A PREALBUMIN ACTIVATOR OF PREKALLIKREIN*

III. Appearance of Chemotactic Activity for Human Neutrophils by the Conversion of Human Prekallikrein to Kallikrein

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The generation of bradykinin in human plasma is initiated by the activation of Hageman factor (1–3). Activated Hageman factor and a series of fragments derived from activated Hageman factor by digestion with the fibrinolytic enzyme plasmin lead to the subsequent conversion of prekallikrein to kallikrein (4, 5). The smallest of the Hageman factor fragments is stable, has a mol wt of approximately 30,000–35,000, and appears to be the primary activator of prekallikrein having six times greater prekallikrein-activating activity than the parent Hageman factor molecule. Cleavage of the substrate kininogen by kallikrein leads to the elaboration of the permeability factor bradykinin (6).

In order to explore further the potential participation of the kinin-generating system in the inflammatory response, certain of the various components were examined for chemotactic activity. The generation of chemotactic activity specific for human neutrophils is shown to result from the conversion of prekallikrein to kallikrein. Evidence is also presented that both the chemotactic activity and bradykinin-generating activity are dependent upon the integrity of the active site of kallikrein.

Materials and Methods

Bradykinin triacetate (Sandoz Pharmaceuticals, Basel, Switzerland, or New England Nuclear Corp., Cambridge, Mass.) was used as the standard for native bradykinin. Antisera to IgG, IgA, IgM, β-lipoprotein, transferrin, albumin, and whole human serum were purchased from Behring Diagnostics Inc., Woodbury, N. Y. Antiserum to human κ and λ Bence Jones proteins was obtained from Melpar Inc., Falls Church, Va. Hexadimethrine (polybrene) was a gift of Dr. Floyd McIntire of Abbott Laboratories, North Chicago, Ill. Plasma thromboplastin antecedent (PTA)[1]-deficient plasma containing less than 1% of normal PTA activ-

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[1] *Abbreviations used in this paper:* DFP, diisopropyl fluorophosphate; ECF-A, eosinophil

ity was supplied by Dr. Anthony Britten (Boston, Mass.). Plasma thromboplastin component (PTC)-deficient plasma containing 2% normal PTC activity was obtained from Sera-Tec Biologicals, New Brunswick, N. J. Diisopropyl fluorophosphate (DFP) was a product of Aldrich Chemical Co., Inc., Milwaukee, Wis. Tosyl-L-lysine chloromethyl ketone (TLCK) was obtained from Cyclo Chemical Division of Travenol Laboratory, Los Angeles, Calif. Ampholine carrier ampholytes for use in isoelectric focusing were purchased from Microbiological Associates Inc., Bethesda, Md. Ovalbumin 5X crystalized was a product of Pentex, Kankakee, Ill.

Fractionation Procedures

Serum was drawn for the isolation of active enzymes of the kinin-generating system and was processed as previously described (4). Diethylaminoethyl (DEAE)-cellulose chromatography, carboxymethyl (CM)-cellulose chromatography, Sephadex G-100 gel filtration (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden), and disc gel electrophoresis at pH 9.3 were performed as previously described (4, 5).

Plasma was prepared for the isolation of proenzymes of the kinin-generating system as follows. 100 ml of blood were collected in plastic tubes containing 9 mg of disodium ethylenediaminetetraacetate (EDTA) and 3.6 mg of hexadimethrine in 0.1 ml of 0.15 M saline for each 10 ml of blood drawn. The tubes were immediately centrifuged at 900 *g* for 20 min at 4°C and the plasma was separated using siliconized pipettes and dialyzed against 4 liters 0.01 M PO_4 buffer, pH 7.8. The dialysis bag was opened hourly, the plasma centrifuged at 900 *g* for 10 min at 4°C to remove any precipitated protein, and the supernatant plasma dialyzed for a total of 6 hr. Samples were concentrated by ultrafiltration using a UM-10 membrane (Amicon Corp., Lexington, Mass.) to 5–10 ml and then further concentrated by wall vacuum using Collodion bags No. 100 (Schliecher and Schuell Inc., Keene, N. H.).

Gel Filtration.—A 2.5 × 150 cm column of Sephadex G-150 superfine was equilibrated with 0.02 M tris (hydroxymethyl) aminomethane (Tris) Cl, pH 7.6. Samples of 2–3 ml were applied and the column run by upward flow at 5 ml/hr. 2.5-ml fractions were collected.

A 5.0 × 100 cm column of Sephadex G-200 was equilibrated with 0.01 M PO_4 buffer, 0.15 M NaCl, pH 7.3. Samples of 4–5 ml were applied and the column run by upward flow at 10 ml/hr. 5-ml fractions were collected.

Quaternary Aminoethyl (QAE) Sephadex Chromatography.—A 5 × 30 cm column of QAE Sephadex was equilibrated with 0.01 M PO_4 buffer, pH 7.8. 30-ml samples dialyzed in 0.01 M PO_4 buffer, pH 7.8, were applied to the column. The column was washed with 500 ml of equilibrating buffer and a linear salt gradient of 2 liters of equilibrating buffer and 2 liters of equilibrating buffer containing 0.3 M NaCl was applied. The column was run at 60 ml/hr and 12-ml fractions were collected.

Sulfoethyl (SE) Sephadex Chromatography.—A 5 × 30 cm column of SE Sephadex was equilibrated with 0.01 M PO_4 buffer, pH 6.0. 30-ml samples dialyzed in 0.01 M PO_4 buffer, pH 6.0, were applied. The column was washed with 500 ml of equilibrating buffer and a linear salt gradient of 2 liters of equilibrating buffer and 2 liters of equilibrating buffer containing 0.5 M NaCl was applied. The columns were run at 60 ml/hr and 12-ml fractions were collected.

