Isolation of angiotensin converting enzyme from testes of *Locusta migratoria* (Orthoptera)

NATHALIE MACOURS1, ANICK VANDINGENEN1, CONSTANT GIELENS2, KORNEEL HENS1, GEERT BAGGERMAN1, LILIANE SCHOOF1 and ROGER HUYBRECHTS1

1Laboratory of Developmental Physiology and Molecular Biology, Katholieke Universiteit Leuven, Naamsestraat 59, B-3000 Leuven, Belgium

2Laboratory of Biochemistry, Katholieke Universiteit Leuven, Celestijnenlaan 200G, B-3001 Leuven-Heverlee, Belgium

**Key words.** ACE inhibitors, cDNA cloning, insects, *Locusta migratoria*, reproduction, testes

**Abstract.** By means of a tracer assay using a labeled synthetic angiotensin converting enzyme (ACE) substrate hip­purylglycylglycine, we have detected high ACE activity in the testes of the African migratory locust, *Locusta migratoria*. Lower, but significant, ACE activity was observed in midgut and hemolymph. In a two-step purification procedure involving anion exchange and gel permeation chromatography, we have purified *LomACE* from the locust testes. The enzyme of approximately 80 kDa shows substantial amino-acid sequence homology with ACE from both vertebrate and invertebrate origin. The ACE identity of the purified enzyme was further confirmed by cDNA cloning of the *Locusta* ACE fragment, which, after *in silico* translation, revealed a mature protein of 623 amino acids with a large structural similarity to other known ACE proteins.

**INTRODUCTION**

As a part of the renin-angiotensin system (RAS), the Zn2+ metalloprotease angiotensin converting enzyme, or ACE (dipeptidyl carboxypeptidase I, EC 3.4.15.1), is an important factor in the regulation of blood pressure and fluid and electrolyte homeostasis of mammals. By cleaving off its C-terminal dipeptide, ACE converts the decapeptidic angiotensin I into the potent vasoconstrictor angiotensin II. In addition, ACE inactivates the potent vasodilator bradykinin by the sequential removal of two C-terminal dipeptides (Corvol et al., 1995). ACE functionality is, however, not restricted to blood pressure regulation. The hemoregulatory peptide N-Ac-Ser-Asp-Lys-Pro, which is an inhibitor of haematosiatic stem cell proliferation, is inactivated through hydrolysis by ACE (Rousseau et al., 1995; Azizi et al., 1996). ACE also exerts a very broad *in vitro* substrate specificity, hydro­lyzing various peptides such as bradykinin, substance P, luteinising hormone-releasing hormone, angiotensin I and angiotensin II (Hooper, 1991). It is, in addition, to its important role in blood pressure, known to be involved in developmental processes. Its exact function in these processes is still under investigation (Ganong, 1995; Vinson et al., 1997; Kessler et al., 2000).

Mammalian ACE exists in two isoforms. The somatic form (sACE) is composed of two highly similar domains, each containing one similar catalytic site. They are called the N- and C-domain, according to their relative position within the protein. The testicular isoform (tACE) has only one domain and is homologous to the C-domain of sACE (Howard et al., 1990; Ehlers et al., 1992). While sACE is widespread throughout the body (Hooper et al., 1991), tACE expression is limited to maturing sperm and spermatoozoa (Vellutri, 1985; Pauls et al., 1999). sACE is responsible for blood pressure regulation, but the function of tACE remains uncertain, although a role in male fertility is hypothesized (Kessler et al., 2000; Hagaman et al., 1998).

