JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ACE Inhibitory Peptides Derived from Enzymatic Hydrolysates of Animal Muscle Protein: A Review

LIESELOT VERCRUYSSE,*,†,‡ JOHN VAN CAMP,‡ AND GUY SMAGGHE†

Laboratory of Agrozoology, Department of Crop Protection, and Laboratory of Food Science and Human Nutrition, Department of Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium

Naturally occurring ACE (angiotensin converting enzyme) inhibitory peptides have a potential as antihypertensive components in functional foods or nutraceuticals. These peptides have been discovered in various food sources from plant and animal protein origin. In this paper an overview is presented of the ACE inhibitory peptides obtained by enzymatic hydrolysis of muscle protein of meat, fish, and invertebrates. Some of these peptides do not only show in vitro ACE inhibitory activity but also in vivo antihypertensive activity in spontaneously hypertensive rats. To focus on new sources of ACE inhibitory peptides, more specifically insects and other invertebrates, we compared the vertebrate and invertebrate musculature and analyzed phylogenetic relationships.

Keywords: Angiotensin converting enzyme inhibitors; bioactive peptides; muscle

INTRODUCTION

The nutritional and functional properties of food proteins have been investigated for many years. The nutritional quality of a protein depends on its amino acid content and on the physiological utilization of specific amino acids after digestion and absorption (*1*). In terms of their functional properties, proteins contribute to the physicochemical and sensory properties of various protein-rich foods (*2*). Furthermore, the possibility to release biologically active peptides from food proteins has gained a lot of interest (*1*, *3*). Bioactive peptides are inactive within the sequence of the parent protein and can be released by proteolytic enzymes during gastrointestinal digestion or during food processing (*3*). Once they are liberated in the body, bioactive peptides can affect numerous physiological functions of the organism. For example, they may act as antihypertensive agents or as opioid agonists or antagonists. Furthermore, immunomodulating, antithrombotic, antioxidative, anticancer, and antimicrobial activity has been reported (*4*). Because of their therapeutic potential for treatment or prevention of disease, bioactive peptides may be used as components in functional foods or nutraceuticals.

Bioactive peptides have been detected in many different food sources (*5*), with milk proteins being the most commonly known source (*3*, *4*). Among the different classes of bioactive peptides, the antihypertensive peptides are the best known (*6*, *7*). The main group of the antihypertensive peptides corresponds to the inhibitors of angiotensin converting enzyme (ACE).

ACE plays an important role in the regulation of blood pressure as well as fluid and salt balance in mammals. It is a dipeptidylcarboxypeptidase which converts the inactive decapeptide, angiotensin I, into a potent vasoconstrictor, the octapeptide angiotensin II. Moreover, ACE inactivates bradykinin, a vasodilatory peptide. Hence, ACE raises blood pressure (*8*, *9*).

Currently, specific inhibitors of ACE are used as pharmaceuticals to treat hypertension, congestive heart failure, and myocardial infarction (*8*). Three kinds of synthetic ACE inhibitors were designed; they are grouped by their ligand for the active site on ACE. Captopril, the major representative of his group, has a sulfydryl moiety, lisinopril and enalapril have a carboxyl moiety, and fosinopril has a phosphorus group (*10*) (**Figure 1**). These synthetic ACE inhibitors are known to have strong side effects, such as cough, which is the most common problem, skin rashes, and angioedema (*11*). In contrast, the ACE inhibitory peptides derived from food proteins have not shown these side effects yet (*12*). ACE inhibitory peptides have been discovered in various food sources such as milk (*13*), gelatine (*14*), maize (*15*, *16*), soybean (*17*), and wheat (*18*). Many studies have also been performed on fish protein, such as sardine (*19*), bonito (*20*, *21*), tuna (*22*, *23*), and salmon (*24*). Recently, ACE inhibitory peptides were purified from porcine muscle (*25*−*28*) and chicken muscle (*29*).

Biochemical properties of regulatory peptides derived from milk proteins have been reviewed by Meisel (*13*), while Korhonen and Pihlanto (*2*) reviewed the biologically active peptides from plant and animal proteins. Yamamoto et al. (*7*) reviewed the ACE inhibitors derived from food proteins. The physiological and pharmacological effects of these ACE inhibitory peptides have been reviewed by Li et al. (*30*).

