Reversibility of Acid-Activation and Cryoactivation of Inactive Renin in Human Plasma

MASATO MATSUNAGA, M.D., KENICHI MORIMOTO, M.D.*, AKIRA HARA, M.D.**
CHUN HO PAK, M.D.*, AND CHUICHI KAWAI, M.D.*

INACTIVE renin in human plasma can be activated by acidification or by cold exposure.¹⁻⁴ A major role has been attributed to plasma kallikrein in the alkaline phase of acid-activation and in the cryoactivation,⁵⁻⁷ while an involvement of acid protease^{1,8} or plasmin^{9,10} has also been proposed. In this study, we re-investigate various facets of activation of inactive renin in normal human plasma and show the following evidences: (1) inactive renin can be acid-activated without dialysis, (2) endogenous acid protease does not participate in the acid-activation, (3) the activation of inactive renin at an acid pH or at a low temperature is reversed by the restoration of physiological pH and/or temperature, when it is not accompanied by an activation of plasma kallikrein, and (4) plasma kallikrein, when activated, activates inactive renin irreversibly.

Renin activity was determined by radioimmunoassay of angiotensin I liberated in the presence of excess sheep renin-substrate.⁷ Plasma kallikrein was measured employing Z-Phe-Arg-methylcoumarinylamide as the substrate.¹¹ Endogenous acid protease activity was assayed by the degradation of hemoglobin.¹²

Acid activation was carried out by both of dialysis and titration methods. In the former, normal human plasma containing EDTA was

dialyzed against 50 mM glycine-HCl buffer, pH 3.3, for 24 hours and then against 50 mM tris-HCl buffer, pH 7.5, for 24 hours at 4°C. In the other method, the plasma was placed at 4°C for 24 hours after adjusting pH to 3.3 with HCl in the presence of 50 mM glycine and then another 24 hours after the readjustment of pH to 7.5 with tris. Though the activation of inactive renin obtained by titration was usually less than that by dialysis, it varied from 41 to 122% of the latter. The titration method activated 25 to 69% of the inactive renin activatable by trypsin (1 mg/ml, at -4°C for 1 hour). This result does not support the claim that dialysis is indispensable for acid-activation of inactive renin.¹³

The activation of inactive renin at pH 3.3 (acid phase) comprised about 70% of the total (acid phase + alkaline phase) acid-activation on the average by either dialysis or titration method. This figure is considerably larger than those reported by others.^{5,6,14} The discrepancy may be due to a large variability of the proportion of acid phase. It varied from 37 to 100% by dialysis and from 54 to 97% by titration of 10 different samples.

Either acid phase or alkaline phase of acid-activation of inactive renin was not suppressed by 1-6 μ M acetylpepstatin which inhibited completely endogenous acid protease activity. This rules out an involvement of endogenous acid protease in the acid-activation. The acid phase of acid-activation was not affected by a serine protease inhibitor, i.e., soy bean trypsin inhibitor (SBTI), lima bean trypsin inhibitor (LBTI) or aprotinin, or by a thiol protease inhibitor, N-

Key Words:

Acid protease
Plasma Kallikrein
Dialysis

College of Medical Technology, Kyoto University, Kyoto, Japan

**The Third Division, Department of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto, Japan*

***The First Department of Internal Medicine, Fukui Medical School, Fukui, Japan*

ethylmaleimide.

The higher was the pH of the plasma in the 24 hours following the treatment at pH 3.3, the greater decrease was observed in the renin activity which had been increased in the acid phase. This was true up to pH 6.5, where no significant activation of plasma kallikrein was detected. When the pH had been raised higher than 7.0, plasma kallikrein was activated and accompanied by a further increase in renin activity (alkaline phase). In the presence of SBTI, LBTI or apro-tinin, the activation of plasma kallikrein was inhibited and the renin activity was decreased even at an alkaline pH. The inhibition of plasma kallikrein activation by LBTI, which does not inhibit plasma kallikrein *activity*, indicates that plasma kallikrein was activated by another alkaline serine protease. When the plasma was titrated to pH 7.4 after the treatment at pH 3.3 and incubated at 37°C immediately (before the initiation of plasma kallikrein activation), the renin activated in the acid phase lost most of its activity in 1 hour. The lost activity was recovered by the repeated treatment at pH 3.3. On the other hand, after the 24-hour alkaline phase activation of renin and plasma kallikrein, no significant change was observed in the renin activity whereas most of plasma kallikrein activity was lost at 37°C in 1 hour.

