Activation and Reinactivation of Inactive Renin in Normal Human Plasma

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SUMMARY Various facets of activation of inactive renin by acidification or cold exposure were investigated in normal human plasma. The acid activation obtained by titration was usually less than that by dialysis method, but varied from 41% to 122% of the latter. The acid phase of acid activation accounted for about 70% of the total activation achieved by the combined effects of the acid and alkaline phases on the average, and was not affected by any of the inhibitors for serine, thiol or carboxyl protease, whereas serine protease inhibitors suppressed the activation of both renin and plasma kallikrein in the alkaline phase of acid activation. A different mode of plasma kallikrein activation suggested some difference in the mechanism between the alkaline phase of acid activation and the cryoactivation. A part of cryoactivation of renin was independent of the action of plasma kallikrein. The renin activated by either acidification or cold exposure without concomitant activation of plasma kallikrein was reinactivated by the reversal of pH and temperature, but recovered by repeating acidification or cold exposure. When active plasma kallikrein had been produced, it activated inactive renin irreversibly. It appears unlikely that irreversible activation of inactive renin is taking place in the normal circulation where practically no active plasma kallikrein is present.

KEY WORDS • acid activation • inactive renin • cryoactivation • plasma kallikrein • dialysis

A less active form of renin in the kidney with a molecular weight of 60,000 (big renin) was converted by acidification to a more active form with a molecular weight of 40,000. It was first considered as the storage form and the precursor of the active smaller renin. However, this view was challenged by the fact that no big renin was found in renin-containing granules, and the smaller active renin was converted to the big form in combining a protein in cytoplasm. A completely inactive form of renin recently found in hog kidney was not activated by acidification. It has been known that renin activity in human plasma or amniotic fluid is increased by acidification, exposure to a cold temperature, or the addition of exogenous proteolytic enzymes.

The discovery of a less active renin with a molecular weight of about 60,000 in human plasma and its activation by acidification led investigators to a view that this is the counterpart of the renal big renin. Later studies, however, have shown that inactive renin in human plasma with a molecular weight of 54,000 to 56,000 is activated with no apparent change in its size by exogenous proteases, acidification or cold exposure. Less active big renin in human plasma was therefore considered to be a mixture of the completely inactive renin and its activated form.

Studies with protease inhibitors indicated a major role of endogenous serine protease(s) in cryoactivation and acid activation of inactive renin in human plasma, while some investigators attributed the acid activation to the action of endogenous acid protease, probably pepsin. Factor XII-plasma kallikrein-
Materials and Methods

Human plasma was obtained from healthy normotensives aged 20 to 52 years. Venous blood was withdrawn with a plastic syringe and transferred into a plastic tube containing ammonium salt of ethylenediamine tetraacetate (EDTA, approximately 2 mg per ml of blood). Plasma was separated immediately by centrifugation at 10°C and stored frozen at -80°C until ready to use. Some of individual specimens were studied separately, and others were pooled.

Acid activation was carried out by either dialysis or titration at 4°C. In the dialysis method, plasma was dialyzed for 24 hours against 50 mM glycine-HCl buffer, pH 3.3, containing 10 mM EDTA-Na₂ and 90 mM NaCl. A portion of this plasma was dialyzed for 24 hours against 50 mM tris-HCl buffer, pH 7.5, containing the same concentrations of EDTA and NaCl as the above. In the titration method, 1 M glycine solution was added to the plasma to make the final concentration of 50 mM, and then the pH was adjusted to pH 3.3 with 1 M HCl. After 24 hours, a portion of the acidified plasma was titrated to pH 7.5 with 2 M tris base and let stand for another 24 hours. In an experiment to examine the effect of pH, the pH in the second step was modified. A portion of the untreated plasma was kept frozen after the volume equivalent was adjusted to that of the treated one by the addition of distilled water, and served as the control. For cryo-activation, plasma was kept at -4°C for 7 days in a methanol bath.

Trypsin-activation of inactive renin in untreated plasma was performed by incubating with 1 mg/ml trypsin (Sigma type III) at -4°C for 1 hour and then terminating the reaction by the addition of 1 mg/ml soybean trypsin inhibitor (SBTI, Sigma type I-S) or lima bean trypsin inhibitor (LBTI, Sigma type II-L). Since trypsin activation always gave higher renin activity than acid activation and cryoactivation, the difference between the activities before and after the trypsin activation was considered as the amount of inactive renin. For trypsin activation following acid activation or cryoactivation, trypsin concentration and incubation time were modified as are described in the text. The renin activity after the trypsin activation (active renin + inactive renin) was referred to as "total renin activity."