The aim of this paper is to present an overview on ACE inhibitory peptides, obtained by enzymatic hydrolysis of animal

* Address correspondence to this author at the Laboratory of Agrozoology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium. E-mail: Lieselot.Vercruysse@UGent.be. Tel.: +32 9 264 61 44. Fax: +32 9 264 62 39.

† Laboratory of Agrozoology, Department of Crop Protection.

‡ Laboratory of Food Science and Human Nutrition, Department of Food Safety and Food Quality.

10.1021/jf0508908 CCC: $30.25
Published on Web 09/21/2005

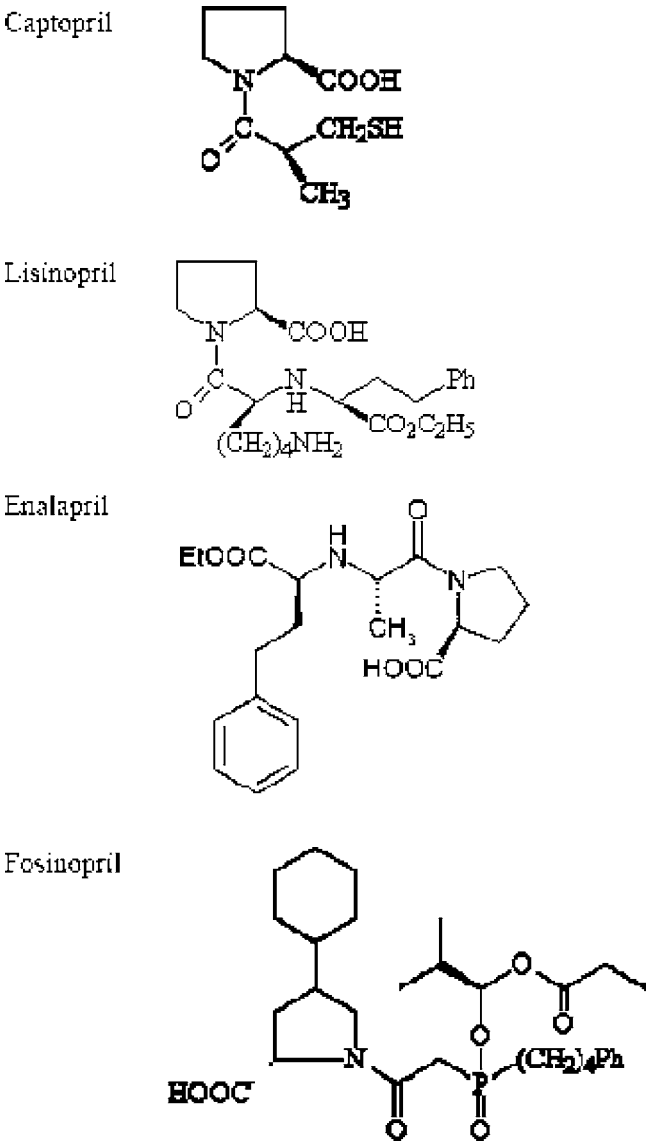

Figure 1. Chemical structure of captopril, lisinopril, enalapril, and fosinopril.

muscle protein, thus, actin, myosin, and collagen from meat, fish, and invertebrates. Furthermore, by comparison of the vertebrate and invertebrate musculature and by analysis of phylogenetic relationships, we discuss the innovative concept to employ new possible sources of ACE inhibitory peptides, specifically from insects and other invertebrates.

MUSCLE PROTEIN OF VERTEBRATES

The force generated by muscles in animals is used to do mechanical work, such as moving objects. Vertebrate muscular tissue can be divided, on both morphological and functional grounds, into two major muscular cell classes: smooth and striated muscle. The latter is subdivided into two types: skeletal muscle (connected to bones) and cardiac muscle (associated with the muscle of the heart). Smooth muscle surrounds internal organs such as the intestines and the blood vessels.

Two muscle proteins responsible for contraction are actin and myosin. Myosin is the primary structural component of thick filaments, while thin filaments are composed of actin, troponin, and tropomyosin. The thick and thin filaments are arranged into sarcomeres, which are the functional units of the muscle. These sarcomeres are longitudinally repeated to form myofibrils. In turn, myofibrils are arranged in parallel and form myofibers. Numerous parallel organized myofibers form muscle fibers (**Figure 2**). Because the proteins are arranged in parallel bands, they give the skeletal and cardiac muscle a stripped or striated appearance (*31*).