ACE homologues have recently been discovered in several insect species (Isaac & Lamango, 1994; Loeb et al., 1998; Schoofs et al., 1998; Ekbote et al., 1999; Zhu et al., 2001a, b; Vandingenen et al., 2002). Although we have indicated that the *Neobellieria bullata* trypsin modulating oostatic factor (NebTMOF) is a putative *in vivo* substrate for *NebACE* (Vandingenen et al., 2001), the exact physiological relevance of insect ACE remains obscure (Isaac et al., 1998; Veelaert et al., 1999; Seinsche et al., 2000). Cloning and/or purification of ACE from the fruitfly *Drosophila melanogaster* (AnACE) (Cornell et al., 1995), the housefly *Musca domestica* (Lamango et al., 1996), the buffalo fly *Haematobia irritans exigua* (HieACE) (Wijffels et al., 1996), and the silk worm *Bombyx mori* (BmACE or ACE-related) (Quan et al., 2001) have contributed to the molecular and biochemical characterization of insect ACE, which is composed of only one domain and shares structural and enzymatic properties with tACE and the C-domain of sACE.

Because most of what is known about insect ACE characteristics originates from dipteran ACE homologues, we have conducted both the purification of *LomACE* from testes of *L. migratoria* and the cloning of its cDNA. The obtained data were used to compare the structural characteristics of orthopteran ACE with those of its dipteran and mammalian counterparts. The purified or expressed *LomACE* will be used in future investigations concerning the functions of insect ACE.

* Corresponding author. Tel: 00 32 (0) 16 / 32 39 00; fax: 00 32 (0) 16 / 32 39 02; e-mail: nathalie.macours@bio.kuleuven.ac.be
MATERIAL AND METHODS

Handling of insects

Locusts were reared as described (Ashby et al., 1972). For collection of hemolymph, animals were anesthetized with CO2 and a leg was amputated. Hemolymph was drawn from the bleeding wound with a pipette and immediately diluted tenfold in ice-cold Hepes buffer (0.05 M Hepes; pH 8.0; 0.3 M NaCl; 0.06 M (NH4)2SO4) to prevent coagulation. After centrifugation of the pooled hemolymph (13,000 rcf, 5 min, 4°C), supernatant (designated as hemolymph sample) was stored at −80°C. Each locust typically yielded 20–100 μl of (undiluted) hemolymph.

For dissection of testes and midgut, animals were anesthetized and head, legs and wings were cut off. The ventral side of the body was slit open; midgut and testes were removed and rinsed with Locusta Ringer solution (9.82 g/L NaCl; 0.48 g/L KCl; 0.19 g/L NaH2PO4; 0.25 g/L NaHCO3; 0.73 g/L MgCl2; 0.32 g/L CaCl2; pH 6.5). Tissues were pooled and homogenized in Tris buffer (Tris–HCl; pH 8.2; 20 mM). The homogenate was centrifuged (10,000 rcf, 30 min, 4°C) repeatedly until pelleting stopped. Supernatants were stored at −80°C.

ACE activity assay

ACE activity measurements were based on the method proposed by Ryan et al. (1977) and modified as in Vandingenen et al. (2001). Typically, reactions were conducted in a final volume of 100 μl in HEPES (see above) buffer. 40 μl of sample, to which 10 μl of buffer was added, was incubated at 37°C with 50 μl of tritiated substrate (2 μl buffered [1H]benzoylarginine p-nitroanilide ([H]-BAPNA) and [1H]benzoyl-arginine ethyl ester ([H]-BAEE) (1)). In a negative control, 10 μl of HEPES buffer (see above) containing 100 μM captopril (Sigma) was added (instead of 10 μl buffer), resulting in a complete inhibition of ACE activity (2). After a desired incubation time, reactions were terminated by adding 1 ml of 0.1 M HCl. The reaction product ([H]-hippurate) was separated from the unhydrolyzed substrate ([H]-Hip-Gly-Gly) by extraction with 1 ml of ethyl acetate. The two phases were mixed by vortexing and the layers were separated by centrifugation (10,000 g, 5 min, 4°C) repeatedly until peletting stopped. Supernatants were stored at −80°C.

