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Naturally occurring active N-domain of human angiotensin I-converting enzyme

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ABSTRACT **Angiotensin I-converting enzyme (ACE, kininase II) is a single-chain protein containing two active site domains (named N- and C-domains according to position in the chain). ACE is bound to plasma membranes by its C-terminal hydrophobic transmembrane anchor. Ileal fluid, rich in ACE activity, obtained from patients after surgical colectomy was used as the source. Column chromatography, including modified affinity chromatography on lisinopril-Sepharose, yielded homogeneous ACE after only a 45-fold purification. N-terminal sequencing of ileal ACE and partial sequencing of CNBr fragments revealed the presence of an intact N terminus but only a single N-domain active site, ending between residues 443 and 559. Thus, ileal-fluid ACE is a unique enzyme differing from the widely distributed two-domain somatic enzyme or the single C-domain testicular (germinal) ACE. The molecular mass of ileal ACE is 108 kDa and when deglycosylated, the molecular mass is 68 kDa, indicating extensive glycosylation (37% by weight). In agreement with the results reported with recombinant variants of ACE, the ileal enzyme is less Cl^--dependent than somatic ACE; release of the C-terminal dipeptide from a peptide substrate was optimal in only 10 mM Cl^-. In addition to hydrolyzing at the C-terminal end of peptides, ileal ACE efficiently cleaved the protected N-terminal tripeptide from the luteinizing hormone-releasing hormone and its congener 6–31 times faster, depending on the Cl^- concentration, than the C-domain in recombinant testicular ACE. Thus we have isolated an active human ACE consisting of a single N-domain. We suggest that there is a bridge section of about 100 amino acids between the active N- and C-domains of somatic ACE where it may be proteolytically cleaved to liberate the active N-domain. These findings have potential relevance and importance in the therapeutic application of ACE inhibitors.**

The widely distributed angiotensin-I-converting enzyme (ACE; kininase II, EC 3.4.15.1) has two main physiological functions (1): conversion of angiotensin I to the vasoconstrictor angiotensin II (2) and, as kininase II, the inactivation of the vasodilator bradykinin (3–6). In addition, ACE cleaves many other peptides such as substance P, luteinizing hormone-releasing hormone (LH-RH), neurotensin, or des-Arg^9-bradykinin, as shown mainly in experiments *in vitro* (1, 7).

Molecular cloning of human endothelial ACE revealed that the enzyme consists of two homologous domains, named N-domain and C-domain, according to their location in the single-chain protein. Each of them contains an active site with a zinc cofactor in the active center (8–10), and this structure is probably derived from the duplication of an ancestral gene. The testicular (germinal) form of ACE is shown, by molecular cloning, to contain the C-domain of endothelial (somatic) ACE (8–13) with a unique N-terminal sequence of 67 amino acids arising from the use of an alternate transcription start site in intron 13 of somatic ACE (11). When the activities of somatic and germinal ACE were examined, all the activity was attributed to the C-domain alone (12). Later, it was found that both domains are indeed active but that the C-domain has most of the angiotensin I-converting activity. The actions of the N-domain were characterized by using recombinant mutant enzymes and enzyme fragments expressed in Chinese hamster ovary (CHO) cells (14). The function of the N-domain *in vivo* is still a puzzle and, until now, no naturally occurring form of a truncated ACE having only an active N-domain has been found.

Our interest in ACE was also stimulated by the very wide clinical application of its inhibitors in high blood pressure and in some diseases of the heart and kidney (15). In this report, we describe the isolation and characterization of a naturally occurring form of human ACE that consists only of the N-domain. This active extensively glycosylated enzyme has the shortest peptide chain of any active ACE found in the body. The enzyme was isolated from human ileal fluid and partially sequenced, and its properties were compared with those reported for the recombinant N-domain of ACE.

MATERIALS AND METHODS

Materials. Ileal fluid was obtained from subjects with ileal stomas after surgical colectomy. These samples were obtained in accordance with the Institutional Review Board of the University of Chicago Hospitals. Recombinant rabbit testicular ACE (13) was a gift from Indira Sen at the Cleveland Clinic, Cleveland, and lisinopril was a gift from Merck, Sharp & Dohme. Sepharose CL-4B and Sephacryl S-200 HR were from Pharmacia. Hepes, LH-RH, and the derivative des-Gly^{10}-LH-RH-ethylamide (-NHET), Hip-His-Leu (where Hip is benzoyl-glycine), *o*-phthalic dicarboxaldehyde, Hip-Gly-Gly, 6-[*N*-(*p*-aminobenzoyl)amino]caproic acid, and trifluoromethanesulfonic acid were from Sigma. Whatman DEAE-cellulose (DE-52) was obtained from Fisher Scientific. [^{3}H]Hip-Gly-Gly was from Amersham. All other chemicals and buffers used were reagent grade or better. Human renal ACE was purified as reported (16).

