Characterization of a Human Angiotensinogen Cleaved in Its Reactive Center Loop by a Proteolytic Activity from Chinese Hamster Ovary Cells*

Jérôme Célérieri‡§, Georg Schmidt¶, Jean-Pierre Le Caër†, Anne-Paule Gimenez-Roqueplo‡, Daniel Bur†, Arno Friedlein¶, Hanno Langen¶, Pierre Corvol‡, and Xavier Jeunemaître‡

From the ‡INSERM U36-Pathologie Vasculaire et Endocrinologie Rénale, Collège de France-Chaire de Médecine Expérimentale et d’Endocrinologie Rénale 3, rue d’Ulm 75005 Paris, France, ¶Hoffmann La Roche Ltd., Pharmaceutical Research Gene Technologies 124, Grenzacherstrasse CH-4070 Basel, Switzerland, and ¶Laboratoire de Neurobiologie et Diversité Cellulaire-CNRS UMR 7637-Ecole Supérieure de Physique et de Chimie Industrielles de la Ville de Paris 10, rue Vauquelin 75231 Paris, France

Angiotensinogen, the renin (E.C. 3.4.23.15) substrate, belongs to the serpins superfAMILY and has been classified as a noninhibitory serpin. Using mass spectroscopy, angiotensinogen purified from Chinese hamster ovary cell supernatant shows a broad spectrum. The absence of protease inhibitors throughout the purification leads to an angiotensinogen cleaved within the reactive center loop. This cleavage does not affect the Ang I generation because kinetic parameters are similar to the values of the full-length angiotensinogen. Although cleavage is complete, the cleaved angiotensinogen migrates after deglycosylation on SDS-polyacrylamide gel electrophoresis as a doublet differing by 4 kDa. To test whether the circulating angiotensinogen is cleaved in the reactive center loop, it was purified from a pool of human plasma and was shown to be uncleaved. Its migration was obviously slower than of cleaved angiotensinogen but also consisted of two bands pointing to a so far unexplained residual heterogeneity. We then compared the heat-induced polymerization of full-length and reactive center loop-cleaved angiotensinogens. Both monomers were able to aggregate, revealing a particular behavior of angiotensinogen distinct from that of reactive center loop-cleaved serpins. Lacking the threedimensional structure of angiotensinogen, we propose and discuss a structural model of the serpin fold within the renin substrate.

Angiotensinogen (AGT)1 is the unique angiotensin I (Ang I) precursor that, when cleaved by the aspartyl proteinase renin (E.C. 3.4.23.15), releases the inactive Ang I decapeptide. Human AGT is a 452-amino acid residue protein delivered in the circulation, mainly from the liver. The other product of renin (E.C. 3.4.23.15), releases the inactive Ang I decapeptide. Hu-

* This work was supported by grants from INSERM, Collège de France, and Hoffmann La Roche (Research Agreement 94128). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: INSERM U36-Col
de France, 3, rue d’Ulm 75005 Paris, France. Tel.: 33.1.44.27.16.75; Fax: 33.1.44.27.16.91; E-mail: jerome.celelier@college-de-france.fr.

‡ The abbreviations used are: AGT, angiotensinogen; hAGT, human AGT; Ang I, angiotensin I; CHO, Chinese hamster ovary; MALDI, matrix-assisted laser desorption ionization; TOF, time-of-flight; RCL, reactive center loop; AT, a1-antitrypsin; Tricine, N-(2-hydroxy-1,1-bis-(hydroxymethyl)ethyl)glycine.
partial C-terminal cleavage (19) could explain why these two bands persist even after extensive chemical or enzymatic deglycosylation (20). We provide here evidence for an in vitro RCL cleavage of human AGT and analyze its biochemical characteristics. Finally, we propose a three-dimensional model of the serpin fold of the renin substrate.

