Rat Angiotensinogen and Des(angiotensin I)angiotensinogen: Purification, Characterization, and Partial Sequencing†

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ABSTRACT: Rat angiotensinogen was completely purified by a six-step procedure including (1) ammonium sulfate precipitation, (2) affinity chromatography on Affi-gel blue, (3) chromatography on DEAE-Sephacel, (4) chromatography on hydroxylapatite, (5) chromatography on Ultrogel AcA 54, and (6) isoelectric focusing. Two peaks of pure angiotensinogen were obtained, distinguishable by their isoelectric points (4.55 and 4.75). Both contained 23 μg of angiotensin I/mg of protein. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the peak with pI = 4.55 revealed two protein bands (respectively M_r 57 000 and 59 000) and a single protein band (M_r 57 000) for the peak with pI = 4.75. The molecular weight of the latter homogeneous form, as determined by sedimentation equilibrium, was 55 000. Only one immunoprecipitin line was observed when antiserum reacted with the heterogeneous angiotensinogen in Ouchterlony gels. The first 17 amino acids of the N-terminal region of the angiotensinogen with pI = 4.75 are reported. The amino acids in positions 10 and 11 which correspond to the renin cleavage site are leucyl-leucyl. The des(angiotensin I)angiotensinogen obtained after hydrolysis of angiotensinogen with pure mouse submaxillary gland renin was found to consist of a single protein band with an M_r of 56 000 as revealed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Only one N-terminal residue (leucyl) was obtained for this des(angiotensin I)angiotensinogen. These findings establish that renin only cleaves angiotensinogen at a single site.

Angiotensinogen, a plasma protein synthesized by the liver (Nasjletti & Masson, 1971), is split by the enzyme renin (EC 3.4.99.19), thus releasing the decapeptide, angiotensin I. The latter is then converted by a dipeptidylcarboxypeptidase to angiotensin II which plays a major role in blood pressure homeostasis and hydromineral metabolism control.

Angiotensinogen is a limiting factor of the renin–substrate reaction in the rat (Mênard & Catt, 1972) and in humans (Gould & Green, 1971). For small concentrations of substrate, the rate of reaction is proportional to the substrate concentration. Moreover, there is a specificity for the substrate since human angiotensinogen is only cleaved by human and primate renins (Braun-Menedez et al., 1946); this may suggest differences in the primary structure of angiotensinogen molecules. Finally, it has been reported that the renin–substrate reaction may also be subject to various activating (Sambhi et al., 1975) or inhibitory plasma factors [see reviews from Poulsen (1973) and Oparil & Haber (1974)]. Thus, the importance of angiotensinogen prompted us to purify it completely in a laboratory animal such as the rat.

Angiotensinogen was purified for the first time from hog plasma (Skeggs et al., 1963). Since then, several attempts have been made to purify angiotensinogen from different species (Rosenthal et al., 1971; Skinner et al., 1975; Eggena et al., 1976; Printz et al., 1977; Morris et al., 1979; Tewksbury et al., 1977), and this was done from human plasma by Dorer et al. (1978) and by Tewksbury et al. (1979) and from rat plasma by Hilgenfeldt et al. (1980). The sequence of the N-terminal amino acids determined for horse (Skeggs et al., 1957) and human angiotensinogen (Tewksbury et al., 1979) made it possible to establish that to release angiotensin I, renin must cleave a leucyl–leucyl bond in the case of horse angiotensinogen and a leucyl–valyl bond for human angiotensinogen.

For a greater insight into the characteristics of angiotensinogen, the aim of this work was to determine the main physicochemical properties of rat angiotensinogen and to prepare and characterize des(angiotensin I)angiotensinogen.

Materials and Methods

Materials

Male Wistar rats (300 g) were bilaterally nephrectomized and bled 24 h later. Blood was taken from the aorta under ether anesthesia and collected in tubes containing Na_2EDTA[1] (final concentration 0.2%) and the plasma separated by centrifugation. Hog renin (specific activity 0.87 Golblatt unit/mg of protein) was partially purified up to step 4 of the method of Skeggs et al. (1967). Pure mouse submaxillary gland renin (specific activity 50 Golblatt units/mg of protein) was prepared in this laboratory according to Cohen et al. (1972). All chemicals were of reagent grade.

