Inhibition of Human Renin by Synthetic Peptides Derived from Its Prosegment

(Received for publication, December 20, 1984)

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The primary structure of human preprorenin has recently been determined from its cDNA sequence. It includes a 46-amino acid NH2-terminal prosegment. Six peptides corresponding to the entire prosegment (9-40), except for the NH2-terminal (1-8) and COOH-terminal (41-46) ends have been synthesized. These peptides were tested for their inhibitory effect on human plasma renin activity. Boc-Tyr-Thr-Thr-Phe-Lys-Arg-Ile-Phe-Leu-Lys-Arg-Met-Pro-OMe (where Boc represents t-butoxy carbonyl and OMe represents methoxy) (h Y(9-20) and its fragment Boc-Leu-Lys-Arg-Met-Pro-OMe h (16-20) were the most potent inhibitors with IC50 values of 2 x 10^-4 and 3 x 10^-5, respectively. Peptides located near the COOH-terminus were less inhibitory.

The inhibitory capacity of h (16-20) was studied further on highly purified human renin acting on either pure human angiotensinogen or a synthetic human tetradecapeptide substrate. In both of these assays its inhibitory potency was about 10-fold greater than that found on plasma renin activity. Peptide h (16-20) was 3-6 times less potent in inhibiting human renin than its mouse counterpart m (15-19) was in inhibiting mouse renin. Kinetic studies carried out with h (16-20) showed a mixed type of inhibition. When human angiotensinogen was used as substrate, K, and K' values were 17.7 ± 3.9 and 2.9 ± 0.9 μM, respectively. These studies showed that human renin, like mouse renin and pepsin, can be inhibited by peptides derived from its prosegment. In addition, as in the case of pepsin, they suggest that the NH2-terminal part of the prosegment interacts more strongly with the active enzyme.

RENIN (EC 3.4.23.3) is a key enzyme in the regulation of blood pressure and electrolyte metabolism (Oparil and Haber, 1974). It cleaves angiotensinogen, an M, 55,000 protein synthesized by the liver to release the decapetide angiotensin I from its NH2 terminus. Angiotensin I is in turn converted to the biologically active octapeptide angiotensin II by a carboxy-dipeptidase. Renin belongs to the class of aspartyl proteases (Inagami et al., 1974; McCown and Gregerman, 1974a) and has a unique specificity for angiotensinogen as well as for the synthetic tetradecapeptide which corresponds to the NH2-terminal 14 amino acids of angiotensinogen.

We have recently shown (Panthier et al., 1982) that mouse submaxillary gland renin is synthesized as a renin precursor, preprorenin, and that the prosegment of renin consists of a 45-amino acid peptide (1-45) (Fig. 1). Further studies showed that synthetic peptides derived from the prosegment of mouse submaxillary renin were able to inhibit the activity of mouse submaxillary renin on the porcine synthetic tetradecapeptide, strongly suggesting that mouse prorenin is inactive. The most potent inhibitor was a pentapeptide corresponding to amino acids 15-19 of the renin prosegment which exhibited a K value in the micromolar range (Evin et al., 1984). In this respect, mouse submaxillary renin again behaves like other aspartyl proteases such as pepsin and chymosin which are processed from the inactive precursors pepsinogen and prochymosin, respectively, to give the active enzyme after release of the prosegment (Harboe and Folman, 1975; Folman et al., 1977). Peptides corresponding to a portion of bovine or porcine pepsin prosegment are also able to inhibit active pepsin (Harboe et al., 1974; Dunn et al., 1978), the most active peptides being located in the NH2-terminal portion of their prosegment.

The complete sequence of the structural gene coding for human renal renin has been reported recently (Imai et al., 1983) and the amino acid sequence of preprorenin deduced. The exact length of the prosegment is not known with certainty but, by analogy with mouse submaxillary and renal renin (Panthier et al., 1982; Holm et al., 1984), it could be a 46-residue peptide (1-46). This prosegment is in part homologous to the mouse submaxillary renin prosegment (Fig. 1). The purpose of this study was to synthesize peptides derived from different portions of this prosegment and to study their inhibitory effect on human plasma renin activity. The pentapeptide h (16-20) was then studied in more detail for its ability to inhibit pure human renin acting on pure natural substrate. The results show that human renin is indeed inhibited by peptides derived from its prosegment, especially those originating from the NH2-terminal region. They also show that human prorenin is most likely inactive.