Smooth muscle cells are composed of thick and thin filaments, thus containing the same proteins as striated muscle, but they are not organized into sarcomeres; so there is no striated appearance.

A myosin molecule is composed of two identical heavy chains, which are bound by their α-helical tails to form a rod with a globular head region at one end of the rod, and four light chains, attached to the head (*32*). An actin filament, called F-actin, is a two-stranded helix formed by a polymerization of globular G-actin molecules. The regulatory proteins: tropomyosin (a two-stranded coiled-coil of α-helices) and troponin are attached to the actin filament (*33*).

During muscle contraction, thin filaments actively slide along between the thick filaments, so the sarcomeres shorten (Sliding Filament Theory) (*31*).

Whereas muscles generate force, the role of force transmission is usually ascribed to the extracellular connective tissue network of muscle. Therefore, each end of the muscle is attached to a bone or to some other structure by a tough strap of connective tissue, called a tendon. The endomysium (surrounding the muscle as a whole), perimysium (surrounding the muscle fibers), and epimysium (surrounding each myofiber) extend beyond the fleshy part of the muscle to form the tendon.

The connective tissue consists predominantly of fibrillar collagen. Collagen contains large quantities of hydroxyproline. In the primary structure of collagen, the repeating sequence glycine-proline-hydroxyproline-glycine-AA is found. At least 12 different forms have been identified, each having a unique sequence of amino acids. In its secondary structure, the molecular chain is arranged as a left-handed helix and three of these combine to form a right-handed super helix, called tropocollagen. The tropocollagen molecules assemble to form fibrils and these aggregate to form fibers (**Figure 3**) (*33*).

ACE INHIBITORY PEPTIDES DERIVED FROM VERTEBRATE MUSCLE PROTEIN

ACE Inhibitory Peptides Derived from Fish Muscle Proteins. The first report of ACE inhibitory peptides derived from fish muscle proteins was made by Suetsuna and Osajima (*34*). They reported that denazyme AP (a protease from *Aspergillus oryzae*) hydrolysates of sardine and hair tail meat contained ACE inhibitory peptides with IC_{50}-values in vitro of 3.79 and 9.01 mg/L. The IC_{50}-value is the concentration of sample that inhibits 50% of the ACE activity. By now, ACE inhibitory peptides have been detected in muscle of salmon, sardine, bonito, and tuna. **Table 1** shows the amino acid sequence of ACE inhibitory peptides derived from muscle proteins of vertebrates, their origin, the enzyme used for hydrolysis, and the IC_{50}-value.

Dried bonito (Katsuobusi), a Japanese traditional food processed from bonito muscle, was hydrolyzed by various proteases and ACE inhibitory activity was detected (*20*). Among the digests, a thermolysin digest showed the most potent inhibitory activity with an IC_{50}-value of 29 μg/mL. Eight peptides were isolated and four of them were found in the primary structure of actin, by searching for sequence homology. One of these 8 peptides, LKPNM ($IC_{50} = 2.4$ μM), was found to be hydrolyzed by ACE, producing another, more potent ACE inhibitory peptide, LKP ($IC_{50} = 0.32$ μM) (*29*). Both peptides, intravenously administrated to spontaneously hypertensive rats