Renin activity was assayed by a modification of Skinner's method. To 0.2 ml of a sample was added the same volume of nephrectomized sheep plasma containing 1 mg/ml EDTA-Na₂ and 5 μl of 5% diisopropyl fluorophosphate (DFP). To make the pH of the mixture 7.4 at 37°C, 40 μl of 0.5 M tris-HCl buffer of an appropriate pH (Sigma T 4378) was added. When the sample had been acidified, the pH was adjusted approximately with 2 M tris base prior to the addition of the buffer at 4°C. The mixture was incubated at 37°C for 30 minutes, and generated angiotensin I was measured by radioimmunoassay according to the method of Haber et al. using a commercial kit (CEA-IRE-SORIN). The activity was expressed in nanograms of angiotensin I generated per 1 hour per 1 ml of plasma (ng/ml/hr).

Plasma kallikrein activity was determined by a modification of the method of Morita et al. using a specific synthetic substrate, carbobenzoxy-L-phenylalanyl-L-arginine 4-methylcoumarinyl-7-amide (Z-Phe-Arg-MCA). Plasma samples were diluted 1 to 10 with distilled water. The reaction was started by the addition of 50 μl of the diluted sample to 1 ml of substrate-buffer solution (0.1 mM Z-Phe-Arg-MCA in 50 mM tris-HCl buffer, pH 8.0, containing 0.1 M NaCl) preincubated at 37°C for 10 minutes. The reaction was carried out at 37°C for 30 minutes and terminated by the addition of 2 ml of 15% acetic acid. Liberated 7-amino-4-methylcoumarin (AMC) was measured by fluorometry. The activity was expressed in terms of micromoles of AMC liberated per 1 hour per 1 ml of plasma (μmole/ml/hr).

Endogenous acid protease activity was measured according to method II for cathepsin D assay described by Barret and Heath. Hemoglobin substrate solution (8% w/v) was prepared from commercial hemoglobin powder (ICN Pharmaceuticals). The incubation mixture contained 0.1 ml of a sample, 0.25 ml of hemoglobin substrate, and 0.25 ml of 1.0 M formate buffer, pH 3.3. The final volume was adjusted to 1.0 ml by adding distilled water. After the incubation at 45°C for 3 hours, the reaction was terminated by the addition of 5.0 ml of 3% trichloroacetic acid. After filtration through Whatman No. 1 paper, 1.0 ml of the filtrate was examined with Folin-Lowry reaction. The activity was expressed in micrograms of tyrosine equivalent to the peptides liberated per 1 hour per 1 ml of the sample (μg/ml/hr).

Among other reagents used, N-acetyl-pepstatin was a gift from Eisai Pharmaceutical Company, Japan.

Statistical analyses were made by Student's t test for paired or unpaired samples. Mean values are given together with 1 standard error of the mean (SEM).
**Figure 1.** Effects of enzyme inhibitors and of pH in the second step on the acid activation of inactive renin and prekallikrein in a pooled plasma. From the left to right: untreated control, after 48 hours at +4°C without any other treatment, after the first step at pH 3.3 for 24 hours; and after the second step for 24 hours at various pHs. White, striped and black columns are mean values with SEM (n = 5) in the presence of no enzyme inhibitor, 5 μM N-acetyl- pepstatin and 1 mg/ml soybean trypsin inhibitor (SBTI), respectively. Open and closed circles are mean values of two experiments in the presence of 1,000 kallikrein inhibitor units (KIU) of aprotinin per ml and 10 mM N-ethylmaleimide (NEM), respectively.