**EXPERIMENTAL PROCEDURES**

**Enzymes, Proteins, and Antibodies—**Human recombinant renin (21) was provided by Hoffmann-La Roche. Endoglycosidase H, neuraminidase, and O-glycosidase came from Roche Molecular Biochemicals; endoglycosidase F, F2, F4, and T, and 0.5 mg/ml gel) phenyl-Sepharose (Amersham Pharmacia Biotech) re- 

**Purification of AGT—**Starting from CHO cell supernatant was discarded, cells were washed twice with 1 liter of 

**RESULTS**

Cleavage of the Peptide Bond Gln111-Gln112 within the RCL of AGT by a Protease from CHO Cells—Starting from CHO cell supernatants in which AGT represents 20–40% of the total protein, the renin substrate was purified 2.5-fold with a yield of around 20% using a non-denaturing procedure. MALDI-TOF mass spectroscopy analysis was performed on each preparation. The spectrum of this glycosylated AGT is broad, expanding from m/z = 55000 to more than 60,000 with a mean m/z around 57,000–58,000 (Fig. 1A). No contaminating proteins were present in the mass range of 20–80 kDa.

In three preparations performed without any protease inhibit-
separation of small peptides in the range 1–20 kDa (27). Reducing and/or heat treatment did not release higher amounts. After transfer onto a polyvinylidene fluoride membrane, the 60-kDa band and the 4-kDa peptide were N terminus-sequence (Fig. 2B). The 60-kDa band revealed only one sequence corresponding to the mature AGT. For the 4-kDa peptide, three N-terminal sequences were detected; the most abundant began at Gln412, the second at Leu413, and the third at Asn414 (Fig. 2A). The 60-kDa band and the 4-kDa peptides were N terminus-sequence (Fig. 2B). Thus the initial site of cleavage was between Gln411 and Gln412, in accordance with the previous results obtained by MALDI-TOF mass spectroscopy (Fig. 1B).

**Influence of RCL Cleavage on Angiotensin I Generation**—Native AGT studied in this experiment has a specific activity of 26 μg of Ang I/mg of protein, whereas the RCL-cleaved AGT has a specific activity of 26.7 μg of Ang I/mg of protein; both values are close to the theoretical Ang I content of AGT, which is 25 μg of Ang I/mg of protein. In addition, initial velocity parameters for renin hydrolysis, determined using the full-length and the RCL-cleaved AGT, showed no significant difference (Table I). This shows that Ang I generation, located in the N terminus, is not affected by the cleavage in its RCL at the opposite end of the molecule.

**Although the RCL Cleavage Was Complete, AGT Migrated as Two Bands**—To detect AGT devoid of its 4-kDa C-terminal peptide following cleavage in the RCL, an AGT peptide antibody against the C-terminal end (C-1350) was produced. This antibody together with a N-terminal Ang I antibody (N-1345) (24) allowed the detection of the full-length AGT but not the RCL-cleaved AGT (Fig. 3A). For an unknown reason, the C-terminal antibody (C-1350) did not recognize the isolated 4-kDa peptide generated following cleavage in the RCL of AGT (data not shown). As expected, both native AGT and the RCL-cleaved AGT were equally responsive to the Ang I antibody, with a slight difference (around 5 kDa) in their respective apparent molecular masses due to the loss of C-terminal peptides. After extensive enzymatic deglycosylation, which in all cases of glycosidases (endoglycosidases F1 and H, neuraminidase, O-glycosidase) according to Gimenez-Roqueplo et al. (26) for 15–16 h at 37 °C in a 100 mM acetate buffer (pH 5.5) containing 1 mM β-mercaptoethanol were resolved by 9% SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting.

**TABLE I**

|                | Recombinant angiotensinogen | Native AGT | RCL-cleaved |
|----------------|----------------------------|------------|-------------|
| Vm (fmol of Ang I/s) | 1.8 ± 0.1 | 1.6 ± 0.1 | 1.0 ± 0.1 |
| Km (μM) | 0.76 ± 0.05 | 0.8 ± 0.1 | 0.8 ± 0.1 |
| kcat/Km (sec⁻¹·μM⁻¹) | 1.2 ± 0.2 | 1.0 ± 0.2 | 1.0 ± 0.2 |