Preparation of Lisinopril-Sepharose. Lisinopril-Sepharose was synthesized by standard methods using a 6-[*N*-(*p*-aminobenzoyl)amino]caproic acid spacer, coupled to epoxy-activated Sepharose CL-4B (17, 18).

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Abbreviations: ACE, angiotensin-I-converting enzyme; LH-RH, luteinizing hormone-releasing hormone; NHET, ethylamide; Hip, benzoyl-glycine.

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Enzyme Purification. ACE was purified from ileal fluid by a combination of ion-exchange chromatography (19), modified affinity chromatography (17, 18), and gel filtration. All steps were carried out at 4°C unless otherwise indicated. Ileal fluid (500 ml) was diluted to 5 liters with water and then batchwise adsorbed by stirring with 10 g of DE-52 (4 h) equilibrated with 50 mM Hepes (pH 7.5). The mixture was centrifuged at 2000 × *g* for 15 min and the sediment was washed three times with 10 mM Hepes (pH 7.5). Enzyme activity was eluted by stirring the cellulose in 100 ml of 10 mM Hepes, pH 7.5/0.15 M NaCl for 4 h followed by centrifugation; ACE in the supernatant was further purified by affinity chromatography.

Although ileal ACE activity bound poorly to lisinopril-Sepharose in buffer containing 0.3 M NaCl, it bound well in high salt buffer. Thus, the ileal enzyme was applied to a 50-ml bed volume of lisinopril-Sepharose in 10 mM Hepes (pH 8.0) containing 0.8 M NaCl and 10 μM $ZnSO_4$. The column was washed with 10 vol of the same buffer and then eluted with 10 mM Hepes, pH 6.0/10 μM $ZnSO_4$. Fractions (10 ml) were collected and active fractions were pooled and then concentrated by Amicon YM10 membrane filtration.

ACE was finally purified by gel filtration in a 1.6 × 95 cm column of Sephacryl S-200 HR in 0.1 M Tris·HCl, pH 7.5/0.1 M NaCl. Fractions of the single-activity peak were pooled, concentrated, and stored in 50% (vol/vol) glycerol at −20°C.

Enzyme Assays. ACE activity was determined using several techniques (7, 10). In a recording spectrophotometric assay, Hip-His-Leu was the substrate. In a fluorometric assay, the dipeptide cleaved from this substrate was coupled to *o*-phthalic dicarboxaldehyde. A radiometric assay utilized [^{3}H]Hip-Gly-Gly (7, 10). The cleavage of longer peptide substrates was assessed by HPLC (20).

Enzyme Inhibition. The inhibition studies were done according to Wei *et al.* (14) with Hip-His-Leu as substrate. Lisinopril, at 9 nM to 22 pM, was incubated in 0.1 M Tris·HCl (pH 8.3) containing 10 μM $ZnSO_4$, ovalbumin (1 mg/ml), 0.3 M NaCl, and either 0.1 nM renal (somatic) or 0.1 nM ileal enzyme.

HPLC. A Waters automated gradient system was used and peptides were detected at 214 nm. Peptides and their hydrolysis products were separated on a Waters μBondapak C_{18} reversed-phase column (0.39 × 30 cm) with an increasing linear gradient of acetonitrile/0.05% trifluoroacetic acid in H_2O/0.05% trifluoroacetic acid (20).

Deglycosylation. Purified ileal ACE was chemically deglycosylated with trifluoromethanesulfonic acid (21).

PAGE and Electroblotting. SDS/PAGE was done in 7.5% or 9.0% slab gels. Protein samples (1–2 μl containing 0.5–1.0 μg of protein) were added to sample buffer (6 μl) with dithiothreitol (1 mg) and then denatured by boiling for 5 min. Proteins were transferred from polyacrylamide gels to nitrocellulose with a semidry blotting apparatus (American Bionetics, Hayward, CA) and detected immunologically using primary antiserum to human kidney ACE and gold-labeled secondary antibody (goat anti-rabbit IgG) followed by silver enhancement.