Protein identification

Tryptic in-gel digestion. The protein band was excised from the Coomassie Blue-stained SDS-PAGE gel and sliced into small pieces, which were destained with two changes (20 min each) of 50 μl 50% acetonitrile (AcN) containing 50 mM NH4HCO3. With one change of 50% AcN, pieces were dehydrated until they became opaque white (+ 5 min). The pieces were dried and covered with 20 μl of 10 mM dithiothreitol (DTT). 100 mM NH4HCO3, reduced at 56°C for 1 h and cooled to room temperature. The DTT was replaced with 20 μl of 55 mM iodoacetamide, 100 mM NH4HCO3, and the pieces were incubated in the dark for 45 min at room temperature with occasional vortexing. Pieces were washed for 10 min with 50 μl of 100 mM NH4HCO3, dehydrated for 10 min by addition of 50 μl of 50% AcN, swollen for 10 min by rehydration in 50 μl of 100 mM NH4HCO3 and shrunk for 10 min by addition of 50 μl of 50% AcN. The liquid phase was removed and pieces were dried in a vacuum centrifuge for 5 min. Twenty-five μl of digestion buffer (50 mM NH4HCO3; 5 mM CaCl2; 25 ng trypsin/μl buffer) were added and the pieces were incubated for 45 min in an ice-bath. The supernatant was removed, replaced by 5 μl of 50 mM NH4HCO3, 5 mM CaCl2 and the pieces were incubated overnight at 37°C. Subsequently, the gel pieces were washed for 20 min with one change of 20 μl of 20 mM NH4HCO3 and peptides were extracted with three changes (20 μl and 20 min for each change) of 5% formic acid in 50% ACN at room temperature and dried.

Desalting and concentrating. The peptide mixture was desorbed in 50 μl of 2% ACN, 0.1% trifluoroacetic acid (TFA) and desalted by means of a ZipTip (Millipore), which is a pipette tip containing 1 μl of C18 beads at the orifice. The entire mixture was loaded onto the ZipTip in 10 μl batches. Subsequently, the tip was washed with 0.1% TFA to remove salts and the peptides were eluted with 3 μl of ACN/water/formic acid (70/0/9/0.1, v/v/v).

CID analysis with ESI-TOF MS. Nanoflow electrospray ionization quadrupole orthogonal acceleration time-of-flight mass spectrometry was performed on a Q-Tof system (Micro-
mass, UK). Two μl of the desalted and concentrated sample was loaded in a metal-coated capillary (Protana L/Q nanoflow needle). This sample was sprayed at a typical flow rate of 30 nl/min, giving extended analysis time in which we acquired an MS spectrum, as well as several MS/MS spectra. During MS/MS or tandem mass spectrometry, fragment ions are generated from a selected precursor ion by collision-induced dissociation (CID) (Morris et al., 1996). Because not all peptide ions fragment with the same efficiency, the collision energy was typically varied between 20 and 35 eV so that the parent ion was fragmented into a satisfying number of different daughter ions. Needle voltage was set at 900 V, cone voltage was 35 V. The obtained fragmentation spectra were combined and transformed into their singly charged state by treatment with the Max-ent3 software (Masslynx 3.5 software; Micromass, UK). Amino acid sequences were determined by calculating the mass differences between adjacent b-type ions and/or y-type ions.

cDNA cloning

Messenger RNA was isolated from Locusta testes according to the “Quickprep mRNA purification Kit” (Pharmacia). 0.8 μg of mRNA was used for single stranded cDNA synthesis (Marathon, Clontech), of which 0.1 μl was used in the PCR reaction. A first Locusta specific ACE (LomACE) cDNA fragment was obtained by PCR with two degenerate primers (SP1F : 5’ CAY YTN YWN GGN AAY ATG TGG GC 3’ and SP2R : 5’ RTC NCC NAC NGC YTC RTG RAA CN 3’) based on the consensus sequences deduced from the open reading frames of ACE sequences of several organisms. The reaction had an annealing temperature of 50°C and contained the following components: 2μg of each primer, 1× advantage 2 polymerase mix (Clontech), 0.5mM dNTP’s, 40 mM Tricine-KOH, 15 mM KOAc, 3.5 mM Mg(OAc)2, 3.75 pg/ml BSA. The PCR products were separated on a 1.2% agarose gel, cloned into the PCR 2.1 TA-cloning vector (Invitrogen) and sequenced. Double stranded cDNA synthesis and further RACE reactions were performed according to the Marathon protocol with 3’ and 5’ RACE primers (P4 : 5’ GAC ATC TCG GTT CCC TTC CCT GGA AAG C 3’ and M1 : 5’ GCT GAG GCA TGG CAT ATC AGT TCT TCC CC 3’) based on the LomACE fragment.