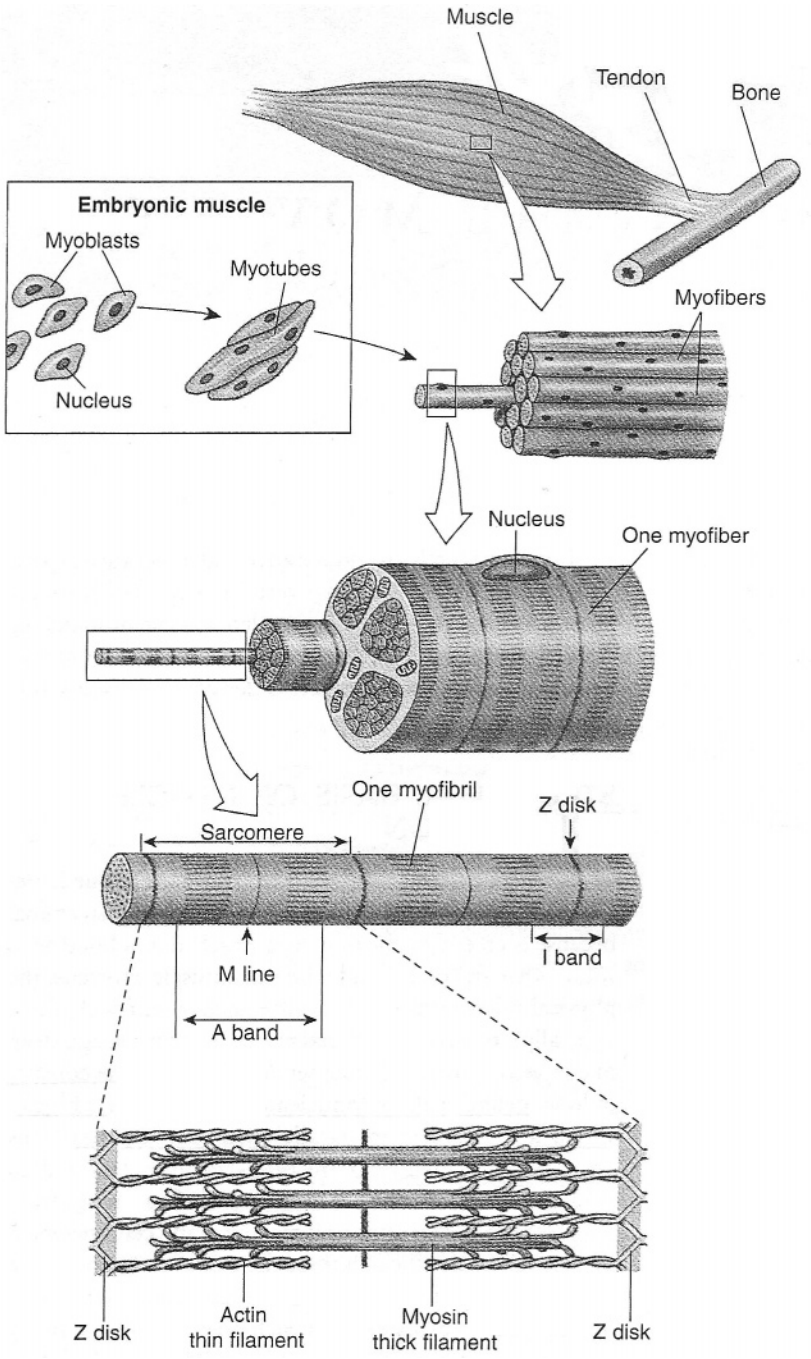

Figure 2. General organization of skeletal muscle tissue (*31*).

(SHR), exerted antihypertensive activity. Oral administration in SHR showed that LKPNM had maximum reduction of blood pressure 6 h after administration, while LKP exerted a maximum decrease after 4 h. LKPNM is a prodrug-type ACE inhibitory peptide which is converted into LKP by ACE in vivo. Therefore, the 2 h delay in maximal activity is explained by the time lag required for enzymatic conversion of LKPNM. In a small scale clinical study, the thermolysin digest evidenced long-lasting antihypertensive activity after oral administration in hypertensive and borderline hypertensive subjects. This digest has been officially approved as a "Food for Specified Health Use" in Japan.

Thermolysin hydrolysate of defatted upstream chum salmon muscle showed high ACE inhibitory activity, with an IC_{50}-value of 27.9 μg protein/mL. After fractionation, 6 dipeptides were identified as WA, VW, WM, MW, IW, and LW, with a respective IC_{50}-value of 277.3, 2.5, 96.6, 9.9, 4.7, and 17.4 μM. When orally administrated, the hydrolysate significantly lowered blood pressure for up to 8 h after administration with a maximum decrease 4 h after administration (*24*).

When sardine muscle is hydrolyzed with alcalase, a *Bacillus licheniformis* alkaline protease, an ACE inhibitory activity is detected with an IC_{50}-value of 260 μg of protein/mL (*35*). This activity is about 2.4-fold higher than that of a peptic hydrolysate (620 μg/mL) of sardine muscle. After fractionation of the alcalase hydrolysate, a more potent inhibitor fraction is observed, with an IC_{50} of 83 μg/mL. The ACE inhibitory activity of this fraction did not change after being treated with gastrointestinal proteases. Thirteen ACE inhibitory peptides were isolated with IC_{50}-values ranging from 1.63 to 330 μM (*36*). VY derived from the alcalase hydrolysate was further investigated in vivo. In SHR, VY decreased the blood pressure after oral administration