Protein Sequencing. Purified ileal ACE samples were concentrated and desalted (22) by adsorption onto Pro-spin filters (Applied Biosystems). The filters were extracted with 0.1% trifluoroacetic acid/20% (vol/vol) methanol and then with methanol/0.1% triethylamine to remove adventitious amino acids. Intact enzyme was sequenced in an Applied Biosystems model 477A peptide sequencer (23).

Enzyme bound to the filter was also subjected to cleavage by CNBr (25 mg/ml) in 70% trifluoroacetic acid followed by sequence analysis, both with and without *o*-phthalic dicarboxaldehyde blockage at selected sequencing cycles.

RESULTS

Enzyme Purification. ACE was purified to homogeneity from the ileal fluids obtained from seven patients who had undergone surgery for diseases of the large intestine and were not being fed orally. The fluid was viscous due to the presence of mucus and was, therefore, diluted 1:10 with water, which also reduced the Cl^- concentration. This was followed by batchwise adsorption on DE-52 ion-exchange resin, affinity chromatography, and gel filtration.

Unlike ACE isolated from a variety of human tissues and fluids (1, 8, 17, 24), ACE activity from ileal fluid did not bind well to lisinopril-Sepharose in the presence of 0.3 M NaCl and was eluted during washing. ACE did bind, however, when 0.8 M NaCl was used, and the activity was eluted with a buffer that had no NaCl.

A typical purification of ileal fluid ACE is summarized in Table 1. Starting with 500 ml of ileal fluid, we obtained 1.6 mg of ACE with a yield of 20% after a 45-fold purification. This indicates that the enzyme accounted for ≈2% of the protein in the fluid.

Characterization of Ileal ACE. After the final purification, the ileal enzyme migrated as a single band in SDS/PAGE (data not shown). The apparent molecular mass of the purified enzyme was considerably lower than that of purified human kidney ACE electrophoresed in the same gel as control, 108 kDa vs. 170 kDa. When electroblotted to a nitrocellulose membrane, the 108-kDa band reacted strongly with polyclonal antiserum raised against human kidney ACE (16, 19).

Deglycosylation of Ileal ACE. Chemical deglycosylation of ileal ACE, followed by SDS/PAGE and immunoblot analysis, yielded a single band with an apparent molecular mass of 68 kDa, indicating that the enzyme is extensively glycosylated, containing ≈37% carbohydrate by weight.

Sequence Analysis. Two samples of purified ileal fluid ACE were subjected to N-terminal sequencing. A single sequence (LDPGLQ-) was obtained in both cases, indicating that the samples were homogeneous. This sequence is identical with the N-terminal sequence of human ACE (8), thus proving that the ileal enzyme contains the N-terminal portion of the molecule. Ileal ACE was then cleaved with CNBr and the peptides in the resulting mixture were simultaneously sequenced (Fig. 1). The numbers obtained by quantitative analysis of the yield of each amino acid in the first four cycles are consistent with the presence of only the first 12 CNBr fragments in the mixture, out of a possible 30. More importantly, four of the potential CNBr fragments (fragments 13–16) contain unique residues that are not found in the same position in any of the first 12 peptides. These residues (Gly^2 in peptide 13; Gln^1-Ile^2-Ala^3-Asn^4 in peptide 14; Glu^1-Thr^2-

Table 1. Purification of ileal fluid ACE

Fraction	Total protein, mg	Total activity, units	Specific activity, units/mg of protein	Purification, fold	Yield, %
Ileal fluid diluted 1:10	360	65,000	181	1	100
DE-52	10	15,173	1517	8	23
Lisinopril-Sepharose	2.2	13,365	6075	34	21
Sephacryl-S200 HR	1.6	12,981	8113	45	20

One unit of activity is 1 nmol of [^{3}H]Hip-Gly-Gly cleaved per min.

