COMPARATIVE CHEMICAL COMPOSITIONS, PHYSICOCHEMICAL AND ENZYMATIC PROPERTIES BETWEEN PULMONARY AND SOLUBLE ANGIOTENSIN I- CONVERTING ENZYMES

Bruno BAUDIN[1,2,*], Abdelkrim TAHRAOUI[1], Bénédicte BENETEAU-BURNAT[2], Daniel ROBIC[1] and Jacqueline GIBOUDEAU[2]

1- Laboratoire de Biochimie et de Glycobiologie - UFR Pharmacie - Université René Descartes (Paris v) - Paris.
2- Laboratoire de Biochimie A – Hôpital Saint Antoine – AP-HP, Paris.
Running title : Comparison between lung and serum angiotensin-converting enzymes.

* Author to whom correspondence should be addressed:
Laboratoire de biochimie A –Hôpital Saint-Antoine
184, rue du Fbg Saint-Antoine – 75571 Paris Cedex 12 – France
Tel : 33.1.49.28.20.13 ; Fax : 33.1.49.28.20.77.

SUMMARY

Angiotensin I-converting enzyme (ACE) was purified from pig lung and serum to compare their physicochemical, catalytic and chemical properties with a particular focus on their sugar moieties; pig and rat pulmonary enzymes were also compared.
For the three forms of ACE the molecular mass was 172 ± 4 kDa on SDS-PAGE. The isoelectric point (pI) was 4.4 – 4.8 for both pulmonary ACEs but lower for serum ACE (4.3 – 4.6) ; neuraminidase increased both pI to 4.8 – 5.2. Serum and lung porcine ACEs exhibited identical Km, Kcat, optimal pH and optimal chloride activating concentration for two synthetic specific substrates. From amino-acid analysis, slight differences were shown between serum and pulmonary ACEs, and between pig and rat, in particular both pulmonary forms contained more hydrophobic amino-acids. From sugar analysis, serum ACE was more glycosylated (11.9%) than pig and rat lung enzymes (8.6% and 8.8%, respectively); neither of the three ACEs contained N-acetylgalactosamine but only the sugars characteristic of N-glycosylation. Pig serum ACE was richer in N- acetylneuraminic acid than the pulmonary from that corroborates with differences in pI; both forms presented sugar molar ratios prediting mixtures of N-acetyllactosaminic and oligomannosidic chains, or hybrid structures, for rat lung ACE also but differently. Thus, membrane-bound and soluble ACEs have different chemical compositions that could correspond to translational and post-translational events in the endothelial cell, in particular for anchoring of the membrane-bound form and for excreting a soluble form protected against hepatic lectins. Nevertheless, the differences on amino-acid and sugar compositions have no influence on ACE catalytic properties.

Key-words: angiotensin I-converting enzyme; endothelium; isoelectric point ; glycoproteins ; membranous enzymes.

The abbreviations used are : ACE, angiotensin I-converting enzyme (peptidyl- dipeptidase, EC 3.4.15.1) ; FAPGG, furylacryloyl-phenylalanyl-glycyl-glycine ; HHL, hippuryl—histidyl-leucine (hippuric acid as benzoylglycine) ; IEF , isoelectric focusing ; SDS-PAGE, sodium dodecylsulfate/ polyacrylamide-gel electrophoresis.

INTRODUTION

Aangiotensin I-converting enzyme (ACE) (or peptidyldipeptide hydrolase EC.3.4.15.1) is a key enzyme in renin- angiotensin and kallikrein-kinin systems, by removing the carboxyterminal dipeptides of angiotensin I and bradykinin, thereby actiavting the former into angiotensin II, a vasopresseur, and degrading the latter, a vasodilator. ACE is a zinc-containing glycoprotein which is expressed in various tissues under different molecular forms, but always with one polypeptide chain. The somatic isoenzyme seems to exist under two molecular forms, a membrane-bound form and a soluble form. The membrane-bound form is mainly located in vascular endothelial cells as an ectoenzyme in particular from pulmonary microvasculature (Caldwell *et al.*, 1976; Ryan *et al.*, 1976) but also on the brush-border of renal tubular and intestinal epithelial cells (Bruneval *et al.*, 1986), and in monocytes and macrophages (Friedland *et al.*,1978). On the other hand, testicular cells synthesize a germinative isoenzyme with a lower molecular mass, around 100 versus around 160 kDa for the somatic enzyme (Kumar *et al.*, 1989;

