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Renin Cleavage of a Human Kidney Renin Substrate Analogous to Human Angiotensinogen, H-Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu–Val–Ile–His–Ser-OH, that Is Human Renin Specific and Is Resistant to Cathepsin D

MARTIN POE,* JOSEPH K. WU,* TSAU-YEN LIN,* KARST HOOGSTEEN,* HERBERT G. BULL,† AND EVE E. SLATER‡

*Department of Biophysics, †Department of Biochemistry, and ‡Department of Biochemical Endocrinology, Merck, Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

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A synthetic tetradecapeptide, H-Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu–Val–Ile–His–Ser-OH, which corresponds to the 13 amino terminal residues of human angiotensinogen plus a carboxy terminal serine to replace a suggested site of carbohydrate attachment, has been shown to be a good substrate for human kidney renin. At pH 7.2 and 37°C the K_M or Michaelis constant was 8.4 ± 2.9 μM, and the V_M or velocity at infinite tetradecapeptide concentration was 11.3 ± 2.4 μmol angiotensin I made per hour per milligram renin. The tetradecapeptide was highly resistant to cleavage by mouse submaxillary renin. The tetradecapeptide was also slowly cleaved by human liver cathepsin D, by rabbit lung angiotensin-converting enzyme, and by reconstituted human serum, but did not yield angiotensin I. Thus, this synthetic renin substrate should permit more specific measurement of human kidney renin activity.

KEY WORDS: renin; renin assay; angiotensinogen; angiotensin I; tetradecapeptide renin substrate; cathepsin D-resistant.

Renin (EC 3.4.99.19) is a carboxyl protease important to the control of blood pressure (1). The natural protein substrate for renin, an α_2 globulin (2) called angiotensinogen of molecular weight 66,000, is present near 1000 ng per ml in the plasma (3). Renin produces angiotensin I from angiotensinogen via proteolytic activity; the decapeptide angiotensin I comprises the amino terminal 10 residues of the natural substrate (4,5). Because current radioimmunoassays for human renin (6) are insufficiently sensitive, renin levels are measured from renin enzymatic activity as measured in an angiotensin I radioimmunoassay. The natural substrate can be used in the study of renin, but because of the difficulties in its purification, only a few laboratories have obtained relatively homogenous preparations (see, for example, Refs. (2,7,8)). Thus, many investigators use anephric, or renin-free, plasma which contains a host of non-renin proteases and inactive renin (9). Because of these problems, a large number of researchers use synthetic peptides that mimic the amino terminal sequence of angiotensinogen as substrates, especially for purified renin preparations. Perhaps the most widely used synthetic renin substrate is a tetradecapeptide (STRS,[1] see Table 1) introduced by Skeggs and coworkers (10) that corresponds to the first 14 residues of horse angiotensinogen. In particular, the scissile Leu^{10}–Leu^{11} bond is characteristic of all subprimate angiotensinogens

[1] Abbreviations used: STRS, synthetic tetradecapeptide renin substrate, H-Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu–Leu–Val–Tyr–Ser-OH; STRS-2, synthetic tetradecapeptide renin substrate No. 2, H-Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu–Val–Ile–His–Ser-OH. In both STRS and STRS-2, all residues are in the L-, or S-, configuration; Ang I, angiotensin I.

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TABLE 1

COMPARISON OF THE AMINO TERMINAL SEQUENCES OF NATURAL AND SYNTHETIC RENIN SUBSTRATES

Material	Sequence
Horse angiotensinogen	H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-Y-
STRS, horse tetradecapeptide	H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH
STRS-2, human tetradecapeptide	H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-Ser-OH
Human angiotensinogen	H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-X-Glu-Ser-Thr-Ser-Glu-Gln-Leu-Ala-Lys-Ala-Asp-

Note. X and Y have not been identified; X has been proposed to be a site of carbohydrate attachment (5).

sequenced to date; this includes horse (2), hog (11), and rat (12) angiotensinogen. STRS was originally produced by trypsin cleavage of horse angiotensinogen (13); it is now available as a synthetic product, both with a free or *N*-α-acetylated amino terminus. This synthetic substrate is cleaved to produce angiotensin I, or *N*-α-acetylated angiotensin I, respectively, with high catalytic efficiency by both human renin (14) and the renins of other vertebrates (15–17). However, it is also cleaved to produce the same products by cathepsin D (18–20) and can be cleaved by other enzymes as well, such as angiotensin-converting enzyme (21).