**Figure 2.** Reinactivation of acid-activated renin in a pooled plasma. From the left to right: untreated control, after 48 hours at +4°C without any other treatment; treated at pH 3.3 for 24 hours, and treated at 37°C for 1 hour immediately after the readjustment of pH to 7.4, then treated again at pH 3.3 for 24 hours, treated at pH 7.4 for 24 hours after the 24-hour treatment at pH 3.3, and kept at 37°C for 1 hour; treated at pH 3.3 for 24 hours and then at pH 7.4 for 24 hours and again at pH 3.3 for 24 hours. White, striped and black columns are mean values with SEM (n = 4) in the presence of no inhibitor, 1 mg/ml lima bean trypsin inhibitor (LBTI) and 1 mg/ml soybean trypsin inhibitor (SBTI), respectively.
Results

Effect of Dialysis on Acid Activation

Acid activation of inactive renin was carried out on seven plasma samples from different individuals and three lots of pooled plasma by both titration and dialysis methods. In general, more activation was obtained by dialysis than by titration. By the dialysis method, 46% to 96% (62.5 ± 5.2%) of the trypsin-activatable inactive renin was activated, while 25% to 69% (43.0 ± 4.4%) was activated by the titration method. However, there was a wide range of variability in the effect of dialysis on the individual plasma. The activation by titration ranged from 41% to 122% of that by dialysis. These were also true in the acid phase alone. In 24 hours at pH 3.3, 21% to 63% (44.6 ± 3.7%) of the trypsin-activatable inactive renin was activated by dialysis, and 18% to 38% (29.5 ± 2.4%) was activated by titration. The acid phase activation by titration ranged from 34% to 121% of that by dialysis.

Acid Phase of Acid Activation

There was a large variability in the proportion of acid phase in the total (acid phase + alkaline phase) acid activation. The activation at pH 3.3 in 24 hours ranged from 37% to 100% of the total acid activation by dialysis and from 54% to 97% of the total by titration. No correlation was observed between the proportions obtained by dialysis and by titration. On the average, the proportion of acid phase activation was 72.9% ± 6.3% by dialysis and 70.3% ± 4.7% by titration. These figures are considerably larger than those reported by other investigators.14, 31, 44

The acid phase activation was not affected by the presence of a serine protease inhibitor, i.e., SBTI (1 mg/ml), LBTI (1 mg/ml), or aprotinin (1000 KIU/ml), or a thiol protease inhibitor, N-ethylmaleimide (NEM, 10 mM) (fig. 1). The acidified plasma showed 111 ± 3 μg/ml/hr (n = 4) of endogenous acid protease activity which was inhibited almost completely by 1 μM N-acetyl-pepstatin, a carboxyl protease inhibitor. On the other hand, presence of 1–6 μM N-acetyl-pepstatin did not suppress the acid phase activation of inactive renin (fig. 1). It was confirmed in a preliminary experiment that this amount of N-acetyl-pepstatin does not affect the renin activity itself in the plasma.

Effects of pH and Enzyme Inhibitors in the Second Step of Acid Activation

The effect of pH in the second step of acid activation was examined mainly by the titration method. After activation at pH 3.3 for 24 hours, the pH was adjusted to various values between 3.3 and 8.0. As is illustrated in figure 1, the renin activity once increased at pH 3.3 was decreased significantly at pHs between 4.5 and 6.5 in 24 hours (p < 0.01). The decrease appeared greater at a higher pH. On the other hand, when the pH in this step was more than 7.0, a further increase in the renin activity was observed (p < 0.05), i.e., the alkaline phase of acid activation. However, in the presence of SBTI (1 mg/ml), LBTI (1 mg/ml) or aprotinin (1,000 KIU/ml), a decrease in the activity (p < 0.01), instead of an increase, was observed at pHs more than 7.0 (figs. 1 and 2). N-acetyl-pepstatin (5 μM) or N-ethylmaleimide (10 mM) did not affect the alkaline phase of acid activation. Similar results were obtained when the acid phase activation had been carried out by the dialysis method.

Activation of Plasma Kallikrein

A part of plasma prekallikrein was activated in the process of the acid activation and cryoactivation of inactive renin. However, no significant activation of prekallikrein was observed unless the pH in the second step of acid activation had been higher than neutral (fig. 1). The activation of prekallikrein at an alkaline pH in the acid activation was time-dependent and significant activation was not observed until 1 hour after the readjustment of pH at 4°C. It was inhibited in the presence of a serine protease inhibitor, e.g., SBTI (1 mg/ml), LBTI (1 mg/ml) or aprotinin (1000 KIU/ml) (figs. 1 and 2). Prekallikrein activation during the cold exposure was completely inhibited by 1 mg/ml SBTI, but not by 1 mg/ml LBTI (figs. 3 and 4). The activa-