Ehlers *et al.*, 1991). The latter has two highly homologous domains each bearing a putative catalytic site, whereas the smaller isozyme from germinative cells has a single domain therefore only one active site and would represent the ancestral, non-duplicated form of the ACE gene (Alhenc-Gelas*et al.*, 1990). A circulating soluble form of ACE is found in blood plasma and is most likely released by the vascular endothelial cells (Hayes *et al.*, 1978; Baudin *et al.*, 1997b). In particular, several recent studies have shown that a specific secretase is able to liberate a soluble ACE form from plasma membrane (Wei *et al.*, 1991; Beldent *et al.*, 1993). The relative role of the plasma enzyme in the mechanisms responsible for the control of the vascular tone has not been clearly established, eventhough it seems important to know in regard of the number of ACE inhibitors used in the treatment of hypertension; in particular, some inhibitors have been described for binding on tissular targets, such as the vascular endothelium (Unger *et al.*, 1984; Baudin and Drouet, 1989). On the other hand, plasma ACE level is an useful marker of endothelial cell injury (Drouet *et al.*, 1988); the clinical interest in plasma ACE also results from its abnormal elevation in granulomatous diseases, such as sarcoidosis in which activated macrophages synthesize and release large amounts of the enzyme (Friedland *et al.*, 1977; Bénéteau-Burnat and Baudin, 1991).
Whether endothelial ACE is released by a specific proteolytic mechanism or by another way, for example incriminating glycosylations, it seemed to us relevant to compare at both physicochemical and enzymological levels ACE released by endothelial cells, the soluble form, and ACE anchored in plasma membrane of endothelial cells, the membranous form. The latter was purified from pig lung as it contains as number of capillaries anchoring membranous ACE; a soluble form of the enzyme was purified from pig serum. We compared their chemical compositions, with a particular focus on their sugar moieties, and some of their enzymatic and physicochemical properties.

MATERIALS AND METHODS

1- Materials and reagents:

Hippuryl-L-histidyl-L-leucine (HHL) was purchased from Bachem (Bubendorf, Switzerland), [glycine-1-^{14}C]-HHL from New England Nuclear (Boston, MA, USA) and furylacryloyl-phenylalanyl-glycyl-glycine (FAPGG) from Sigma (Saint Louis, USA). All other reagents of analytical grade were provided by Merck (Darmstadt, Germany) or Prolabo (Paris, France).

2- Purification of lung and serum ACEs:

Tissues were collected from freshly slaughtered normal Large White pigs. Lung and serum ACEs were purified by a protocol including DEAE-Spherodex ion-exchange (for lung only), lisinopril-Sepharose affinity chromatography and superose 12 size-exclusion chromatography as previously described (Baudin *et al.*, 1991).
ACE was also purified from rat lung (Wistar) using a similar protocol as for pig lung and as previously described (Bénéteau-Burnat *et al.*, 1994). Each purified preparation was extensively dialyzed against 2 mM Tris, 0.1 M NaCl, 1 mM $ZnCl_2$, pH=8.0 buffer for complete restoring of ACE activity.

3- Determination of ACE activity:

ACE activity was determined on two substrates : i) a radiometric assay used an isotopic dilution of the radiolabelled substrate [^{14}C-glycine]-HHL (Baudin *et al.*, 1990); ii) a spectrophotometric assay used the substrate FAPGG with a continuous kinetic measure (Bénéteau-Burnat *et al.*, 1986) for both assays one unit is defined as the quantity of enzyme that hydrolyses 1µmol of substrate per minute at 37°C. Proteins were measured by the Lowry's method (1951) or by spectrophotometric determination at 280 nm using the coefficient of absorption which we previously measured (Baudin *et al.*, 1995).

4- Electrophoreses:

Sodium dodecylsulfate- polyacrylamide electrophoresis (SDS-PAGE) isoelectric focusing (IEF) were performed on LKB-Pharmacia (Uppsala, Sweden) systems and using Biorad (Richmond, USA) or Pharmacia materials, respectively, as previously described (Baudin *et al.*, 1991; Bénéteau-Burnat *et al.*, 1994).