(1): LDPGLQPGNFSADEAGAQLFAQSYNSSAEQVLFQSVAASWAHDTNITAENARRQEEA
ALLSQEFAEAWGQKAKELYEPIWQNFTDPQLRRIIGAVRTLGSANLPLAKRQQYNALLSN
M- (2): SRIYSTAKVCLPNKTATCWSLDPDLTNILASSRSYAM- (3): LLFAWEGWHNAAGI
PLKPLYEDFTALSNEAYKQDGFTDTGAYWRSWYNSPTFEDDLEHLYQQLEPLYLNLHAF
VRRALHRRYGDRYINLRGPIPAHLLGDM- (4): WAQSWENIYDM- (5): VVPFPDKPNLDV
TSTM- (6): LQQGWNATHM- (7): FRVAEEFFTSLELSPM- (8): PPEFWEGSM- (9): LEKPA
DGREVVCHASAWDFYNRKDFRIKQCTRVTM- (10): DQLSTVHHEM- (11): GHIQYYLQY
KDLPVSLRRGANPGFHEAIGDVLALSVSTPEHLHKIGLLDRVTNDTESDINYLLKM- (12):
443
ALEKIAFLPFGYLV*DQWRWGVFSGRTPPSRYNFDWWYLRTKYQGICPPVTRNETHFDAGA*
KFHVPNVTPYIRYFVSFVLQFQFHEALCKEAGYEGPLHQCDIYRSTKAGAKLRKVLQAGSSR
559
PWQEVLKDM- (13): VGLDALDAQPLLKYFQPVTQWLQEQNQQNGEVLGWPEYQWHPP
LPDNYPEGIDLVTDEAEASKFVEEYDRTSQVVWNEYAEANWNYNTNITTETSKILLQKN
M- (14): QIANHTLKYGTQARKFDVNQLQNTTIKRIIKKVQDLERAALPAQELEEYNKILLD
M- (15): ETTYSVATVCHPNGSCLQLEPDLTNVM- (16): ATSRKYEDLLWAWEGWRDKA
GRAILQFYPKYVELINQAARLNGYVDAGDSWRSM- (17): YETPSLEQDLERLFQELQPLY
LNLHAYVRRALHRHYGAQHINLEGPIPAHLLGNM- (18): WAQTWSNIYDLVVPFPSAPSM
- (19): DTTEAM- (20): LKQGWTPRRM- (21): FKEADDFFTSLGLLPVPPEFWNKSM- (22):
LEKPTDGREVVCHASAWDFYNGKDFRIKQCTTVNLEDLVVAHHEM- (23): GHIQYFM-
(24): QYKDLPVALREGANPGFHEAIGDVLALSVSTPKHLHSLNLLSSEGGSDEHDINFLM-
(25): KM- (26): ALDKIAFIPFSYLVDQWRWRVFDGSITKENYNQEWWSLRLKYQGLCPPV
PRTQGDFDPGAKFHIPSSVPYIRYFVSFIIQFQFHEALCQAAGHTGPLHKCDIYQSKEAGQR
LATAM- (27): KLGFSRPWPEAM- (28): QLITGQPNM- (29): SASAM- (30):
LSYFKPLLDWLRTENELHGEKLGWPQYNWTPNSARSEGPLPDSGRVSFLGLDLDAQQAR
VGQWLLLFLGIALLVATLGLSQRLFSIRHRSLHRHSHGPQFGSEVELRHS

FIG. 1. Peptides potentially released by CNBr cleavage of human ACE. Peptides are numbered sequentially as they are referred to in the text. Sequences definitely identified in ileal fluid ACE are underlined and those not found in the experiments, thus absent in ileal ACE, are double underlined. Based on the sequencing data, the C terminus of ileal fluid ACE lies somewhere between Asp^{443} and Met^{559} (italic type) (for details, see text).

Thr^3 in peptide 15; and Thr^2-Ser^3-Arg^4 in peptide 16) were absent in the corresponding sequencing cycles (Fig. 1). This was examined in more detail by using an *o*-phthalic dicarboxaldehyde blocking procedure that derivatizes all primary amines, allowing only peptides containing N-terminal proline at a given sequencing cycle to be sequenced further. In one experiment, the CNBr peptides were blocked at position 4, which should allow further sequencing of only peptides 9, 17, and 22, which have proline in this position (Fig. 1). The sequence for peptide 9 was clearly present (PADGREVVCHASA) and those for peptides 17 and 22 were absent (Fig. 1). The same strategy was used with another sample, blocking at cycle 9, to determine the presence or absence of peptides 12, 26, and 27. Only the sequence for peptide 12 (PFGYLV) was found (Fig. 1). Finally, the absence of peptide 13 (the only CNBr fragment containing proline at position 10) was confirmed by the inability to obtain any sequence after blocking with *o*-phthalic dicarboxaldehyde in cycle 10. These data indicate that ileal-fluid ACE contains only the N-domain active site, being truncated somewhere between Asp^{443} and Met^{559} (Fig. 1), with a calculated protein molecular mass between 49 kDa and 61 kDa. The larger size agrees well with the mass of the deglycosylated enzyme determined by gel electrophoresis.