**Fig. 2. N-terminal sequences of the 4-kDa peptides from the RCL-cleaved AGT.** A, G-250 Coomassie staining of a Tris-Tricine 10–20% gradient gel. The recombinant RCL-cleaved AGT (5 μg/lane) was analyzed. Reduced sample was unfolded in the presence of 10 mM dithiothreitol, and the heated sample was incubated at 100 °C for 3 min just before loading. Molecular mass standards are shown in kDa. B, N-terminal sequencing of the 60-kDa band and the 4-kDa band of RCL-cleaved AGT. Pure AGT (150 pmol) was loaded onto a Tris-Tricine 10–20% gradient gel and transferred onto a microporous Immobilon PSQ 0.1-μm membrane (Millipore). The membrane was Amido Black-stained, and the spots corresponding to the 60-kDa and 4-kDa peptides were cut out and submitted to the Edman degradation using a 494 protein sequencer (Perkin-Elmer). The amount of each detected amino acid sequence is expressed as a percentage.

**Fig. 3. C-terminal immunological responses of the native and the RCL-cleaved AGTs before and after deglycosylation.** Western blot of purified recombinant AGTs using our pair of terminal end polyclonal antibodies, N-1345 and C-1350, on glycosylated (A) and deglycosylated (B) AGTs. Untreated AGTs (100 ng/lane) and treated AGTs (250 ng/lane), which were extensively deglycosylated using a mixture of glycosidases (endoglycosidases F1 and H, neuraminidase, and O-glycosidase) according to Gimenez-Roqueplo et al. (26) for 15–16 h at 37 °C in a 100 mM acetate buffer (pH 5.5) containing 1 mM β-mercaptoethanol were resolved by 9% SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting.
bands were observed. For the full-length AGT, bands at 54 and 50 kDa were both responsive to N-1345 as well as C-1350, and for AGT cleaved within the RCL, bands at 48 kDa and 44 kDa were responsive only to N-1345. Our data reveal that human recombinant AGT migrates on SDS-polyacrylamide electrophoresis gels as a doublet, differing by 4 kDa even if it is more than 95% C terminus-cleaved.

Furthermore, we used this C terminus antibody to detect the step at which the RCL cleavage occurred during purification. CHO-produced AGT was intact in the serum-free medium because it was N and C termini, and the C-terminal response of AGT was lost during the third step of our purification (data not shown), suggesting the co-purification of the protease and AGT.

The Circulating Renin Substrate Was Not Cleaved in Its RCL—To test whether a RCL-cleaved AGT may exist in vivo, human plasma AGT was purified and characterized. Plasma AGT was purified 200 times with a yield about 12% (Fig. 4A). It was analyzed using a Tris-Tricine gel (Fig. 4B) and the pair of end-terminal AGT polyclonal antibodies (N1345 (Fig. 4C) and C1350 (Fig. 4D)). In our conditions of purification, no RCL cleavage of circulating AGT was observed because (i) no 4-kDa peptide was detected (Fig. 4B) and (ii) both 60-kDa bands were recognized by the N terminus as well as the C-terminal antibody before (Fig. 4C) and after deglycosylation (Fig. 4D). Although this circulating AGT was not cleaved in the C terminus, a doublet (54–50 kDa) was still consistently observed, even after N- and O-deglycosylation and after removal of sialic acids residues. This pattern of migration was consistent with that of the recombinant native AGT (Fig. 3).

Formation of SDS-resistant Aggregates on Heating RCL-cleaved AGT—Heating native (i.e. full-length) serpins generally leads to the formation of loop-sheet polymers that migrate as a ladder on nondenaturing gels (29). We tested this property by heating the recombinant AGTs at 65 °C (Fig. 5). Unexpectedly, the RCL-cleaved AGT had kept its ability to polymerize, suggesting a mechanism of polymerization different from the much-studied loop-sheet interactions for the RCL-cleaved serpins (29). Monomers of AGT disappeared after 15 min of incubation; a longer incubation time had no further effect (data not shown).