5- Amino-acid analysis:

Quantitative amino-acid analysis was carried out by ion-exchange chromatography with analyzer model 119CL (Beckman-Fullerton, USA) after hydrolysis with 5.6 N HCl in evacuated sealed glass vials for 24 hours at 110°C of 200 µg of purified ACE.
Amino-acids were eluted by increasing pH gradient of sodium citrate buffer and quantified by ninhydrin reaction. The standard was 2.5 M mixture of free amino-acids (Beckman). Cysteine was determined as hemi-cysteinyl; tryptophan and methionine were not measured. The hydrophobic amino-acid content was calculated according to Heller (1968).

6- Sugar analysis:

The molar composition in oligosaccharides was determined by gas chromatography of trimethylsilylated methyl-glycosides according to Kamerling *et al.* (1975) modified by Montreuil *et al* Respectively 42 µg and 55 µg of purified lung and serum ACE were treated and mesoinositol was used

as internal standard. Gas chromatography was carried out on Girdel chromatographe model 300 with capillary column (0.32 mm ID x 30 m) coated with OV101 as stationnary phase. A gradient of temperature was applied from 100 to 240°C with a temperature rate of 2°C/min. The gaz vector was helium.

Neuraminidase, from *Clostridium Perfringens* (type X – Sigma), was used in 0.05 M citrate/phosphate (sodium salts), 0.02 M $CaCl_2$, pH= 5.6 buffer at 1 unit per 100 µg of purified ACE and at 37°C.

RESULTS

Comparison of physicochemical and catalytic properties between porcine lung and serum ACEs :

2.5 mg of pulmonary ACE and 0.8 mg of serum ACE were isolated from 300 grams of lung tissue and 2 liters of blood, respectively, and with specific activities of 24 and 19 U-HHL/mg of protein, respectively. The purity of the preparations was assessed by SDS-PAGE where one single band characterized each enzyme with an identical *Mr* of 172000 ± 4000 and even when both enzymes were mixed (Figure 1) ; the absence of reduction reagent in buffer did not modify their electrophoretic mobilities (not shown).

Both enzymes were studied by isoelectric focusing which showed a more acidic isoelectric point (pI) for serum ACE (4.3 – 4.6) than for the pulmonary enzyme (4.4 -4.8); each isoform appeared as a multiband pattern recovering all the ACE activity fluorimetrically measured in the gel; after neuraminidase treatment, pI of ACE increased to 4.8 – 5.2 for both forms and still with several bands recovering the enzymic activity (Figure 2).

Both purified enzymes exhibited very close Michaelis kinetic properties, i.e. identical Km, Kcat, optimal pH and optimal chloride activating concentration, for HHL as well as for FAPGG. The only discrepancy was a catalytic efficiency (Kcat/Km) slightly higher for serum ACE than for the pulmonary form (+ 23% with HHL, + 12 % with FAPGG) (Table I).

Chemical composition of the purified enzymes :

From the determination of amino-acid content, glutamate and aspartate were the most represented residues in both porcine ACEs and cysteine content was low for both. As the accuracy of the method of determination of amino-acids was near 10 %, the most stricking differences were shown for arginine, histidine and tyrosine which are more represented in lung ACE, and glycine, serine and lysine which are in greater amount in serum ACE (Table II). Heller's ratio was 41.3 % and 37.6 % for lung and serum ACE,, respectively.

From the determination of sugar content, pig lung ACE appeared less glycosylated than the serum enzyme (8.6 % and 11.9 %, respectively). The latter was essentially richer in both N-acetylneuraminic acid and mannose, whereas pulmonary ACE was substantially richer in fucose (Table III). They did not contain any N-acetylgalactosamine. The establishment of sugar molar ratio on the basis of three mannoses showed that both glycoproteins had only slightly different molar ratios for N-acetylglucosamine and galactose; pulmonary ACE again appeared more fucosylated and serum ACE more sialylated.

ACE was also purified from rat lung and with identical pI and Mr to those of pig lung ACE (not shown); but its chemical composition showed some discrepancies from pig lung ACE: in particular rat ACE was slightly richer in serine, proline, isoleucine and above all in cysteine residues, but poorer in glutamic acid, leucine, alanine and arginine (Table II) nevertheless Heller's ratio was not different (41 %). Rat ACE contained substantially less fucose and N-acetylglucosamine but more sialic acid, whereas its total sugar content (8.8 %) was near that of pig lung ACE (Table III).