Effect of Cl^- Concentration. The effect of Cl^- concentration on the hydrolysis of Hip-His-Leu by the ileal ACE and renal somatic ACE was different (Fig. 2). The ileal enzyme reached nearly optimal activity in only 10 mM NaCl. The kidney enzyme had only 10.5% activity in 10 mM NaCl (reflecting primarily the contribution of the N-domain active site) and reached maximum at about 0.8 M NaCl due to the activity of the C-domain. On a molar basis, the renal enzyme was ≈13 times more active with Hip-His-Leu in 0.8 M NaCl than the ileal enzyme. These results are similar to those studies comparing the activity of the recombinant N-domain of ACE with wild-type ACE (14, 25, 26).

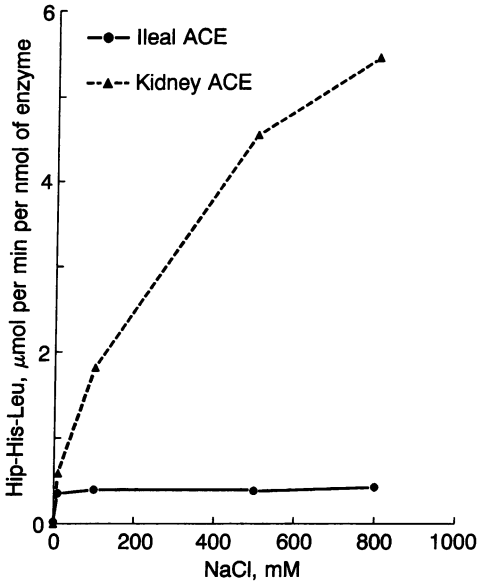

FIG. 2. Effect of Cl^- concentration on the hydrolysis of Hip-His-Leu by the ileal and kidney enzymes. The initial linear rate of substrate hydrolysis was determined by a continuously recording spectrophotometric assay. Reaction mixtures contained 1.0 mM substrate, 0–800 mM NaCl, and either 70 nM ileal enzyme or 20 nM kidney enzyme in 0.1 mM Hepes (pH 8.0).

Hydrolysis of LH-RH and des-Gly^{10}-LH-RH-NHET. ACE releases the protected N-terminal tripeptide of LH-RH and that of its derivative (26, 27), and this activity is attributed mainly to the N-domain of ACE (28, 29). We therefore compared the hydrolysis of these substrates by ileal ACE and a recombinant testicular ACE, which has only the C-domain active site (13). Equimolar amounts (3 nM) of either human ileal or recombinant rabbit testicular ACE were incubated with 0.4 mM LH-RH or des-Gly^{10}-LH-RH-NHET for 1 h at 37°C in 50 mM Hepes (pH 7.4) containing either 25 mM or 300 mM NaCl. The extent of the release of <Glu^1-His^2-Trp^3 was determined by HPLC. As shown in Table 2, these peptides were hydrolyzed more efficiently by the ileal enzyme than by the testicular enzyme. The largest difference was seen with des-Gly^{10}-LH-RH-NHET; ileal ACE cleaved this peptide 31-fold faster in 25 mM Cl^- and 16-fold faster in 300 mM Cl^- than testicular ACE. LH-RH was also cleaved more readily by the ileal enzyme (≈12- or 6-fold faster; Table 2). The results are in good agreement with those obtained with purified or recombinant ACE containing active N- and/or C-domains (25–29).

Enzyme Inhibition. The IC_{50} value of lisinopril was 0.47 nM for kidney ACE and 2.2 nM for ileal ACE. These values agree with those reported for wild-type recombinant enzyme and for the recombinant N-domain of ACE (25, 26).

