Vol. 129, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1985 Pages 76-81

THE EFFECT OF TRYPTASE FROM HUMAN MAST CELLS ON HUMAN PREKALLIKREIN[1]

Lawrence B. Schwartz[2], M.D., Ph.D., Timothy Bradford and John H. Griffin, Ph.D.

Departments of Medicine and of Microbiology and Immunology,
Virginia Commonwealth University, Medical College of Virginia, Richmond, VA

Department of Immunology,
Scripps Clinic and Research Foundation, La Jolla, CA

Received April 18, 1985

Tryptase, the dominant protease in human mast cells, was examined for its effect on human prekallikrein. Tryptase in the presence and absence of heparin failed to activate prekallikrein as shown in a spectrophotometric assay for kallikrein employing benzoyl-pro-phe-arg-p-nitroanilide. Treated prekallikrein was converted to active kallikrein by bovine trypsin. Prekallikrein cleavage products were analyzed by electrophoresis in polyacrylamide gels under denaturing conditions (± reduction). Tryptase caused no apparent cleavage under conditions where trypsin caused complete cleavage. Thus, tryptase, which has previously been shown to lack kallikrein and kininase activities, neither activates nor destroys prekallikrein.

Tryptase is the dominant neutral protease and protein component in secretory granules of human lung mast cells (1,2). It is preferentially present in and appears to be a specific marker for human mast cells (3), but little is known of its function(s) *in vivo*. A role for tryptase in the generation of bradykinin has been considered because kinin activity has been detected in nasal secretions of allergen-challenged atopic subjects (4); IgE-dependent activation of human lung fragments results in the secretion kininogenase (5), prekallikrein activating (6), and Hageman factor (7) activities; and plasma taken from patients undergoing systemic anaphylaxis

[1]This work was supported by Grants AI-20487 and HL-21544 from the National Institutes of Health.
[2]To whom correspondence should be addressed at The Medical College of Virginia, Box 263, Richmond, VA 23298.

Abbreviations used in this paper were: TAMe, tosyl-L-arginine methyl ester; BPPA-pNA, benzoyl-L-pro-phe-arg-p-nitroanilide; TN buffer, 0.05 M Tris, pH 7.6, containing 0.12 M NaCl; SDS-PAGE, electrophoresis in polyacrylamide gels containing SDS.

0006-291X/85 $1.50

shows consumption of high molecular weight kininogen with normal levels of prekallikrein and Hageman factor (8).

However, prior studies on plasma kininogens, the major precursors of bradykinin (9-12), have shown that purified tryptase rapidly destroys human high molecular weight kininogen (13) and has no effect on human low molecular weight kininogen (14). The capacity of tryptase to activate plasma prekallikrein to kallikrein (EC3.4.21.8) is examined in the present report. The prekallikrein proenzyme circulates in plasma as an inactive gammaglobulin of which two forms, I (88,000 MW) and II (85,000 MW), have been reported (15). Activated Hageman factor (factor XIIa) is the principal known physiologic activator of prekallikrein. Activation results in kallikreins that have a 52,000 MW heavy chain and a light chain of 36,000 MW for type I and 33,000 MW for type II (15). The present study indicates that tryptase neither activates nor destroys prekallikrein.

METHODS

Tryptase was purified to homogeneity from human lung mast cells by ammonium sulfate fractionation and sequential chromatography on decyl-agarose, diethyl amino ethyl-Sephadex, and heparin-agarose as described (1,3). Specific tosyl-L-arginine methyl ester (TAMe) esterase activity of the purified enzyme ranged from 96 units/mg to 102 units/mg protein, where one unit cleaves one umole of TAMe/min at 22°C (1). Prekallikrein was purified to homogeneity from human plasma by sequential chromatography on diethyl amino ethyl-Sephadex and sulfopropyl-Sephadex followed by affinity chromatography as described (16). Purified prekallikrein had a specific activity of 20 clotting units/mg; one clotting unit is the amount of activity present in 1 ml of citrated normal human plasma. Less than 1% of the maximal trypsin induced kallikrein activity was detected in the prekallikrein preparation using benzoyl-pro-phe-arg- p-nitroanilide (BPPA-pNA) as substrate. Both tryptase and prekallikrein were stored at -75°C. Bovine trypsin (160 TAMe units/mg) was obtained from Worthington Biochemical Corp. (Freehold NJ). Commercial porcine heparin was obtained from Sigma Chemical Co (St. Louis MO) and purified by chromatography on Dowex 1-X2, dialysed against 0.15 M NaCl, adjusted to 1 mg/ml, and stored at -75°C.

Kallikrein activity was quantified by cleavage of 0.1 mM BPPA-pNA in 0.6 ml of 0.05 M Tris, pH 7.6, containing 0.12 M NaCl (TN buffer). One unit of enzyme activity cleaves 1 umole substrate/min at 37°C. All references to units of enzyme activity refer to this assay unless stated otherwise. BPPA-pNA concentration was determined by its absorbance at 316 nm (molar extinction coefficient = 12,700). The net absorbance change upon cleavage of substrate was followed at 405 nm where the molar extinction coefficient was 8,800. Samples of prekallikrein (160 µg/ml) were incubated at 37°C in TN buffer with tryptase, trypsin, or TN buffer alone in the absence and presence of heparin for up to 135 min. At various times samples were removed from the incubation mixtures and assessed for kallikrein activity or were subjected to denaturation with SDS (± reduction with 5 mM DTT) and electrophoresis in a 5% to 17% (w/v) polyacrylamide gradient slab gel as described previously (9,10).