Isoelectric Focusing in Gels.—Isoelectric focusing in 4% polyacrylamide gels containing from pH 7 to 10 ampholytes was performed as described by Righetti and Drysdale (7). Samples of 200 μl were each electrofocused in a series of gels; the ampholytes of one gel were removed by electrophoresis and the gel was stained with Coomassie blue in 20% ethanol and 7% acetic acid. The others were cut in 5-mm slices and eluted in 0.2 ml of distilled water overnight at 4°C. The pH of each slice was then measured and the eluates assayed.

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chemotactic factor of anaphylaxis; PTA, plasma thromboplastin antecedent; PTC, plasma thromboplastin component; QAE, quaternary aminoethyl; SE, sulfoethyl; TLCK, tosyl-L-lysine chloromethyl ketone.

Preparation and Assay of Components of the Bradykinin-Forming System.—Activated Hageman factor was prepared by sequential fractionation of plasma on QAE Sephadex, Sephadex G-100, CM-cellulose,and elution from disc gels after electrophoresis at pH 9.3 as previously described (5, 8). The prealbumin Hageman factor fragments were prepared by sequential chromatography of serum on DEAE-cellulose, Sephadex G-100, CM-cellulose, and elution from disc gels after electrophoresis at pH 9.3 as previously described (5, 8). Preparation of prekallikrein and kallikrein are described in the Results section.

Bradykinin was routinely determined by bioassay utilizing the isolated guinea pig ileum (9) or by radioimmunoassay (10). Kallikrein, prekallikrein, and the prealbumin Hageman factor fragments were determined as previously described (4). Heat-inactivated plasma utilized as a source of the substrate kininogen was prepared as described by Jonasson and Becker (11).

The shortening of the partial thromboplastin time of PTA-deficient plasma was used as an assay for PTA. Activated PTA was determined by incubating 0.05 ml of PTA source with 0.05 ml cephalin reagent (12) and 0.05 ml of PTA-deficient plasma for 2 min at 37°C. 0.05 ml of 0.05 M $CaCl_2$ was added and the clotting time determined at room temperature. The tubes were tilted each minute and the end point defined as the time interval required for the clot to adhere to the glass tube. Unactivated PTA was determined by incubation of 0.025 ml of PTA source with 0.025 ml purified activated Hageman factor for 5 min at 37°C. 0.05 ml cephalin-kaolin reagent and 0.05 ml of PTA-deficient plasma were then added and the mixture further incubated at 37°C for 2 min. 0.05 ml 0.05 M $CaCl_2$ was added and the clotting time determined.

Measurement of Chemotaxis.—Chemotaxis of human leukocytes was assayed by a modification of the Millipore technique of Boyden as previously described (13). Blood was drawn into plastic syringes and transferred to plastic tubes containing 50 units of heparin/ml of blood. The red blood cells were allowed to settle for 90 min at 37°C, after which the leukocytes were removed and centrifuged for 5 min at 100 *g*. The cells were washed once in Hanks' solution and resuspended in Hanks' solution containing 0.5% ovalbumin at pH 7.3. The cell count was adjusted to 1.5×10^6 leukocytes/ml. Chemotactic experiments were performed using a 3.0 μ pore size; except where stated otherwise, donors of peripheral blood leukocytes were normal volunteers. The 0.5% ovalbumin solution used as a suspending medium in the chemotactic chambers did not contain active kallikrein or a substrate for kallikrein.

An anaphylactic diffusate from passively sensitized human lung challenged with ragweed antigen E containing histamine, slow reacting substance of anaphylaxis (SRS-A), and an eosinophil chemotactic factor of anaphylaxis (ECF-A) was prepared as described (14). In experiments comparing the selective chemotactic activity of kallikrein and ECF-A, Tyrode's solution in 0.5% ovalbumin was used as suspending medium both in the test and cell compartments. Most preparations of kallikrein were dialyzed at 4°C for 6–12 hr against Hanks' solution. Some kallikrein preparations, contained in Tris HCl buffer, were assayed directly, the molarity of the solution in the test compartment being adjusted to that of Hanks' solution.

RESULTS

Chemotactic Activity Associated with Plasma Kallikrein.—Human serum clotted in glass so as to activate Hageman factor is known to possess chemotactic activity (15, 16). In order to assess whether any of the chemotactic activity so obtained was associated with the appearance of serum kallikrein, 5 ml of human serum were fractionated by Sephadex G-200 gel filtration as shown in Fig. 1. Three major peaks of chemotactic activity were observed, the first located between the IgM and IgG peaks, the second along the descending limb of the IgG peak, and the third with low molecular weight moieties eluting subsequent to albumin. Assay of the column for kallikrein activity

indicated a peak which was superimposable upon the second major peak of chemotactic activity. A more highly purified preparation of kallikrein was therefore prepared in order to further assess this association.

