and porcine pancreatic elastase were purchased from Calbiochem–Behring (San Diego, CA, U.S.A.). Human urokinase was provided by Dr. Kazuaki Shimamoto (Sapporo Medical College, Sapporo, Japan). Purified human plasma kallikrein was a gift from Dr. Allen P. Kaplan (State University of New York at Stony Brook, NY, U.S.A.). Purified human thrombin was a gift from Dr. Alan Cardin (University of Cincinnati, College of Medicine, Cincinnati, OH, U.S.A.), and was originally provided by Dr. John W. Fenton (Division of Laboratories and Research, New York State Department of Health, Albany, NY, U.S.A.). Human urinary kallikrein and antiserum to human urinary kallikrein were prepared as described previously (Shimamoto *et al.*, 1980). Human α_1 -antitrypsin in serum was purified as described previously (Pannell *et al.*, 1974). Elastase and kallikrein were labelled with ^{125}I by using the lactoperoxidase method (Shimamoto *et al.*, 1980).

Binding assays with ^{125}I -labelled human tissue kallikrein or ^{125}I -elastase

^{125}I -labelled human tissue kallikrein or ^{125}I -labelled elastase (sp. radioactivity $\sim 70 \mu\text{Ci}/\mu\text{g}$) (10000 c.p.m./reaction) was incubated with $1 \mu\text{l}$ of human serum or $6 \mu\text{g}$ of purified α_1 -antitrypsin at 37°C for 90 min in a buffer with the final concentration of 5 mM-sodium phosphate (pH 7.0) and a total volume of $40 \mu\text{l}$. The binding reaction was stopped by the addition of $40 \mu\text{l}$ of extraction buffer [0.125 M-Tris/HCl buffer, pH 6.8, containing 30% (v/v) glycerol, 0.04 M-dithiothreitol and 5% (w/v) SDS] and the mixture was heated at 100°C for 5 min.

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In time-course studies, reactions were stopped after different incubation times. For non-SDS/PAGE, reactions were stopped by adding the extraction buffer omitting SDS and dithiothreitol and the sample immediately electrophoresed.

Polyacrylamide-gel electrophoresis

Samples from binding reactions were analysed by SDS/PAGE under reducing conditions in 7.5–15%-(w/v)-acrylamide linear-gradient slab gels or by non-SDS/PAGE in 7.5% slab gels. The gel thickness was 1.5 mm. Electrophoresis was performed at 3 mA/cm until the Bromophenol Blue front reached the bottom of the gel. Gels were stained with 0.2% Coomassie Brilliant Blue in acetic acid/ethanol/water (2:9:9, by vol.) and destained in acetic acid/methanol/water (2:5:20, by vol.)

Autoradiography

Slab gels were dried and exposed at -70°C for 18–24 h using Kodak XAR-5 films with a du Pont Cronex Lightening Plus intensifying screen. The quantity of the complexes was measured either by cutting the autoradiogram spots from gels and counting the spots for radioactivity in a γ -radiation counter or by densitometer scanning of the exposed gel.

Immunoprecipitation of human urinary kallikrein–kallikrein-binding protein complexes

Human serum (0.2 ml) was preincubated with 2.5 μg of human urinary kallikrein in 0.35 ml of 10 mM-sodium phosphate buffer, pH 7.0, at 37°C for 2 h and then at 4°C for 16 h. Undiluted rabbit anti-(human urinary kallikrein) antiserum (50 μl) was added and the mixture was incubated at 4°C for 16 h. Immunoprecipitates were collected by centrifugation (10000g) for 30 min at 4°C in a Microfuge, washed once with 0.14 M-NaCl/0.01 M-sodium phosphate buffer, pH 7.3, and re-centrifuged. A 100 μl portion of extraction buffer [0.06 M-Tris/HCl buffer, pH 6.8, containing 15% (v/v) glycerol, 0.02 M-dithiothreitol and 2.5% (w/v) SDS] was then added and the samples were boiled for 5 min. Proteins (50 μl) were run on a 7.5–15% linear-gradient polyacrylamide gel containing 0.1% SDS as described previously (Chao *et al.*, 1983).

Kallikrein–agarose affinity chromatography

Human serum was diluted 1 in 3 in 0.2 M-NaCl/0.01 M-sodium phosphate buffer, pH 7.0, and applied to a human kallikrein–Affigel 15 column (1.5 cm $\times$ 15 cm) equilibrated with the same buffer. Kallikrein–kallikrein-binding protein complexes were eluted with 0.1 M-acetic acid. The eluted fractions were adjusted to pH 7.0 with 1 M-NaOH. The protein concentration of each fraction was measured by the absorbance at 280 nm.