RESULTS

ACE activity in different tissues of L. migratoria

Testes, midgut and hemolymph samples could effectively hydrolyze the ACE substrate ³H-Gly-Gly-Gly (Fig. 1). Testicular tissue clearly contains the highest ACE activity, while hemolymph and midgut exhibit substantially less ACE activity.

Purification and identification of Lom-ACE from the locust testes

A 52 equivalent testes sample was subjected to anion exchange chromatography (Fig. 2). The majority of ACE activity eluted from fraction 75 to 95, corresponding to a NaCl concentration ranging from 0.18 M to 0.22 M (Fig. 2A). The maximum hydrolysis level reached ≈ 80% and the peak had a slight trailing tendency. As shown by the absorbance measurements, the bulk of the proteins present in the testes sample eluted in the first 50 (0–0.12 M NaCl) and the last 10 fractions (rinsing of the column with 1.00 M NaCl), while considerably fewer proteins eluted in the other fractions, especially in the region of ACE activity (Fig. 2B).

Active fractions of the Q-Sepharose chromatography (fractions 75–94) were pooled and concentrated by ultrafiltration. A hundred-fold dilution of the retentate (containing proteins > 10 kDa) exhibited 65% hydrolysis of ³H-Gly-Gly, while the filtrate was completely devoid of ACE activity. Part of the retentate (2 ml, corresponding to about 1/3 of the material) was submitted to gel chromatography on Ultrogel AcA34 (Fig. 3). ACE activity was only present in fractions 80 to 90. This activity was, however, not stable and decreased within 5 days. As indicated by the absorbance profile at 230 nm (data not shown), the bulk of the protein material in the retentate eluted at the salt volume of the column (proteins < 20 kDa). From its elution volume, the Mₗ of the ACE protein was estimated...
Fig. 3. ACE activity profile of gel permeation Ultrogel AcA34 chromatography of pooled active fractions of the Q-Sepharose chromatography. The elution positions of calibration proteins are indicated by arrows: Hc, hemocyanin (eluting at the void volume); SA₂ – serum albumin dimers; SA – serum albumin monomers; OA – ovalbumin; Mb – myoglobin.

to be 81,000, making use of a plot of log $M_r$ versus elution volume constructed with calibration proteins (Andrews, 1965). This value was in fair agreement with the result obtained by SDS-PAGE analysis. Indeed, the active fractions from the Ultrogel column revealed a $\approx 78$ kDa protein band of apparent homogeneity (Fig. 4). Non-active fractions did not contain this protein band.

To investigate whether this protein band corresponded to the enzyme that exerts ACE activity, the band in fraction 85 was excised from the SDS-PAGE gel and trypsinolyzed. The eluted peptides were subjected to a CID analysis with ESI-TOF MS. Amino acid sequences of 10 of the 12 identified peptide fragments were 100% identical with sequences within the LomACE amino acid sequence as predicted by the cDNA cloning of LomACE (Fig. 5). This alignment proves that the enzyme isolated from locust testes exerting ACE activity was visualized on an SDS-PAGE as a single protein band of $\approx 78$ kDa, of which the amino acid sequence displays sequence similarities with ACE orthologues from animal species of vertebrate and invertebrate origin.

The two other peptide fragments display no significant similarity with ACE sequences (data not shown).