DISCUSSION

This report contains the finding of a naturally occurring ACE having only the N-domain active site. After fragmentation with CNBr, sequencing studies showed that the ileal-fluid ACE contains at least 442 amino acids but not more than 559 residues. These data are consistent with the molecular mass determined for the glycosylated and deglycosylated ileal enzyme. This ileal ACE molecule represents the active

Table 2. Hydrolysis of LH-RH and des-Gly^{10}-LH-RH-NHET by ileal and testicular ACE at low and high Cl^- concentrations

Substrate	Cl^-, mM	Activity		
		Ileal ACE	Testicular ACE	Ratio of ileal/testicular ACE
LH-RH	25	4.86	0.40	12.2
	300	5.66	0.98	5.8
des-Gly^{10}-LH-RH-NHET	25	2.82	0.09	31.3
	300	6.95	0.43	16.2

Final concentration of each substrate was 0.4 mM. Activity is expressed as μmol of <Glu^1-His^2-Trp^3 formed per h per nmol of enzyme. Values are the mean of two determinations done in duplicate.

N-domain of the human enzyme, which, to our knowledge, has not been found separately before in the body. Although ileal ACE contains at most only 44% of the total ACE sequence, it has 8–10 of the 17 potential Asn-linked glycosylation sites (9). This is consistent with the finding that carbohydrate makes up ≈37% of its molecular mass.

The Cl^- dependence of ileal ACE was much less than that of somatic ACE; maximum activation was observed in 10 mM NaCl vs. 800 mM NaCl for the somatic ACE. By taking these differences in Cl^- sensitivity into consideration, we simplified the purification of ACE on the inhibitor column. In contrast to some other reported procedures, we did not elute with an ACE inhibitor (17), but instead varied the NaCl concentration in the eluting buffer. In 0.8 M NaCl, both ileal and somatic ACE were adsorbed on the lisinopril-Sepharose column. In 0.3 M NaCl, the somatic but not the ileal enzyme was bound, and Cl^--free buffer eluted both enzymes. It was reported that the recombinant N-domain of ACE could not be purified because it does not bind to lisinopril-Sepharose in the usual buffer containing 0.3 M NaCl (9), and this observation provided the first clue that ileal ACE contained only the N-domain active site.

The hydrolysis of the N-terminal tripeptide of LH-RH and its derivative was catalyzed efficiently by the ileal enzyme, as expected for an enzyme consisting of only the N-domain. This was clearly different from the slow hydrolysis observed with the testicular (C-domain) ACE (refs. 28 and 29; Table 2).

ACE is widely distributed and is mainly bound to plasma membranes of endothelial (1, 30), epithelial (1, 31), and neuroepithelial (1, 31) cells. Microvilli of the brush border in the kidney, intestine, placenta (1, 7, 31–34), and choroid plexus (35) are particularly rich in ACE. ACE on the cell membrane of vascular endothelial cells cleaves circulating peptides (36). Besides that, it is present on the epithelial lining of various conduits in the body. The high concentration of ACE in these structures has been known for a long time; for example, in the human kidney, the activity per weight is 5–6 times higher than in the lung (19). The role of ACE in transport functions of these epithelial cells is not clear yet. In the small intestine, ACE may cleave off dipeptides of digested protein fragments that are then easily absorbed by enterocytes (34, 37).

Very likely, the ileal enzyme is liberated by a proteolytic cleavage of intact somatic ACE either while attached to the brush border membrane at its C-terminal anchor peptide or, alternatively, after its release into the fluid (26, 38, 39). The release of the intact ACE from the membrane could occur by cleavage in the C-domain between Arg^{1137} and Leu^{1138} as shown for the soluble plasma ACE (40). Subsequent release of the active N-domain is unlikely to be caused by trypsin (19, 41) or by the metalloprotease described by Oppong and Hooper (42), because these enzymes liberate full-size ACE; besides, the latter enzyme is absent from the intestine. ACE probably contains a frangible bridge region between the N- and C-domain susceptible to enzymatic cleavage. Based on these results, it is estimated to be between residues 442 and 559 in the somatic enzyme. The N-terminal end of ileal ACE is intact and thus appears to be resistant to proteases and peptidases in the ileal fluid, whereas the C-domain is probably rapidly metabolized, since we could find no evidence for its presence. It is also possible that in patients who are not fed orally, the N-domain is released from the membrane-bound enzyme by cleavage in the frangible bridge region, leaving the C-domain behind on the membrane. Up to 90% of the ACE present in ileal fluid has only the N-domain (P.A.D., B.M., R.A.S., and E.G.E., unpublished data).

Another possibility for biogenesis of ileal ACE would involve alternate splicing of its mRNA, yielding a single N-domain enzyme directly, although ACE purified from human and rat intestinal tissue is the full-size enzyme (43–45).