We recently commissioned the synthesis of a tetradecapeptide that corresponds to the recently determined amino terminal sequence of human angiotensinogen (5) and which differs from the sequence of horse angiotensinogen in particular at positions 11 to 13 (see Table 1). Presented herein is evidence that the human tetradecapeptide (STRS-2) is cleaved to produce angiotensin I by human kidney renin with high catalytic efficiency and that STRS-2 is resistant to cleavage by cathepsin D, mouse submaxillary gland renin, angiotensin-converting enzyme, and human serum.

MATERIALS AND GENERAL METHODS

Human angiotensin I and STRS were purchased from US Biochemical Corporation, Cleveland, Ohio. Human angiotensin II and H-Leu-Val-Tyr-Ser-OH were from Vega. A 50-mg batch of STRS-2 was prepared by Peninsula Laboratories, Inc., Belmont, Calif. This peptide was found to be homogenous in three thin-layer chromatographic systems: chromatography on cellulose-coated plates (E. Merck) in 15:10:3:12 (v/v) *n*-butanol:pyridine:acetic acid:water, in 42:24:4:30 *n*-butanol:pyridine:acetic acid:water, and in 4:1:5 *n*-butanol:acetic acid:water, with visualization by ninhydrin staining. Quantitative amino acid analysis following hydrolysis for 20 h in 6 N HCl at 110°C *in vacuo* found the STRS-2 preparation to be 66% peptide (with the rest of the mass salt and H_2O) for a formula weight of 1733.24 with the following contents (relative to Phe): His (3) 2.59; Arg (1) 1.09; Asp (1) 0.88: Ser (1) 0.97; Pro (1) 1.02; Val (2) 1.64; Ile (2) 1.51; Leu (1) 1.08; Tyr (1) 0.88; Phe (1) 1.00. Two high-pressure liquid chromatography (HPLC) analyses were done and showed the STRS-2 to be 96.8% one species. One HPLC analysis was on a Beckman Model 344 liquid chromatograph fitted with a 4.6-mm × 25-cm Zorbax CN column at a flow rate of 1 ml/min for a linear gradient from 0.05 M NaH_2PO_4 to 6:4 (v/v) acetonitrile: 0.05 M NaH_2PO_4 developed over 40 min at ambient temperature, injecting 40 μg STRS-2 in 10 μl, 0.05 M, NaH_2PO_4 with detection at 210 nm; STRS-2 eluted at 23.6 min under these

conditions. These elution conditions were also used for a 5-mm × 25-cm Altex C-18 column on an Altex Model 332 liquid chromatograph.