Figure 3. Cryoactivation and re-inactivation of inactive renin and prekallikrein in a pooled plasma. From the left to right, untreated control; kept at −4°C for 7 days, and treated at 37°C, pH 7.4 for 1 hour, then treated again at −4°C for 7 days, kept at −4°C for 14 days. White, striated and black columns are mean values with SEM (n = 5) in the presence of no enzyme inhibitor, 1 mg/ml lima bean trypsin inhibitor (LBTI) and 1 mg/ml soybean trypsin inhibitor (SBTI), respectively.
Cryoactivation and Effects of Enzyme Inhibitors

The 7-day cold exposure activated approximately one-half of the trypsin-activatable inactive renin in the plasma. This figure was fairly constant (51.7% ± 2.7%, n = 10). The presence of 1 mg/ml LBTI exhibited no significant effect on the cryoactivation of inactive renin. This result is contrasted to the report of Atlas et al. On the other hand, 1 mg/ml SBTI inhibited 50%-80% of the cryoactivation, although a significant proportion of inactive renin was cryoactivated (p < 0.0001) even in the presence of SBTI (figs. 3 and 4).

Inactivation of the Activated Renin at a Higher Temperature

When the plasma treated at pH 3.3 for 24 hours was brought to around pH 7.4 by titration and immediately placed at 37°C, the once-increased renin activity
was markedly decreased \((p < 0.001)\) in a short period (figs. 2 and 5). Similarly, the cryoactivated renin in the presence of SBTI, i.e., without activation of plasma kallikrein, rapidly lost its activity \((p < 0.0001)\) when kept at \(37^\circ\text{C}, \text{pH} 7.4\) (figs. 3 and 5). The lost activities were recovered by repeating acidification or cold exposure (figs. 2 and 3).

On the other hand, no significant change in renin activity was observed by keeping the plasma at \(37^\circ\text{C}\) after completion of the alkaline phase of acid activation, where active plasma kallikrein had been produced (fig. 2). The renin cryoactivated with the concomitant activation of plasma kallikrein did not lose its activity at \(37^\circ\text{C}\) (fig. 3). The plasma kallikrein activity was markedly decreased during the treatment at \(37^\circ\text{C}\) (figs. 2 and 3). Inhibition of plasma kallikrein activity by adding \(1\ \text{mg/ml}\) SBTI at the time of the \(37^\circ\text{C}\) treatment did not affect the renin activity.

Only a slight or no significant decrease was observed in the renin activity of the control plasma at \(37^\circ\text{C}\).

**Reexamination of Enzyme Assay Methods**

The results obtained above prompted us to reexamine the condition for renin assay, because it appeared probable that the renin activated by acidification or cold exposure in the absence of plasma kallikrein activation may be reinactivated during the incubation at \(37^\circ\text{C}, \text{pH} 7.4\) for the determination of the activity, and therefore underestimated. The situation was also the same in the plasma kallikrein assay.

If this was the case, the rate of liberation of angiotensin I or AMC would decline with time during the incubation. However, the rate of angiotensin I liberation showed no significant decline up to 60 minutes of incubation employing plasma samples treated at \(\text{pH} 3.3\) for 24 hours or treated at \(-4^\circ\text{C}\) for 7 days in the presence of SBTI, which showed the rapid re-inactivation of renin when the plasma alone was kept at \(37^\circ\text{C}\) (fig. 6 upper graph). The AMC-liberating plasma kallikrein reaction also proceeded linearly up to 60 minutes in the study on the plasma samples after the alkaline phase of acid activation or the 7-day cryoactivation (fig. 6 lower graph). Although the reason why the renin activity is preserved under this condition is not clear, it is probable that sheep renin-substrate in the incubation mixture may protect renin from the reactivation. The addition of DFP alone to the plasma had no significant effect against the reactivation of renin. The enzyme stabilizing effect of substrate and the \(1/210\) dilution of the sample in the incubation mixture may contribute to the preservation of plasma kallikrein activity during the enzyme assay.