DISCUSSION

Numerous authors have studied at biochemical level the relationships between tissular ACE, namely the membranous form anchored in luminal plasma membrane of the vascular endothelium, and soluble ACE, i.e. a soluble form circulating in blood stream (Soffer *et al.*, 1974; Weare *et al.*, 1982 Harris and Wilson, 1982). When many of their results are heterogeneous or ambiguous in term of animal origin, neither of them has shown stricking differences between both forms. In particular no evidence for a difference in the length of their polypeptide chain could be detected. Nevertheless, such a hydrophobic segment could be very short, certainly less than 10 kDa as deduced from molecular biology data (Soubrier *et al.*, 1988), and then not visible by the usual biochemical methods. New data showing a specific secretase activity for membrane-bound ACE are in accordance with the existance of such an anchoring fragment (Hopper *et al.*, 1987; Wei *et al.*, 1991). Our data on the pig show that membranous and soluble ACEs are very close at both physicochemical and enzymological levels: (1) they exhibit the same Mr on SDS-PAGE, (2) the identical Stockes radius in size-exclusion chromatograph (not shown) and previous studied on pulmonary ACE (Baudin *et al.*,1996) (3), also the same reactivity against antiACE antibody in western-blot analysis (personal communication), and (4) identical enzymic parameters, i.e. Km, Kcat, optimal pH and optimal chloride activating concentration.

The main differences were encountered at the chemical level. The establishment of amino-acid composition shows that the pulmonary endothelial form contains significantly more hydrophobic amino-acids than the soluble form that agrees with a more hydrophobic polypeptide which could be the anchorage fragment. Moreover, close amino-acid compositions were determined for human kidney ACE (Weare *et al.*,1982), bovine lung ACE (Harris and Wilson, 1982) and here for rat lung ACE. From our study, glutamate and aspartate are the most represented residues in all the purified ACEs, that easily explains their acidic isoelectric point (pI); the multiband patterns on isoelectric focusing could be related to microheterogeneities in the primary structure or in the glycanic chains. The difference on pI between both porcine forms cannot be explained by a difference in dicarboxylic acid content but more easily in sialic acid content as we confirm by chemical analysis, and as did Das *et al.* (1977) for rabbit ACE. Curiously, ACE purified from rat lung contains more sialic acid than pig lung ACE, whereas their pI are very close. The role of sialic acid on pI was specified by the treatement of pig lung and serum ACEs by neuraminidase, which increased both pI, remaining acidic but now identical. But neuraminidase treatement did not abolish the multiband pattern on isofocalisation suggesting that all the microheterogeneities are not related to the variation observed on sialylation.

About the total sugar content of ACE, widely variable values were reported, often by indirect estimations: from 8 % for rat lung (our data) to almost 30 % for bovine lung, human serum (Harris and Wilson, 1982) and human kidney ACE (Weare *et al.*, 1982); 16 % was reported for both canine (Conroy *et al.*, 1978) and rabbit (Soffer *et al.*, 1974) lung ACEs. By direct quantification, we found a value near 8 % for pig lung ACE and also the rat enzyme, but pig serum ACE clearly is more glycosylated than the pulmonary form; in a previous work we already found 8 % of sugars for porcine lung ACE using the same methods but with another ACE preparation (Baudin *et al.*, 1988). All the ACE preparations seemed to be only N-glycosylated since N-acetylgalactosamine could not be detected, this osamine being specific for O-glycosylation. Our previous studies on lectin-affinity chromatography have confirmed N-glycosylation of pig lung ACE as it completely bound onto concanavalin A, a lectin recognizing polymannose features and biantennary N-acetyllactosaminic or hybrid structures, whereas the binding onto wheat germ agglutinin was less effective that agrees with a relatively poor content in heavily sialylated structures (Baudin *et al.*, 1997a). The establishement of sugar molar ratio could not define a characteristic type of N-glycans. For pulmonary ACE, the ratio of 3/3/2.5 between N-acetylglucosamine, mannose and galactose is close to that of biantennary N-acetyllactosaminic glycans; but only some chains would be sialylated whereas all of them would contain one fucose residue. These results correlate with Hartley and Soffer's data (1978) characterizing a lactosaminyl glycopeptide in a pronase digest of rabbit pulmonary ACE. For the serum enzyme, the sugar ratio is 2.4/3/2.4 that suggests a mixture of oligomannosidic and N-acetyllactosaminic types, or hybrid, glycanic chains. But we cannot discard the hypothesis that plasma soluble ACE could be a mixture of both solubilized membrane-bound ACE and specifically secreted ACE. It is also possible that different cell lines could produce ACE forms with differential glycosylations, for example as described by Hooper and Turner (1987) in the pig brain. Recent findings of Ripka *et al.* (1993), based on enzymatic deglycosylations and ConA affinity, indicated that ACE preparations purified from human, rat, rabitt and guinea pig tissues would be heterogeneous in terms of numbers of N-glycosylated sites and types of structure of oligosaccharides, but these authors did not compare plasma and lung ACEs from the same species.

