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Specific Antibody to Human Renal Renin and Its Cross-Reactivity with Inactive Human Plasma Prorenin (40897)

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Abstract. A specific antibody to human renin was produced employing a pure human renal renin as antigen. This antibody inhibited the activity of human renal renin but did not inhibit the activities of hog renal renin, rat renal renin, and mouse submaxillary gland renin. Using this antibody an immunoaffinity column was constructed. The gel-bound antibody was used to demonstrate its capability to neutralize inactive prorenin in human plasma as well as active plasma renin, indicating that inactive plasma renin contains structures common to renal renin.

Attempts have been made in several laboratories to raise antibodies to human renin using partially purified renal renin preparations as antigens (1–4), and their reactivities with renins from other mammalian species (1) and human plasma (3, 4) have been examined. Due to the impurity of the antigens, these antibodies possessed only limited degree of specificity, which restricted their usefulness in application. Studies conducted using these antibodies did not produce definitive information on the immunologic identity of renin-like enzymes and related substances.

Recently we have purified human renal renin to homogeneity (5). Availability of this material enabled us to prepare specific antibody and to measure the cross-reactivity with renins obtained from other mammalian species and human plasma.

The presence of inactive renin in human plasma and its activation by several methods have been reported (6). Inactive renin has often been called prorenin. Immunologic relationship of prorenin to active renin has not been studies. Using a completely inactive human prorenin which has been separated from active renin (7), the cross-reactivity of prorenin with antibody to pure renal renin was demonstrated.

Materials and methods. Human renal renin to be used as antigen was purified as previously reported (5). Renins from hog kidney (8), rat kidney (9), and mouse submaxillary gland (10) were prepared employing previously published methods.

Inactive renin and active renin were prepared from human plasma according to the method of Yokosawa *et al.* (7). Activation of inactive plasma renin by trypsin was carried out as previously reported (7).

Renin activity was determined using a modification of the method of Haber *et al.* (11) for the radioimmunoassay of angiotensin I. Hog substrate was obtained from the plasma of nephrectomized hog and partially purified (12). Human substrate was purified through ammonium sulfate precipitation and column chromatography on DEAE–Sephacel followed by pepstatin–aminohexyl-Sepharose.

Pure human renin was insolubilized with glutaraldehyde (Sigma, St. Louis, Mo.) according to the method of Hirose *et al.* (13), emulsified with an equal volume of Freund's complete adjuvant (Miles, Elkhart, Ind.), and injected intradermally at multiple sites on the back of three Dutch-belted rabbits. An initial injection of 30 μg of renin was followed by a booster of 10 μg at the 5th week. At the 6th week and each subsequent week, blood was drawn from rabbit ear artery. A titer of antibody increased gradually after the 8th week,

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reached a plateau at about the 12th week, then decreased. Antibody obtained from the rabbit at the 12th week of immunization was used for the experiments reported in this communication. Preimmune plasma from the same rabbits was used for control.

The rabbit anti-renin IgG was isolated by affinity chromatography on protein A–Sepharose (Pharmacia, Uppsala, Sweden) by the method of Goding (14). Rabbit anti-human renin plasma (4 ml) was applied to a 5-ml column of protein A–Sepharose equilibrated with 0.02 *M* phosphate buffer, pH 7.4, containing 0.13 *M* NaCl. After exhaustive wash with the same buffer, IgG was eluted with 0.1 *M* acetic acid, containing 0.15 *M* NaCl and the pH of the eluate was quickly adjusted to neutrality with 1 *M* glycine–NaOH, pH 10.5. The IgG fractions (16 mg) were coupled to 12 ml of cyanogen bromide-activated Sepharose 4B-CL beads in 0.2 *M* citrate buffer, pH 7.0, at 4°C for 24 hr with gentle agitation essentially by the method of Walsh *et al.* (15). The yield of coupling of IgG was 85%.

A solution containing inactive renin in 0.02 *M* Tris–acetate buffer, pH 6.8, containing 0.3 *M* KCl was applied to a 1-ml column of the anti-renin–Sepharose column previously equilibrated with the same buffer. The column was washed with the same buffer followed by 0.05 *M* glycine–HCl buffer, pH 3.3, containing 0.1 *M* NaCl. Prorenin was eluted by 0.2 *M* acetic acid or 4 *M* $MgCl_2$ in 0.02 *M* Tris–acetate buffer, pH 6.7, containing 0.3 KCl. Elution was performed either by an acid or 4 *M* $MgCl_2$ after exhaustive wash as detailed in the legend to Fig. 2.