**Trypsin-Activation after Various Treatments**

Since protease inhibitors in human plasma is destroyed by acidification and an excessive treatment with trypsin destroys renin, the concentration and incubation time were reduced for trypsin-activation of inactive renin in the acid-treated plasma. Incubation with \(0.1\ \text{mg/ml}\) trypsin for 30 minutes at \(-4^\circ\text{C}\) was found to be appropriate in a preliminary experiment. After keeping the acid-treated plasma at \(37^\circ\text{C}, \text{pH} 7.4\), for 1 hour, an increase in trypsin inhibitor capacity was found by the method of Eriksson, and \(0.5\ \text{mg/ml}\) trypsin-activation for 30 minutes was appropriate.

Trypsin activation of the acid-treated plasma before and after the \(37^\circ\text{C}\) treatment yielded equivalent total renin activities, indicating no significant destruction of active and inactive renins by keeping the plasma at
FIGURE 7. Total renin activities after various treatments. White and black columns show mean values with SEM (n = 4) of renin activities before and after the trypsin-activation, respectively. Upper Graph. Acid-treated plasma by titration (T) and by dialysis (D): (1) untreated control, (2) treated at pH 3.3 for 24 hours at +4°C, (3) treated at 37°C, pH 7.4 for 1 hour after the acid treatment, (4) treated at pH 7.4 for 24 hours at +4°C after the acid treatment. Lower Graph. Cold-treated plasma: (1) untreated control, (2) treated at −4°C for 7 days with no enzyme inhibitor, (3) treated at −4°C for 7 days in the presence of 1 mg/ml SBTI, (4) treated at 37°C for 1 hour after the cold treatment in the presence of SBTI.

Discussion

There has been some controversy about the mechanism of activation of inactive renin in human plasma by acidification or cold exposure in vitro. Most investigators in this field attributed it to an action of endogenous proteolytic enzyme(s). Kotchen et al. stated that denaturation of a circulating renin inhibitor may contribute to the increased renin activity after acidification or cold exposure of human plasma. Eggena et al. did not obtain any significant activation of inactive renin by the acid treatment without at least one step of dialysis, while they failed to find the evidence for a dialyzable renin inhibitor in the plasma. On the other hand, Shulkes et al. and Kotchen et al. obtained considerable activation of inactive renin by the acid treatment without dialysis.

The results of the present study were compatible with those of the latter groups. At least 25% of trypsin-activatable inactive renin was acid-activated by the titration method without dialysis. More activation was usually obtained by the dialysis method. This is consistent with the observation by Kotchen et al., and implies that some dialyzable factor(s) may interfere with the process of the activation of inactive renin. Alternatively, the less effectiveness of the titration method may be due to some destruction of active or inactive renin during the acidification, because titration is more drastic a method than dialysis. However, there was a large variance among individual samples: the titration method was as effective as or more effective than the dialysis method in some samples while the effect of dialysis was remarkable in some others. The discrepancy between our results and those of Eggena et al. may be due to the variability or to a difference in the destruction of renin by titration. Although they used human substrate while Shulkes et al. and we used sheep substrate for the determination of renin activity, this is not likely the cause of the discrepancy since Kotchen et al. obtained similar results to ours by using human substrate.

A large variability was also observed in the proportion of acid phase in the total effect of acid activation of renin. This variance could not be attributed to an interexperimental error, because several specimens from different individuals were dialyzed at the same time in a same container. In spite of the variability, the acid phase accounted for a major part (about 70% on the average) of the acid activation. Atlas et al. reported that only about 30% was activated at pH 3.3 in the acid activation. The proportion of the acid phase described by Derkx et al. was also small. Although the reason for the discrepancy between the results of Atlas et al. or Derkx et al. and ours in the estimation of the acid phase is not clear, we could suspect some possibilities. First, the wide range of variability may be responsible for the discrepancy. Second, if the acid-treated plasma had been kept at a higher pH (e.g., pH 5.7) for a long time prior to the determination of renin activity, or preincubated before mixing with the substrate, the reinactivation may have...
An involvement of plasma kallikrein in the mecha-
nantly during the process of cryoactivation of renin.

Serine proteases and thiol proteases cannot partici-
pate in the acid-phase activation, since the activation
was not inhibited by the inhibitors of these enzymes.
Endogenous acid protease is not likely responsible for
the acid phase activation, because N-acetyl-pepstatin,
which was enough to inhibit the endogenous acid pro-
tease activity almost completely, could not suppress
the acid phase activation of inactive renin.