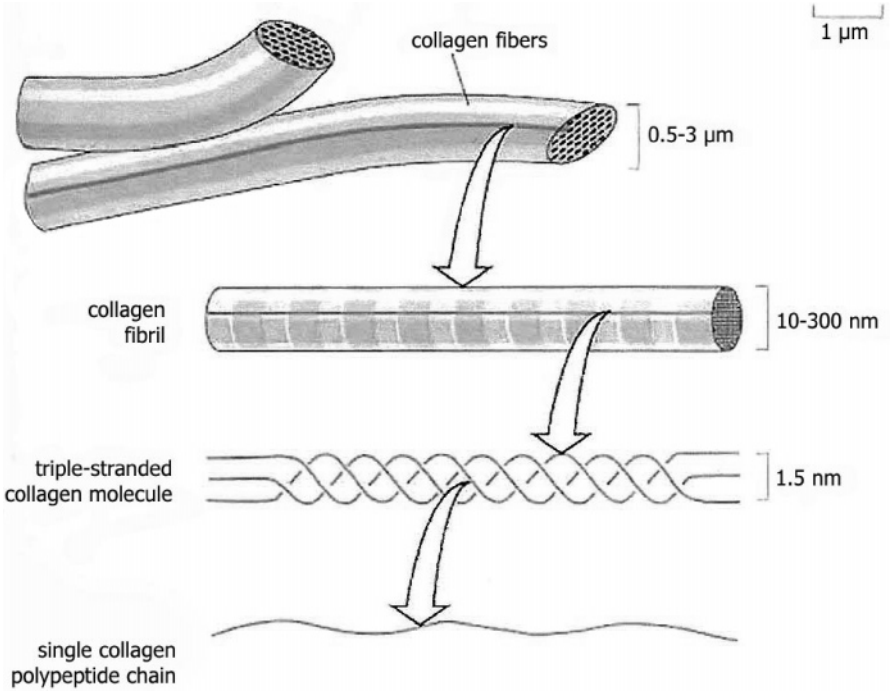

Figure 3. Structural organization of collagen (*60*).

(*37*). A randomized double-blind placebo-controlled study, carried out on 29 volunteers, presented a significant antihypertensive effect on mild hypertensive subjects via ACE inhibition (*38*).

Kohama et al. (*22*) purified an ACE inhibitory octapeptide from the extract of tuna muscle (*Neothunnus macropterus*). The amino acid sequence of the peptide was established as PTHIKWGD. The peptide was chemically synthesized and inhibited the ACE activity to the substrates hippuryl-His-Leu and angiotensin I with K_i values of 1.7 and 5.7 μM, respectively (*39*). Further research revealed various analogues of the octapeptide and determined that PTHIKW is important in the biological activity of the tuna peptide (*23*).

Moreover, proteolytic digestion of gelatine extracts from Alaska pollack (*Theragra chalcogramma*) skin brought about ACE inhibitory activity (*40*). Five proteases were used to digest the skin gelatine in a batch reactor: alcalase, α-chymotrypsin, neutrase, pronase E, and trypsin. A continuous-type process was also conducted on the skin gelatine. A first digestion with alcalase was followed by digestion with pronase E and a hydrolysis with collagenase ended the continuous system. Among the hydrolysates prepared in the batch reactor, the alcalase and pronase E hydrolysates revealed high ACE inhibitory activity with IC_{50}-values of 629 and 649 μg/mL, respectively. Subsequent hydrolysis with alcalase and pronase E increased the ACE inhibitory activity slightly ($IC_{50} = 555$ μg/mL). After digestion in the continuous system and separation by chromatographic methods, 2 ACE inhibitory peptides were isolated: GPL and GPM, showing IC_{50}-values of 2.65 and 17.13 μM, respectively.

Ohba et al. (*41*) studied the physiological functions of enzymatic hydrolysates of collagen or keratin contained in livestock and fish waste. After hydrolysis of collagen waste, called meat meal, an ACE inhibitory activity was detected with IC_{50}-values ranging from 600 to 2800 μg/mL.

ACE Inhibitory Peptides Derived from Muscle of Domestic Animals. ACE inhibitory peptides derived from muscle protein of domestic animals have only been examined in pork and chicken (**Table 1**).