The very high concentration of this truncated ACE in the ileal fluid (up to 2% of total protein) suggests functional importance. Some ACE inhibitors given therapeutically are in a pro-form with the crucial carboxyl group esterified, and activation presumably occurs after absorption from the gastrointestinal tract (46). Other ACE inhibitors, such as captopril or lisinopril, are administered in their fully active form, thus binding of active inhibitors by ileal ACE may influence their absorption and excretion.

If proteolytic cleavage releases the active N-domain of ileal ACE, this could possibly happen elsewhere in the body, for example, as a consequence of the activation of the blood-borne proteases. Hypothetically, such a truncated ACE may stay in circulation because some of its carbohydrate components can prevent uptake by the liver lectins and also its removal by glomerular filtration due to the added molecular mass. Because it can dissociate faster, such a truncated ACE may form a less-stable complex with an inhibitor than the intact somatic enzyme.

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1. Skidgel, R. A. & Erdös, E. G. (1993) in *The Renin Angiotensin System*, eds. Robertson, J. I. S. & Nicholls, M. G. (Gower Medical Publ., London), Vol. 1, pp. 10.1–10.10.
2. Skeggs, L. T., Jr., Marsh, W. H., Kahn, J. R. & Shumway, N. P. (1954) *J. Exp. Med.* **99**, 274–282.
3. Erdös, E. G. & Yang, H. Y. T. (1967) *Life Sci.* **6**, 569–574.
4. Yang, H. Y. T. & Erdös, E. G. (1967) *Nature (London)* **215**, 1402–1403.
5. Yang, H. Y. T., Erdös, E. G. & Levin, Y. (1970) *Biochim. Biophys. Acta* **214**, 374–376.
6. Yang, H. Y. T., Erdös, E. G. & Levin, Y. (1971) *J. Pharmacol. Exp. Ther.* **177**, 291–300.
7. Erdös, E. G. & Skidgel, R. A. (1986) *Hypertension* **8**, Suppl. I, I34–I37.
8. Soubrier, F., Alhenc-Gelas, F., Hubert, C., Allegrini, J., John, M., Tregear, G. & Corvol, P. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9386–9390.