The activation of plasma prekallikrein in the alka-
line phase of acid activation is consistent with the view
that plasma kallikrein is responsible for the alkaline
phase of activation of inactive renin.19-22 However, no
significant activation of prekallikrein was observed in
the second step of acid activation when the pH was
lower than neutral. The prekallikrein activation at an
alkaline pH was inhibited by SBTI or other serine
protease inhibitors. The inhibition of prekallikrein ac-
tivation by LBTI which does not inhibit plasma kalli-
krein activity indicates that the activation is brought
about by an action of another serine protease (prob-
ably factor XIIa), which is active at an alkaline
range.

Plasma kallikrein was also activated concomi-
tantly during the process of cryoactivation of renin.
An involvement of plasma kallikrein in the mecha-
nism of cryoactivation was already indicated in our
earlier work.13,17 However, enough concentration of
SBTI to completely inhibit the activation of plasma
kallikrein could not suppress 1/4 to 1/2 of the activa-
tion of inactive renin, implying that a part of inactive
renin can be cryoactivated independent of the action
of plasma kallikrein. Incomplete inhibition of cryo-
activation of renin by SBTI, aprotinin or DFP was
also observed in our previous work.13 Contrary to the
report of Atlas et al.,14 1 mg/ml LBTI did not sup-
press the cryoactivation of either renin or plasma
kallikrein. Wilczynski and Osmond40 observed some
inhibition of cryoactivation of inactive renin in normal
human plasma by 1.5 mg/ml LBTI, but much less
than that by SBTI. Both inhibitors did not prevent
cryoactivation in prekallikrein deficient plasma. These
results suggest some difference in the mechanism be-
tween the alkaline phase of acid activation and cryo-
activation.

We could not detect any appreciable activation of
plasmin during the acid activation and cryoactivation
of inactive renin. Some hydrolysis of H-D-valyl-L-
leucyl-L-lysine p-nitroanilide (Kabi S-2251), a syn-
thetic substrate for plasmin,43 was observed by the
plasma after the alkaline phase of acid activation or
after the regular cryoactivation, but not inhibited by
LBTI which was enough to inhibit an equivalent ac-
tivity of human plasmin (Kabi) (unpublished data).

The involvement of endogenous plasmin in acid ac-
tivation of inactive renin has been excluded by Sealey
et al.44

Of interest is the re-inactivation of a once activated
renin under a nearly physiological condition. Restora-
tion of pH to a higher value decreased the renin ac-
tivity which had been increased at pH 3.3, when there
was no significant activation of prekallikrein, indicat-
ing that the activation of inactive renin at an acid pH
is reversible by the reversal of pH. Similar reduction
of renin activity can also be seen in a figure in a paper
of Derkx et al.21 at pHs between 4.5 and 6.5 in the sec-
ond step of acid activation, although they did not men-
tion anything about it. The re-inactivation was accel-
erate and made more complete by raising the tem-
perature to 37°C. The reversal of the temperature to
37°C also re-inactivated the renin which was activated
at a cold temperature in the absence of active plasma
kallikrein.

The renin activity lost at 37°C, pH 7.4 was recov-
ered by repeating the acidification or the cold ex-
posure. This recovery might be attributed to the activa-
tion of the inactive renin which had remained inactive
during the previous activation procedure whereas once
activated renin had been destroyed. However, this is
not likely the case because no significant reduction of
total (active + inactive) renin activity was observed
after the re-inactivation (fig. 7) and the prolongation of
the acid treatment to 48 hours or of the cold exposure
to 14 days increased the renin activity only slightly as
compared with the regular treatment (figs. 1 and 3).
The renin activity was fairly stable at 37°C, pH 7.4, in
the untreated control plasma and when activated in
association with the prekallikrein activation.

These results indicate that at least one part of in-
active renin in human plasma is activated under an
unphysiological condition, such as low pH or low tem-
perature, but re-inactivated by restoring the physi-
ological condition in the absence of active plasma kalli-
krein. The present data may not be sufficient to
answer the question whether this process is really
reversible, i.e., whether the re-inactivated renin is iden-
tical to native inactive renin. However, the re-inacti-
ved renin resembles native inactive renin at least in
the activatability by trypsin, acidification, and cold
exposure. Some decrease in the trypsin susceptibility
observed after the re-inactivation of acid-activated
renin can be explained by a production of some un-
identified antitryptic substance during the treatment
at 37°C, although a possibility that the re-inactivated
renin may be more resistant to trypsin than the native
inactive renin cannot be excluded.