RESULTS

Prekallikrein (160 ug/ml) in buffer (± 37 ug of heparin/ml) had an amidolytic activity of 0.008 ± 0.002 (s.d., n = 3) units/ml (Table I), and no autoactivation of prekallikrein was detected with incubation in quartz cuvettes at 37°C for up to 135 min. Tryptase (4.3 ug/ml) and trypsin (1.7 ug/ml) each showed 0.007 units of activity/ml. Upon incubation of prekallikrein with tryptase (± 37 ug heparin/ml), as shown in Table I, kallikrein activities detected in the incubation mixtures were not significantly different from buffer controls over the 135 min incubation time. In contrast, incubation of prekallikrein (160 ug/ml) with trypsin (1.7 ug/ml) produced a time dependent rise in kallikrein activity that was maximal at 1.24 units/ml by 30 min and had declined to 1.03 units/ml by 135 min.

In order to determine whether tryptase destroyed prekallikrein without production of kallikrein, trypsin (2.7 ug/ml) was incubated for 15 and 45 min with prekallikrein that had first been incubated with buffer or tryptase (± heparin) for 135 min as above. In this case, kallikrein activity increased

TABLE I

ACTIVATION OF PREKALLIKREIN BY TRYPTASE AND TRYPSIN[a]

Time of Incubation (min)	TN Buffer	Tryptase (4.3 µg/ml)	Tryptase (4.3 µg/ml) + Heparin (37 µg/ml)	Trypsin (1.7 µg/ml)
0	0.008±0.002	0.008±0.003	0.013±0.011	0.22±0.10
15	0.007±0.001	0.009±0.002	0.010±0.001	0.79±0.34
30	0.008±0.002	0.008±.002	0.010±0.001	1.24±0.46
60	0.010±0.001	0.011±0.008	0.010±0.001	1.24±0.46
135	0.010±0.001	0.009±0.002	0.010±0.001	1.03±0.42
After addition of 2.7 µg trypsin/ml of each fraction				
15	1.15±0.26	1.15±0.26	1.12±0.26	0.89±0.36
45	1.24±0.43	1.21±0.36	1.17±0.39	0.73±0.33

a. Kallikrein amidolytic activity is expressed as BPPA-pNA units/ml (± s.d., n=3). Each initial incubation mixture contained 160 µg prekallikrein/ml.

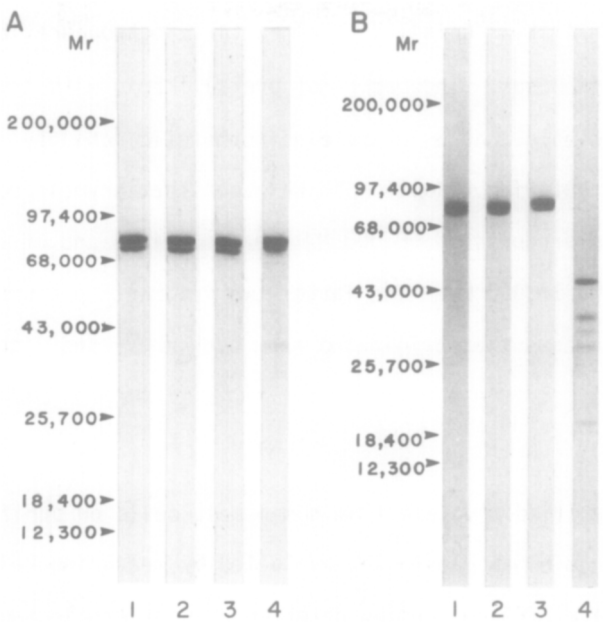

Figure 1. SDS-PAGE of prekallikrein treated with buffer (lane 1), tryptase (lane 2), tryptase together with heparin (lane 3), and trypsin (lane 4) without (A) and with (B) reduction.

from 0.01 units/ml to 1.15 units/ml at 15 min and 1.24 units/ml at 45 min. Similarly, when preincubation with tryptase or tryptase together with heparin preceeded the addition of trypsin, kallikrein activity increased from 0.01 unit/ml in each case to respective values of 1.15 units/ml and 1.12 units/ml at 15 min and to 1.21 units/ml and 1.17 units/ml at 45 min. Thus, there was no apparent functional destruction or activation of prekallikrein by tryptase.