Methods

Purification of Angiotensinogen. All purification steps were carried out at 4 °C. Plasma (250 mL) was diluted with 250 mL of distilled water in the presence of 2 mM phenylmethanesulfonyl fluoride (PMSF) and 5 mM sodium tetrathionate. Silicic acid was added to the plasma as described by Hilgenfeldt et al. (1980). The fraction containing angiotensinogen precipitated between 1.5 and 2.4 M ammonium sulfate, and the precipitate, after having been dissolved in water, was dialyzed for 16 h against 5 L of water containing 10 mM Na_2EDTA. Insoluble material was then removed by centrifugation.

The protein solution was applied to a blue dextran–Sepharose column (Affi-gel blue, Bio-Rad) equilibrated with 0.05 M Tris-HCl, pH 8.0, with 0.1 M NaCl (Tewksbury et al.,

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[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; Tris, tris(hydroxymethyl)-aminomethane; $NaDodSO_4$, sodium dodecyl sulfate.

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1977). Fractions of 12 mL were eluted with the same buffer, and those containing the highest specific activity of angiotensinogen were pooled and applied to a DEAE-cellulose ion-exchanger column (DEAE-Sephacel, Pharmacia) equilibrated with 0.05 M Tris-HCl, pH 8.0, with 0.1 M NaCl. The column was eluted with a linear gradient of NaCl from 0.1 to 0.35 M in 0.05 M Tris-HCl, pH 8.0, as described by Tewksbury et al. (1977). The peak fractions were pooled and applied to a hydroxylapatite column (Hypatite C, Clarkson Chemical) as described by Tewksbury et al. (1977). Elution was performed with 0.035 M phosphate buffer, pH 6.80. The fractions with the highest specific activity were concentrated by ultrafiltration to 1.6 mL and applied to an agarose–polyacrylamide gel (Ultrogel AcA 54, LKB) column (1 × 100 cm) equilibrated and eluted with 0.1 M phosphate buffer, pH 6.5. Peak fractions were pooled, dialyzed against 1 mM phosphate buffer, pH 7.0, and submitted to preparative isoelectric focusing in an LKB column (110 mL) with 1% Ampholine, pH 4–6 (Vesterberg & Svenson, 1976); 500 V were applied for 36 h at 5 °C. The column was emptied by using a pump, and 1-mL fractions were collected. The pH, optical density, and angiotensinogen concentration were determined in all fractions.

Preparation of Des(angiotensin I)angiotensinogen. Pure angiotensinogen (0.6 mg of protein) was incubated with 0.5 μg of pure mouse submaxillary gland renin for 180 min at 37 °C in 0.2 M phosphate buffer, pH 6.5, containing 10 mM Na_2EDTA and 3 mM PMSF. Angiotensin I was completely removed by dialysis, as verified by the addition of [^{125}I]angiotensin I as tracer. The amount of renin used was sufficient since subsequent hydrolysis under the same conditions no longer released any angiotensin I.

Physicochemical Characterization. Polyacrylamide gel electrophoresis was performed with Buchler equipment. Acrylamide and bis(acrylamide) (Sigma) were recrystallized from acetone. The tracking dye was bromophenol blue (Aldrich). Proteins in the gel were stained with Coomassie blue R 250 (Eastman) (Vesterberg, 1971). Glycoproteins were stained by the method of Zaccharius et al. (1969). For the identification and quantification of angiotensinogen, gels were cut transversely into 0.5-mm slices with a gel fractionator (Gilson), and angiotensinogen was eluted by diffusion overnight at 4 °C in 1 mL of 0.2 M phosphate buffer, pH 6.5. $NaDodSO_4$–gel electrophoresis was performed as described by Weber & Osborn (1969) in 10% acrylamide gels. Angiotensinogen or des(angiotensin I)angiotensinogen was incubated at 37 °C for 2 h with 1% mercaptoethanol and 1% $NaDodSO_4$ before application to the gel. Calibrating curves for molecular weight determinations were constructed with standard proteins (Galen et al., 1979).

Analytical Gel Filtration. For determination of the molecular weight of angiotensinogen, it was cochromatographed with 4 mg of albumin, ovalbumin, and α-chymotrypsinogen on an Ultrogel column (AcA 54, LKB). The column was developed in 0.1 M phosphate buffer, pH 6.5, and fractions of 1.0 mL were collected for protein and angiotensinogen determination.