There is another possibility that the acid treatment
may not only destroy plasma protease inhibitors but
also render the remaining inactive renin more sensi-
tive to trypsin, and the re-inactivated renin may have
regained the trypsin resistance. The mechanism of the
apparently reversible activation of inactive renin is not
clear from the present study and requires further in-
vestigation. It may be due to a change in the con-
formation of renin molecules or to the dissociation
and association of renin-inhibitor complex. A struc-
tural alteration of inactive renin by acidification was recently suggested by Hsueh et al.

On the other hand, no or only slight decrease was detected in the renin activity at 37°C, pH 7.4, when it had been activated along with the concomitant production of active plasma kallikrein. Therefore it is probable that plasma kallikrein activates inactive renin irreversibly. Since the activity of plasma kallikrein was rapidly lost at 37°C, pH 7.4 and the addition of SBTI after the plasma kallikrein activation did not affect the stability of renin, it is not likely that the presence of active plasma kallikrein by itself protects renin activity. The apparent alkaline phase of acid activation may be the difference between the activation by plasma kallikrein and the reinactivation of the renin activated in the acid phase.

The fact that the renin activated in the absence of active plasma kallikrein is inactivated at the physiological pH and temperature leads us to a speculation that inactivation of an active form of renin, rather than activation of inactive renin, may be occurring in the normal circulation where practically no active form of plasma kallikrein is present. It has not been proved whether the inactive renin is released into circulation in the form as it was in the tissue or secreted only after activation. Whether the inactive renin is a single entity or a complex has not been elucidated either. If we assume that renin is released into circulation not only in an irreversibly activated form but also in a reversibly activated form similar to that observed after acidification or cold exposure, the latter would be inactivated soon after the release to form an inactive renin. Thus it is quite possible that at least a part of inactive renin in human plasma may be an inactivation product of renin released as an active form from the kidney or some other tissues.

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Addendum

During the preparation of this paper, observations similar to ours concerning the reversibility of the acid phase of acid activation of inactive renin were reported by Leckie and McGhee (Leckie BJ, McGhee NK. Reversible activation-inactivation of renin in human plasma. Nature 288: 702, 1980).

References

31. Haber E, Koerner T, Page LB, Kilman B, Purnode A: Application of a radioimmunoassay for angiotensin I to the physio-
logic measurement of plasma renin activity in normal human
32 Morita T, Kato H, Iwanaga S, Takada K, Kimura T, 
Sakakibara S: New fluorogenic substrates for α-thrombin, fac-
tor Xa, kallikreins and urokinase. J Biochem 82: 1495, 1977
33 Barrett AJ, Heath MF. Lysosomal enzymes. In Lysosomes,
edited by Dingle JT. Amsterdam-New York-Oxford: North 
Holland, 1977, p 19
34 Atlas SA, Sealey JE, Laragh JH: “Acid” and “cryo”-acti-
35 Sealey JE, Atlas SA, Laragh JH, Oza NB, Ryan JW: Activa-
tion of a prorenin-like substance in human plasma by trypsin 
and by urinary kallikrein. Hypertension 1: 179, 1979
Scand 177 (suppl 432): 1, 1965
37 Kotchen TA, Welch WJ, Talwalkar RT: Modification of the
enzymatic activity of renin by acidification of plasma and by 
exposure of plasma to cold temperatures. Hypertension 1: 190, 
1979
38 Eggena P, Barrett JD, Wiedman CE, Sambhi MP. Studies on 
renin activation in normal human plasma. Hypertension 1: 523, 
1979
39 Friberger F, Knös M, Gustavsson S, Aurell L, Claeson G. 
Methods for determination of plasmin, antiplasmin and 
plasminogen by means of substrate S-2251. Haemostasis 7: 
138, 1978
40. Hsuhe WA, Carlson EJ, O’Connor D, Warren S. Renn re-
tains a structural alteration prior to activation by renal kalli-
41. Schachter M. Kallikreins (kinogenases) — a group of serine 
proteases with bioregulatory actions. Pharmacol Rev 31: 1, 
1980
Activation and reinactivation of inactive renin in normal human plasma.
M Matsunaga, K Morimoto, A Hara, C H Pak and C Kawai

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