EXPERIMENTAL PROCEDURES AND RESULTS

DISCUSSION

Peptides corresponding to different regions of the prosegment (9-40) of the human renin precursor have been synthetized.
A prosegment is designated by line segments under the sequence of the renin prosegment. The one-letter code for amino acids is as follows:

- A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; X, norleucine.

Peptides located in the COOH-terminal region have been shown to inhibit pepsin activity. Peptides both native and synthetic peptides of the parent pepsinogen.

If ten peptides overlapped each other, we were able to locate short potent inhibitor of the four synthesized peptides. These peptides were studied separately and compared to peptide h (1-8) which was due to the sequence of the pentapeptide h (15-19). It appears that most of the inhibitory activity of h (15-19) was the most potent of this latter group, whereas h (16-20) which inhibited renin activity by 65% where h (9-15) and h (16-20) inhibited renin activity by 11 and 57%, respectively. Thus, it appears that most of the inhibitory activity of h (9-20) was due to the sequence of the pentapeptide h (16-20). Peptides corresponding to the sequences of region 1-17 were less inhibitory, h (31-37) was the most potent of this latter group, producing an inhibition of 40% at 3.5 x 10^{-4} M.

We had previously shown that peptides related to region 11-19 of the prosegment of mouse submaxillary renin were inhibitors of mouse renin activity but no systematic study had been made with peptides from other parts of this prosegment. However, it is interesting to point out that the pentapeptide Boc-Leu-Lys-Arg-Met-Pro-OMe, referred to as m (15-19) and which is homologous to h (16-20), was the most potent inhibitor of the four synthesized peptides.

A notable homology exists between the prosegment sequences of renin precursors and those of other known aspartyl protease zymogens, particularly bovine and porcine pepsinogens (Fig. 3). Some peptides of the pepsin prosegment have been shown to inhibit pepsin activity. Peptides (1-17) (Dunn et al., 1978) and (27-38) (Harboe et al., 1974) obtained on enzymatic activation of bovine pepsinogen were found to inhibit bovine pepsin activity in a milk clotting assay, at pH 5.5, whereas peptide (18-25) had no inhibitory effect. Dunn et al. (1978) have shown that porcine pepsin is inhibited by both native and synthetic peptides of the parent pepsinogen. They found that peptides of the NH2-terminal region (1-16, 1-13, 1-11) were 10-20-fold more potent inhibitors than peptides located in the COOH-terminal region (25-41 and 17-44). Peptide (1-11) which is located in the region corresponding to 9-20 in human renin prosegment was a particularly potent inhibitor with a K, value of 0.5 μM. Dunn et al. (1978) suggested that electrostatic interactions could be involved in the inhibition. The NH2-terminal portion of porcine pepsin prosegment has more positive charges than the COOH terminus which could explain why peptides corresponding to the NH2 terminus bind more tightly than those from the COOH terminus. The same type of binding could occur between human renin and prosegment peptides: h Y(9-20) also possesses several positively charged residues, namely Lys 12, Arg 13, Lys 17, Arg 18. This interaction could be an explanation for the reversible acid activation of inactive plasma renin (Hsueh et al., 1981; Atlas et al., 1978; Leckie and McGhee, 1980) which we have shown to be immunologically identical to prorenin (Bouhnik et al., 1985). Electrostatic bonds existing at neutral pH between renin and prosegment basic residues would be reversibly broken by acidification.