Human serum was dialyzed against 0.01 M PO_4 buffer, pH 7.8, and applied to a column of DEAE-cellulose. The effluent is known to contain IgG (17), kallikrein (18, 19), and activated PTA (20). Further fractionation by Sephadex G-150 gel filtration permitted partial separation of these three proteins and the fractions so obtained were assessed for chemotactic activity (Fig. 2). The single peak of optical density at 280 mμ represented primarily IgG as assessed by immunoelectrophoresis using anti-whole human serum. The peak of acti-

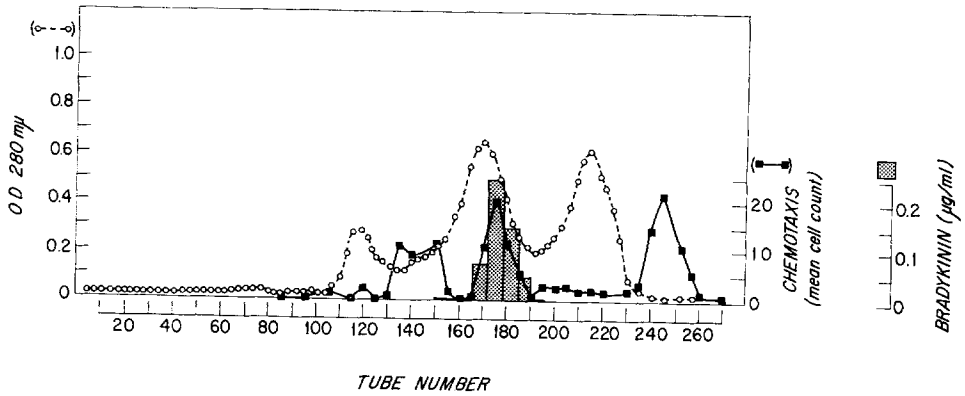

FIG. 1. Chromatography of human serum on Sephadex G-200. After a preliminary assessment for kallikrein activity, the negative fractions were pooled into 100-ml lots, concentrated to 5 ml, and assayed for kinin-generating activity while the positive region extending from tubes No. 165 to 190 was divided into four portions, each concentrated to 5 ml and assayed for bradykinin-generating activity as indicated. Mean cell count refers to the average of the cell counts in five high power fields.

vated PTA was found in the ascending limb of the IgG peak and had an estimated mol wt of 170,000–175,000. The peak of kallikrein activity clearly followed the IgG peak and had an estimated mol wt of 130,000. For chemotactic studies, the column fractions were pooled as follows: 1–40, 41–80, 81–120, 121–150, 151–157, 158–190, 191–220, 221–245, 246–300 and concentrated to 10 ml each so that the peak of activated PTA would be essentially free of kallikrein. The chemotactic activity was found to overlap the peak of kallikrein activity.

When the kallikrein-rich concentrate (Fig. 2) was assessed by disc gel electrophoresis, the Coomassie blue stain revealed a single broad band in the γ-globulin region (Fig. 3). An unstained gel was sliced in 2-mm sections, eluted in 0.5 ml of 0.15 M NaCl, dialyzed against Hanks' solution, and assayed for kallikrein activity and for chemotactic activity; both were located in the first two slices as shown in Fig. 3. In addition, some chemotactic activity was found in slice 7.

The majority of the cells which migrated in response to the various kallikrein preparations in the experiments described above were neutrophils, only an occasional eosinophil being noted. The chemotactic activity of kallikrein for neutrophils was further investigated using peripheral blood leukocytes obtained from a patient who had an eosinophilia of 70% in association with seropositive rheumatoid arthritis. The chemotactic response to kallikrein was compared to that obtained with the anaphylactic diffusate containing ECF-A. As shown in Fig. 4, a dose response was obtained with each chemotactic factor; however while ECF-A attracted primarily eosinophils, the kallikrein preparation attracted only the patient's neutrophils. Bradykinin in concentrations of 1.0, 0.1, and 0.01 μg was not chemotactic for either cell type.

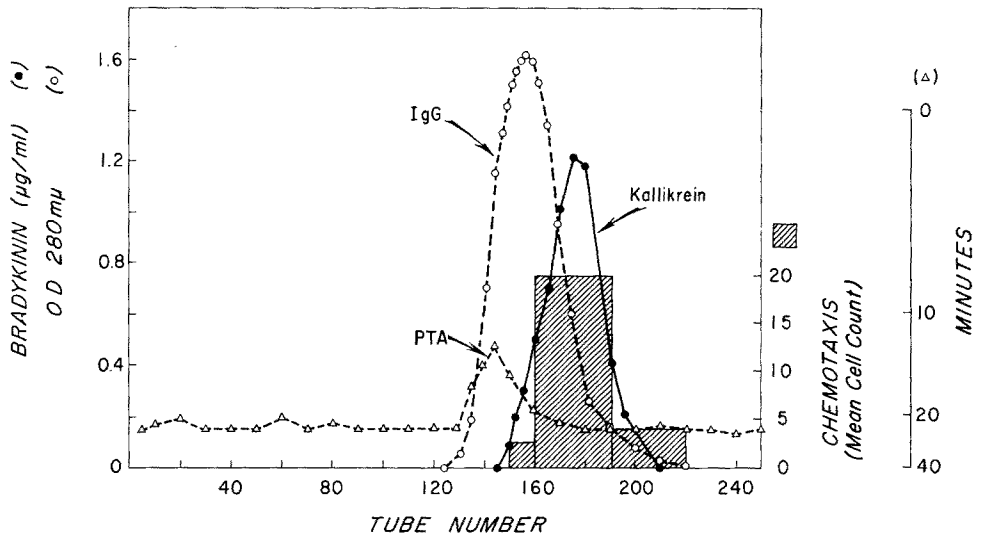

FIG. 2. Sephadex G-150 superfine gel filtration of the DEAE-cellulose effluent obtained with 0.01 M PO_4 buffer, pH 7.8.