Human kidney renin (M_r 50,000) was from Slater and Strout (14). Mouse submaxillary gland renin was prepared according to Poe *et al.* (22). The mouse renin was homogenous in polyacrylamide gel electrophoresis, both with and without sodium dodecyl sulfate. The purified mouse renin yielded 0.14 mmol angiotensin 1 per h per mg renin when assayed at pH 5.6 and 37°C with 5.7 μM horse tetradecapeptide [STRS] (22). Rabbit lung angiotensin-converting enzyme [EC 3.4.15.1] was homogenous in sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The converting enzyme preparation used had a specific activity of about 180 s^{-1} for cleavage of furanacryloyl-L-phenylalanyl–glycine–glycine at pH 7.5 and 25°C in the assay conditions of Holmquist *et al.* (23). This specific activity was about 57% of the value reported by Holmquist *et al.* (23). The concentration of converting enzyme used in our assays was standardized by activity in the assay of Holmquist *et al.* (23), assuming that fully active, pure rabbit lung converting enzyme had a turnover number of 317 s^{-1} and a Michaelis constant of 0.30 mM. Human liver cathepsin D (EC 3.4.23.5) was purified to homogeneity according to the method of Barrett (24). One of the three forms separated by preparative isoelectric focussing, the gamma form which has a p*I* of 6.4, was used in this study. This enzyme reacted with an antiserum against human liver cathepsin D provided by Dr. A. J. Barrett (Strangeways Research Laboratory, Cambridge, England). This cathepsin D preparation was a single component when analyzed by analytical isoelectric focussing and polyacrylamide gel electrophoresis. The specific activity measured on methyl-[^{14}C]-glycinated hemoglobin was about 0.7 g substrate degraded per g enzyme per min at pH 4.0 and 37°C. The cathepsin D preparation cleaved pyroGlu–D-Phe–Pro–Phe–Phe–Val–Trp-OH at the Phe–Phe bond (25). Processed human plasma was from Clinical Assays, Cambridge, Mass. The lyophilized plasma (labeled "renin activity control") had 0.02 M sodium azide when reconstituted with 3 ml 50 mM triethylamine phosphate, pH 5.92; this solution had a pH of 6.36.

Fluorometric enzyme assays. Enzyme incubations with STRS-2 were at 37°C in 0.1 M citrate phosphate, pH 7.2, for mouse and human renin, with the appearance of new amino termini measured fluorometrically after fluorescamine reaction by the procedures of Corvol *et al.* (26) and Galen *et al.* (27), as described elsewhere (22). To demonstrate the linear dependence upon incubation time and enzyme concentration of the cleavage of the human tetradecapeptide (STRS-2) by human kidney renin, incubations were done for 0- to 120-min with 230 ng human kidney renin and were done with 0 to 230 ng renin for 120 min, both with 3500 nM STRS-2. The cleavage of STRS-2, as measured fluorimetrically, was linear with time and enzyme concentration (data not shown). For the determination of the Michaelis constant K_M and maximal velocity V_M of STRS-2 cleavage by human kidney renin, multiple assays were done for 120 min with 230 ng renin with 0, 1.9, 3.8, or 5.7 μM STRS-2. Cleavage of 10 to 40% of the STRS-2 was noted in the various incubations. The inverse of the difference in fluorescence between the renin incubation solution and a corresponding enzymeless control was plotted versus the inverse of the STRS-2 concentration and fitted manually to the standard hyperbolic equation: $1/v = (1/V_M)(1 + [K_M/S])$ where v was the rate of STRS-2 cleavage, and S was STRS-2 concentration (22). The cleavage of STRS by human cathepsin D was measured at 37°C and pH 3.2 in 1 ml 0.25 M sodium formate. The appearance of new amino termini for STRS upon cleavage by human cathepsin D was measured fluorometrically as for STRS-2 cleavage by the renins after neutralization with 1 ml 0.25 M Na_2HPO_4. In 1 ml of the formate buffer with 0.028 mM STRS-2 and 450 ng enzyme, the linearity of the assay with time was measured for 0 to 75 min. The linearity of the assay with enzyme concentra-

tion was measured for 0 to 2250 ng enzyme with 0.028 mM STRS; the assay was linear with time and with enzyme concentration over these ranges.

