Cleavage of prorenin's prosegment causes irreversible formation of renin. In contrast, renin activity is reversibly exposed when prorenin is acidified to pH 3.3. Nonetheless, acidification of plasma results in irreversible activation of prorenin, because endogenous proteases cleave the prosegment of acid-activated prorenin. Chilling of plasma results in irreversible cryoactivation of prorenin. In this study we investigated whether cryoactivation of purified prorenin is reversible.

The intrinsic renin activity of recombinant human prorenin was measured by an enzyme kinetic assay using partially purified human angiotensinogen as substrate. Results are expressed as a percent (mean ± S.E.) of the maximal activity exposed after limited proteolysis by trypsin. The intrinsic renin activity of two pools (0.3 and 0.06 Goldblatt units/ml) was 1.5% ± 0.3 and 1.2% ± 0.6 at 37°C. Activity increased to 19% ± 0.3 and 26% ± 0.5 after incubation at 0°C and to 5.4% ± 0.5 and 2.1% ± 1.2 at room temperature. Cryoactivation did not occur in buffers containing more than 1 M NaCl. It took 8 min at 37°C or 180 min at room temperature for cryoactivated prorenin to lose half of its intrinsic renin activity. It took 48 and 26 h, respectively, at 0°C for the two pools of prorenin at 37°C to regain half of their maximum intrinsic activity at 0°C. A direct immunoradiometric assay that detects active renin but not prorenin was able to detect cryoactivated prorenin.

These results show that human prorenin can be reversibly cryoactivated in buffers of low ionic strength and has greater intrinsic activity at room temperature than at 37°C.

Renin, an aspartyl protease, is synthesized and stored in the juxtaglomerular cells of the kidney (1). Prorenin is the biosynthetic precursor of renin (2-4). Several characteristics make prorenin an unusual precursor. It circulates together with renin but at 10-fold higher concentrations (5-7). It is the major product of renin gene expression in the kidney. Prorenin is packaged into granules to be secreted on demand but at 10-fold higher concentrations (5-7). It is the major product of renin gene expression in the kidney. Prorenin is interesting, since it opens the possibility that prorenin could be reversibly activated in vivo at its target site and could evoke properties of angiotensin II that were previously thought to occur at physiological concentrations.

Prorenin is reversibly activated in human plasma that has been acidified to pH 3.3 and returned to neutral pH (19,20) or that has been incubated at -4°C (21). Irreversible acid activation and cryoactivation of prorenin do not occur in plasma that is devoid of factor XII or prekallikrein (22,23). Acid activation of purified prorenin is reversible (24,25). The phenomenon of cryoactivation has not yet been studied with purified reagents. The purpose of this study was to investigate the effects of temperatures from 37 to 0°C and sodium chloride concentration on the intrinsic catalytic activity of purified recombinant human prorenin.

**EXPERIMENTAL PROCEDURES**

**Materials**

**Chemicals**—L-1-Tosylamide-2-phenylethylchloromethyl ketone (TPCK)1-treated trypsin (Type XIII); bovine serum albumin (BSA), crystalline and lyophilized essentially globulin-free; sodium azide; soybean trypsin inhibitor, and phenylmethanesulfonyl fluoride were purchased from Sigma. Benzamidine hydrochloride was purchased from Kodak. All other chemicals were of reagent grade or higher. Buffers were heat treated to 85°C for 30 min and filtered.

**Prorenin**—Recombinant human prorenin was secreted by Chinese hamster ovary cells and purified as described (8). The synthesis, purification, and properties of prorenin have been described elsewhere (8). The abbreviations used are: TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; BSA, bovine serum albumin; GU, Goldblatt unit; IRMA, immunoradiometric assay; AI, angiotensin I.
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hamster ovary cells transfected with a vector containing cDNA for preprorenin and was purified by affinity chromatography (26). It was a gift from the Upjohn Company. Prorenin concentration was determined by standardization against human renal renin after trypsin activation (described below); 1 Goldblatt unit (GU) of renin germinates 120 pmol of angiotensin I/1h with human renin substrate (angiotensinogen) at K m. A stock solution (determined to have a prorenin concentration after activation with trypsin of 115 GU/ml in 50 mM Tris-Cl, pH 7.5, containing 5 mM benzamide hydrochloride, was stored in 50-pl aliquots at -70°C.