In conclusion, pulmonary and serum ACE seem to be very close at both structural and enzymatic levels. The differences shown in amino-acid content are in agreement with the recent findings which demonstrate the presence in membrane-bound ACE of an anchorage fragment which can be cleaved by a specific mechanism. It could be especially the case for the ACE form facting the lumen of the endothelium, but no result has assessed that, *in vivo*, a specific hydrolytic process is physiological or effective in every physiopathological situation. In particular, our findings on sugar content rather agree with a differential processing: one ACE form could be specialized for direct secretion form the endothelial cell to blood stream, thus without the anchorage segment but with glycanic chains rich in sialic acid for protection against hepatic lectins; another form could be assigned to membrane anchorage, thus richer in hydrophobic residues, amino-acid and sugar. Taken together with reports of potential N-glycosylation sites, our data indicate that pulmonary and serum ACEs differ in terms of degree of glycosylation, structures of bound oligosaccharide chains and may be also sites of glycosylation since both forms partially differ in amino-acid content.

REFERENCES

Alhenc-Gelas F., Soubrier F., Hubert C., Allegrini J., Lattion A. L. and Corvol P. (1990) The angiotensin I-converting enzyme (kininase II): Progress in molecular and genetic structure. *J. Cardiovasc. Pharmacol.* 15,S25-S29.

Baudin B. and Drouet L. (1989) In vitro interactions between ramiprilat and angiotensin I-converting enzyme in endothelial cells. *J. Cardiovasc. Pharmacol.*14, S37-S42.

Baudin B., Alves N., Pilon A., Bénéteau-Burnat B. and Giboudeau J. (1997a) Structural and biological roles of glycosylations in pulmonary angiotensin I-converting enzyme. *Glycobiology* 7,565-570.

Baudin B., Bénéteau-Burnat B., Baumann F.C. and Giboudeau J. (1990) A reliable radiometric assay for the determination of angiotensin I-converting enzyme activity in urine. *J. Clin. Biochemie.* 28, 857-861.

Baudin B., Bérard M., Carrier J.L., Legrand Y. and Drouet L. (1997b) Vascular origin determines angiotensin I-converting enzyme expression in endothelial cells. *Endothelium* 5, 73-84.

Baudin B., Mario N., Gaba S. and Giboudeau J. (1995). Spectrocospic studies on angiotensin I-converting enzyme. *Enzyme Protein* 48, 265-274.

Baudin B., Mario N., Robic D. and Giboudeau J. (1996). Angiotensin I-converting enzyme molecular mass by size-exclusion high performance liquid chromatography and capillary electrophoresis. *Int. J. Biochromatogr.* 2, 109-118.

Baudin B., Tahraoui A. Baumann F.C., Robic D., Drouet L. and Legrand Y. (1991) Purification and analysis of lung and plasma angiotensin I-converting enzyme by high-performance liquid chromatography. *Protein Expr. Purif.* 2, 412-419.

Baudin B., Timmins P.A., Drouet L., Legrand Y. and Baumann F. C. (1988) Molecular weight and shape of angiotensin I-converting enzyme. A neutron scattering study. *Biochem. Biophys. Res. Commun.* 154, 1144-1150.

Beldent V., Michaud A., Wei L., Chauvet M. T. and Corvol P. (1993). Proteolytic release of human angiotensin-converting-enzyme. *J.Biol. Chem.* 168: 26428-26434.

Bénéteau-Burnat B. and Baudin B. (1991) Angiotensin-converting enzyme: clinical applications and laboratory investigations on serum and other biological fluids. *Crit. Rev. Clin. Lab. Sci.* 28, 337-356.

Bénéteau-Burnat B., Baudin B., Morgant G., and Giboudeau J. and Baumann F.C. (1986) Automated kinetic assay of angiotensin-converting enzyme in serum. *Clin. Chem. 32, 884-886.*

Bénéteau-Burnat B., Tahraoui A., Barbut F., Giboudeau J. and Baudin B., (1994) Physicochemical and immunological comparisons between angiotensin I-converting enzymes purified from different mammalian species. *Comp. Biochem. Physiol.* 109B, 623-635.