HPLC enzyme assays. The products of STRS cleavage by human cathepsin D and of STRS-2 cleavage by human kidney renin, by mouse renin, by human liver cathepsin D, by human serum, and by rabbit lung angiotensin-converting enzyme were analyzed by reverse-phase HPLC in an isocratic system [system I in (22)] and a gradient system [system II in (22)]. The isocratic elution was with 79:21 (v/v) 50 mM H_3PO_4 (adjusted to pH 3.50 with triethylamine):acetonitrile at ambient temperature (21°C) at a flow rate of 1.0 ml per min; elution was from a 3.9-mm × 30-cm Waters Microbondapak (10 μm) column. The gradient elution used the same column with a linear gradient from 100 of 1% (v/v) H_3PO_4 (adjusted to pH 3.00 with triethylamine) to 5:95 (v/v) triethylamine phosphate (pH 3.00):acetonitrile. The gradient elution was at room temperature (21°C) at a flow rate of 2.0 ml per min. In the HPLC analyses of STRS-2 cleavage products, all reagents and solvents were HPLC grade. It was important to use fresh, high-quality triethylamine in the gradient system [system II in (22)]. In the enzyme incubations for HPLC analysis, the following conditions were used, all at 37°C: human kidney renin, 0.181 mM STRS-2 in 50 mM triethylamine phosphate, pH 6.01, and 0.011 mg/ml enzyme; human liver cathepsin D, 0.187 mM STRS-2 in 0.25 M sodium formate, pH 3.21, with 7800 ng/ml enzyme; and 0.279 mM STRS in 0.25 M sodium formate, pH 3.20, with 7500 ng/ml enzyme; human serum, 0.19 mM STRS-2 in lyophilized human serum reconstituted with 50 mM triethylamine phosphate, pH 5.92 (the serum was reconstituted to its original volume, and contained 20 mM NaN_3; its pH was 6.36); rabbit lung angiotensin-converting enzyme, 0.0238 mM STRS-2 in 0.10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) adjusted to pH 7.50 with NaOH, 0.3 M KCl, 0.1 mM $ZnCl_2$, and 0.01 mM bovine serum albumin (Pentex monomer standard, Miles) with 0.011 mg/ml enzyme. Enzyme incubations were terminated either by freezing at −20°C or by injection onto the HPLC column.

RESULTS

The tetradecapeptide analogs of horse angiotensinogen (STRS) and human angiotensinogen (STRS-2) were readily cleaved by human kidney renin. Summarized in Table 2 are the Michaelis constants, K_M, and the velocity at infinite substrate concentrations, V_M, for the two substrates at 37°C and pH 7.2. The K_M for STRS-2 was 2.7-fold higher than for STRS but the maximal velocity was 6.8-fold higher. Thus the catalytic efficiency (28), herein defined as V_M/K_M, was 2.5-fold higher for STRS-2 than for STRS. Summarized in Fig. 1 are the results of a series of experiments

TABLE 2

KINETIC CONSTANTS FOR STRS AND STRS-2 AT 37°C

Enzyme	Substrate	V_M (μmol substrate cleaved per h per mg)	K_M (μM)	Catalytic efficiency V_M/K_M
Human kidney renin	STRS	1.66 ± 0.28	3.1 ± 0.9	0.54
Human kidney renin	STRS-2	11.3 ± 2.4	8.4 ± 2.5	1.35
Human cathepsin D[a]	STRS	30 ± 3	37 ± 7	0.81
Mouse submax. renin[b]	STRS	1280	36	36

[a] Measured at pH 3.2 in 0.25 M sodium formate. Other determinations at pH 7.2 in 0.1 M citrate phosphate.
[b] See Ref. (22).