Chemotactic Activity Resulting from the Activation of Prekallikrein.—

Preparation and Properties of Human Prekallikrein.—30 ml of dialyzed plasma containing hexadimethrine were applied to a column of QAE Sephadex. The effluent contained IgG, as assessed by immunoelectrophoresis, had no detectable active kallikrein and had only trace quantities of activated PTA. When 5 μl of the effluent were incubated with 10 μl of the purified Hageman factor fragments and then assayed for bradykinin-generating activity after incubation with 0.2 ml of heat-inactivated plasma, 100 ng of bradykinin were generated, indicating that the preparation contained prekallikrein. Incubation of 25 μl of the QAE Sephadex effluent with 25 μl of activated Hageman factor shortened the partial thromboplastin time of PTA-deficient plasma to 2.0 min, indicating the presence of unactivated PTA in the

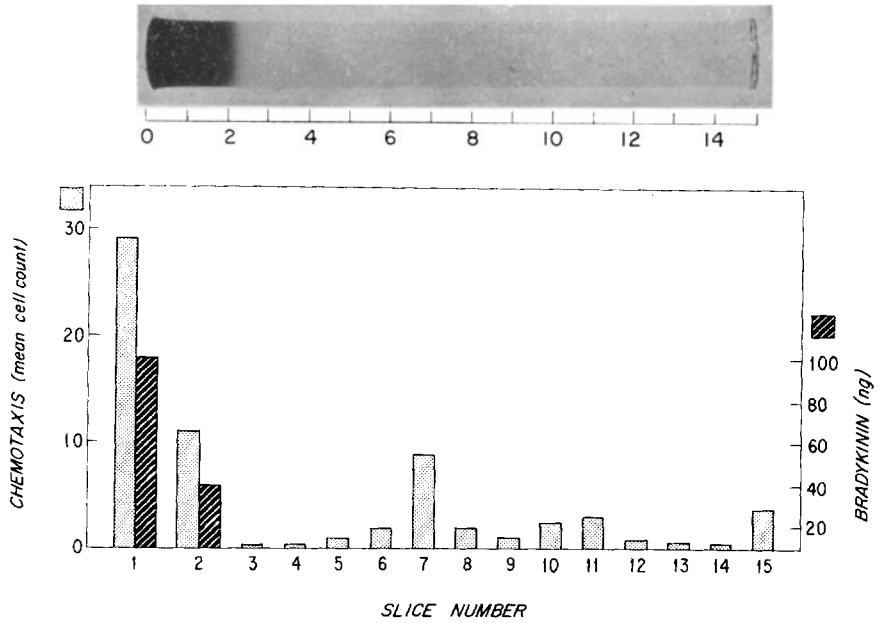

FIG. 3. Disc gel electrophoresis of the kallikrein-rich concentrate obtained from Sephadex G-150. An unstained gel run simultaneously was sliced as indicated into 15 equal sections, eluted, and assayed for kallikrein and for chemotactic activity.

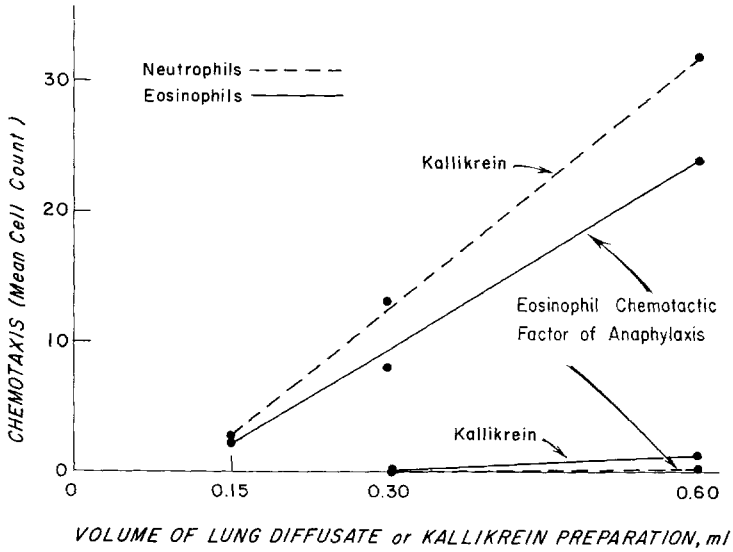

FIG. 4. Comparative chemotactic assay of kallikrein and ECF-A utilizing the peripheral blood leukocytes of a patient who had a 70% eosinophilia.

effluent. The addition of activated Hageman factor alone to the PTA-deficient plasma shortened the partial thromboplastin time from the control time of 28 min to 16 min. Addition of the effluent plus activated Hageman factor mixture in the same amounts as noted above to PTC-deficient plasma gave no shortening of the partial thromboplastin time.

The QAE Sephadex effluent containing IgG, prekallikrein, and unactivated PTA was then subjected to chromatography on SE Sephadex as shown in Fig. 5, resulting in separation of prekallikrein from the majority of IgG and from unactivated PTA. Prekallikrein eluted between 0.17 and 0.19 M NaCl and was