**Structure of the LomACE sequence**

Sequence analysis of the RACE-PCR products revealed an ACE fragment of 623 AA’s, which represents the full mature protein sequence. Comparison with ACE protein sequences from several invertebrate organisms predicts that the first 10–20 AA’s, representing the signal peptide, are missing (Fig. 6). Several attempts to gain this 5’ sequence information failed. However, the full sequence of the mature ACE protein is identified. There is a good consensus site for cleavage of the presumed signal peptide between AA 3 and 4 (L-D), which results in a mature protein of 623 AA’s, with a calculated mass of 72 kDa deduced from its longest ORF. There are 4 possible consensus sites for N-glycosylation, from which 3 are conserved in *Drosophila* AnCE. The region with the highest similarity to other ACE proteins is situated around the conserved active site (His$^{317}$, His$^{364}$, Glu$^{388}$). The aspartic acid residue, Asp$^{388}$, believed to have a role in the positioning of the first zinc binding residue and the Glu$^{358}$, which is involved in catalysis (Corvol et al., 1995), are also conserved in the *Locusta* ACE sequence.

**DISCUSSION AND CONCLUSIONS**

ACE activity, defined as a captopril-inhibitable dipeptidyl carboxypeptidase activity towards $^3$H-Hip-Gly-Gly, was detected in testes, midgut and hemolymph of the African migratory locust, *Locusta migratoria*. These results are in agreement with the general tissue distribution of ACE in insects. Although this distribution is not identical in different species, ACE seems to be concentrated in certain tissues, including reproductive tissues, brain, midgut and hemolymph (Cornell et al., 1995; Ekbote et al., 1999; Vandingenen et al., 2001; Vandingenen et al., 2002), suggesting that the enzyme is of physiological importance in these tissues. Indeed, numerous reports substantiate the involvement of insect ACE in reproduction, pro-hormone processing and regulation of peptide titers (Isaac et al., 1994; Tatei et al., 1995; Wijffels et al., 1996; Lamango et al., 1997). Since ACE activity was highest in the testes, which is consistent with the former observation that very high ACE activity is found in locust adult testis (Isaac et al., 1998), this particular tissue was chosen for isolation of the enzyme.

An $\approx 80$ kDa enzyme displaying ACE activity was isolated from testes of *L. migratoria* by means of sequential anion exchange and gel permeation chromatography, two techniques that were also included in the purification procedure of ACE from leech and buffalo fly (Laurent & Salzet, 1996; Wijffels et al., 1996). Because 10 tryptic digestion products of this protein were 100% identical to the amino acid *LomACE* sequence as predicted by the partial cDNA cloning and displayed significant similarity with ACE sequences from other organisms, this dipeptidyl carboxypeptidase was identified as *LomACE*. The presence in the digest of two remaining peptide fragments, which could not be aligned with the ACE sequences, could indicate the presence of (an) equally sized contaminating protein(s) co-eluting with *LomACE*
The purification of LomACE from a testes sample, homogenized without the use of detergents and deprived of membranes, suggests that LomACE protein of 623 amino acids. The active site regions of mammalian and invertebrate ACE are conserved in the deduced amino acid sequence of Locusta migratoria angiotensin converting enzyme. The 10 peptides of the tryptic digest of the ≈78 kDa isolated protein are underlined. Possible N-glycosylation sites are indicated with an asterisk. The active site is shown in bold.
LomACE. The predicted translation product showed the largest identity and similarity with the Bombyx mori homologue of ACE. The difference in protein size between the purified band on the SDS-PAGE gel and the size as predicted by the deduce amino acid sequence can be attributed to the 3 N-glycosylation sites present in the LomACE sequence. Based upon the available information of invertebrate ACE sequences (Fig. 6), we expect a secretion signal at the 5' end of LomACE. We did not succeed in getting the full sequence information, so only the last two amino acids before the predicted cleavage site of this signal peptide (confirmed by N-terminal sequencing of the LomACE protein) are identified. However, sufficient cDNA information has been retrieved to identify the complete mature protein sequence and can be used in recombinant expression experiments.

In conclusion, a ~ 80 kDa soluble ACE homologue was purified to apparent homogeneity from testes of L. migratoria that shared high structural similarity with dipteran ACE, BmACE, the C-domain of mammalian sACE and with mammalian tACE. Whether these similarities imply any functional conservation remains to be determined. The presence of LomACE activity in testes, hemolymph, and midgut, however, suggests a physiological role for ACE in these tissues. The exact function of insect ACE remains unclear and will be investigated in future experiments using the purified ACE or the expression of recombinant LomACE.