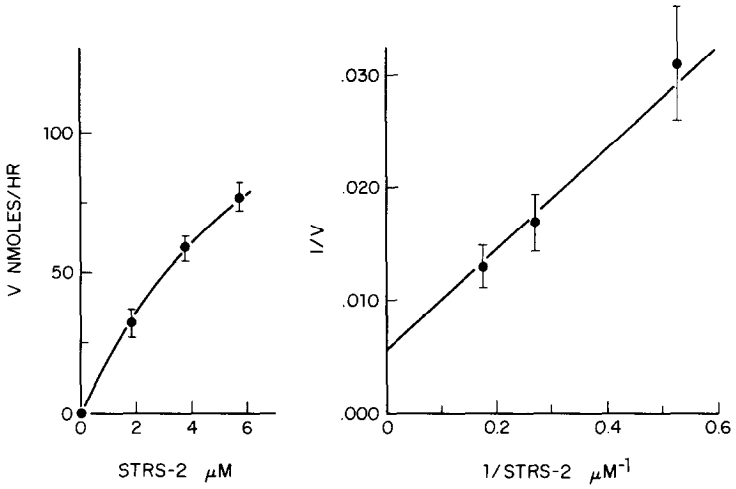

FIG. 1. Dependence of the rate v of cleavage of STRS-2 by human kidney renin upon STRS-2 concentration. Left-hand side is normal plot; right-hand side is inverse plot of $1/v$ versus inverse of STRS-2 concentration. Assays for 120 min at 37°C in 0.10 M citrate phosphate, pH 7.20, with 230 ng renin. The solid line connecting the data points is a theoretical curve for a K_M of 8400 nM and V_M of 185 nmol per hour.

used to measure the kinetic constants for the cleavage of STRS-2 by human kidney renin. Each experimental data point represented the average of two to five determinations. The solid line was a theoretical curve for a K_M of 8.4 μM and a V_M of 185 nmol/h.

Mouse submaxillary renin has a catalytic efficiency for STRS of 36 (see Table 2 and Ref. (22)), which is about 67-fold higher than for human kidney renin at pH 7.2 and 37°C. The V_M of mouse renin is 1280 μmol/h/mg and its K_M is 36 μM. Mouse renin was found to be inactive versus STRS-2. The fluorometric assay for STRS-2 cleavage was repeated with mouse submaxillary renin, and no significant cleavage was seen. Less than 0.13 nmol of STRS-2 were cleaved in 72 h at 37°C with 0.127 mM STRS-2 in 0.1 M citrate phosphate, pH 7.2, by 13,700 ng mouse renin. The specific activity of ≤0.13 nmol STRS-2 cleaved/h/mg renin was less than one-millionth the activity of mouse renin with 0.127 mM STRS [140,000 nmol/h/mg (22)].

Human cathepsin D was quite efficient in cleavage of the horse tetradecapeptide (STRS). Kinetic analyses by the fluorometric assay showed that the dependence of the rate of STRS cleavage by human cathepsin D upon STRS concentration could be well fit by the usual hyperbolic equation. The minor reaction that accounted for about 10% of the STRS cleavage (see HPLC analyses below) apparently did not interfere with the K_M and V_M determinations reported in Table 2. This lack of interference could have been due to the minor reaction having similar K_M and V_M values to the major reaction (which produced angiotensin I), or perhaps due to the minor reaction having a higher K_M for STRS so as to be less noticeable at the 10-fold lower STRS concentrations used in the fluorimetric assays, or possibly due to simply being lost in the experimental error.

The cleavage of STRS-2 by human kidney renin produced angiotensin I. An example of an experiment to demonstrate that angiotensin I is produced from STRS-2 by human kidney renin is replotted on Fig. 2. In the upper trace, 50 μl of 0.19 mM STRS-2 in 50 mM triethylamine phosphate, pH 6.01, was injected onto the HPLC column. The large, broad peak centered at 12.4 min corresponded to STRS-2; the small peaks at 3.0 and 3.6 min were buffer and a STRS-2 contaminant, respec-

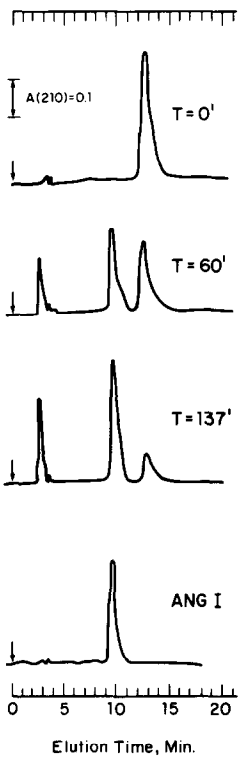

FIG. 2. HPLC analysis of STRS-2 cleavage by human kidney renin. Upper trace, STRS-2 before enzyme addition. Middle traces, STRS-2 after cleavage by human kidney renin for 60 and 137 min, respectively. Lower trace, human angiotensin I. Injections at arrow. HPLC analysis by reverse-phase chromatography on C18 Waters Microbondapak column in 79:21 triethylamine phosphate (pH 3.50):acetonitrile at room temperature.