In the enzymatic hydrolysates of the water-insoluble protein fraction from porcine skeletal muscle (containing mainly actin, myosin, and collagen) and of myosin (*25*) ACE inhibitory peptides were released. Digestion was carried out by one of eight types of proteases (pepsin, α-chymotrypsin, trypsin, papain, ficin, pronase E, proteinase K, and thermolysin), among which the thermolysin hydrolysate showed the highest ACE inhibitory activity. Two peptides were purified from the thermolysin digest of myosin, namely, myopentapeptide A (MNPPK) and myopentapeptide B (ITTNP), and their IC_{50}-values were 945.5 and 549.0 μM, respectively. These pentapeptides were synthesized together with 6 tripeptides containing sequence parts of the myopentapeptides and their IC_{50}-values were measured (**Table 1**). The tripeptide, MNP, showed a much higher ACE inhibitory activity than the other tripeptides.

The thermolysin hydrolysates of the water-insoluble proteins and myosin from porcine skeletal muscle exhibited antihypertensive activities after single oral administration to SHR (*42*). The synthetic penta- and tripeptides were dosed at 1 mg of peptide/kg of body weight. Six hours after administration, myopentapeptide A, myopentapeptide B, MNP, and PPK significantly lowered systolic blood pressure. Twenty-four hours after administration, only myopentapeptide B, MNP, and PPK caused a significant decrease in systolic blood pressure.

ACE inhibitory peptides have been generated not only from porcine myofibrillair structural proteins but also from regulatory proteins such as tropomyosin and troponin. Although crude troponin did not exhibit ACE inhibitory activity, the peptic hydrolysate of troponin showed relatively strong activity (*28*). Two fragments were purified, a 9mer (RMLGQTPTK) and a partial peptide of this 9mer, RMLGQTP, showing IC_{50}-values of 34 and 503 μM, respectively. When coming in contact with ACE, the 9mer was slowly hydrolyzed, while the 7mer was rapidly hydrolyzed and inactivated by ACE. Incubation with pepsin, α-chymotrypsin or trypsin revealed that 9mer exerts relatively high resistance to digestive proteases. Thus, it might be expected that 9mer works well in vivo as an ACE inhibitor.

Next to porcine muscle, also chicken muscle was hydrolyzed in order to search for ACE inhibitory peptides (*29*). Digestion

Table 1. ACE Inhibitory Peptides Derived from Muscle Protein of Vertebrates: Source, Amino Acid Sequence, Parent Protein, Enzyme Used for Hydrolysis, and IC_{50}-Value

source	amino acid sequence	parent protein	enzyme	IC_{50} (μM)	ref
		Vertebrates			
bonito	IKPLNY	muscle[a]	thermolysin	43	*20*
	IVGRPRHQG	actin	thermolysin	2.4	*20*
	IWHHT	actin	thermolysin	5.8	*20, 21*
	ALPHA	actin	thermolysin	10	*20*
	FQP	actin	thermolysin	12	*20*
	LKPNM	muscle	thermolysin	2.4	*20, 21*
	IY	actin	thermolysin	2.31	*20, 21*
	DYGLYP	muscle	thermolysin	62	*20*
	LKP	muscle	thermolysin	0.32	*21*
	IWH	actin	thermolysin	3.5	*21*
	IKP	muscle	thermolysin	6.9	*21*
	IVGRPR	actin	thermolysin	300	*29*
salmon	WA	muscle	thermolysin	277.3	*24*
	VW	muscle	thermolysin	2.5	*24*
	WM	muscle	thermolysin	96.6	*24*
	MW	muscle	thermolysin	9.9	*24*
	IW	muscle	thermolysin	4.7	*24*
	LW	muscle	thermolysin	17.4	*24*
sardine	MF	muscle	alcalase	44.7	*36*
	RY	muscle	alcalase	51	*36*
	MY	muscle	alcalase	193	*36*
	LY	muscle	alcalase	38.5	*36*
	YL	muscle	alcalase	82	*36*
	IY	muscle	alcalase	10.5	*36*
	VF	muscle	alcalase	43.7	*36*
	GRP	muscle	alcalase	20.0	*36*
	RFH	muscle	alcalase	330	*36*
	AKK	muscle	alcalase	3.13	*36*
	RVY	muscle	alcalase	205.6	*36*
	GWAP	muscle	alcalase	3.86	*36*
	KY	muscle	alcalase	1.63	*36*
	VY	muscle	alcalase	10	*38*
tuna	PTHIKWGD	muscle	acid	–[b]	*22*
Alaska pollack	GPL	skin	alcalase + pronase + collagenase	2.65	*40*
	GPM	skin	alcalase + pronase + collagenase	17.13	*40*
pork	ITTNP	myosin	thermolysin	549.0	*25*
	MNPPK	myosin	thermolysin	945.5	*25*
	MNP	myosin	synthesized	66.6	*25*
	NPP	myosin	synthesized	290.5	*25*
	PPK	myosin	synthesized	>1000	*25*
	ITT	myosin	synthesized	678.2	*25*
	TTN	myosin	synthesized	672.7	*25*
	TNP	myosin	synthesized	207.4	*25*
	RMLGQTPTK	troponin C	pepsin	34	*28*
	RMLGQTP	troponin C	pepsin	503	*28*
chicken	LKA	creatine kinase	thermolysin	8.5	*29*
	LKP	aldolase	thermolysin	0.32	*29*
	LAP	muscle	thermolysin	3.2	*29*
	FQKPKR	myosin	thermolysin	14	*29*
	IVGRPRHQG	actin	thermolysin	2.4	*29*
	FKGRYYP	creatine kinase	thermolysin	0.55	*29*
	IKW	muscle	thermolysin	0.21	*29*
		Invertebrates			
Antartic krill	KLKFV	muscle	pepsin + trypsin	30	*53*
oyster	LF	muscle	denazyme AP	126	*51*
	FY	muscle	alcalase	–	*52*
	AW	muscle	alcalase	–	*52*
	VW	muscle	alcalase	–	*52*
	GW	muscle	alcalase	–	*52*