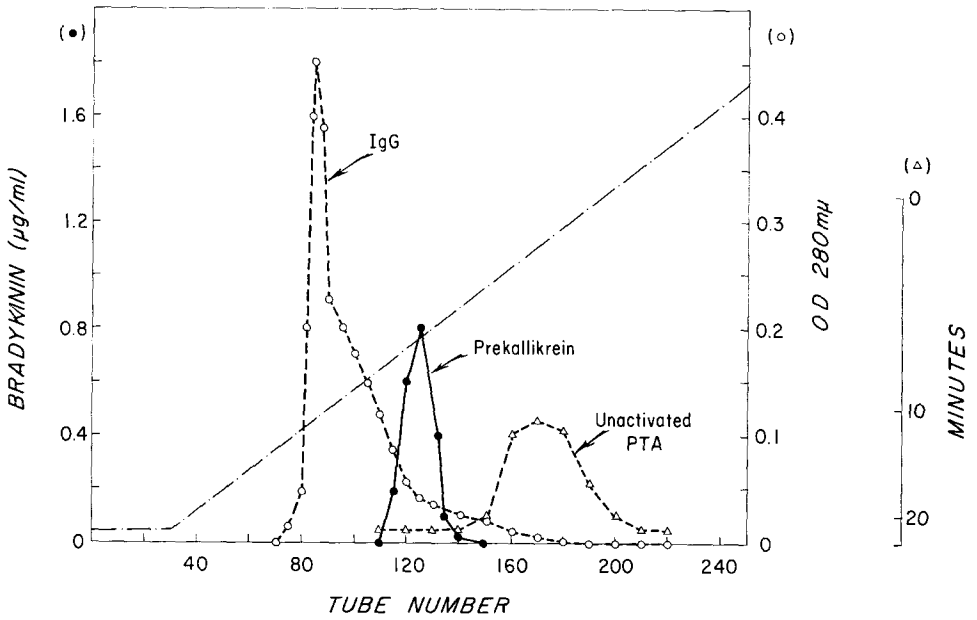

FIG. 5. SE Sephadex chromatography of the QAE Sephadex effluent obtained with 0.01 M PO_4 buffer, pH 7.8.

activated by incubation with the Hageman factor fragments. The prekallikrein peak was then pooled, concentrated, and further fractionated by Sephadex G-150 gel filtration as shown in Fig. 6. When the prekallikrein peak was pooled and concentrated, neither unactivated PTA nor activated PTA was detectable. IgG, however, was still detectable by gel diffusion using anti-IgG or anti-κ chain antisera. This preparation was then examined by disc gel electrophoresis and isoelectric focusing in gels. Analysis of the preparation by disc gel electrophoresis revealed a faint broad band in the γ-globulin region. When an unstained disc gel run simultaneously was sliced in 2-mm slices, eluted, and assayed for prekallikrein, the activity was found only in the first two slices at the top of the gel. When the same preparation was assessed by isoelectric

focusing in polyacrylamide gels, a faint broad band was recognized extending from pH 7.5 to 8.9, shown in Fig. 7. No other discrete bands were visible. When an unstained gel run simultaneously was sliced, eluted, and assayed for prekallikrein, prekallikrein was found between slices 5 and 9, which corresponded to an isoelectric point ranging from pH 8.5 to 8.9, the peak being at pH 8.7 (Fig. 7).

Chemotactic Activity.—500 μl of the Hageman factor fragments were incubated with 250 μl of prekallikrein obtained at the SE Sephadex step (Fig. 5) for 5 min at 37°C, diluted to 1 ml with Hanks' solution, and dialyzed against Hanks' solution for 12 hr. 100 μl of the mixture incubated with 200 μl of heat-inac-

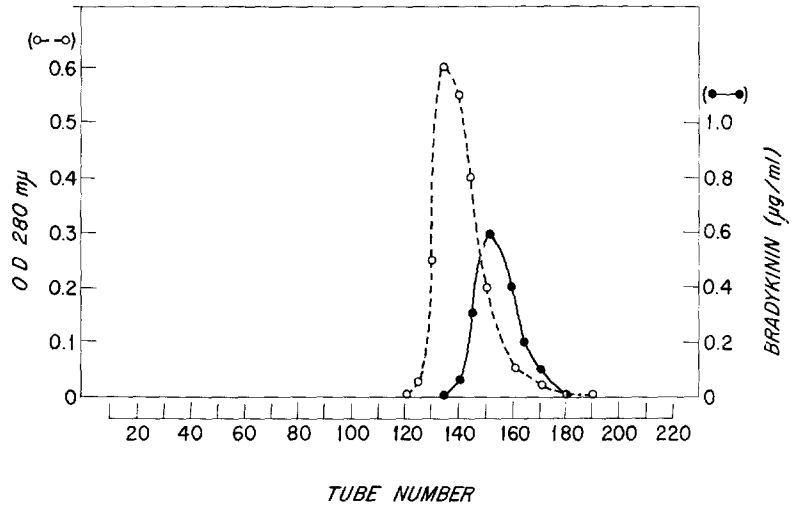

FIG. 6. Sephadex G-150 gel filtration of prekallikrein obtained from SE Sephadex (Fig. 5).

tivated plasma generated 200 ng of bradykinin indicating conversion of the prekallikrein to kallikrein. The same mixture was chemotactic for human neutrophils as shown in Table I, Experiment 1. Controls consisting of 250 μl of prekallikrein incubated with 500 μl of normal saline and 500 μl of the Hageman factor fragments incubated with 250 μl of normal saline, processed identically, did not generate bradykinin from heat-inactivated plasma and had no significant chemotactic activity. Experiment 2 shows a similar result utilizing prekallikrein obtained from the Sephadex G-150 gel filtration step (Fig. 6).