The heat-induced polymerization of both AGTs was studied and compared after 1 h of incubation in 2 different conditions: acidic (pH 5.5 in acetate buffer) and basic (pH 8.0 in Tris-HCl buffer). Each was measured at various temperatures ranging from 4 °C to 100 °C (Fig. 6). The acidic condition corresponded to that previously used to obtain the latent form with partial insertion of the loop of the native plasminogen activator inhibitor-1 (30). The formation of aggregates was followed by their ability to generate Ang I after extensive renin hydrolysis (Fig. 6A) and by Coomassie Blue staining after migration on an SDS-polyacrylamide electrophoresis gels (Fig. 6B). When the heat stability of both AGTs was tested in the acidic condition, it was increased by 12 °C, as indicated by the shift to the left of the corresponding transition curves. At pH 8.0, although the two curves cannot be completely superimposed, they showed a nonsignificant difference in $T_m$ value. In fact, $T_m$ was around 56 °C when native and RCL-cleaved AGTs were incubated at pH 8.0 and 68 °C when both proteins were incubated in acetate buffer. At both pH values, the activity was totally abolished after 1 h at 75 °C (Fig. 6A). The dramatic loss of the renin substrate activity was accompanied by the formation of SDS-resistant aggregates for the native as well as the RCL-cleaved AGT (Fig. 6B). The formation of aggregates started around 70 °C when the pH was acidic and at 60 °C with a basic pH. Lowering the pH significantly increased the stability of the monomeric AGT ($M$). The mechanism of AGT polymerization was characterized by the transient formation of dimers ($D$) leading to the final formation of large aggregates ($A_g$) that were so big that they did not enter in the running gel. After 1 h at 100 °C, no monomers were stained; thus, all the AGT had been
changed into higher molecular weight aggregates. The presence of free cysteines in the AGT sequence could explain this particular behavior of a serpin when heated. In fact, the aggregates were dissociated when a reducing agent was added before loading (data not shown).

**DISCUSSION**

We have presented evidence for a new cleavage site in human AGT. Such a cleavage does not affect the generation of Ang I by renin and does not change the tendency of AGT to aggregate. Cleavage of recombinant AGT was observed between Gln 411 and Gln 412 during a nondenaturing purification, generating a major 4-kDa peptide that was N terminus-degraded, presumably by an aminopeptidase. The residue 415, a proline, stops its action. No other AGT peptides were detected from the purified recombinant AGT by mass spectroscopy or in Tris-Tricine gel, showing that the full-length protein can be specifically proteolysed in its RCL. In addition, we have shown that the generated C-terminal 4-kDa peptides remain associated with the rest of the protein unless AGT has been unfolded by SDS.

This site of proteolysis, between two consecutive glutamine residues, is very unusual. Our data allow the assignment of a new P1-P1′ cleavage site in the RCL of human AGT and prompt a reexamination of the comparison of the reactive center sequences of others serpins (Fig. 7A). According to this alignment, the doublet of glutamine residues Gln 411-Gln 412 is conserved in marmoset, rat, and mouse but not in sheep. Thus, it may constitute a physiological P1-P1′ site, the target of an unknown protease. For inhibitory serpins, the P1 residue plays an important role in determining the target specificity of the protease involved (31). In this regard, it should be noted that the P1 residue in human AGT is a glutamine, although it is very unlikely that it can form an acyl-enzyme with the target protease. Nevertheless, our alignment also showed the conservation of a proline residue in P6 position, except in sheep, and the strict conservation of residues present at position P3, P5′, and P6′. They may have a role in the presentation of the P1-P1′ peptide bond for cleavage.

Most importantly, human AGT has a large charged residue, a glutamate, in P14 in accordance with its inability to insert the reactive center loop (12, 13). Indeed, the presence of an arginine charged residue in P14 in ovalbumin has been proposed to explain the absence of loop insertion (32).

A number of other alignments of this C terminus part of human AGT have been previously described (14, 33, 34). In addition, Patston and Gettins (14) have recently proposed a classification of the serpins based on the prediction of the secondary structure outside the RCL. A requirement for an alanine residue between P9 and P12 rather than a structural requirement for an α-helix was proposed for loop insertion. However, AGT did not match with these predictions, especially in the β-sheet, which immediately precedes their putative RCL cleavage site, Pro 416-Glu 417.