Monoclonal-antibody development

The procedures for hybridoma development were similar to that described for tissue kallikreins (Chao & Chao, 1987). Briefly, 6-week-old female BALB/c mice were immunologically primed with an intraperitoneal injection of 50 μg of the purified human α_1 -antitrypsin emulsified in complete Freund's adjuvant and boosted at an interval of 3–4 weeks. At 3 months after the first injection, blood was collected from the tail vein, and the antibody-binding titres to α_1 -antitrypsin in the immunized mouse sera were determined by e.l.i.s.a. Clones producing antibody were subcloned by limited dilution with 0.5 cell plated per well, and ascitic fluid was produced in syngenic mice.

Western-blot analysis

Samples were subjected to SDS/PAGE on a 7.5–15% gradient slab gel and then electroblotted on to nitrocellulose membranes. The immunoblotting procedures using antigen overlay were as

described previously (Lämmle *et al.*, 1986; Chao *et al.*, 1989). Briefly, the nitrocellulose filters were blocked with BLOTTO [non-fat dried milk; 5% in 0.01 M-sodium phosphate (pH 7.4)/0.14 M-NaCl/1 μM -*p*-amidinophenylmethanesulphonyl fluoride/thimerosal (1 mg/l)/NaN₃ (200 mg/l)/0.01% antifoam A] (Johnson *et al.*, 1984) for 1 h at 30°C and then incubated with rabbit anti-(human urinary kallikrein) antiserum (1:600 in BLOTTO) or mouse monoclonal anti-(human α_1 -antitrypsin) ascitic fluid (1:500 in BLOTTO). After a 3 h incubation at 30°C with gentle shaking, the nitrocellulose membranes were washed three times with BLOTTO and then incubated with either ¹²⁵I-human tissue kallikrein or ¹²⁵I-rabbit anti-mouse IgG (250 000 c.p.m./ml; sp. radioactivity $\sim 70 \mu\text{Ci}/\mu\text{g}$ of kallikrein) for 1.5 h at 30°C . The nitrocellulose membranes were washed three times with BLOTTO and once with phosphate-buffered saline (0.01 M-sodium phosphate/0.14 M-NaCl, pH 7.4), air-dried, and exposed to Kodak X-Omat film for 16 h at -70°C .

RESULTS

Kallikrein complex-formation and effects of heparin

Human tissue kallikrein can form a ~ 92 kDa SDS-stable complex with kallikrein-binding protein or α_1 -antitrypsin; however, the interactions of these two serum proteins are different. When ¹²⁵I-kallikrein ($M_r \sim 38000$) is incubated with human serum (Fig. 1*a*, i) or purified α_1 -antitrypsin (Fig. 1*a*, ii), a 92 kDa SDS- and heat-stable complex formed. Heparin at 0.2 unit (lane 2), 1 unit (lane 3), 5 units (lane 4) and 10 units (lane 5) completely inhibits the complex-formation between kallikrein and the serum binding protein, whereas the complex-formation between kallikrein and α_1 -antitrypsin is totally resistant to inhibition at these heparin concentrations (Fig. 1*a*, ii, lanes 2–5). On SDS/PAGE the complex formed when kallikrein is added to serum has a molecular mass identical with that of the complex of kallikrein and purified α_1 -antitrypsin. However, in non-SDS/PAGE the electrophoretic mobility of the kallikrein–serum binding protein complex is retarded as compared with that of the kallikrein– α_1 -antitrypsin complex (Fig. 1*b*). The complex-formation between kallikrein and the serum binding protein is also susceptible to heparin inhibition, as shown in the non-SDS gel (Fig. 1*b*, ii, upper band), whereas the complex-formation between tissue kallikrein and α_1 -antitrypsin in serum (Fig. 1*b*, i, lower band) or with purified α_1 -antitrypsin (Fig. 1*b*, ii) is unaffected by a wide range of heparin concentrations.

Kallikrein complex-formation in α_1 -antitrypsin-deficient sera

On non-SDS/PAGE the quantity of complex formed between kallikrein and binding protein in one individual serum (Fig. 1*b*, i, upper band) is closely correlated with the quantity of the SDS-stable 92 kDa complex (Fig. 1*a*). When tissue kallikrein is added to human sera of various α_1 -antitrypsin phenotypes (ZZ, MZ or MS), the amount of kallikrein complex formed is independent of the serum concentration of α_1 -antitrypsin (Fig. 2). For example, in serum sample C, the α_1 -antitrypsin concentration is 0.95 mg/ml, a level one-third of the α_1 -antitrypsin concentration in sample F (2.50 mg/ml); however, the amount of complex formed between kallikrein and the binding protein in sample C is 15% higher than that of the complex formed in sample F.