Results. The ability of antibody to neutralize renin from various species was examined by measuring inhibition of the renin activity of angiotensin I generation by serial dilutions of antibody. As shown in Fig. 1, the antibody inhibited the activity of human renal renin. On the other hand, hog, rat, and dog renal renin, and mouse submaxillary gland renin were inhibited only very slightly even at a high concentration of the antibody. The greatly diminished cross-reactivity was observed with antisera from all three rabbits immunized with human renin, indicating structural divergence of human renin from those of other species.

To ascertain whether inactive prorenin from human plasma was antigenetically similar to renal renin, cross-reactivity of inactive prorenin with antibody produced against renal renin was determined with or without trypsin activation.

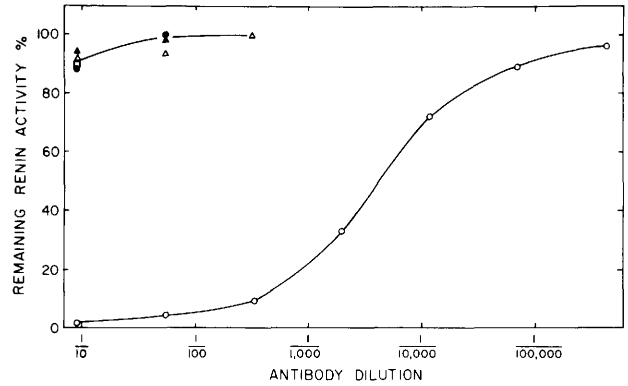

FIG. 1. Neutralization of renin from different species by anti-human renin antibody measured by the renin inhibitory activity. 25 μl of purified renins (150 pg) was incubated with 50 μl of antibody diluted as indicated on the abscissa in 0.01 *M* Tris–acetate buffer, pH 7.4, containing 0.1% BSA for 20 hr at 4°C. Residual renin activity was then measured by allowing the renin–antibody mixture to react with hog renin substrate in 0.1 *M* maleate buffer, pH 6.0, containing 7 m*M* EDTA and 2 m*M* phenylmethanesulfonyl fluoride. For each species of renin the angiotensin I generated in the absence of anti-renin antibody was set equal to 100%. (○) Human renin, (△) rat renin, (▲) hog renin, (●) mouse submaxillary gland renin, and (□) crude dog renal cortical extract.

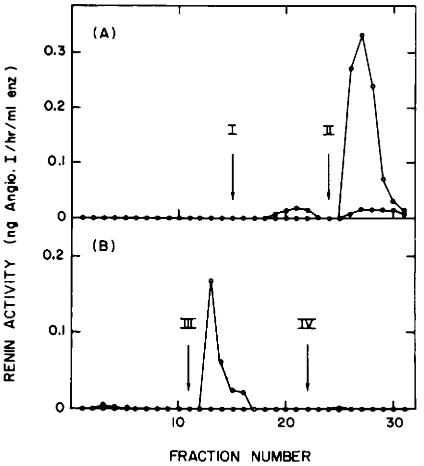

FIG. 2. Chromatography of inactive renin from human plasma on anti-renin globulin–Sepharose column. At arrow I, 0.05 *M* glycine–HCl buffer, pH 3.3, containing 0.1 *M* NaCl (A, arrow I) was applied to wash the column and at arrow II, prorenin was eluted with 0.2 *M* acetic acid containing 0.3 *M* KCl. In a separate chromatography (B), 4 *M* $MgCl_2$ in 0.02 *M* Tris–acetate buffer, pH 6.7, containing 0.3 *M* KCl was applied at arrow III followed by 0.2 *M* acetic acid containing 0.3 *M* KCl (arrow IV). The activity of inactive renin with (open circle) or without (closed circle) activation by trypsin at room temperature for 5 min was assayed at pH 6.0 in 0.2 *M* maleate buffer containing 7 m*M* EDTA and 2 m*M* phenylmethanesulfonyl fluoride using human renin substrate.

Table I shows parallel antibody inhibition experiment of activities of trypsin-activated inactive plasma prorenin, active plasma renin, and renal renin. The degree of inhibition of three types of renin was equivalent, indicating that the activated form of prorenin cross-reacts with anti-renal renin antibody in a similar manner as renal renin.