AGT is heavily glycosylated, leading to a heterogeneous protein, which migrates as a fuzzy band around 60 kDa in SDS-polyacrylamide electrophoresis gels (19). This heterogeneity of the circulating renin substrate has been well documented (16–19); a partial C-terminal cleavage has been invoked to explain the presence of two bands, differing by 4 kDa, which remain after deglycosylation. In our experiments, human plasma AGT
purified by a new nondenaturing method was not cleaved in its C terminus. Nevertheless, after removal of N- and O-glycans and sialic acid residues, it migrates as two equal intensity bands (54–50 kDa), both stained by Coomassie Blue. This result is in accordance with our previous data showing a similar behavior for deglycosylated wild type recombinant AGT (52–50 kDa) detected by antibody in serum-free medium (26). Moreover, both proteins showed the same thermal stability when incubated at pH 8.0 and pH 5.5. In summary, we have demonstrated that the heat-induced inactivation of human AGT is concomitant with the formation of SDS-resistant polymer, independently of its RCL cleavage. As distinct from the vast majority of native serpins that are able to form noncovalent aggregates, we describe AGT as able to form noncovalent aggregates added after translation. The complete cleavage of the AGT in its C terminus does not lead to the disappearance of the doublet, suggesting that this compound may be linked to the rest of the protein and not to the C-terminal peptides.

An unexpected result shows that the RCL-cleaved protein does not differ from the intact AGT in its ability to polymerize on heating. We used native and also denaturing gel to characterize the polymerization of AGT. Neither experiment revealed any significant difference between the native and the RCL-cleaved AGT. In our conditions, both AGTs were able to polymerize on SDS-polyacrylamide electrophoresis gels, leading to a dramatic loss of their ability to serve as a substrate for human renin. Moreover, both proteins showed the same thermal stability when they were incubated at pH 8.0 and pH 5.5. In summary, we have demonstrated that the heat-induced inactivation of human AGT is concomitant with the formation of SDS-resistant polymer, independently of its RCL cleavage. As distinct from the vast majority of native serpins that are able to form noncovalent aggregates, we describe AGT as able to form SDS-resistant aggregates. We suspect a predominant role for free cysteines (Cys$^{232}$ and/or Cys$^{308}$) (24) in this mechanism of polymerization.

Finally, our results lead us to propose a structural model for human AGT. A search in a data base for proteins with known three-dimensional structures revealed highest similarity between human AGT and AT (36). We have constructed a three-dimensional model of the renin substrate using AT as a template to build a C-terminal part of the AGT. The model was built using the Roche in-house modeling package Moloc (46). Because of the rather low sequence identity between template and target sequence, we refrained from introducing any side chains. The final model comprises Cα-positions of amino acid 70–452 of human AGT. Some key residues are indicated as bigger balls: Ala$^{70}$, the first residue, and Ala$^{452}$, the last residue of the mature protein; three out of the four AGT cysteines; and the Met$^{235}$ residue. Gln$^{411}$ and Gln$^{415}$ in the middle of the RCL and the first residue of each of the four strands of the central $\beta$-sheet A are indicated. The C-terminal peptide (Gln$^{412}$-Ala$^{452}$) is represented as a bolder line. The figure was generated using RasMol 2.6.

**FIG. 7. Human AGT: comparison of the RCL sequences and modeling its serpin structure.** A, human AGT sequence in the RCL region is reported and compared with the four other cloned mammalian AGTs and with sequences from a number of serpins. The nomenclature P14-PI0 is from Schechter and Berger (43). AGT sequences from human (hAGT) (4), rat (rAGT) (3), mouse (mAGT) (44), and sheep (sAGT)(45) were previously reported. The marmoset AGT sequence (mAGT) is from Olivier Valdennair (personal communication). The alignment between the five mammalian AGTs was made using the TREEALIGN software. Amino acid sequences of the reported serpins and their classification were taken from Patston and Gettins (14).