Time course of interaction between kallikrein and binding protein or α_1 -antitrypsin

The time course of complex-formation between kallikrein and binding protein in serum (*a*) or purified α_1 -antitrypsin (*b*) is shown in Fig. 3. Complexed kallikrein is detectable in serum after 5 min incubation at 37°C , and the complex-formation reaches a plateau after 10 h, with a half-maximal binding at

Fig. 1. Effect of heparin on the binding of human tissue kallikrein to serum binding protein and α_1 -antitrypsin

(a) ^{125}I -human tissue kallikrein (10000 c.p.m.) was incubated with human serum (1 μl) or purified human α_1 -antitrypsin (6 μg) in the absence (lane 1) or presence of heparin (lane 2, 0.2 unit, lane 3, 1 unit; lane 4, 5 units; lane 5, 10 units) for 90 min at 37 °C in 0.01 M-sodium phosphate, pH 7.0. The binding assay was stopped by boiling the mixture in SDS extraction buffer at the times indicated. The tracer and the complex were resolved by SDS/7.5–15%-linear-gradient PAGE under reducing conditions and autoradiographed. (b) The experimental conditions were the same as for (a), except that at the end of incubation period the reaction was stopped by adding the extraction buffer, omitting SDS and dithiothreitol, and the mixtures were separated by non-SDS/7.5%-PAGE and autoradiographed. *, Complex of tissue kallikrein and kallikrein-binding protein; **, complex of tissue kallikrein and α_1 -antitrypsin.

Fig. 2. Comparison of α_1 -antitrypsin concentration (▨) kallikrein complex-formation in human serum (■)

The experimental procedures are described in the legend to Fig. 1(a) (90 min incubation). Human serum was from individual subjects (lanes A–G) and the α_1 -antitrypsin concentration is as indicated. Note that the kallikrein complex-formation in human serum is unrelated to α_1 -antitrypsin concentrations.

Fig. 3. Time-dependence of complex-formation between ^{125}I -kallikrein and human serum (a) or purified human α_1 -antitrypsin (b)

^{125}I -kallikrein was incubated with human serum (1 μl) or α_1 -antitrypsin (6 μg) at 37 °C in 0.01 M-sodium phosphate, pH 7.0. The binding reaction was stopped by boiling in SDS extraction buffer at times indicated. The samples were then electrophoresed in a SDS/polyacrylamide gel (7.5–15%) under reducing conditions and autoradiographed.

~ 30 min. However, complex-formation between kallikrein and purified α_1 -antitrypsin is much slower, with half-maximal binding at 6–7 h. The rate of kallikrein complex-formation in serum is nearly 12 times higher than that between kallikrein and purified α_1 -antitrypsin. We also compared kallikrein complex-formation in serum with the complex-formation between kallikrein and α_1 -antitrypsin purified from the same subject's serum. The amount of kallikrein complex formed with α_1 -antitrypsin purified from the individual serum was < 5% of that formed in serum containing the same α_1 -antitrypsin concentration. In contrast, elastase formed complexes with serum and with purified α_1 -antitrypsin in relatively equal quantities (results not shown).

Finally, since elastase is the target enzyme of α_1 -antitrypsin in serum, we compared the rates of the complex-formation between kallikrein and α_1 -antitrypsin with elastase and α_1 -antitrypsin. Fig. 4 shows that ^{125}I -elastase forms an SDS-stable ~ 79 kDa complex with both α_1 -antitrypsin in serum (a) and purified α_1 -antitrypsin (b) in a time-dependent manner. The rates of elastase complex-formation with α_1 -antitrypsin or serum are indistinguishable, with an identical half-maximal binding of less than 2 min, compared with 6–7 h for kallikrein and α_1 -antitrypsin.