Interaction of inactive renin with anti-renal renin antibody was measured using an immobilized anti-renin antibody. As shown in Fig. 2, inactive prorenin was adsorbed by anti-renin antibody–Sepharose column and eluted by either an acidic pH or 4 *M* $MgCl_2$ at a neutral pH. Renal active renin was also adsorbed by this column and was eluted under the same conditions. Thus, it was clear that inactive prorenin and its activated form cross-reacted with anti-renal renin antibody.

Discussion. Isolation of human renal renin in a pure and stable form (5) has enabled us to produce monospecific antibody to this enzyme. Antibodies are useful for many applications such as specific inhibition and identification of its antigen and its isolation by specific precipitation or immunoaffinity chromatography. While antibodies prepared with impure renin preparations were useful for limited purposes and the validity of conclusion obtained from such application may be limited, the present monospecific antibody will be useful for much more extensive application and will provide definitive conclusions for the identification of renin.

Potter *et al.* (16) have reported that antibody prepared with partially purified

TABLE I. INHIBITION OF RENIN ACTIVITY BY ANTIBODY TO HUMAN RENAL RENIN[a]

Anti-human renin plasma	Activity (%)		
	Trypsin-activated inactive plasma prorenin[b]	Active plasma renin	Renal renin
Control	100	100	100
1:26,000	81	93	97
1:4,400	68	82	76
1:730	22	36	32
1:120	7	2	4
1:20	0	0	1

[a] 120 μl of solution of identical renin activities (1 ng angiotensin I generated/hr/ml) was incubated with 50 μl of serially diluted antibody in 0.07 *M* Tris–acetate buffer, pH 7.4, containing 0.3% BSA for 20 hr at 4°C. Residual renin activity was then measured in 0.15 *M* maleate buffer, pH 6.0, containing 6 m*M* EDTA and 2 m*M* phenylmethanesulfonyl fluoride using human renin substrate.

[b] Inactive plasma prorenin was activated with 2 μg of trypsin in 110 μl of 0.1 *M* Tris–acetate buffer, pH 7.4, containing 0.5% BSA for 10 min at room temperature. Activation was stopped by adding 100 μg of lima bean trypsin inhibitor.

human renin used as immunogen inhibits dog renin. This finding was contrary to the report of Haas *et al.* (1) that anti-human renin antibodies induced with partially purified human renin or its acetylated derivatives did not cross-react with renin from other mammalian species. The present study carried out with antibody raised with a pure human renin preparation showed concentration dependent but greatly diminished immunoreactivity with pure renin from rat and hog kidneys, mouse submaxillary gland, or crude dog renin extracted from dog kidney cortex is essentially in agreement with the results of Haas *et al.* (1) and Galen *et al.* (17).

The greatly diminished immunologic cross-reaction between human renin and renins of other species seems to be reciprocal. Lamfrom *et al.* (18) have shown that while antibodies against hog renin produced in human or dog serum inhibit renins from rabbit, pig, dog, beef, sheep, and rat, the same antibodies do not inhibit human renin. Hirose *et al.* (13) using monospecific antibody produced with pure hog renin have demonstrated cross-reaction with pure rat and mouse renin but not with human renin. These findings indicate that the structure of human renin is unique compared with renin from other species. On the other hand renins of nonhuman mammals seem to be closely related.

Day and Luetscher (19) have shown that activation of big renin did not produce low-molecular-weight renin. Yokosawa *et al.* (7) have observed that high-molecular-weight inactive prorenin in human plasma does not produce low-molecular-weight renin upon activation. These observations have suggested the possibility that the low-molecular-weight enzyme may not be related to the high-molecular-weight form. Definitive demonstration of their relationship by antibody-dependent inhibition of enzyme activity was not possible as the prorenin is enzymatically inactive. Use of monospecific antibody in the immunoaffinity chromatography permitted us to demonstrate for the first time that inactive prorenin in human plasma and the product of trypsin activation of prorenin is bound to the column as tightly as renin in its active form. It indicates that prorenin and active renin share common structural features. Immunological cross-reactivity of prorenin with active renin, as well as its activation by endogeneous protease (7), strongly suggests the possiblity that inactive prorenin in the plasma is the zymogen of active renin. However, the present result alone does not rule out the alternative explanation that inactive prorenin is a renin-inhibitor complex. The successful application of the antibody–Sepharose column for adsorbing prorenin will permit the purification of prorenin from various human tissues and fluids. Such studies will eventually clarify the possible zymogenenzyme relationship between prorenin and active renin.

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