9. Wei, L., Alhenc-Gelas, F., Corvol, P. & Clauser, E. (1991) *J. Biol. Chem.* **266,** 9002–9008.
10. Wei, L., Alhenc-Gelas, F., Soubrier, F., Michaud, A., Corvol, P. & Clauser, E. (1991) *J. Biol. Chem.* **266,** 5540–5546.
11. Lattion, A.-L., Soubrier, F., Allegrini, J., Hubert, C., Corvol, P. & Alhenc-Gelas, F. (1989) *FEBS Lett.* **252,** 99–104.
12. Howard, T. E., Shai, S.-Y., Langford, K. B., Martin, B. M. & Bernstein, K. E. (1990) *Mol. Cell. Biol.* **10,** 4294–4302.
13. Sen, I., Samanta, H., Livingston, W., III, & Sen, G. C. (1991) *J. Biol. Chem.* **266,** 21985–21990.
14. Wei, L., Clauser, E., Alhenc-Gelas, F. & Corvol, P. (1992) *J. Biol. Chem.* **267,** 13398–13405.
15. Gavras, I. & Gavras, H. (1987) in *Angiotensin Converting Enzyme Inhibitors*, eds. Kostis, J. B. & DeFelice, E. A. (Liss, New York), pp. 93–122.
16. Weare, J. A., Gafford, J. T., Lu, H. S. & Erdös, E. G. (1982) *Anal. Biochem.* **123,** 310–319.
17. Bull, H. G., Thornberry, N. A. & Cordes, E. H. (1985) *J. Biol. Chem.* **260,** 2963–2972.
18. Pantoliano, M. W., Holmquist, B. & Riordan, J. F. (1984) *Biochemistry* **23,** 1037–1042.
19. Stewart, T. A., Weare, J. A. & Erdös, E. G. (1981) *Methods Enzymol.* **80,** 450–460.
20. Jackman, H. L., Tan, F., Tamei, H., Beurling-Harbury, C., Li, X.-Y., Skidgel, R. A. & Erdös, E. G. (1990) *J. Biol. Chem.* **265,** 11265–11272.
21. Edge, A. S., Faltynek, C. R., Hof, L., Reichert, L. E., Jr., & Weber, P. (1981) *Anal. Biochem.* **118,** 131–137.
22. Morris, P. W. (1992) *6th Annual Symposium of the Protein Society, July 25–29, 1992* (Fed. Am. Soc. Exp. Biol., Bethesda, MD), S85 (abstr.).
23. Tan, F., Morris, P. W., Skidgel, R. A. & Erdös, E. G. (1993) *J. Biol. Chem.* **268,** 16631–16638.
24. Erdös, E. G. (1979) in *Bradykinin, Kallidin and Kallikrein: Handbook of Experimental Pharmacology*, ed. Erdös, E. G. (Springer, Heidelberg), Suppl. to Vol. 25, pp. 427–487.
25. Soubrier, F., Hubert, C., Testut, P., Nadaud, S., Alhenc-Gelas, F. & Corvol, P. (1993) *J. Hypertens.* **11,** 471–476.
26. Soubrier, F., Wei, L., Hubert, C., Clauser, E., Alhenc-Gelas, F. & Corvol, P. (1993) *J. Hypertens.* **11,** 599–604.
27. Skidgel, R. A. & Erdös, E. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1025–1029.
28. Ehlers, M. R. W. & Riordan, J. F. (1991) *Biochemistry* **30,** 7118–7126.
29. Jaspard, E., Wei, L. & Alhenc-Gelas, F. (1993) *J. Biol. Chem.* **268,** 9496–9503.
30. Caldwell, P. R. B., Seegal, B. C., Hsu, K. C., Das, M. & Soffer, R. L. (1976) *Science* **191,** 1050–1051.
31. Defendini, R., Zimmerman, E. A., Weare, J. A., Alhenc-Gelas, F. & Erdös, E. G. (1983) *Neuroendocrinology* **37,** 32–40.
32. Ward, P. E., Erdös, E. G., Gedney, C. D., Dowben, R. M. & Reynolds, R. C. (1976) *Biochem. J.* **157,** 643–650.
33. Hall, E. R., Kato, J., Erdös, E. G., Robinson, C. J. G. & Oshima, G. (1976) *Life Sci.* **18,** 1299–1303.
34. Ward, P. E., Sheridan, M. A., Hammon, K. J. & Erdös, E. G. (1980) *Biochem. Pharmacol.* **29,** 1525–1529.
35. Igić, R., Robinson, C. J. G. & Erdös, E. G. (1977) in *Central Actions of Angiotensin and Related Hormones*, eds. Buckley, J. P. & Ferrario, C. M. (Pergamon, New York), Suppl. to *Biochem. Pharmacol.*, pp. 23–27.
36. Aiken, J. W. & Vane, J. R. (1972) *Circ. Res.* **30,** 263–273.
37. Yoshioka, M., Erickson, R. H., Woodley, J. F., Gulli, R., Guan, D. & Kim, Y. S. (1987) *Am. J. Physiol.* **253,** G781–G786.
38. Erdös, E. G. & Gafford, J. T. (1983) *Clin. Exp. Hypertens.* **A5,** 1251–1262.
39. Hooper, N. M., Keen, J., Pappin, D. J. C. & Turner, A. J. (1987) *Biochem. J.* **247,** 85–93.
40. Beldent, V., Michaud, A., Wei, L., Chauvet, M.-T. & Corvol, P. (1993) *J. Biol. Chem.* **268,** 26428–26434.
41. Nishimura, K., Yoshida, N., Hiwada, K., Ueda, E. & Kokubu, T. (1976) *Biochim. Biophys. Acta* **483,** 398–408.
42. Oppong, S. Y. & Hooper, N. M. (1993) *Biochem. J.* **292,** 597–603.
43. Hayakari, M., Amano, K., Izumi, H. & Murakami, S. (1987) *Adv. Exp. Med. Biol.* **247B,** 365–370.
44. Naim, H. Y. (1992) *Biochem. J.* **286,** 451–457.
45. Erickson, R. H., Suzuki, Y., Sedlmayer, A., Song, I. S. & Kim, Y. S. (1992) *Am. J. Physiol.* **263,** G466–G473.
46. Patchett, A. A. & Cordes, E. H. (1985) in *Advances in Enzymology and Related Areas of Molecular Biology*, ed. Meister, A. (Wiley, New York), Vol. 57, pp. 1–84.