**Abbreviations.** ACE – angiotensin converting enzyme; ACER – ACE-related; ACN: acetonitrile; AnCE – angiotensin converting enzyme; BmACE – Bombyx mori ACE; BmACER – Bombyx mori ACE-related; BmACER (accession AB026110.1) Drosophila melanogaster (AnCE, accession NM_057698.3) and Haematobia irritans (accession Q10715).

**Fig. 6. Alignment of LomACE with ACE homologues from Bombyx mori (BmACER, accession AB026110.1) Drosophila melanogaster (AnCE, accession NM_057698.3) and Haematobia irritans (accession Q10715).**
Hie — Haematothia irritans exiguia; Hip-Gly-Gly — Hip-
pyrrolglycylglycine; Lom — Locusta migratorina; Neb — Neobelli-
teria bullata; SACE — somatic ACE; tACE — testicular ACE;
TFA — trifluoroacetic acid; TMOF — trypsin modulating oostac
factor.

ACKNOWLEDGEMENTS. The authors wish to thank J. Putte-
mans and M. Christiaens for technical assistance. This project
was sponsored by the Flemish Science Foundation (FWO,
GO356.98 and GO187.00) and by the Research Foundation of

REFERENCES

ANDREWS P. 1965: The gel filtration behaviour of proteins
related to their molecular weights over a wide range.
Biochem. 1. 96: 595—606.

ASHBY G.J. 1972: Locustis. In: UFAW (ed.): The UFAW Hand-
book on the Care and Management of Laboratory Animals.

AZIZI M., ROUSSEAU A., EZAB E., GUYENE T.T., MICHELET S.,
GRONGET J.M., LENFANT M., CORVOL P. & MENARD J. 1996:
Acute angiotensin-converting enzyme inhibition increases the
plasma level of the normal stem cell regulator N-acetyl-seriy-

CORNELL M.J., WILLIAMS T.A., LAMANO N.S., COATES D.,
CORVOL P., SOUBREUR F., HOIBERG J., LEHRACH H. & ISAAC
R.E. 1995: Cloning and expression of an evolutionary con-
served single-domain angiotensin converting enzyme from

CORVOL P., WILLIAMS T.A. & SOUBREUR F. 1995: Peptidyl dipep-
tidase A: Angiotensin I-converting enzyme. Methods
Enzymol. 248: 283—305.

DEDDISH P.A., WANG J., MICHEL B., MORRIS P.W., DAVIDSON N.
active N-domain of human angiotensin I-converting enzyme.

N-terminal sequence of testis angiotensin-converting enzyme is
heavily O-glycosylated and unessential for activity or sta-

EDEBTE U., COATES D. & ISAAC R.E. 1999: A mosquito
(Anopheles stephensi) angiotensin I-converting enzyme (ACE)
is induced by a blood meal and accumulates in the

GAMONG W.F. 1995: Reproduction and the renin-angiotensin

HAGMAN J.R., MOYER J.S., BACHMAN E.S., SHROY M., MAGYAR
P.L., WELCH J.E., SMITHEIS O., KRIDGE J.H. & O’BRIEN D.A.

HOOPER N.M. 1991: Angiotensin converting enzyme: implica-
tions from molecular biology for its physiological functions.
Int. J. Biochem. 23: 641—647.

HOWARD T.E., SHAI S.Y., LANGLEORD K.G., MARTIN B.M. & BERN-
STEIN K.E. 1990: Transcription of testicular angiotensin-
converting enzyme (ACE) is initiated within the 12th intron

ISAAC R.E. & LAMANGO N.S. 1994: Peptidyl dipeptidase a
22: 2925.

ISAAC R.E., COATES D., WILLIAMS T.A. & SCHOFOS L. 1998:
Insect angiotensin-converting enzyme: comparative biochem-
Recent Advances in Arthropod Endocrinology. Soc. Exp.