Competition of kallikrein and elastase in complex-formation in serum

Competition assays showed that the binding of kallikrein to serum binding protein is not inhibited by excess elastase. When a fixed amount of ^{125}I -human tissue kallikrein was incubated with serum in the presence of increasing amounts of ^{125}I -elastase (molar ratio of elastase to kallikrein up to 8-fold), kallikrein

Fig. 4. Time-dependence of complex-formation between ^{125}I -elastase and human serum (a) or purified human α_1 -antitrypsin (b)

^{125}I -elastase was incubated with human serum (1 μl) or human α_1 -antitrypsin (6 μg) at 37 $^\circ\text{C}$ in 0.01 M-sodium phosphate, pH 7.0. At indicated times the binding was stopped by boiling in SDS extraction buffer. The samples were then subjected to SDS/7.5–15%-PAGE under reducing conditions and autoradiographed.

complex-formation was not decreased. However, elastase complex-formation with serum α_1 -antitrypsin increased proportionally with increasing elastase concentrations (Fig. 5a). Similarly, when fixed amounts of ^{125}I -elastase were incubated with serum in the presence of increasing amounts to 8-fold higher ratio of kallikrein to elastase (Fig. 5b), there was no inhibition of elastase complex-formation by kallikrein. Excess plasma kallikrein or urokinase did not inhibit elastase complex-formation, but thrombin slightly inhibited it (results not shown). However, the binding of ^{125}I -elastase to α_1 -antitrypsin in serum or to purified α_1 -antitrypsin is inhibited by excess unlabelled elastase.

Western-blot analysis

We have developed a monoclonal antibody to human α_1 -antitrypsin which binds to purified α_1 -antitrypsin or α_1 -antitrypsin in human plasma in a quantitative immunoblot assay. The specificity and sensitivity of the monoclonal anti-(human α_1 -antitrypsin) antibody in the immunoblot assay has been established. The antibody against α_1 -antitrypsin recognizes only one species of protein in human serum or plasma with the same molecular mass as that of the purified human α_1 -antitrypsin (54 kDa). Detection of immunoreactive α_1 -antitrypsin in human serum is dose-dependent, with a minimal limit of 0.03 μl of normal human serum. In addition, the monoclonal antibody can also recognize the higher-molecular-mass α_1 -antitrypsin-protein complexes (results not shown). The 92 kDa kallikrein complex in serum is not recognized by this monospecific anti-(α_1 -antitrypsin) antibody (Fig. 6). Neither endogenous kallikrein complex isolated from human serum by a kallikrein-affinity column nor the

Fig. 5. Competition by (a) excess elastase of the kallikrein-serum complex-formation and (b) excess kallikrein of the elastase-serum complex-formation

(a) Lane 1, ^{125}I -elastase (10,000 c.p.m.); lane 2, ^{125}I -human kallikrein (10,000 c.p.m.); lane 3, human serum (1 μl) was incubated with ^{125}I -human kallikrein (5000 c.p.m.); lanes 4–8 are the same as lane 3, but contained 2500, 5000, 10,000, 20,000 and 40,000 c.p.m. of ^{125}I -elastase respectively. Experimental procedures were as described in the legend to Fig. 1(a). (b) Lane 1, ^{125}I -human kallikrein (10,000 c.p.m.); lane 2, human serum (1 μl) was incubated with ^{125}I -elastase (5000 c.p.m.); lanes 3–8 are the same as lane 2, but contained 2500, 5000, 10,000, 20,000, 40,000 and 80,000 c.p.m. of ^{125}I -kallikrein respectively. Experimental procedures are described in the legend to Fig. 1(a).

exogenous kallikrein complex formed by the addition of excess purified human tissue kallikrein to human serum is recognized by the monoclonal antibody to human α_1 -antitrypsin (Fig. 6b, lanes 2 and 3). However, the 92 kDa protein is a complex recognized by anti-(human tissue kallikrein) antiserum (Fig. 6a, lanes 2 and 3).

DISCUSSION

The present studies demonstrate that tissue kallikrein forms complexes with α_1 -antitrypsin and a serum binding protein distinct from α_1 -antitrypsin. This conclusion is based on several observations. First, the electrophoretic mobility of the kallikrein-serum binding protein complex is distinguishable from that of kallikrein- α_1 -antitrypsin complex by non-SDS/PAGE. Secondly, the kallikrein complex-formation in serum is extremely susceptible to heparin inhibition, whereas complex-formation between kallikrein and α_1 -antitrypsin is unaffected by heparin. Thirdly, the quantity of kallikrein complex-formation in serum is unrelated to serum α_1 -antitrypsin concentration. Fourthly, the rate at which kallikrein complexed with the serum binding protein is more than 10-fold higher than that with α_1 -antitrypsin.

Fig. 6. Western-blot analysis of endogenous and exogenous kallikrein complexes in human serum

(a) Immunoblotting with rabbit polyclonal anti-(human urinary kallikrein) antibody (Anti-Kal). (b) Immunoblotting with mouse monoclonal anti-(human α_1 -antitrypsin) (Anti- α_1 -AT) ascitic fluids. Lane 1, purified human urinary kallikrein (1 μ g); lane 2, endogenous kallikrein and kallikrein-binding protein complexes purified from human serum by a kallikrein-agarose affinity column; lane 3, immunoprecipitated complexes of human urinary kallikrein and kallikrein-binding protein from human serum; and lane 4, purified human α_1 -antitrypsin.