Results obtained with h Y(16-20) could not be strictly compared with those previously found with its mouse counterpart m (15-19) because they were not studied in the same type of assay (Table II): m (15-19) was tested solely on pure mouse renin acting on a hog synthetic substrate. A better comparison was made possible by studying both pentapeptides on renin acting on plasma, on a synthetic tetradecapeptide substrate or on a pure natural substrate. For both renin species, the inhibitory potency of the prosegment peptides was about 10-fold lower on plasma renin activity than in pure assays. This could be due to degradation during the assay or binding to plasma proteins. The binding of the inhibitor to plasma renin could also be disturbed by the presence of fatty acids (Poe and Liesch, 1983) or proteins (Ueno et al., 1981) bound to the enzyme. When both pentapeptides were studied on pure renin acting on either angiotensinogen or synthetic substrate, IC50 values were comparable. Peptide h Y(16-20) was 3-6 times less potent in inhibiting human renin than m Y(15-19) was in inhibiting mouse renin. Interestingly, no significant differences was found in the IC50 values for the same inhibitor and the same enzyme whatever the substrate examined.

The enzymatic characteristics of h (16-20) was further studied with pure human renin acting on pure angiotensinogen. Both plots were in agreement with a mixed type of inhibition (K, > K+). The kinetic constants found showed a stronger inhibition with the enzyme-substrate or enzyme-product complexes than with the enzyme alone. Several peptides described above have been used as antigens. Notably antibodies have been raised against h Y(28-40) and found to

**Table II**

| Inhibitory effect of peptides derived from the renin prosegment of human and mouse renin in different assays |
|----------------------------------------------------------------------------------------------------------------|
| **Human assay (1)** | **Mouse assay (2)** |
|---------------------|-------------------|
| Plasma renin activity | 290 | 25 |
| Angiotensinogen | Human | 16.6 | 2.6 |
| Rat | Synthetic substrate | DRVYIHPFHLVHT | N-ac-DRVYIHPFHLVLYS |
| | | 10.5 | 3 |

*The abbreviations used are: Boc, t-butoxycarbonyl; OMe, methoxy.*
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In addition to different sources (Bouhnik et al., 1985). Our results support the hypothesis that inactive renin is prorenin.

Acknowledgments—We thank N. Braure for typing the manuscript, A. Boisquillon for preparing artwork, and Dr. Roisin (Faculté de Médecine St Antoine) for her helpful contribution to amino acid analyses. We are grateful to Dr. Longjou (St Louis, Mo) for critically reading the manuscript. We are indebted to Dr. Tewksbury and Dr. Bouhnik for the gift of pure human and rat angiotensinogen, respectively.

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Supplementary material for:

EXPERIMENTAL PROCEDURES

METHODS

1. Synthesis and analysis of fragments related to the human renin gene

2. Peptides derived from the renin gene

3. Inhibition of human renin by synthetic peptides

4. Human renin activity

5. Human renin activity

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Table 1 shows that these peptides inhibited human renin at concentrations in the 10^{-11} M range. The IC_{50} values, determined at varying concentrations of renin (0, 0.1, 1, 10, and 100 ng/ml), were found to be 3.25 x 10^{-10}, 3.45 x 10^{-10}, and 6.29 x 10^{-10} M, respectively. Between the 10^{-10} M and 10^{-7} M concentrations, it was impossible to detect inhibition. (IC_{50} values could not be determined for peptide A, Z, or B.)

In order to compare the inhibitory potency of all the different peptides, we studied their inhibitory effect at the same concentration (3.45 x 10^{-10} M). We found that peptides B, Z, and A inhibited the reaction more than the other peptides tested. Peptide B (10-23) is a strong inhibitor of human renin, as is peptide A (10-23) which is a part of B (10-23) and has the same potency. We also tested the inhibitory potency of peptides A (10-23) and B (10-23) at a concentration of 3.45 x 10^{-10} M. Inhibition was found to be 74% and 89%, respectively.

The inhibitory potency of peptide A (10-23) was found to be 0.25 for the reaction of human renin with human renin as substrate. The IC_{50} value for this reaction was 4.25 x 10^{-10} M. The IC_{50} value for the reaction of human renin with human renin as substrate was 6.25 x 10^{-10} M for peptide A (10-23) and 5.5 x 10^{-10} M for peptide B (10-23).

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