Two prorenin pools (0.3 and 0.06 GU/ml) were prepared in 50 mM sodium phosphate buffer, pH 7.4, containing 0.1 M NaCl, 10 mM benzamide hydrochloride, 3 mM Na 2EDTA, 0.1% sodium azide, and 0.5% BSA. Both pools were equilibrated at 0°C for 7 days and stored in 50-pl aliquots at -70°C. Two other sources of recombinant human prorenin, gifts from California Biotechnology, Inc. (Cal Bio) (27) and Pfizer, Inc. (28), were also diluted to 0.3 and 0.06 GU/ml and stored at -70°C.

Renin Inhibitor—Hui Peptide 27, a gift from Dr. K. Y. Hui, 20 nM was used to stop the action of renin on angiotensinogen (29).

Angiotensinogen—Angiotensinogen was partially purified from human plasma that had been heat-treated (50°C for 30 min) to inactivate renin (30). Heat-denatured proteins were removed by centrifugation (10,000 × g for 30 min at 4°C). The supernatant was diluted 1:2 with 50 mM Tris-Cl, pH 8.0, and subjected to ammonium sulfate precipitation. The proteins precipitating between 30 and 65% saturation were removed by centrifugation. The pellet was dialyzed and purified by ion exchange chromatography on DEAE-Sepharose. Angiotensinogen was eluted with a 0.07 M NaCl to 0.4 M NaCl gradient in 50 mM Tris-Cl, pH 8.0, containing 3 mM Na 2EDTA. The final substrate preparation was able to generate 20,000 ng of Al/ml during incubation with excess renin (17 GU/liter).

Methods

Time Course of Cryoactivation—0.3 and 0.06 GU/ml pools of prorenin were preincubated for either 1 h at 37°C or 3 h at room temperature and then placed in a 0°C ice bath for 5 days. Samples were removed and frozen at 6-h intervals for the first 24 h and then at 24-h intervals for up to 5 days. Additional samples were removed in duplicate at 0, 2, 5, and 15 days; one was incubated for 1 h at 37°C to determine if cryoactivation was reversible, the other was trypsin-activated (see below) to determine if total renin concentration remained stable. Activity was determined by generation of angiotensin I (see below).

Time Course of Reversal of Cryoactivation—Reversal of cryoactivation was studied at room temperature and at 37°C. Samples of the 0.3 and 0.06 GU/ml pools which had been incubated for 5 days at 0°C were rapidly thawed and then incubated at room temperature or in a 37°C water bath. Samples incubated at room temperature were frozen at 0, 15, 30, and 60 min and then at hourly intervals for 8 h. Samples incubated at 37°C were frozen at 5-min intervals for the first 20 min and then at 60 and 120 min. Additional samples were taken in duplicate, at 2, 4, and 8 h which were either re-incubated at 0°C for 4 days to determine if cryoactivation could still occur or trypsin-activated to determine if total renin concentration remained stable.

Effect of Sodium Chloride Concentration on Cryoactivation—The time course of cryoactivation was examined using the buffer in which the renin pools were prepared (described above) to which was added 0.4–4 mM sodium chloride. The final concentrations of sodium and chloride in the buffers was measured using ion-sensitive electrodes (Astra, Beckman).

Trypsin Activation of Prorenin—Total renin activity was determined by activation of 0.3 ml of sample with 3 μl of 10 mg/ml TPCK-treated trypsin (final concentration 100 μg/ml) for 1 h at room temperature. The reaction was stopped by the addition of 3 μl of 20 mg/ml soybean trypsin inhibitor (final concentration 200 μg/ml) and incubation for 15 min at room temperature (31).

Intrinsic renin activity by generation of Angiotensin I—Renin-like activity was assessed by incubating samples for 5 min at 37°C, pH 7.4, in the presence of 3 mM Na 2EDTA, 3 mM phenylmethylsulfonyl fluoride, and 1330 ng of AI equivalents/ml (K m) human angiotensinogen. The reaction was stopped abruptly by addition of an equal volume of 20 mM renin inhibitor (K m) to the incubation mixture which was then centrifuged to minimize the reversal of cryoactivation of prorenin. Trypsin-activated samples were incubated for 1 h at 37°C to generate AI. Angiotensin I was quantitated by radioimmunoassay (32). Results were expressed as nanograms AI/ml/h and then calculated as a percent of the maximal activity determined by limited proteolysis with trypsin.