Cold exposure of normal human plasma at -4°C for 7 days activated $51.7 \pm 2.7\%$ ($n = 10$) of the trypsin-activatable inactive renin. The cryoactivation of renin was accompanied by an activation of plasma kallikrein. The presence of 1 mg/ml LBTI did not affect the cryoactivation of either renin or plasma kallikrein, indicating no significant involvement of plasmin which is inhibited by LBTI. A part of cryoactivation of renin was not suppressed by 1 mg/ml SBTI which inhibited completely the activation of plasma kallikrein. Similar to the acid-activated renin, the cryoactivated renin in the presence of SBTI lost its activity at 37°C, which was restored by repeating the cold exposure. However, the cryo-activated renin accompanying the concomitant activation of plasma kallikrein showed no significant change at 37°C.

These results indicate that the acid phase of acid-activation and a part of cryoactivation of plasma inactive renin are independent of the action of plasma kallikrein and reversed by the restoration of physiological pH and/or temperature, whereas plasma kallikrein activates inactive renin irreversibly in the alkaline phase of acid-

activation and in the cryoactivation. It is not likely that inactive renin is irreversibly activated in the normal circulation where practically no active plasma kallikrein exists. Instead, it is possible that at least a part of plasma inactive renin may be an inactivation product of a reversible type of active renin similar to that observed after acidification or cold exposure.

REFERENCES

1. REID IA, MORRIS BJ, GANONG WF: The renin-angiotensin system. *Ann Rev Physiol* **40**: 377, 1978
2. SKINNER SL, CRAN EJ, GIBSON R, TAYLOR R, WALTERS WAW, CATT KJ: Angiotensins I and II, active and inactive renin, renin substrate, renin activity and angiotensinase in human liquor amnii and plasma. *Am J Obst Gynecol* **121**: 626, 1975
3. OSMOND DH, ROSS LJ, SCAIFF KD: Increased renin activity after cold storage of human plasma. *Canad J Physiol Pharmacol* **51**: 705, 1973
4. SEALEY JE, LARAGH JH: "Prorenin" in human plasma? *Circ Res* **36** (Suppl II): II-10, 1975
5. DERKX FHM, BOUMA BN, SCHALEKAMP MPA, SCHALEKAMP MADH: An intrinsic factor XII-prekallikrein-dependent pathway activates the human plasma renin-angiotensin system. *Nature* **280**: 315, 1979
6. SEALEY JE, ATLAS SA, LARAGH JH, SILVERBERG M, KAPLAN AP: Initiation of plasma prorenin activation by Hageman factor dependent conversion of plasma prekallikrein to kallikrein. *Proc Natl Acad Sci* **76**: 5914, 1979
7. MORIMOTO K, MATSUNAGA M, HARA A, KAWAI C: Studies on the activation and molecular weight of inactive renin in human plasma. *Hypertension* **2**: 680, 1980
8. SHULKES AA, GIBSON RR, SKINNER SL: The nature of inactive renin in human plasma and amniotic fluid. *Clin Sci Mol Med* **55**: 41, 1978
9. OSMOND DH, LO EK, LOH AY, ZINGG EA, HELDIN AH: Kallikrein and plasmin as activators of inactive renin. *Lancet* **2**: 1375, 1978
10. DERKX FHM, BOUMA BN, TANG-TJIONG HL, SCHALEKAMP MADH: Activators of inactive renin ('prorenin') in human plasma: Their connection with kinin formation, coagulation and fibrinolysis. *Clin Sci* **57** (Suppl 5): 89s, 1979
11. MORITA T, KATO H, IWANAGA S, TAKADA K, KIMURA T, SAKAKIBARA S: New fluorogenic substrates for α -thrombin, factor Xa, kallikreins and urokinase. *J Biochem* **82**: 1495, 1977
12. BARRETT AJ, HEATH MF: Lysosomal enzymes, *In* Lysosomes, ed by DINGLE JT, North Holland, Amsterdam, 1977, p 19
13. EGGENA P, BARRETT JD, WIEDMAN CE, SAMBHI MP: Studies on renin activation in normal human plasma. *Hypertension* **1**: 523, 1979
14. ATLAS SA, SEALEY JE, LARAGH JH: "Acid-" and "cryo-" activated inactive plasma renin. *Circ Res* **43** (Suppl I): I-128, 1978