Bruneval P., Hinglais N., Alhenc-Gelas F., Tricotet V., Corvol P., Ménard J., Camillieri J.P. and Bariéty J. (1986) Angiotensin I-converting enzyme in human intestine and kidney. Ultrastructural immunohistochemical localization. *Histochemistry* 85, 73-80.

Caldwell P.R., Seegal B.C., Hsu K.C., Das M. and Soffer R.L. (1976) Angiotensin -converting enzyme : Vascular endothelial localization. *Science* 191, 1050-1051.

Conroy J.M., Hartley J.L. and Soffer R.L. (1978) Canine pulmonary Angiotensin -converting enzyme. Physicochemical, catalytic and immunological properties. *Biochim. Biophys. Acta* 524, 403-412.

Das M., Hartley J.L. and Soffer R.L. (1977) Serum-angiotensin -converting enzyme. Iolation and relationship to the pulmonary enzyme. *J. Biol. Chem.* 252, 1316-1319.

Drouet L., Baudin B., Baumann F.C. and Caen J.P. (1988) Serum-angiotensin -converting enzyme : an endothelial cell marker. Application to thromboembolic pathology. *J. Lab. Clin. Med.* 112, 450-457.

Ehlers M.R.W., Chen .N.P. and Riordan J.F. (1991) Spontaneous solubilization of membrane-bound human testis Angiotensin -converting enzyme expressed in Chinese hamster ovary cells. *Biochemistary* 88, 1009-1013.

Friedland J., Setton C. and Silverstain E. (1977) Angiotensin -converting enzyme : induction by steroids in rabbit alveolar macrophages in cultures. *Science,* 197, 64-66.

Friedland J., Setton C. and Silverstain E. (1978) Induction of angiotensin -converting enzyme in human monnocytes in cultures. *Biochem. Biophys. Res. Commun.* 83, 843-849.

Harris R.B. and Wilson I.B. (1982) Physico-chemical characteristics of homogeneous bovine lung angiotensin I -converting enzyme. Comparison with human serum enzyme. *Int. j. Peptid Protein Res.* 20, 167-176.

Hartley J.L. and Soffer R.L. (1978) On the oligosaccharide moiety of angiotensin -converting enzyme. *Biochem. Biophys. Res. Commun.* 83, 1545-1552.

Hayes L.W., Goguen C.A., Ching S.G. and Slakey L.L. (1978) Angiotensin -converting enzyme :

accumulation in medium from culture of endothelial cells. *Biochem. Biophys. Res. Commun.* 82, 1147-1153.

Heller J. (1968) Structure of visual pigments. I. Purification, molecular weight and composition of bovine pigment 500. *Biochemistry* 7, 2906-2913.

Hooper N.M. and Turner A.J. (1987) Isolation of two differentially glycosylated forms of peptidyl-dipeptidase A (angiotensin -converting enzyme) from pig brain: a re-evaluation of their role in neuropeptide metaboloism. *Biochem. J.* 241, 625-633.

Hooper N.M., Keen J., Pappin D.J.C. and Turner A.J. (1987) Kidney angiotensin -converting enzyme. Purification and characterization of amphiphatic and hydrophilic forms of the enzyme establishes C-terminal anchorage to theplasma membrane. *Biochem. J.* 247, 85-93.

Kamerling J.P., Gerwig G.J., Vliegenthart J.F.G. and Clamp J.R. (1975) Characterization by gas liquid chromatography-mass spectrometry and proton magnetic resonance spectroscopy of pertrimethylsilyl glycosides obtained in the methanolysis of glycoproteins and glycolipids. *Biochem. J.* 151, 491-495.

Kumar R.S., Kusari J., Roy S.N., Soffer R.L. and Sen G.C. (1989) Structure of testicular angiotensin -converting enzyme. A segmental mosaic isoenzyme. *J.Biol. Chem.* 28, 16754-16758.

Lowry O.H., Rosebrough N.J., Farr A.L. and Randall E.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.

Montreuil J., Bouquelet S., Debray J., Fournet B., Spik G. and Strecker G. (1986) Glycoproteins. In: *Carbohydrate analysis: a practical approach* (Chaplin MF, Kennedy JF) I.R.L. Press, Oxford, Washington: pp 143-204.