Effect of Cryoactivated Prorenin on a Direct Renin Radioimmunoassay—A direct immunoradiometric assay (IRMA) for active renin (Renin radioimmunoassay, Institut Pasteur, Paris) is unable to detect prorenin in plasma when the assay is performed at room temperature. To determine if prorenin was detectable after cryoactivation, the IRMA was also run at 4°C. Before assay 0.3 GU/ml prorenin samples were cryoactivated at 0°C for 7 days followed by (a) no further treatment, (b) re-equilibration at room temperature for 24 h, (c) re-equilibration at 37°C for 1 h, or (d) trypsin activation to determine the total enzyme present. Samples were diluted using the diluent supplied with the kit.

Results

Temperature Dependence of the Intrinsic Activity of Human Prorenin—The level of intrinsic renin-like activity of recombinant human prorenin was dependent on the temperature at which the prorenin was equilibrated (Table I). After equilibration at 0°C the 0.3 and 0.06 GU/ml prorenin pools were, respectively, 19% ± 0.3 and 26% ± 0.3 of maximal activity. The difference in activity between the two pools was statistically significant (p < 0.05 by Student’s t test). After 8 h at 37°C reaction temperature the levels of activity were 5.4% ± 0.5 and 2.1% ± 0.2 of maximal activity (not significant). Prorenin equilibrated at room temperature or 37°C always exhibited an increase in activity when re-equilibrated to 0°C. There was no permanent change in the activity of the pools that would suggest that the prorenin had been irreversibly converted to renin. There was no change in the level of activity measured after trypsin activation that would suggest any change in the total amount of prorenin present.

Time Course of Cryoactivation—Recombinant human prorenin that had been pre-equilibrated at 37°C (Fig. 1) or at room temperature (Fig. 2) exhibited increased catalytic activity when incubated at 0°C. This increase in activity was completely reversible at 37°C. Extrapolation analysis (33) revealed differences between the two different concentrations of prorenin. In samples pre-equilibrated at 37°C, maximum activity was calculated to be 21% for the 0.3 GU/ml pool and 35% for the 0.06 GU/ml pool. Similarly, the maximal activity was 22 and 38%, respectively, for the two pools pre-equilibrated at room temperature. K m to maximal activity was different between the two pools. When pre-equilibrated at 37°C, it took 26 h for the 0.3 GU/ml pool and 48 h for the 0.06 GU/ml pool. When pre-equilibrated at room temperature these times were 23 and 58 h, respectively. In fact, equilibrium was not reached in the current studies which lasted a maximum of 5 days (120 h).

Samples taken at 0, 48, and 120 h decreased in activity.

Table I

| Temperature | Equilibration temperature | Prorenin concentration | 37°C (n = 4) | 1.5% ± 0.3 | 1.2% ± 0.6 |
|-------------|---------------------------|------------------------|-------------|------------|------------|
| 0°C (n = 3) | 19% ± 0.3                 | 26% ± 0.5              |
| Trypsin-treated | 100% = 3331 ± 88 | 100% = 603 ± 38 |

Data expressed as percent (mean ± S.E.) of the maximal activity measured after limited proteolysis by trypsin. Samples were equilibrated for either 2 h at 37°C, 8 h at room temperature, or 5 days at 0°C.
Reversible Cryoactivation of Human Prorenin

**Figure 1.** Time course of cryoactivation of recombinant human prorenin. Two pools of recombinant human prorenin, 0.3 and 0.06 GU/ml, were preincubated at 37°C for 1 h. They were then incubated at 0°C for up to 120 h (6 days). Renin activity was measured as the hourly rate of generation of angiotensin I in the presence of partially purified human angiotensinogen. The incubation was carried out at 37°C for 5 min. Results are expressed (mean ± S.E.) as a percentage of the maximal activity generated by limited proteolysis by trypsin. Maximal activity was 3331 ± 88 ng AI/ml/h in the 0.3 GU/ml pool and 603 ± 38 ng AI/ml/h in the 0.06 GU/ml pool. Filled circles indicate the activity in cryoactivated samples. Open circles indicate the activity in samples that were first cryoactivated and then incubated at 37°C for 1 h. A dashed line connects these samples to their cryoactivated counterpart. The intrinsic activities were 3.0 and 3.2% for the 0.3 and 0.06 GU/ml pools, respectively. Extrapolation analysis showed that the maximal activities were 21 and 33% and the t_1/2 to maximal activity were 25 and 48 h, respectively.