KESLER S.P., ROWE T.M., GOMOS J.B., KESLER P.M. & SEN
G.C. 2000: Physiological non-equivalence of the two iso-
forms of angiotensin-converting enzyme. J. Biol. Chem. 275:
26259—26264.

LAEMMLI U.K. 1970: Cleavage of structural proteins during the
assembly of the head of the bacteriophage T4. Nature 227:
680—685.

LAMANGO N.S., SARD M. & ISAAC R.E. 1996: The endopeptidase
activity and the activation by Co2+ of angiotensin-converting
enzyme is evolutionarily conserved: purification and prop-
erties of an angiotensin-converting enzyme from the housefly,

LAMANGO N.S., NACHEMAN R.J., HAYES T.K., STREY A. & ISAAC
R.E. 1997: Hydrolysis of insect neuropeptides by an
angiotensin-converting enzyme from the housefly, Musca
domestica. Peptides 18: 47—52.

LAURENT V. & SALZET M. 1996: Biochemical properties of
the angiotensin-converting-like enzyme from the leech Thero-
myzon tessulatum. Peptides 17: 737—745.

LOEB M.J., DE LOOF A., SCHOFOS L. & ISAAC R.E. 1998: Angio-
tensin II and angiotensin-converting enzyme as candidate
compounds modulating the effects of testis ecdysiotropin in
testes of the gypsy moth, Lymantria dispar. Gen. Comp.
Endocrinol. 112: 232—239.

MORRIS H.R., PAXTON T., DELL A., LANGHORNE J., BERG M., BOR-
DOLI R.S., HOYES J. & BATeman H. 1996: High sensitivity
collisionally-activated decomposition tandem mass spec-
ometry on a novel quadrupole/orthogonal-acceleration time-
10: 889—896.

PAULS K., FINK L. & FRANK F. 1999: Angiotensin-converting
enzyme (CD143) in neoplastic germ cells. Lab. Invest. 79:
1425—1434.

QUAN G.X., MITA K., OKANO K., SHIMADA T., UGAKI N., XIA Z.,
GOTO N., KANKE E. & KAWASAKI H. 2001: Isolation and
expression of the ecdysiotropin-inhibiting angiotensin-
converting enzyme-related gene in wing discs of Bombyx

ROUSSEAU A., MICHAUD A., CHAUDET M.T., LENFANT M.
& CORVOL P. 1995: The hormone regulatory peptide Acetyl-Ser-
Asp-Lys-Pro is a natural and specific substrate of the
N-terminal active site of human angiotensin-converting

radioassay for angiotensin-converting enzyme. Bio-

SCHOFOS L., VEELAERT D., DE LOOF A., HUYBRECHTS R. & ISAAC
E. 1998: Immunocytochemical distribution of angiotensin
I-converting enzyme-like immunoreactivity in the brain and

SEINSCHE A., DUKER H., LÖSEL P., BACKHAUS D. & SCHEREN-
BECK J. 2000: Effect of helicokins and ACE inhibitors on
water balance and development of Heliothis virescens larvae.
J. Insect Physiol. 46: 1423—1431.

SOBREUR F., HUBERT C., TESTUT P., NADAUD S., ALHENC-GELAS F.
& CORVOL P. 1993: Molecular biology of the angiotensin I
converting enzyme: Biochemistry and structure of the gene.
J. Hypertens. 11: 471—476.

51: 157—168.

VANDENGENEN A., HENS K., MACOIRS N., SCHOFOS L., DE LOOF
A. & HUYBRECHTS R. 2002: Presence of angiotensin con-
verting enzyme (ACE) interactive factors in ovaries of the grey
27—35.

VANDENGENEN A., HENS K., MACOIRS N., ZHU W., JANSEN I.,
BREUER M., DE LOOF A. & HUYBRECHTS R. 2001: Captorpril, a

---

473


Received January 13, 2003; revised March 20, 2003; accepted June 26, 2003