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ON THE SPECIFICITY OF HUMAN RENIN

STUDIES WITH PEPTIDE INHIBITORS

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Summary

The amino acid sequence around the renin substrate site is known to be identical to the N-terminal tetradecapeptide: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser. Renin (EC 3.4.99.19) from both primates and non-primates cleaves this peptide at the leucylleucine bond. Several analogs of the octapeptide segment: His-Pro-Phe-His-Leu-Leu-Val-Tyr of this tetradecapeptide act as competitive inhibitors for human renin with inhibition constants down to 1 μM. The same peptides were shown, however, to have no or only slight affinity for non-primate renin. The substrate site has been preserved throughout evolution whereas the enzyme site for human renin is different from that of non-primate renins. The findings suggest that species-specific peptides must be developed for both studies of renin inhibition and for renin purification.

Introduction

Renin substrates from different species vary markedly in their susceptibility to cleavage by renin (EC 3.4.99.19) from various species [1]. Human renin substrate is not cleaved at all by non-primate renin. Human renin, however, reacts with renin substrate from both primates and other animal species, with a few exceptions [1,2].

The amino acid sequence around the substrate site is known to be the N-terminal tetradecapeptide: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser. This peptide is cleaved off from purified equine renin substrate by tryptic digestion [3] and from hog plasma by alcoholic NaOH treatment [4]. Renin from both primates and other animals cleaves the tetradecapeptide at the

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leucylleucine bond to give the decapeptide angiotensin-I as the product. The structure of angiotensin-I except for an interchange of isoleucine and valine at position 5, is identical in different species. The N-terminal sequence around the cleavage site is therefore believed to be identical in renin substrates from different species, and the rest of the protein renin substrate molecule responsible for the species specificity.

The shortest peptide fragment with a reasonable cleavage rate and affinity for renin is the octapeptide His-Pro-Phe-His-Leu-Leu-Val-Tyr (octapeptide) found between residues 6 and 13 [5]. Synthetic analogs of this octapeptide have proved to be excellent inhibitors of human renin [6,7]. The present paper demonstrates, by the use of these inhibitors, that not only the protein renin substrate but also the conformation of the enzymatic cleavage site of human renin is different from that of other species.

Methods

Human renin. This was of the International Standard from Division of Biological Standards, Holly Hill, London [8].

Rat renin. This was purified from rat kidneys using a slight modification of procedure C kindly given by Dr. Haas [9]. The specific activity was 1.3 G.U./mg *, when standardized against the research standard for hog renin obtained from the Division of Biological Standards.

Hog renin. This was purchased from Nutrition Biochemical Corporation, Cleveland and standardized against the research standard for hog renin.

Tetradecapeptide. Tetradecapeptide was purchased from Schwartz/Mann. Composition and concentration were checked by amino acid analysis and by its conversion to angiotensin-I, which was quantified by radioimmunoassay.

Human substrate. This consisted of plasma (+ 3 mM EDTA) from a normal person receiving an estrogen-containing medication for birth control [6].

Rat substrate. This consisted of plasma (+ 3 mM EDTA) from 24-h nephrectomized rats which, prior to nephrectomy, were treated with diethylstilbestrol to increase further the renin substrate concentration [10].

Inhibitory peptides. Inhibitory peptides were synthesized by solid phase synthesis and characterized as previously described [6,7].

Plasma samples. Blood from humans, rats, dogs, rabbits, mice, guinea pigs was taken in ice-cold tubes containing 3 mM EDTA as an anticoagulant. The plasma was separated immediately at 4°C.

Michaelis constants (K_m) and inhibition constants (K_I) were determined as previously described [6,7] using weighted Lineweaver-Burk plots. All experiments were performed at least twice.

Plasma renin activity and angiotensin-I concentrations were measured with the radioimmunological antibody-trapping method of Poulsen and Jørgensen [10].

Results

The reactions of human, rat and hog renin with various protein renin substrates as well as the tetradecapeptide were evaluated in the same assay system at pH 7.5.

* Goldblatt Units.

TABLE I

SPECIES SPECIFICITY OF THE REACTION BETWEEN RENIN AND ITS SUBSTRATES

K_m values μM pH 7.5.

Renin	Protein renin substrate		Tetradecapeptide substrate
	Human	Rat	
Human	0.5	none	25
Rat	none	3	330
Hog	none	0.4	7

The K_m values were determined and the results are given in Table I. In contrast to the reaction with protein renin substrate, the tetradecapeptide is a substrate for all three renins. The K_m values with tetradecapeptide substrate were, however, 1—2 orders of magnitude greater than those obtained with the homologous protein renin substrates. The protein part of the substrate molecule presumably carries information defining both specificity and affinity.