Sedimentation Equilibrium. Studies were performed with a Spinco Model E analytical ultracentrifuge equipped with interference optics. The temperature was controlled by an RTIC unit. Angiotensinogen was exhaustively dialyzed for 40 h against 0.050 M sodium phosphate buffer, pH 6.80, containing 0.15 M sodium chloride, before the experiments. Equilibrium measurements were made at 23 150 rpm and 20 °C until the data did not change with time. The results reported in this paper were obtained after 48 h of centrifugation. Data were plotted as ln c vs. r^2, and molecular weights were calculated according to

$$M_r = \left(\frac{2RT}{(1 - \bar{v}\rho)\omega^2}\right)\left(\frac{\mathrm{d} \ln c}{\mathrm{d}r^2}\right)$$

Partial specific volume $\bar{v}$ was assumed to be 0.720 as reported for the human angiotensinogen, a glycoprotein (Tewksbury et al., 1978).

Amino-Terminal Sequence Analysis. A 9–27 nmol sample of angiotensinogen or des(angiotensin I)angiotensinogen was analyzed by automated Edman degradation in a Beckman Model 890C sequencer. Polybrene and glycylglycine were submitted to three complete degradation cycles before sample loading (Klapper et al., 1978; Hunkapiller & Hood, 1978). The degradation program was the one previously described (Brauer et al., 1975) using 0.1 M Quadrol and 5% PITC with single cleavage. Complete conversion of the phenylthiazolinone derivatives was obtained with 20% trifluoroacetic acid. The phenylthiohydantoin derivatives were identified by high-pressure liquid chromatography on Waters apparatus equipped with a C18 μBondapak using a sodium acetate–methanol gradient (Zeeuws & Strosberg, 1978).

Productions of Antibodies and Immunodiffusion. Antibodies were raised against pure angiotensinogen (peak C, Figure 2) in three Fauve de Bourgogne rabbits according to the method of Vaitukaitis et al. (1971). Every second month, 50 μg of angiotensinogen, diluted in 0.5 mL of 0.9% NaCl and mixed with an equal volume of Freund's complete adjuvant for the first injection and incomplete adjuvant for the subsequent injections, was injected intradermally at 15–20 sites on the back and neck of the rabbits. Double immunodiffusion was performed according to Ouchterlony (1953).

Iodination of Angiotensinogen. Pure angiotensinogen (3 μg) was iodinated according to Greenwood et al. (1963). Chloramine T was used as an oxidizing agent, and sodium metabisulfite was added to prevent any subsequent iodination. Iodinated angiotensinogen (specific activity 31 μCi/μg) was separated from free iodine on a Sephadex G-25 (Pharmacia) column and applied to an Ultrogel AcA 54 (LKB) column. A single symmetrical peak was found with an apparent molecular weight of 59 000. Angiotensinogen binding to antiserum was tested in phosphate buffer, pH 7.5. [^{125}I]Angiotensinogen was incubated with various dilutions of rabbit antiserum for 24 h at 4 °C, and precipitation of bound angiotensinogen was accomplished in the presence of 1 mg of bovine γ-globulin (Sigma) by using 1.0 mL of a 20% solution of poly(ethylene glycol) 6000 (Fluka) in 0.1 M phosphate buffer, pH 7.5.

Optimum pH of Renin for Pure vs. Unpurified Angiotensinogen. The pH dependency of rat renin was determined on pure angiotensinogen by comparison with a pool of bilaterally nephrectomized rat plasma. Buffer pH ranged from 4 to 6.5 (100 mM citrate–phosphate) and from pH 7 to 9 (100 mM phosphate). The renin source was a plasma from water-deprived rats with a renin content 5 times that of normal animals. Incubations were carried out at 37 °C for 1 h with Na_2EDTA and PMSF as indicated below.

Angiotensinogen Assays. Angiotensinogen was determined by enzymatic assay. Samples were incubated for 60 min at 37 °C with 0.5 mg of hog renin in 0.2 M phosphate buffer containing 0.05 M Na_2EDTA (pH 6.5), and 2.8 mM PMSF, and liberated angiotensin I was measured by radioimmunoassay (Mēnard & Catt, 1972).