**Figure 2.** Cryoactivation of recombinant human prorenin equilibrated at room temperature. See legend to Fig. 1 for details. Maximal activity was 3445 ± 104 ng AI/ml/h in the 0.3 GU/ml pool and 642 ± 43 ng AI/ml/h in the 0.06 GU/ml pool. Filled triangles indicate samples equilibrated at 0°C. Open circles indicate samples re-equilibrated at room temperature for 3 h. Filled triangles indicate samples re-equilibrated to 37°C for 1 h. The intrinsic activities were 7.5 and 7.8% for the 0.3 and 0.06 GU/ml pools, respectively. Extrapolation analysis showed that the maximal calculated activities were 22 and 38% and the t_1/2 values to maximal activity were 23 and 58 h, respectively.

when they were incubated for 3 h at room temperature but the activity did not return to base line, because 3 h at room temperature was not long enough for the prorenin to reach a new equilibrium (Fig. 4 indicates that at least 8 h were needed to reach equilibration at room temperature).

**Cryoactivation of Two Additional Sources of Prorenin—** Recombinant human prorenin prepared by California Biotechnology, Inc. (Cal Bio) (27) and by Pfizer, Inc. (28) showed reversible cryoactivation (Fig. 3). Two pools of Cal Bio prorenin, 0.3 and 0.06 GU/ml were incubated for 168 h at 0°C (Fig. 3, upper panels). The activity in Cal Bio prorenin reached 49 and 67%, respectively, after 7 days at 0°C. 0.3 and 0.06 GU/ml pools of Pfizer prorenin were incubated at 0°C for 144 h (Fig. 3, lower panels) and reached 29 and 45% of maximal activity, respectively. Both sources of prorenin showed reversal of cryoactivation, but activity did not return to base line. The greater increase in activity of both the Cal Bio and Pfizer pools, the lack of a plateau and the failure to return to base line were most likely due to protease contamination of the buffers (see "Technical Problems" below). Like the Upjohn prorenin the 0.3 GU/ml pool of both Cal Bio and Pfizer prorenins cryoactivated to a lesser degree then the 0.06 GU/ml pool. Cryoactivation was reversed at 37°C.

**Time Course of Reversal of Cryoactivation—** Inactivation of cryoactivated prorenin occurred quickly (Fig. 4). The half-life (t_1/2) is defined as the time needed for the activity to decrease to one-half of its initial level. The t_1/2 was 8 min for both pools at 37°C (Fig. 4, upper panels) and 120 min for both pools at room temperature (Fig. 4, lower panels). Following reversal of cryoactivation it was possible to recryoactivate both pools.

**Effect of Sodium Chloride Concentration on Cryoactiva-**
Reversible Cryoactivation of Human Prorenin

**Fig. 3.** Cryoactivation of two additional sources of recombinant human prorenin from California Biotechnology, Inc. (Cal Bio) and Pfizer, Inc. See legend to Fig. 1 for details. Maximal activity in the prorenin preparation from Cal Bio was 4188 and 750 ng AI/ml/h, respectively, in the 0.3 and 0.06 GU/ml/pools. Maximal activity in the prorenin preparation from Pfizer was 3119 and 563 ng AI/ml/h in the 0.3 and 0.06 GU/ml pools, respectively. Neither source exhibited complete reversal of activation after reincubation at 37 °C, most likely because of protease contamination of the samples (see “Technical Problems”).

**Fig. 4.** Reversal of cryoactivation of human recombinant prorenin. Two pools of human recombinant prorenin were equilibrated for 5 days at 0 °C and then incubated at either 37 °C for up to 2 h (upper panels) or at room temperature for up to 8 h. Activity is expressed (mean ± S.E.) as a percentage of the maximal activity generated by limited proteolysis by trypsin. Maximal activity in the 0.3 GU/ml pool was 3325 ± 89 ng AI/ml/h. Maximal activity in the 0.06 GU/ml pool was 654 ± 13 ng AI/ml/h. Filled circles indicate the activity in samples incubated at 37 °C or at room temperature. Open circles indicate the activity in samples that were subsequently cryoactivated for 4 days at 0 °C.

Cryoactivation was completely blocked by high sodium chloride concentration (Fig. 5). All samples were diluted to a sodium chloride concentration of 0.1 M prior to assay for renin activity. The degree of cryoactivation was reduced in buffers containing more than 0.5 M NaCl and did not occur in buffers with more than 1 M sodium chloride.