Inhibition of Chemotactic Activity of Kallikrein.—The generation of bradykinin by human kallikrein is dependent upon an enzymatic site which is inhibitable by DFP (19, 21) through the action of this agent upon the hydroxyl group of a serine residue. The effect of DFP as well as an active site histidine inhibitor, TLCK, upon the chemotactic activity and the bradykinin-generating activity

of kallikrein was examined. 300 μl of kallikrein were made 10^{-3} M in DFP or 10^{-3} M in TLCK, the mixture incubated for 15 min at 37°C and dialyzed three times against 500 ml of Hanks' solution for 12 hr. 700 μl of Hanks' solution were then added to each sample and the samples assayed for chemotactic and kinin-generating activity. As a control, an equal quantity of DFP and TLCK

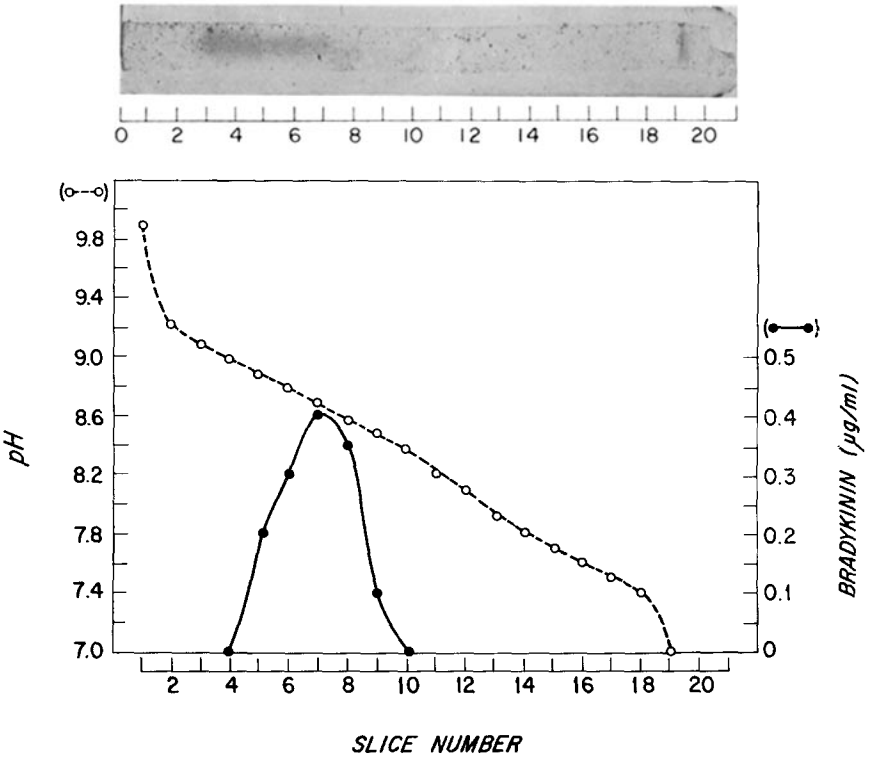

FIG. 7. Isoelectric focusing in 4% polyacrylamide gels of the prekallikrein-rich concentrate obtained from Sephadex G-150 gel filtration (Fig. 6). An unstained gel run simultaneously was sliced into 21 equal sections of 5 mm each and assayed for pH and prekallikrein.

were each added to 100 μl of 0.02 M Tris Cl, pH 7.6, the buffer in which the kallikrein preparation was stored, and dialyzed three times against 500 ml of Hanks' solution for 12 hr. 300 μl of kallikrein were then added, incubated for 15 min at 37°C, the volume brought to 1.0 ml with Hanks' solution, and assayed for chemotactic and kinin-generating activity. As shown in Fig. 8, DFP treatment of kallikrein abolished both kinin-generating and chemotactic activity (*B*), while removal of DFP from the buffer by dialysis before the introduction of kallikrein had no effect upon these two functions (*D*). No effect was seen with 10^{-3} M TLCK (*C*).

A dose response experiment for inhibition of chemotaxis and bradykinin generation by TLCK and DFP is shown in Fig. 9. The experiment was performed as described above except the incubation time of kallikrein with each

TABLE I

Generation of Chemotactic Activity for Human Neutrophils by Activation of Prekallikrein

	Prekallikrein	Hageman factor fragments	Prekallikrein + Hageman factor fragments
Experiment 1*			
Neutrophil chemotaxis (mean cell count)	0	1.5	14.2
Bradykinin generated from heat-inactivated plasma (ng)	0	0	200
Experiment 2‡			
Neutrophil chemotaxis (mean cell count)	0	0	15
Bradykinin generated from heat-inactivated plasma (ng)	0	0	175

* Prekallikrein obtained from SE Sephadex step.

‡ Prekallikrein obtained from Sephadex G-150 step.

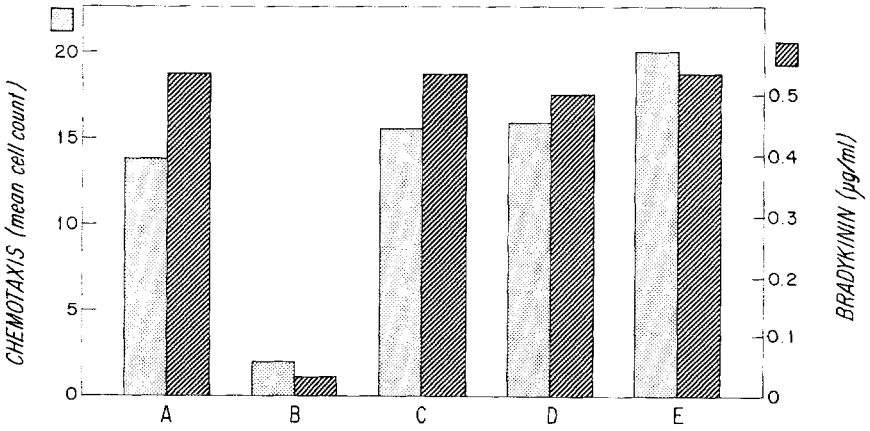

FIG. 8. Effect of 10^{-3} M DFP and 10^{-3} M TLCK upon the chemotactic and bradykinin-generating activity of kallikrein. (*A*) Kallikrein control. (*B*) Kallikrein made 10^{-3} M in DFP. (*C*) Kallikrein made 10^{-3} M in TLCK. (*D*) Kallikrein added to dialyzed DFP solution. (*E*) Kallikrein added to dialyzed TLCK solution.

inhibitor was 30 min. Longer incubation at this temperature resulted in non-specific loss of both kallikrein functions. No inhibition of either activity is obtained with TLCK until a 10^{-2} M concentration is used. A dose response inhibition of both activities was obtained with DFP between 0.25×10^{-4} M and 1.0×10^{-3} M.