Protein Assays. Proteins were measured by the method of Lowry et al. (1951). At protein concentrations below 200

Table I: Purification of Rat Angiotensinogen[a]

step	total protein (mg)	angiotensinogen: μg of AI/mg of P	angiotensinogen: total AI (μg)	recovery (%)	purification factor
nephrectomized rat plasma	19000	0.06	1102	100	1
1. ammonium sulfate precipitation	6184	0.17	1033	94	3
2. Affi-gel blue	944	0.68	641	58	12
3. DEAE-Sephacel	200	2.52	484	44	43
4. hydroxylapatite	23	11.10	256	23	191
5. Ultrogel AcA 54	6.8[b]	17.17	117	11	296
6. isoelectric focusing, pH 4–6	3.4[c]	23.0	80	7	405

[a] Summary of a purification run out of six. AI, angiotensin I. [b] Protein concentration measured by Lowry's method. [c] Protein concentration measured by fluorescamine method.

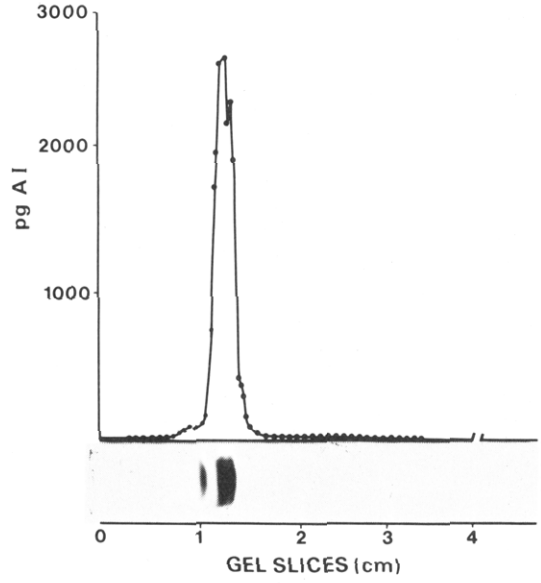

FIGURE 1: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of rat angiotensinogen during purification after step 5, gel filtration on Ultrogel AcA 54. (Lower part) Gel stained with Coomassie blue showing three protein bands; (upper part) the corresponding angiotensin I content of 0.5-mm gel slices after incubation with hog renin. Only the two fastest bands contain angiotensin I.

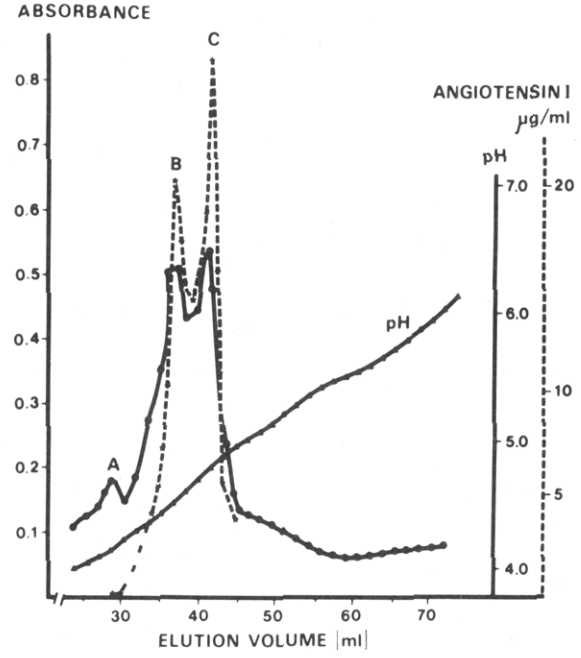

FIGURE 2: Preparative isoelectric focusing of rat angiotensinogen. The sample obtained after Ultrogel AcA 54 filtration was applied to an LKB column with 1% Ampholine (pH 4–6). After electrophoresis, 1-mL fractions were collected for determination of pH (▲), angiotensin I content after incubation with renin (---), and absorbance at 280 nm (●). High background absorbance was due to the presence of ampholine.

μg/mL, the fluorescamine (Fluram, Roche) method was used (Undenfriend et al., 1972; Galen et al., 1979).

Results

Angiotensinogen Purification. As shown in Table I, pure angiotensinogen was obtained after a 400-fold purification with an overall yield of 7%. After the fifth step (Ultrogel filtration), the specific angiotensin I content of angiotensinogen was 17.2 μg/mg of protein. The analysis by $NaDodSO_4$–polyacrylamide gel electrophoresis of this fraction revealed two main proteins and another larger molecular weight protein (Figure 1). Elution of these proteins from the gel slices and determination of their angiotensin I content after incubation with renin showed that only the two lower molecular weight proteins produced angiotensin I.