Fifthly, competition assays show that the serum binding protein is specific for tissue kallikrein, since kallikrein complex-formation is not inhibited by excess unlabelled elastase, thrombin, urokinase, plasma kallikrein (results not shown). Finally, a monoclonal antibody to human α_1 -antitrypsin does not recognize complexes of kallikrein with the serum binding protein.

Among the serpin (serine proteinase inhibitor) superfamily, only four members, namely, antithrombin III, heparin cofactor II, protein C inhibitor and protease nexin are known to contain potential heparin-binding sites (Tollefsen *et al.*, 1982; Scott & Baker, 1983; Blackburn *et al.*, 1984; Koide *et al.*, 1984; Heidtmann *et al.*, 1986; Suzuki *et al.*, 1987). The interactions of these four serpins with their respective target serine proteinases are enhanced by heparin, whereas the inhibition of serine proteinases by α_1 -antitrypsin is not affected by heparin. In contrast, the complex-formation between kallikrein and its serum binding protein is completely inhibited by heparin. Since no heparin-binding site has been identified in the primary structures of human or porcine tissue kallikreins (Tschesche *et al.*, 1976; Lu *et al.*, 1989), a potential heparin-binding site on the kallikrein-binding protein molecule is implicated.

There is a marked difference between the rate of kallikrein complex-formation with the serum binding protein and that with α_1 -antitrypsin. The period for half-maximal binding of kallikrein to its binding protein is ~ 30 min, whereas that of kallikrein to α_1 -antitrypsin is greater than 6 h. In our studies the amount of kallikrein- α_1 -antitrypsin complex measured after 90 min incubation at 37 °C appears to be insignificant (Fig. 3). The rate of the binding of kallikrein to purified α_1 -antitrypsin in our study (half-maximal binding of 6–7 h) is consistent with the reported rate of human tissue kallikrein inhibition by α_1 -antitrypsin ($t_{1/2}$ of ~ 9 h) (Geiger *et al.*, 1985). The slow progressive binding or inhibition of kallikrein by α_1 -antitrypsin suggests that their interaction probably has very little physiological relevance.

Our previous studies showed that a polyclonal antiserum to human α_1 -antitrypsin recognizes purified α_1 -antitrypsin or α_1 -antitrypsin in serum, but does not recognize the complex formed between kallikrein and serum binding protein on Western-blot

analysis (Chao *et al.*, 1986a). Moreover, kallikrein-binding protein is not immunoprecipitated by antisera against α_2 -macroglobulin, inter- α -trypsin inhibitor, C₁-inactivator and anti-thrombin III (Chao *et al.*, 1986a). The present studies show that a monoclonal antibody against human α_1 -antitrypsin does not cross-react with the partially purified kallikrein-binding protein or the kallikrein-binding protein complexes (Fig. 6). Altogether these results strongly suggest that human kallikrein-binding protein in serum is immunologically distinct from α_1 -antitrypsin. Our study also provides evidence that the kallikrein-binding protein in serum is different from protein C inhibitor (also known as urokinase inhibitor or plasminogen activator inhibitor-III), protease nexin, or heparin cofactor II, for the following reasons. First, competitive binding assays showed that the complex-formation between 125 I-kallikrein and the binding protein is not inhibited by excess urokinase or thrombin (Chao *et al.*, 1986a). Secondly, heparin enhances the complex-formation of protein C inhibitor with activated protein C, protein C inhibitor with urokinase, heparin cofactor II with thrombin, and protease nexin with thrombin (Tollefsen *et al.*, 1982; Scott & Baker, 1983; Heeb *et al.*, 1987), whereas the complex-formation of kallikrein and the binding protein is inhibited by heparin (Fig. 1). Whether the kallikrein-binding protein we have identified in human serum can inhibit tissue kallikrein activity remains to be determined. Earlier evidence suggested that this binding protein may contribute to the clearance of active kallikrein from the circulation, since the protein binds to active kallikrein but not latent (inactive) kallikrein (Chao *et al.*, 1988). If the binding protein regulates kallikrein activity, delivery or degradation, it will probably have an important role in the functioning of the kallikrein-kinin system.

This work was supported by U.S. Public Health Service Grants HL29397 and HL33552.

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Received 1 September 1989; accepted 31 October 1989