**Effect of Cryoactivated Prorenin on a Direct Renin Radioimmunoassay**—Cryoactivated prorenin was detected by a direct IRMA developed to detect active renin but not prorenin (Table II). The IRMA normally requires samples to be incubated at room temperature for a total of 5 h. Since this may cause reversal of cryoactivation, we also performed the IRMA at 4 °C. Immunoreactive renin detected in the 4 °C IRMA was greatest in the cryoactivated sample (12% ± 1.2 of maximal), lowest in the samples equilibrated at 37 °C (2.2% ± 0.5 of maximal), and intermediate in samples pre-equilibrated at...
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Fig. 5. Cryoactivation of recombinant human prorenin is inhibited by high concentrations of sodium chloride. A 0.3 GU/ml pool of recombinant human prorenin was incubated at 0 °C for 0, 6, and 18 h in the presence of increasing concentrations of sodium chloride in a 50 mM sodium phosphate buffer, pH 7.4, containing 10 mM benzamidine HCl, 3 mM Na3EDTA, 0.1% sodium azide, and 0.5% BSA. The sodium concentration (measured with a sodium-sensitive electrode) is indicated on the ordinate. The intrinsic renin activity is expressed (mean ± S.E.) as a percentage of the maximal activity generated by limited proteolysis by trypsin. Maximal activity was 39.1 ± 45 ng A1/ml/h. The inset graph shows the difference in the degree of activation after 18 and 6 h at 0 °C at the various sodium concentrations.

Table II
Detection of cryoactivated recombinant human prorenin by a direct IRMA for active renin (n = 3)

| Prorenin equilibration temperature | Prorenin | Prorenin | Prorenin | Prorenin |
|-----------------------------------|----------|----------|----------|----------|
| Prorenin incubation temperature   | Room temperature | 4 °C | Room temperature | 4 °C |
| Prorenin equilibration temperature | Nanograms /ml | % total | Nanograms /ml | % total |
| 7 days, 0 °C                      | 13.2 ± 3.7 | 5.4 ± 1.6 | 24.2 ± 2.3 | 12 ± 1.2 |
| 24 h, RT*                        | 4.8 ± 0.3 | 2.0 ± 0.1 | 8.7 ± 0.6 | 4.3 ± 0.3 |
| 1 h, 37 °C                       | 3.7 ± 0.2 | 1.6 ± 0.1 | 4.3 ± 1.1 | 2.1 ± 0.5 |
| Total renin*                     | 244 ± 19 | 100 | 203 ± 25 | 100 |

* RT, room temperature.

room temperature (4.3% ± 0.3 of maximal). Lower levels of immunoreactive renin were detected in the IRMA carried out at room temperature.

Technical Problems—An early difficulty with these studies was caused by the fact that the purified recombinant prorenin was susceptible to irreversible activation, perhaps because of inadvertent contamination of buffers with proteases. In some experiments the activity of the cryoactivated samples did not return to base line after a 37 °C incubation (Fig. 3). This problem was solved when buffers were made with high grade crystalline BSA heat-treated and sterile-filtered.

The renin assay is an enzyme kinetic assay that is carried out at 0 °C, and because cryoactivation of prorenin is reversed at 37 °C, we chose a 5-min incubation time at 37 °C for generation of angiotensin I to minimize the exposure of prorenin to the elevated temperature. Nonetheless since the t1/2 of reversal at 37 °C is only 8 min we must have slightly underestimated the maximum degree of cryoactivation that actually occurred.

For consistency we pre-equilibrated all pools of prorenin together at 0 °C. They were then frozen. Samples were thawed rapidly in front of a fan and equilibrated to room temperature or 37 °C as needed. This transition to and from the frozen state resulted in some variability of the base-line estimation of activity. This is illustrated in Fig. 4 where the initial level of cryoactivated prorenin is actually lower than in the samples that were re-cryoactivated after exposure to 37 °C and room temperature. It is also illustrated in Figs. 1–3 where the baseline values of the samples before cryoactivation are actually higher than those in the samples used to test reversal of cryoactivation.

Discussion

In this study we showed that human prorenin can be reversibly cryoactivated. After 5 days at 0 °C, prorenin had close to 20% of the maximal activity formed after cleavage of the prosegment by trypsin. At room temperature 3.5% of maximal activity was measured, whereas only 1.4% was detected at 37 °C. The intrinsic renin activity of prorenin could be repeatedly changed by altering the temperature. There was no increase in activity over that measured at 37 °C when prorenin was incubated at 0 °C in the presence of 3.6 M sodium. Antibodies directed against renin’s active site that do not recognize prorenin were able to detect cryoactivated prorenin.