The sixth step (preparative isoelectric focusing) separated two peaks of angiotensinogen (peaks B and C, Figure 2) from the contaminating protein (peak A, Figure 2). The mean isoelectric points for six different preparations were 4.55 ± 0.23 for peak B and 4.75 ± 0.27 for peak C. After step 6, the theoretical angiotensin I content (23 μg/mg of protein) for a pure angiotensinogen molecule was obtained for both.

Physicochemical Properties of Angiotensinogen. Molecular Weight. The apparent molecular weight as determined by gel filtration of angiotensinogen (peaks B and C, Figure 2) was 59 000. Each of these peaks (B and C) was analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis. Angiotensinogen with a higher isoelectric point (peak C) appeared as a single protein band with a molecular weight of 57 000 (Figure 3). However, angiotensinogen with a lower isoelectric point (peak B) exhibited two protein bands, one with a molecular weight of 59 000 and the other with a molecular weight of 57 000. When the gels were stained for glycoprotein, all the angiotensinogen proteins were visualized. The molecular weight of the form with pI = 4.75 (peak C) obtained by sedimentation equilibrium was 55 000 ± 3000. A good linearity of the slope was observed when ln c vs. r^2 was plotted. Since the angiotensinogen with pI = 4.75 was homogeneous, all further studies were carried out with this preparation.

Amino-Terminal Sequence. The N-terminal region of angiotensinogen was determined on four separate preparations. Analysis of the first 17 steps is indicated in Figure 4. A comparison with the published NH_2-terminal sequences for horse and human is given.

Immunological Assays. Antisera selected for immunological studies gave 50% binding of iodinated angiotensinogen (75 pg) at a final dilution of 1/100000. An excess of antiserum (1/5000) led to total binding. Only a single immunopreci-

FIGURE 3: $NaDodSO_4$–polyacrylamide gel electrophoresis of purified angiotensinogen with a p*I* of 4.75 (peak C of Figure 2). Coomassie blue staining reveals a single protein band (M_r 57 000).

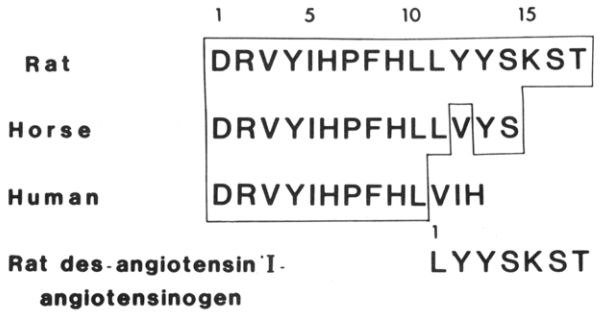

FIGURE 4: Comparison of the N-terminal sequences of rat (this work), horse (Skeggs et al., 1963), and man (Tewksbury et al., 1979) angiotensinogen. Note that angiotensin I, which comprises the first 10 residues, is identical in the three species. (Last line) Rat des(angiotensin I)angiotensinogen. The new amino acid in the N-terminal sequence of des(angiotensin I)angiotensinogen is leucine.

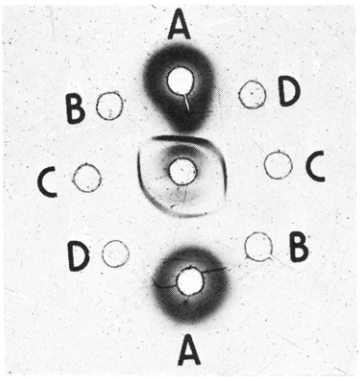

FIGURE 5: Ouchterlony method. The antiserum was in the center well; the surrounding wells contained (A) binephrectomized rat plasma, (B) nothing, (C) pure angiotensinogen, and (D) angiotensinogen during purification (step 5).

pitation line was observed when antiserum reacted with angiotensinogen in Ouchterlony gels (Figure 5).

Optimum pH of rat renin was the same (6.5) for pure angiotensinogen and for unpurified angiotensinogen corresponding to bilaterally nephrectomized rat plasma (Figure 6).