In order to determine whether the inhibition of DFP is related to the active site of kallikrein, an experiment was performed in which the action of DFP upon prekallikrein and kallikrein was compared. 1 ml of prekallikrein obtained from plasma by QAE Sephadex, SE Sephadex, and Sephadex G-150 chromatog-

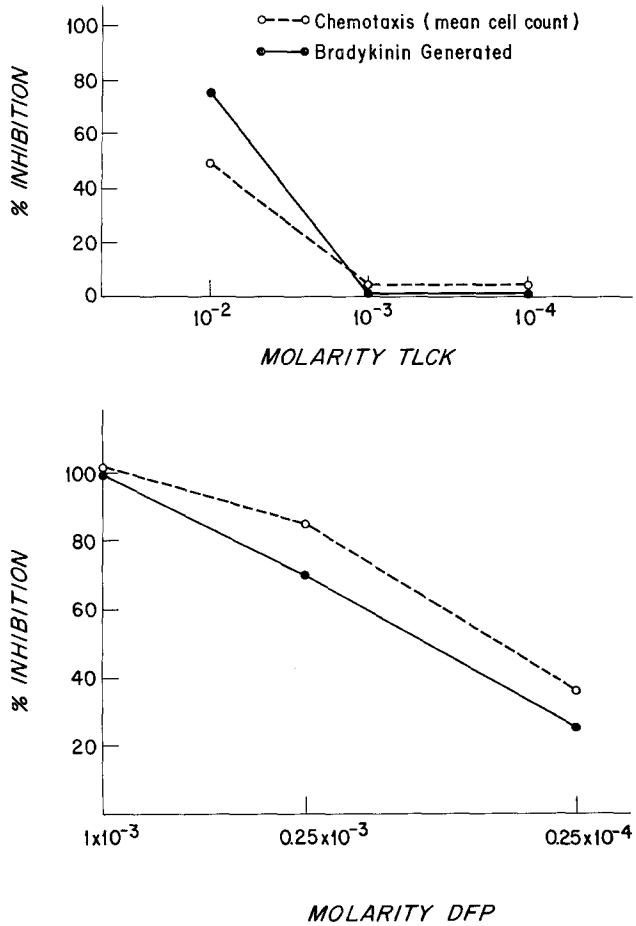

FIG. 9. Dose response inhibition of both chemotactic and bradykinin-generating activity of kallikrein utilizing TLCK and DFP.

raphy was incubated with 100 μl of Hageman factor fragments for 20 min at 37°C. The mixture was then divided in half, one portion was made 10^{-3} M in DFP and both were further incubated at 37°C for 15 min, brought to 1 ml volume with Hanks' solution, and dialyzed three times against 500 ml of Hanks' solution for 12 hr. Two 500 μl aliquots of prekallikrein were first made 10^{-3} M in DFP, incubated at 37°C for 15 min, and dialyzed three times against

500 ml of Hanks' solution for 12 hr. One portion was then activated by addition of 50 μl of the Hageman factor fragments, the mixture incubated for 20 min at 37°C, and both aliquots were then brought to 1 ml volume with Hanks' solution. Controls consisting of prekallikrein or the Hageman factor fragments alone, each brought to the same final concentrations utilized above by the addition of normal saline, were subjected to each incubation and dialysis step. Each preparation was then assayed for bradykinin-generating and chemotactic activity. As shown in Fig. 10, the prekallikrein preparation (*A*), the Hageman factor fragments (*B*), and the mixture of prekallikrein and the Hageman factor fragments which was then exposed to 10^{-3} M DFP (*D*) generated no bradykinin

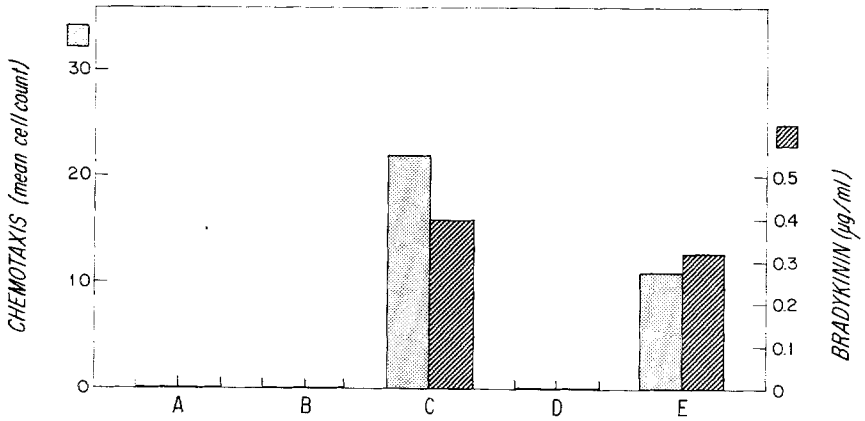

FIG. 10. Comparison of the effect of DFP upon kallikrein and prekallikrein. (*A*) Prekallikrein preparation. (*B*) Hageman factor fragments. (*C*) Prekallikrein activated with the Hageman factor fragments. (*D*) Prekallikrein activated with the Hageman factor fragments and made 10^{-3} M in DFP. (*E*) Prekallikrein made 10^{-3} M in DFP, dialyzed, then activated with the Hageman factor fragments.

from heat-inactivated plasma and possessed no chemotactic activity. Exposure of prekallikrein alone to 10^{-3} M DFP generated no bradykinin from heat-inactivated plasma and possessed no chemotactic activity. Exposure of prekallikrein to 10^{-3} M DFP followed by dialysis to remove the DFP and subsequent conversion to kallikrein by incubation with the Hageman factor fragments (*E*) revealed 80% of the kinin-generating activity and 50% of the chemotactic activity of prekallikrein which was activated without prior exposure to DFP (*C*).