As previously demonstrated [6,7] amino acid substitutions could be made around and at the cleavage site. Properties of the octapeptide inhibitors are given in Table II. The two most effective inhibitors Pro(Phe^{5}Phe^6) octapeptide and Pro(Phe^6) octapeptide have inhibitor constants 1—2 orders of magnitude smaller than the Pro$_5$ octapeptide. They also have about the same inhibitor constants (K_I) whether competing with tetradecapeptide or human protein substrate for human renin. When, however, the same peptides were investigated as inhibitors for rat renin and hog renin (Table II) only Pro(Phe^{5}Phe^6) octapeptide had a measurable (although low) affinity for hog renin of 30 μM, while the affinity for rat renin was lower. The remaining peptides had very low affinity for rat and hog renin. The exact inhibitor constants, if any, escaped detection because of lack of solubility.

Pro(Phe^{5}Phe^6) octapeptide neutralized the generation of angiotensin-I by endogenous renin and substrate in human plasma (Table III). However, inhibition was not observed in rat, dog, rabbit, mouse or guinea pig plasma.

TABLE II

INHIBITOR CONSTANTS FOR SYNTHETIC SUBSTRATE ANALOGS USING DIFFERENT RENINS

K_I values μM at pH 7.5.

	Human renin		Rat renin, rat substrate	Hog renin, rat substrate
	Tetradecapeptide	Human renin substrate		
Pro(Phe^{5}Phe^6) octapeptide	1	1.3	>75	30
Pro(Phe^6) octapeptide	4	9	>940	>150
(Pro)$_3$(Phe^6) octapeptide	3	— *	>150	>150
Pro(Tyr^6) octapeptide	12	—	>550	>550
Pro(Phe^8) octapeptide	71	—	>85	>85
(Pro)$_5$ octapeptide	140	—	>480	>130
(Pro)$_5$(D-Leu^6) octapeptide	500	—	>1080	>1080

* Not performed.

TABLE III

INHIBITION OF PLASMA RENIN ACTIVITY IN PLASMA FROM DIFFERENT SPECIES (n = 5).

Plasma	Pro(Phe^{5}Phe^6) octapeptide, % inhibition at		
	4μM	10μM	75μM
Human	30%	60%	88%
Rat	none	none	none
Dog	none	none	none
Rabbit	none	none	none
Mouse	none	none	none
Guinea pig	none	none	none

Discussion

The K_m value for the reaction between renin and substrate depends on pH, ionic strength, the medium and the assay procedure used. For the reaction between human renin and human renin substrate K_m values spanning the range from 70 nM to immeasurably high values have been reported [11]. There is, however, general agreement about the species specificity of the reaction [1,2]. There is also support for the finding that the K_m value is greater for the reaction between human renin and tetradecapeptide than for the reaction with homologous protein renin substrate [12,13]. No difference could, however, be found when hog renin was used [5].

Even though the K_m value was 50 times higher for the reaction between human renin and tetradecapeptide than for the reaction with human renin substrate, the K_I values for the best inhibitors were almost identical when tested in the two systems (Table II). These results are in accordance with this peptide being a simple competitive inhibitor. When the same inhibitors were tested for inhibition in the reaction between rat or hog renin inhibition could be demonstrated, only with Pro(Phe^{5}Phe^6) octapeptide which had a K_I of 30 μM for hog renin. The affinities between hog or rat renin and rat substrate, which the inhibitors should compete with, were equal to or lower (expressed as $1/K_m$) than that of the human system. This should have favoured inhibition of rat and hog renin.

In order to ensure the stability of the peptides in the rat substrate this was acidified to pH 3.6 at 25°C for 20 min before adjusting to pH 7.5, which greatly reduced peptidase activity [1,11]. The rat substrate was also diluted at least 15-fold. Addition of the peptidase inhibitors EDTA, 8-hydroxyquinoline and 2,3-dimercaptoethanol did not change the result. It is thus unlikely because of precautions taken to neutralize proteolytic enzymes that the lack of inhibition is due to rapid destruction of the inhibitor peptides.

Species specificity demonstrated by the inhibitors could be due to modifications either in the N-terminus or at the cleavage site of the octapeptide. Simple extension of the length of octapeptide is less likely to be the basis of species specificity because of addition of five residues as in the case of the tetradecapeptide does not confer such specificity.

Although the conformation of the substrate site has been preserved during evolution the enzyme site of human renin has changed. Implications of this are twofold. The effective inhibitors modelled for human renin have so little effect on renin from the common laboratory animals that an in vivo effect cannot be demonstrated. To realize this will require the synthesis of other peptides. Secondly, the species specificity of renin will also influence the choice of peptide used for purification of renin by affinity chromatography. The previously published (D-Leu^6) octapeptide has been used for purification of hog and human renin [14], which both have affinity for the peptide at pH 5.5 but not at pH 7.5. This is in accordance with the finding that the Pro_5(D-Leu^6) octapeptide (Table II) had no measurable affinity for hog renin at pH 7.5.

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