Preparation and Characterization of Des(angiotensin I)-angiotensinogen. The hydrolysis of pure angiotensinogen by pure mouse submaxillary gland renin was complete since a second incubation with the same renin did not release further angiotensin I. $NaDodSO_4$–polyacrylamide gel electrophoresis

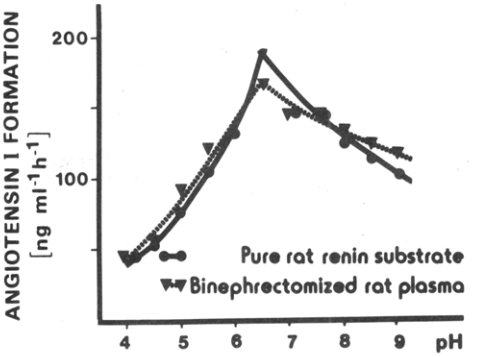

FIGURE 6: Determination of optimum rat renin pH for pure (●) and unpurified (▼) angiotensinogen. Plasma with a high renin content from water-deprived rats (2.5 μL) as source of renin was incubated with 20 μg of purified angiotensinogen or with 0.2 mL of binephrectomized rat plasma at all the specified pHs for 1 h at 37 °C.

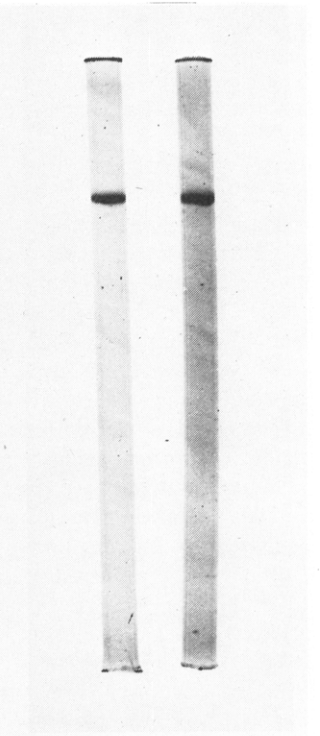

FIGURE 7: $NaDodSO_4$–polyacrylamide gel electrophoresis of rat angiotensinogen (left side) and des(angiotensin I)angiotensinogen (right side). Des(angiotensin I)angiotensinogen was obtained after angiotensinogen incubation with pure mouse submaxillary gland renin. A single protein band (M_r 56 000) appeared with Coomassie blue staining.

of des(angiotensin I)angiotensinogen revealed a single protein band with a molecular weight of 56 000 (Figure 7).

N-Terminal sequence analysis confirmed that the first residue of des(angiotensin I)angiotensinogen corresponding to the 11th residue of the intact angiotensinogen molecule is leucine and that the next 6 amino acids were the same as for this angiotensinogen molecule (Figure 4). A single immunoprecipitin line was observed when antiserum reacted with des(angiotensin I)angiotensinogen in Ouchterlony gels (Figure 8).

Discussion

The six-step procedure used in this work allowed us to purify rat angiotensinogen completely. Bilaterally nephrectomized rat plasma offers the advantage of starting with a renin-free plasma with an angiotensinogen content 3–4 times that of normal animals. The purification procedure followed differs

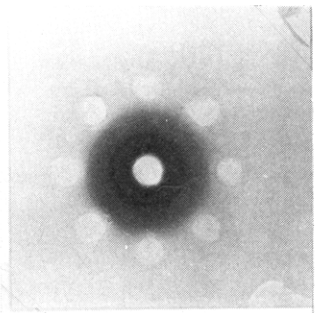

FIGURE 8: Ouchterlony method. The antiserum was in the center well; the surrounding wells contained des(angiotensin I)angiotensinogen.

notably from that described by Hilgenfeldt et al. (1980). Hydroxylapatite used by Tewksbury et al. (1977) proved very effective. The last step of purification was isoelectric focusing which allowed separation of two angiotensinogen peaks with an angiotensin I content of 23 μg/mg of protein as determined by RIA. This value is quite close to the theoretical value of 22.7 expected for an angiotensinogen with a molecular weight of 57 000.