The recombinant human prorenin used in this study had a small degree of residual renin-like activity at 37 °C and in the presence of high concentration of sodium chloride. This preparation contains a small proportion of truncated prorenin (26). Amino acids at positions 9–14 are reported to be needed to keep prorenin inactive (26). We found previously that the source of recombinant human prorenin synthesized by Carilli et al. (27) had undetectable basal activity after incubation at 37 °C (14). This same preparation had 5–10% activity when tested several months later in this study, perhaps because of bacterial contamination of the buffers.

Cryoactivation of prorenin occurred slowly over days. Inactivation of prorenin, by comparison, occurred quickly (8 min at 37 °C and 180 min at room temperature). The difference in the time course of activation versus inactivation may be due to several factors. First, the greater kinetic energy at higher temperatures would favor a more rapid change in three-dimensional structure. Second, based on pepsinogen as a model, activation of prorenin may occur in two stages (34). The prosegment of pepsinogen sits in, or near, the active cleft.
of the enzyme (35). When activated by acid pH, pepsinogen’s prosegment moves away from the body of the protein and then the N-terminal amino acid residues of pepsin move to become part of a six-strand anti-parallel β sheet (34, 36), common to renin and other aspartyl proteases. Inactivation may occur by reversal of either of these two steps and thereby could be faster.

The prosegments of aspartic protease zymogens are rich in basic amino acids (37, 38), which may interact with carboxylate groups of the enzyme, holding the prosegment in the active site (38). At acid pH protonation of the carboxylate groups releases the prosegment of pepsin (37, 38). At neutral pH a lysine residue at position 36 of the prosegment of pepsin, a highly conserved region of aspartic proteases, is hydrogen-bonded to 1 of the aspartic acid residues in the active site and is stabilized by other hydrogen bonds and dipole interactions (38). Amino acids P9–13 of pepsinogen (corresponding to amino acids P9–14 of prorenin) form a hydrophobic core that fits into the substrate binding site (38). Hydrophobic interactions, a major force in protein folding, are disrupted by low temperatures which perhaps explains why prorenin can be cryoactivated (39). High ionic strength strengthens hydrophobic interactions, and this may explain the lack of cryoactivation of human prorenin in the presence of 3 M sodium chloride.

The direct IRMA for human renin uses a monoclonal antibody directed against renin’s active site. We found that cryoactivated prorenin is detected in this assay, suggesting that the prosegment has moved away from the active site, making it accessible to the antibody. Movement of either the N-terminal amino acid residues of renin or of the prosegment could result in prorenin that is inactive yet not in its native three-dimensional state. This improperly folded prorenin may retain its antigenic site and bind to the monoclonal antibody used to detect renin. The current studies do not support that concept, since the direct IRMA detected a lower proportion of the cryoactivated prorenin than the enzyme kinetic assay, perhaps because the assay was carried out at 4 °C rather than 0 °C.

Cryoactivation of prorenin was first discovered in human plasma (40, 41). Human plasma stored cold but not frozen will develop higher renin levels with time. This increase is irreversible, because plasma serine protease inhibitors are inactivated in the cold allowing activation of the enzymes of the coagulation and fibrinolytic pathways (22). Both kallikrein and plasmin are capable of cleaving the prosegment of prorenin (5). It is likely that cryoactivation initially is reversible in plasma. As the prosegment moves away from the active site, however, prorenin may be more susceptible to enzymatic cleavage, as is pepsinogen when its prosegment shifts away from its active site (36). We have shown that reversibly acid-activated prorenin is more susceptible to cleavage by kallikrein than inactive prorenin.2

Demonstrating that prorenin can have intrinsic activity at neutral pH suggests that prorenin could have intrinsic renin activity in vivo under certain circumstances. For example, the prosegment may preferentially bind to other proteins rather than to the main body of the renin molecule. Such an interaction might lead to exposure of prorenin’s active site at localized target sites. This could allow angiotensin II in higher concentrations than that produced by circulating renin. High concentrations of angiotensin II can cause vasodilation, not vasoconstriction, which may explain the association of prorenin with increased blood flow and vasodilation (17). The reversibility of activation of prorenin may be the key to understanding its role in vivo.

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