tively. Upon incubation of 0.181 mM STRS-2 with 0.011 mg/ml human kidney renin for 60 min (see second trace in Fig. 2) at 37°C, appreciable cleavage of STRS-2 has occurred, as shown by the decrease in size of the peak at 12.4 min. Concomitant with the decrease in the 12.4-min peak, there appeared two large peaks centered at 2.6 and 9.6 min. As shown in the lower trace in Fig. 2, the 9.6-min peak corresponded to angiotensin I. The lower trace was 50 μl of 0.144 mM angiotensin I in the enzyme buffer. The 2.6-min peak may be H-Val–Ile–His–Ser-OH. After enzyme incubation for 137 min, appreciably less STRS-2 was seen, and more of the 2.6- and 9.6-min peaks was seen. No other major products were seen, and the amount of angiotensin I formed was quantitatively consistent with the amount of STRS-2 cleaved.

A second HPLC system, featuring a gradient elution from the Waters C18 column, also revealed that angiotensin I was produced during STRS-2 cleavage by human kidney (data not shown).

Human liver cathepsin D showed a slight ability to cleave STRS-2, but did not produce any detectable angiotensin I. Using the same HPLC system used in Fig. 2 to analyze for angiotensin I, human liver cathepsin D was shown to produce less than 6.2 pmol angiotensin I per hr per mg enzyme at 37°C in 0.25 M sodium formate pH 3.2. The results of a 60-min incubation of 0.187 mM STRS-2 with 7800 ng/ml enzyme have been replotted as trace A in Fig. 3. This trace was identical to the one obtained at the start of the incubation. The cleavage of STRS-2 was 4.1 nmol per h per mg enzyme, with 0.127 mM STRS-2. In HPLC system II, there were four principal products of STRS-2 cleavage which eluted at 8.1, 8.5, 9.3, and 11.5 min. The latter product co-chromatographed with human angiotensin II in this HPLC system. The other three products have not been identified. Thus, the human liver cathepsin D had a much lower rate of cleavage of STRS-2 than did human kidney renin.

The human tetradecapeptide, STRS-2, was resistant to cleavage by human serum. Replotted in traces B and C were 0- and 60-min incubations at 37°C of 0.19 mM STRS-2 in reconstituted human serum, respectively. Clearly, there was no detectable change (within ±5%) in the area under the peak eluting at 12.4 min (which corresponded to STRS-2) during the incubation, despite appreciable changes in the peaks between 2 and 4 min.

The incubation of STRS with pure human cathepsin D led to two cleavages, as may be seen in the HPLC data replotted for Fig. 4. The upper and lower traces in Fig. 4, respectively, corresponded to 0- and 60-min incubations. The broad peak centered near an elution time of 51 min corresponded to 0.279