DISCUSSION

When human blood is clotted in glass, thereby activating Hageman factor, the serum obtained has been shown to possess chemotactic activity (15). This activity is partially destroyed by heating for 30 min at 56°C. Since activation

of Hageman factor not only initiates the intrinsic pathway of blood coagulation (22, 23) and the fibrinolytic system (24, 25) but also the kinin-forming sequence, it seemed appropriate to evaluate the components of the kinin-forming system as possible contributors to the chemotactic activity of normal serum. Fractionation of normal serum on Sephadex G-200 revealed several peaks of chemotactic activity of which one coincided with the peak of kallikrein (Fig. 1), the most heat-labile component of the kinin-forming system (26).

The evidence that kallikrein is chemotactic for human neutrophils includes the superposition of kinin-generating and chemotactic activity when partially purified kallikrein is further fractionated by Sephadex G-150 gel filtration (Fig. 2) and by electrophoresis in alkaline disc gels (Fig. 3). Further evidence that chemotactic activity is a function of active kallikrein is provided by the finding that conversion of prekallikrein to kallikrein by the Hageman factor fragments is associated with the appearance of chemotactic activity whereas neither prekallikrein nor the Hageman factor fragments alone exhibited such a property (Figs. 5, 6, Table I). Although it seemed possible that the chemotactic activity observed might be due to a small fragment formed during conversion of prekallikrein to kallikrein, attempts to dissociate such a fragment from kallikrein by rechromatography on G-75 at pH 3.5 failed to reveal chemotactic activity except in association with the kallikrein peak. Furthermore, the capacity of DFP to inhibit both chemotaxis and kinin generation (Figs. 8, 9) is consistent with chemotaxis being an intrinsic function of the kallikrein molecule itself.

Lewis has shown that intradermal injections of relatively high concentrations of bradykinin (1–100 μg/ml) in the rabbit promotes the migration of leukocytes (27). Of the components of the kinin-forming system thus far examined, the Hageman factor fragments, prekallikrein, kallikrein, and bradykinin, only kallikrein was chemotactic. Furthermore, kallikrein attracted neutrophils but not eosinophils from a mixed leukocyte population, even when the suspension contained 70% eosinophils (Fig. 4). In contrast, an eosinophil chemotactic factor released from human lung sensitized with IgE and challenged with specific antigen attracted eosinophils but not neutrophils under the same experimental conditions (14) (Fig. 4).

The activation of Hageman factor by a variety of biologic materials such as collagen (28, 29), sodium urate crystals (30), pyrophosphate crystals (30), L-homocystine crystals (31), and human articular cartilage (32), and the capacity of activated Hageman factor to convert prekallikrein to kallikrein either directly or through its prealbumin fragments (5) offers a nonimmunologic, readily activatable mechanism for the generation of a chemotactic principle independent of the complement system. It is noteworthy that kallikrein is not only chemotactic for human neutrophils but also leads to the generation of the permeability factor bradykinin; two other chemotactic factors, fragments from the third and fifth components of complement both promote leukocyte migra-

tion in vitro (33, 34) and also increase vascular permeability by virtue of their anaphylatoxic activity (35, 36). It may be that a change in vascular permeability plays a role in determining whether or not a chemotactic principle can express this capability in an in vivo situation.

A series of esterases present in rabbit neutrophils, which play an essential role in the chemotactic response, have been described (37–39). That the active site of kallikrein is required for the expression of its chemotactic activity could be explicable either by direct activation of one of these esterases or through a substitution which bypasses an activation step utilized by nonenzymatic chemotactic factors. The relatively high concentration of DFP utilized in the inhibition experiments was necessitated by the requirement to inactivate kallikrein at 37°C within a 15 min period; incubation of kallikrein beyond this interval was associated with a loss in activity which reached 100% in 2 hr. The histidine inhibitor TLCK, however, was inactive at the same concentration and conditions (Fig. 9). The findings that the precursor form of the enzyme, prekallikrein, tolerated a dose of DFP which completely inactivated kallikrein (Fig. 10) is consistent with studies of other serine esterases in which the precursor enzyme was resistant to inactivation (40, 41). Since both the Hageman factor fragments and kallikrein are sensitive to inactivation by DFP (19, 21, 42), the partial loss of activity experienced by the prekallikrein could be attributed to residual DFP despite the extensive dialysis.

SUMMARY

Human plasma kallikrein has been shown to directly and selectively attract human neutrophils from a mixed leukocyte population. The capacity of plasma kallikrein to be chemotactic and to generate the nonapeptide bradykinin was maintained during progressive purification. While neither highly purified prekallikrein nor the prealbumin Hageman factor fragments were chemotactic alone, their interaction so as to convert prekallikrein to kallikrein yielded both chemotactic and kinin-generating activity. Both functions of kallikrein were inhibited by treatment with diisopropyl fluorophosphate, indicating an essential role for the active site of the enzyme in the expression of its chemotactic activity.

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