The two peaks of angiotensinogen differ in their isoelectric points 4.55 and 4.75 and in their M_r as determined by NaDodSO$_4$–polyacrylamide gel electrophoresis. The peak with pI = 4.55 contained two protein bands with M_r 57 000 and 59 000, and the peak with pI = 4.75 contained a single protein band with M_r 57 000. This might be due to differences in their carbohydrate content which could produce a slight change in molecular weight since carbohydrates greatly affect molecular weight determination (Andrews, 1965). Hilgenfeldt et al. (1980), who also started from bilaterally nephrectomized rat plasma, only obtained one form of angiotensinogen. However, the number of components found may largely depend on the resolving power of the technique used, as isoelectric focusing which was not employed by Hilgenfeldt et al. (1980) separated the angiotensinogen into two peaks in this work. Such focusing has been used to demonstrate the presence of multiple forms of hog (Printz et al., 1977), rat (Faiers et al., 1977), rabbit (Printz et al., 1977), sheep (Printz et al., 1978), and human (Lentz et al., 1978; Faiers et al., 1978) plasma angiotensinogens. Importantly, these multiple forms have been demonstrated in both partially purified preparations of angiotensinogen and unfractionated plasmas, thus confirming that they are not artifacts of the purification procedure. Moreover, Gordon et al. (1976) in normal human plasma and Eggena et al. (1977) in pregnant woman plasma detected slower migrating forms by using polyacrylamide gel electrophoresis.

The characterization of rat angiotensinogen and amino acid sequence of the N-terminal region were determined on the form with pI = 4.75, because it appeared as a single protein band. The physical properties of rat angiotensinogen proved similar to those reported for hog and human molecules. Thus, the molecular weight around 57 000 reported by Skeggs et al. (1963) for hog, Tewksbury et al. (1978) for man, Hilgenfeldt et al. (1980) for rat, and Skinner et al. (1975) for a semipurified sheep angiotensinogen preparation is the same as that found here (M_r 55 000 by sedimentation equilibrium or M_r 57 000 by NaDodSO$_4$ electrophoresis). However, Eggena et al. (1976) reported a higher molecular weight (M_r 110 000) for human angiotensinogen. This may well depend upon the experimental techniques. All angiotensinogens examined so far are glycoproteins. The amino acid composition of angiotensinogen is similar for rat (Hilgenfeldt et al., 1980), hog (Skeggs et al., 1963), and man (Tewksbury et al., 1978). However, there are important differences in its primary structure. The NH_2-terminal sequence of the first 17 amino acids for rat angiotensinogen is described here for the first time. The initial 11 amino acids are the same as those found by Skeggs et al. (1957) for horse renin substrate. In the present work, leucine was the 11th amino acid determined by sequencing angiotensinogen, and it was also the N-terminal of the des(angiotensin I)angiotensinogen obtained by incubation of rat angiotensinogen with purified submaxillary gland renin. Moreover, the fact that a single N-terminal residue is obtained for des(angiotensin I)angiotensinogen and that the M_r of this product is close to 56 000 establish beyond doubt that renin cleaves angiotensinogen at one site only.

For human angiotensinogen, the 1–10 sequence is the same as for horse, hog, and rat, but the 11th amino acid is a valine residue (Tewksbury et al., 1979). This difference may explain why rat angiotensinogen, which contains the same Leu^{10}–Leu^{11} bond as hog angiotensinogen, is well hydrolyzed by hog renin and human angiotensinogen is hydrolyzed by this renin only very slowly. The strict conservation of the sequence corresponding to angiotensin I strikingly contrasts with the variability observed after position 10 (Figure 4). In all cases studied the renin cleavage site is constituted by hydrophobic amino acids surrounded on both sides by charged amino acids.

Several authors have suggested the existence in plasma of either renin activators (Sambhi et al., 1975) or more often of inhibitors. Thus, it was reported that human renin was inhibited by various plasma substances such as proteins and peptides (Workman et al., 1974), α-1 antitrypsin (Scharpé et al., 1976), prostaglandins A_2 and E_2 (Kotchen et al., 1974; Eggena et al., 1975), and acetone-soluble lipids (Ostrovsky et al., 1967; Kotchen et al., 1975). However, Poulsen (1971), who studied the reaction between renin and its substrate in untreated plasma, did not find evidence of inhibitors or activators. In the present work, in which pure angiotensinogen was compared with nephrectomized rat plasma containing an equivalent quantity of angiotensinogen, no difference was found in the optimal pH of renin or in angiotensin I formation at each pH.

Thus, the availability of pure angiotensinogen and des(angiotensin I)angiotensinogen opens up a new field for investigation of the renin system by allowing kinetic studies with pure proteins. In addition, the production of highly specific antibodies against angiotensinogen makes possible the passive transfer of antibodies into rats.

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