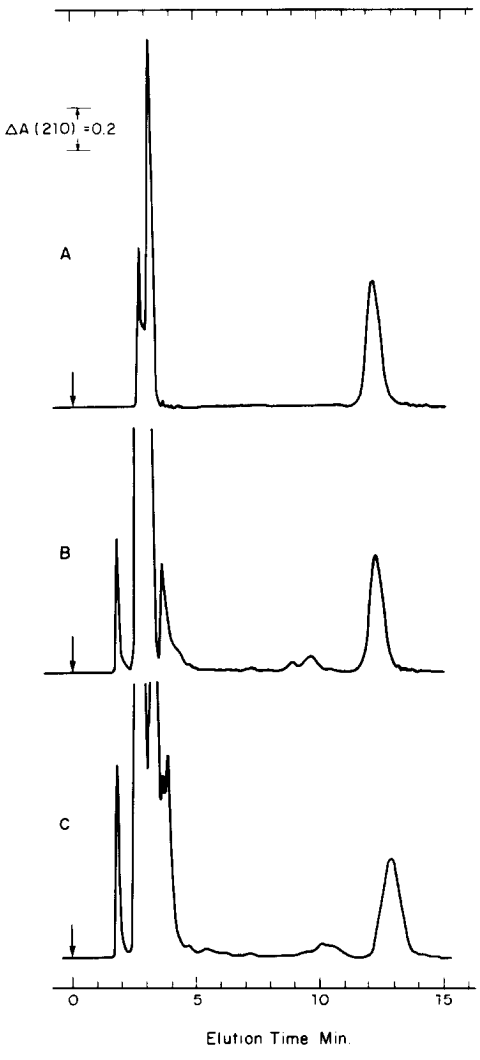

FIG. 3. HPLC analyses of cleavage of STRS-2 by human liver cathepsin D and reconstituted human serum. Upper trace (A), HPLC analysis of 60-min incubation of 0.187 mM STRS-2 with 0.011 mg/ml human liver cathepsin D in 0.25 M sodium formate at pH 3.21 at 37°C. Middle (B) and lower (C) traces, respectively, HPLC analyses of 0- and 60-min incubation of 0.19 mM STRS-2 in lyophilized human serum reconstituted with 50 mM triethylamine phosphate; incubations at pH 6.36 and 37°C. HPLC analyses as in Fig. 2.

mM STRS, while the peaks centered at 2.9 and 3.1 min corresponded to enzyme and formate buffer. After the 60-min incubation, angiotensin I (8.9 min) and H-Leu–Val–Tyr–Ser-OH (3.6 min), which were the products of renin cleavage of STRS (22), were produced in quantities consistent with the fluorometric enzyme assay data. Another cleavage product was seen which eluted at 25 min. This small peak probably represented material intermediate in size between STRS and angiotensin I, but remained unidentified due to the lack of appropriate authentic peptides. This unknown material had an area under its elution peak 12% of that of the angiotensin I peak. Since this material probably had a molar extinction at 210 nm slightly larger than the molar extinction at 210 nm of angiotensin I, it was estimated that 90% of the cleavage of STRS by human cathepsin D went to angiotensin I and H-Leu–Val–Tyr–Ser-OH in this experiment.

The HPLC analyses of incubations of STRS-2 with rabbit lung angiotensin-converting enzyme revealed a slow cleavage of STRS-2, at an initial rate of 0.21 ± 0.04 mmol/h/g enzyme. No detectable angiotensin I was seen (data not shown). In one experiment, less than 76 nM angiotensin I was seen after cleavage of 13,000 nM STRS-2 by the converting enzyme. This lack of detectable angiotensin I can probably be attributed to the high rate of angiotensin I cleavage by converting enzyme, near 400 mmol/h/g enzyme (29,30) at pH 7.8 in 50 mM Tris–HCl 30 mM NaCl 0.4 mM angiotensin I at 37°C, which would thus rapidly cleave any angiotensin I produced from STRS-2. Since the rabbit lung and human (31) converting enzyme have similar kinetic properties, it seemed likely that human-converting enzyme also would not produce much angiotensin I from STRS-2.

DISCUSSION

It was clear that the synthetic tetradecapeptide renin substrate STRS-2, which contained the same 13 amino-terminal residues as human angiotensinogen (5) but had a carboxy terminal serine to replace a suggested site of carbohydrate attachment, was a good substrate for human kidney renin. Quinn and Burton (32) have made a tridecapeptide renin