Ripka J.E., Ryan J.W., Valido F.A., Chung A.Y.K., Peterson C.M. and Urry R.L. (1993) N-glycosylation of forms of angiotensin -converting enzyme from four mammalian species. *Biochem. Biophys. Res. Commun.* 196,503-508.

Ryan U.S., Ryan J.W., Whitaker C. and Chin A. (1976) Localization of angiotensin -converting enzyme (kininase II). II. Immunocytochemistry and immunofluorescence. *Tissue Cell* 8, 125-146.

Soffer R.L., Reza R. and Caldwell P.R.B. (1974) Angiotensin -converting enzyme from rabbit pulmonary particles. *Proc. Nalt. Acad. Sci. USA,* 71, 1720-1724.

Soubrier F., Alhens-Gelas F., Hubert C., Allegrini J., Tregear G. and Corvol P. (1988) Two putative active centers in human angiotensin I-converting enzyme revealed by molecular cloning. *Proc. Nalt. Acad. Sci.USA* 85, 9386-9390.

Unger T., Ganten D., Lang R.E. and Schölkens B.A. (1984) I s tissue converting enzyme Inhibitors? Studies with two different compounds, Hoe-498 and MK-521, in spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.* 6, 872-880.

Weare J.A., Gafford J.T., Lu H.S. and Erdös E.G. (1982) Purification of human kidney angiotensin I-converting enzyme using reverse-immunoadsorption chromatography. *Anal. Biochem.* 123? 310-319.

Wei E., Alhens-Gelas F., Soubrier F., Michaud A., Corvol P. and Clauser E. (1991) Expression and characterization of recombinant human angiotensin I-converting enzyme. Evidence for a C-terminal transmembrane anchor and for a proteolutic processing of the secreted recombinant and plasma enzymes. *J. Biol. Chem.* 266, 5540-5546.

LEGENDS FOR FIGURES

Figure 1 : Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of ACE purified from pig tissues, Lane T, molecular weight standard ; Lane 1, pig lung ACE ; Lane 2, pig serum ACE. Lane 3, equimolar mixture of both samples. All the samples were submitted to dithiothreitol reduction ; Coomassie blue staining.

Figure 2: Polyacrylamide gel isoelectric focusing of purified porcine ACEs in pH range 4.0-6.5 ; a- Lane 1: serum ACE, Lane 2: lung ACE (Coomassie blue staining); b- Effect of desialylation on pig lung ACE, Lane 3 : after neuraminidase, Lane 4 : native ACE, Lane 5 : equimolar mixture of native and desialylated ACEs, Lane 6 : pI markers (silver nitrate staining after Coomassie blue staining and destaining) ; c- Specific ACE zymography with hippuryl-histidyl-leucine a substrate and fluorescence revelation, Lanes 7 and 8: lung ACE, Lanes 9 and 10: serum ACE, 8 and 10: after neuraminidase.

Table I – comparative kinetic properties of purified lung and serum ACEs as judged on HHL and FAPGG

	HHL		FAPGG	
	Lung	Serum	Lung	Serum
Km (mM)	1.16	1.09	0.66	0.64
Kcat (µmol/min/mg)	245	285	400	433
Kcat/Km	211	261	606	676
Optimal pH	8.3	8.3	8.2	8.2
Optimal [Cl^-] (mM)	375	375	350	350

Table II – Comparative amino-acid composition between lung and serum ACEs

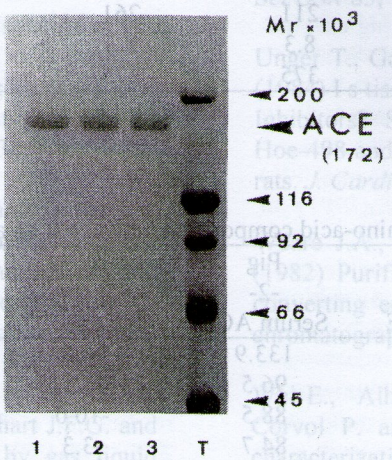
	Pig -1- Lung ACE	Pig -2- Serum ACE	-Δ2/1- (%)	Rat -3- Lung ACE	-Δ3/1- (%)
Glutamic acid	135.1*	133.9	-0.8	118.4	**-14.1**
Aspartic acid	102.4	96.5	-6.1	113.9	+10.0
Leucine	97.3	88.5	-10.0	86.6	**-11.4**
Alanine	87.5	84.7	-3.3	74.9	**-16.8**
Glycine	75.9	98.4	**+29.5**	82.0	+7.4
Serine	62.3	73.8	**+18.4**	74.0	**+15.8**
Proline	61.2	57.2	-7.0	72.8	**+15.9**
Threonine	61.0	64.6	+5.8	55.6	-9.7
Valine	59.6	62.8	+5.4	54.0	-10.4
Lysine	50.9	60.7	**+19.2**	48.1	-5.8
Phenylalanine	47.3	43.5	-8.7	45.9	-3.0
Arginine	45.3	38.9	**-16.6**	40.1	**-13.0**
Histidine	42.1	32.6	**-29.2**	40.1	-5.0
Isoleucine	34.1	31.7	-7.4	41.5	**+17.8**
Tyrosine	31.8	25.3	**-25.6**	30.0	-6.0
1/2-Cysteine	6.3	6.7	+6.4	29.4	**+78.6**