ACT, $\alpha$-anti-chymotrypsin; ATIII, antithrombin III; PEDF, pigment epithelium-derived factor; TBG, thyroxin binding globulin; CBG, corticosteroid binding globulin; PAI-1, plasminogen activator inhibitor-1; PAI-2, plasminogen activator inhibitor-2; HC-II, heparin cofactor II.
is inserted into the core of the protein. The two consecutive glutamines (Gln411-Gln412), although not localized at the very top of the RCL, are in quite a good position for cleavage by a proteinase. In addition, our model predicts that the Cys235-Met236 region is solvent-accessible. Our previous work has shown that this region bearing the M235T polymorphism is localized at the outside of the protein (37) and thus able to interact with the proform of eosinophil granule major basic protein (24). For other natural mutations of the AGT gene (2), only the T174M and the L359M changes could affect the stability of the predicted b-sheets A, which seems to be essential for the serpin fold of hAGT. However, it is very unlikely that these two amino acid substitutions would change the noninhibitory into an inhibitory angiotensinogen. For other natural side of the protein (37) and thus able to interact with the proform of the RCL, are in quite a good position for cleavage by a proteinase from the C-terminal end led to the production of truncated proteins still bearing the neurite-promoting activity (40). Contrary to that, we tried to express a truncated AGT (Asp1–Glu403) in which the serpin RCL and the C-terminal peptide were lacking and failed to produce any expressed AGT in COS cells (data not shown), suggesting a predominant role of the serpin part in the overall folding of the renin substrate.

In conclusion, relationships between AGT and the serpin family can be summarized as a combination of Ang I generation site has not yet been identified. A Reactive Center Loop-cleaved Angiotensinogen

\[ \text{A Reactive Center Loop-cleaved Angiotensinogen} \]