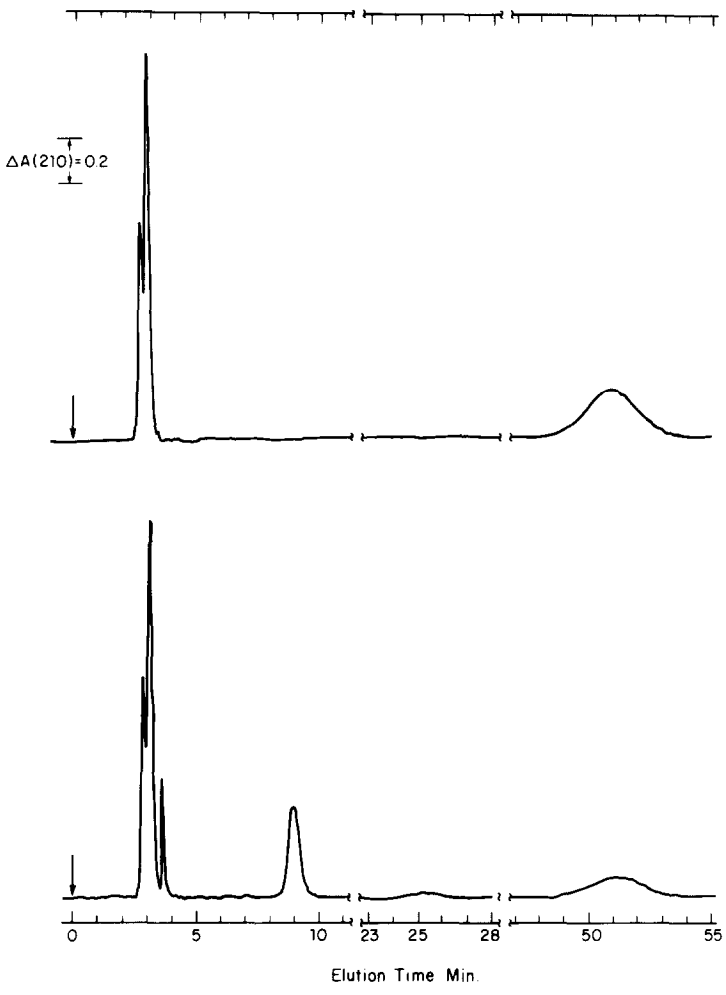

FIG. 4. HPLC analyses of STRS cleavage by human liver cathepsin D. Upper and lower traces, respectively, analyses of 0- and 60-min incubation of 0.279 mM STRS with 7800 ng cathepsin D per ml in 0.25 M sodium formate, pH 3.2, at 37°C. The HPLC traces between 11 and 23 min and between 28 and 47 min showed no peaks and are omitted here. HPLC analyses as in Fig. 2.

substrate corresponding to the amino terminal 13 residues of human angiotensinogen; their substrate had the same K_M and V_M when cleaved by human renin as for the horse tetradecapeptide, i.e., the same catalytic efficiency. Their tridecapeptide was highly resistant to cleavage by dog renin. Quinn and Burton (32) did not test for cleavage of their tridecapeptide by cathepsin D or angiotensin-converting enzyme, so it was not clear whether their tridecapeptide had the same enzyme specificity as STRS-2.

Angiotensin I was generated by human kidney renin action upon STRS-2 with a catalytic efficiency 2.5-fold higher than for the commonly used synthetic tetradecapeptide renin substrate, STRS. Like human angiotensinogen (33), STRS-2 was not susceptible to cleavage by mouse renin. STRS-2 would therefore not be expected to be cleaved by other non-primate renins.

One of the difficulties associated with the use of STRS as a renin substrate is that it is readily cleaved by cathepsin D to form angiotensin I (18–20). This was confirmed in our own work (see Table 2 and Fig. 4). STRS is also cleaved by other enzymes like angiotensin-converting enzyme (21) to form other

products. Since cathepsin D is difficult to separate from renin (10), it is useful to have a renin substrate resistant to its action. The human tetradecapeptide is much more resistant to attack by human cathepsin D than the horse tetradecapeptide. Furthermore, no detectable angiotensin I is produced upon incubation of the human tetradecapeptide with human cathepsin D, with reconstituted human serum, or with rabbit liver angiotensin-converting enzyme.

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