Direct determination of prekallikrein cleavage was performed by SDS-PAGE under denaturing conditions without (A) and with (B) reduction as shown in Figure 1. Samples of prekallikrein (9.6 ug) that had been incubated in 0.03 ml with TN buffer for 0 min (lane 1), tryptase (0.26 ug) for 60 min (lane 2), tryptase (0.26 ug) together with heparin (2.2 ug) for 60 min (lane 3) and trypsin (0.10 ug) for 30 min (lane 4) were examined. Purified prekallikrein without reduction migrated as two bands with apparent molecular weights of 87,000 and 81,000, and with reduction again migrated as two bands with apparent molecular weights of 87,000 and 84,000, corresponding to Types I and II prekallikrein, respectively. Incubation with tryptase or tryptase with

heparin for 60 min at 37°C failed to alter the electrophoretic mobilities of the prekallikrein bands. Incubation of prekallikrein with trypsin for 15 min caused no appreciable change in the electrophoretic mobility in the absence of reducing agent, but with reduction it was seen that trypsin completely cleaved prekallikrein to products of 50,000 MW (heavy chain), and of 37,500 MW, 33,000 MW, 29,000 MW and 17,000 MW. The latter two fragments presumably include degradation fragments that originated from both heavy and light chains.

DISCUSSION

The effect of purified tryptase from human mast cells on purified prekallikrein from human plasma was evaluated by measuring kallikrein activity with the substrate BPPA-pNA and by determining whether cleavage products of prekallikrein were formed as assessed by SDS-PAGE. Bovine trypsin served as a positive control. Tryptase at 2.6-fold higher amounts than trypsin based on weight and 1.6-fold higher based on TAMe esterase activity caused no detectable activation or cleavage of prekallikrein. Thus, purified tryptase, the major neutral protease and only TAMe esterase thus far detected in human mast cells, does not activate prekallikrein and presumably can not account for the prekallikrein activator (6) or kinin generating (5) activities reported to be released by an IgE-dependent mechanism from human lung fragments in vitro or the kinin activity detected in nasal washings from allergen-challenged individuals (4). Identification of the cells involved in these kinin generating activities, however, is not resolved, particularly in light of observations that human alveolar macrophages (17) and peripheral blood eosinophils (18) can be activated to secrete mediators by an IgE-dependent mechanism involving low affinity receptors for this antibody class. Indeed, a previous report showed that systemic reactions of anaphylaxis (presumably due to mast cell degranulation) apparently caused consumption of high molecular weight kininogen without significant changes in levels of Hageman factor and prekallikrein (8). This is consistent with the known in vitro activities of tryptase that include destruction of high molecular weight kininogen (13) and

the absence of any effect on prekallikrein or on low molecular weight kininogen (14).

REFERENCES

1. Schwartz, L.B., Lewis, R.A. and Austen, K.F. J. Biol. Chem. (1981) 256:11939-11943.
2. Schwartz, L.B., Lewis, R.A., Seldin, D. and Austen, K.F. (1981) J. Immunol. 126:1290-1294..
3. Schwartz, L.B. (1985) J. Immunol. 134:526-531.
4. Schleimer, R.P., MacGlashan, D.W., Peters, S.P., Naclerio, R., Proud, D., Atkinson, N.F. and Lichtenstein, L.M. (1984) J. Allergy Clin. Immunol. 74:473-481.
5. Newball, H.H., Meier, H.L., Kaplan, A.P. Revak, D.S., Cochrane, C.G. and Lichtenstein, L.M. (1980) Fed. Proc. 39:3356.
6. Meier, H.L., Kaplan, A.P. Lichtenstein, L.M., Revak, S., Cochrane, C.G. and Newball, H.H. (1983) J. Clin. Invest. 72:574-581.
7. Meier, H.L., Revak, S.D., Kaplan, A.P., Cochrane, C.G., Lichtenstein, L.M. and Newball, H.H. (1980) Clin. Res. 28:354A.
8. Smith, P.L., Kagey-Sabotka, A., Bleecher, E.R., Traystman, R., Kaplan, A.P., Gralnick, H., Valentine, M.D., Permutt, S. and Lichtenstein, L.M. (1980) J. Clin. Invest. 66:1072-1080.
9. Kerbiriou, D.M. and Griffen, J.H. (1979) J. Biol. Chem. 254:12020-12027.
10. Schiffman, S., Mannhalter, C. and Tyler, K.D. (1980) 255:6433-6438.
11. Kerbiriou , D.M., Bouma, B.N. and Griffen, J.H. (1980) 255:3952-3958.
12. Maier, M., Austen, K.F. and Spragg, J. (1983) J. Anal. Biochem. 134:336-346.
13. Maier, M., Spragg, J., and Schwartz, L.B. (1983) J. Immunol. 130:2352-2356.
14. Schwartz, L.B., Maier, M., and Spragg, J. (in press) Adv. Exp. Med. Biol.
15. Mandle, R., Jr. and Kaplan, A.P. (1977) J. Biol. Chem. 252:6097-6104.
16. Vander Graaf, F., Tans, G., Bouma, B.N. and Griffin, J.H. (1982) J. Biol. Chem. 257:14300-14305.
17. Joseph, M., Tonel, A.B., Torpier, G., Capron, A., Arnoux, B., and Benveniste, J. (1983) J. Clin. Invest. 71:221-230.
18. Capron, M., Spiegelberg, H.L., Prin., L., Bennich, H., Butterworth, A.E., Pierce, R.J., Ouassi, M.A., and Capron, A. (1984) 132:462-468.