REFERENCES

1. Gould, A. B., and Green, D. (1971) Cardiovasc. Res. 5, 86–89
2. Corvol, P., and Jeunemaitre, X. (1997) Endocrinol. 138, 662–677
3. Ohkubo, H., Kageyama, R., Ujihara, M., Hirose, T., Inayama, S., and Nakashima, S. (1984) Proc. Natl. Acad. Sci. U.S.A. 80, 2196–2200
4. Kageyama, R., Ohkubo, H., and Nakashima, S. (1984) Biochemistry 23, 3603–3609
5. Doolittle, R. F. (1983) Science 222, 417–419
6. Takita, T., Ohkubo, H., and Nakashima, S. (1984) J. Biol. Chem. 259, 8063–8065
7. Gaillard, I., Clauser, E., and Corvol, P. (1989) DNA (N.Y. ) 18, 87–99
8. Hill, R. E., and Hastie, N. D. (1987) Nature 326, 96–99
9. Stratikos, E., and Gettins, P. G. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 453–458
10. Stratikos, E., and Gettins, P. G. (1999) Proc. Natl. Acad. Sci. U.S.A. 96, 4808–4813
11. Stein, P. E., Tewksbury, D. A., and Carrell, R. W. (1989) Biochem. J. 262, 103–107
12. Mast, A. E., Englund, J. J., Pizzo, S. V., and Salvesen, G. (1991) Biochemistry 30, 1723–1730
13. Patston, P. A., and Gettins, P. G. (1996) FEBS Lett. 383, 87–92
14. Stein, P. E., Leslie, A. G., Finch, J. T., Turnell, W. G., McLaughlin, P. J., and Carrell, R. W. (1990) Nature 347, 99–102
15. Tewksbury, D. A., Frome, W. L., and Dumas, M. L. (1978) J. Biol. Chem. 253, 1357–1380
16. Tewksbury, D. A., and Dart, R. A. (1979) Mol. Cell. Biochem. 27, 47–56
17. Tewksbury, D. A. (1983) Fed. Proc. 42, 2724–2728
18. Tewksbury, D. A. (1990) in Hypertension, Pathophysiology, Diagnosis, and Management (Laragh, J. J., and Brenner, B. M., eds.) Vol. 1, pp. 1197–1216, Raven Press, Ltd., New York.
19. Campbell, C. J., Charlton, P. A., Grunham, C. J., McQueen, C. J., and Periodicity, J. E. (1987) Biochem. J. 243, 121–126
20. Mathews, S., Dobel, P., Pruschky, M., Bossar, R., D’Arcy, A., Oefner, C., Zalauf, M., Gente, R., Breu, V., Matile, H., Schlaeger, J., and Fischli, W. (1996) Protein Expression Purif. 7, 81–93
21. Grueninger-Leitch, F., D’Arcy, A., and Chene, C. (1996) Protein Sci. 5, 2617–2622
22. Genain, C., Bouhnik, J., Tewksbury, D. Corvol, P., and Menard, J. (1984) J. Clin. Endocrinol. Metab. 59, 475–484
23. Gimenez-Roquepo, A. P., Celerier, J., Schmid, G., Corvol, P., and Jeunemaitre, X. (1984) J. Biol. Chem. 239, 34480–34487
24. Wei, L., Gaillard, I., Corvol, P., and Clauser, E. (1988) Biochemistry. Biophys. Res. Commun. 156, 1103–1110
25. Gimenez-Roquepo, A.-P., Leconite, I., Cohen, P., Simon, D., Guenye, T. T., Celerier, J., Pau, B., Corvol, P., Clauser, E., and Jeunemaitre, X. (1986) J. Biol. Chem. 261, 9838–9844
26. Schagger, H., and Von Jagow, G. (1987) Anal. Chem. 166, 368–379
27. Menard, J., and Catt, K. J. (1972) Endocrinology 90, 422–430
28. Lomas, D. A., Elliott, P. R., Chang, W. S., Wardell, M. R., and Carrell, R. W. (1995) J. Biol. Chem. 270, 5282–5288
29. Declerck, P. J., De Mol, M., Vaughan, D. E., and Collen, D. (1992) J. Biol. Chem. 267, 11693–11698
30. Doolitle, R. F. (1983) J. Mol. Biol. 172, 676–681
31. Huber, R., and Corvol, P. (1989) Biochemistry 28, 8866–8871
32. Aertgeerts, K., De Bondt, H. L., De Ranter, C. J., and Declerck, P. J. (1995) J. Clin. Endocrinol. Metab. 80, 3055–3051
33. Cumin, F., N’Guyen, D., Castro, B., Meudel, J., and Corvol, P. (1987) Biochem. Biophys. Acta. 913, 10–19
34. Aertgeerts, K., De Bondt, H. L., De Ranter, C. J., and Declerck, P. J. (1995) J. Biol. Chem. 270, 25992–25999
35. Mathialagan, N., and Hansen, T. R. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 13653–13658
36. Komiyama, T., Ray, C. A., Pickup, D. J., Howard, A. D., Thornberry, N. A., Petersen, G. P., and Salmon, G. (1994) J. Biol. Chem. 269, 13931–13937
37. Schechter, I., and Berger, A. (1967) Biochem. Biophys. Res. Commun. 27, 157–162
38. Clouston, W. M., Evans, B. A., Haralambidis, J., and Richards, R. I. (1988) Genomics 2, 240–248
39. Nagase, M., Suzuki, F., Fukumizu, A., Takeda, N., Takeuchi, K., Murakami, K., and Nakamura, Y. (1994) Biosci. Biotechnol. Biochem. 58, 1884–1885
40. Gerber, P. R., and Muller, K. (1995) J. Comput. Aided Mol. Des. 9, 251–268
Characterization of a Human Angiotensinogen Cleaved in Its Reactive Center Loop by a Proteolytic Activity from Chinese Hamster Ovary Cells

Jérôme Célérié, Georg Schmid, Jean-Pierre Le Caer, Anne-Paule Gimenez-Roqueplo, Daniel Bur, Arno Friedlein, Hanno Langen, Pierre Corvol and Xavier Jeunemaitre

J. Biol. Chem. 2000, 275:10648-10654.
doi: 10.1074/jbc.275.14.10648

Access the most updated version of this article at http://www.jbc.org/content/275/14/10648

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 46 references, 18 of which can be accessed free at http://www.jbc.org/content/275/14/10648.full.html#ref-list-1