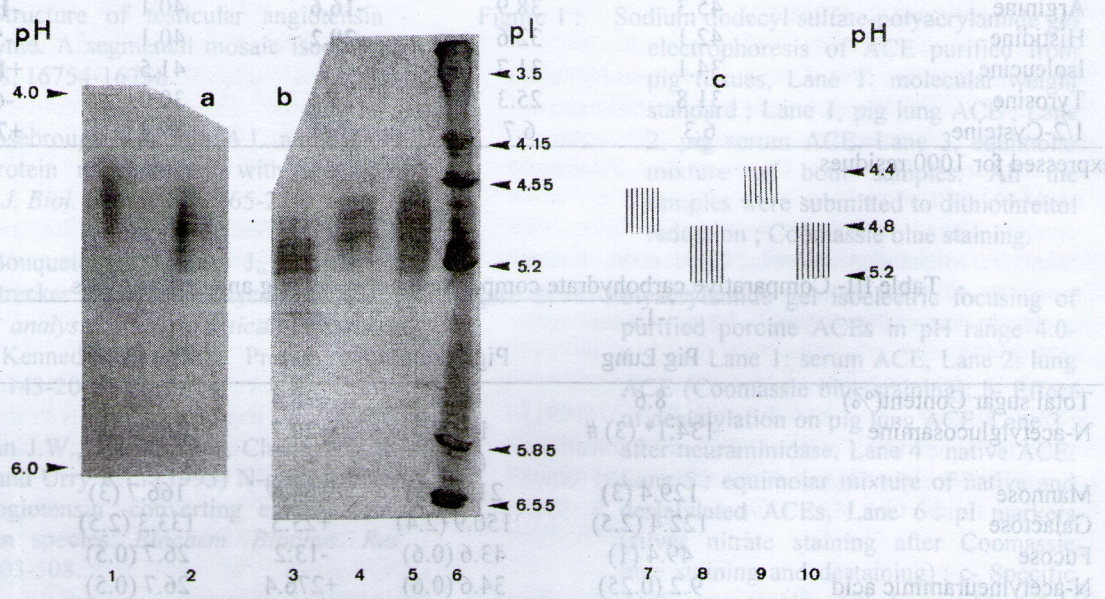
*expressed for 1000 residues

Table III- Comparative carbohydrate composition between lung and serum ACEs

	-1- Pig Lung	-2- Pig Serum	-Δ2/1- (%)	-3- Rat Lung	-Δ3/1- (%)
Total sugar Content(%)	8.6	11.9	+38.4	8.8	+2.3
N-acetylglucosamine	134.1* (3) #	161.8* (2.4)#	+20.7	100* (2) #	-34.1
Mannose	129.4 (3)	212.7 (3)	+64.4	166.7 (3)	+22.3
Galactose	122.4 (2.5)	150.9 (2.4)	+23.3	133.3 (2.5)	+8.2
Fucose	49.4 (1)	43.6 (0.6)	-13.2	26.7 (0.5)	-85.0
N-acetylneuraminic acid	9.2 (0.25)	34.6 (0.6)	+276.4	26.7 (0.5)	+65.5

*expressed as nmoles of monosaccharide per millgram of protein
#molar ratio on the basis of 3 Mannoses


Mr x10³
200
ACE
(172)
116
92
66
45
1 2 3 T


pH
4.0
6.0
a
b
pI
3.5
4.15
4.55
5.2
5.85
6.55
1 2 3 4 5 6
c
pH
4.4
4.8
5.2
7 8 9 10