[a] "Muscle" indicates that the parent protein was not specified in the cited paper. [b] "-" indicates that the IC_{50} value was not reported.

of chicken muscle with thermolysin leads, after fractionation, to seven ACE inhibitory peptides. ACE inhibitory activity of these peptides was measured in vitro, showing IC_{50}-values ranging from 0.21 to 14 μM. In vivo studies on SHR, both intravenous and by oral administration, were conducted. Intravenous administration, dosed at 10 mg of peptide/kg, of IKP, IKW, LAP, and LKP, showed antihypertensive activity. In contrast, FKGRYYP and IVGRPRHQG failed to exert antihy-

pertensive activities under these conditions, although they did exhibit activity in vitro. An oral dose of 60 mg of peptide/kg revealed the same result; some peptides significantly reduced blood pressure, while others failed. It should be noted that IVGRPRHQG showed antihypertensive activity after oral administration, while it did not lower blood pressure after intravenous administration. As IVGRPRHQG is a prodrug-type ACE inhibitor; it is converted by trypsin into the active peptide IVGRPR when administrated orally. An intravenous administration does not exert this conversion, so no ACE inhibitory activity is observed.

INVERTEBRATE MUSCLE PROTEIN

Invertebrates muscle cells comprise two major cell classes: striated and smooth muscle. The striated muscle can be subdivided, according to the type of striation, into transversely striated (like that of the vertebrate striated muscle) and obliquely striated. Obliquely striated muscles have myofilaments that are not perpendicular but oblique to the Z lines. Transversely striated muscles have either continuous or discontinuous Z lines. The muscle cell types are associated with a well-defined zoological group. For example, the flight muscle of *Drosophila melanogaster* is striated transversely muscle with continuous Z lines (*43*). Transversely striated muscle with discontinuous Z lines is found in the heart muscle of the gastropods, such as the snail, *Helix aspersa* (*44*). The obliquely striated muscle appears in nematodes, annelids, molluscs, brachiopods, and chaetognathes, for example, body wall muscle cells from the earthworm *Eisenia foetida* (*45*). Smooth muscle is present in coelenterates, annelids, molluscs, brachiopods, and echinoderms. The retractor muscle of the snail *H. aspersa* consists of this sort of muscle (*46*).

Arthropod musculature is exclusively formed by transversely striated muscle with continuous Z lines, which is among all invertebrate muscle the most similar to vertebrate skeletal muscle. The arthropod muscle is subdivided into three anatomical types of muscles: skeletal (somatic) muscles, visceral muscles (digestive tract and hemolymphatic vessels), and flight muscles. These muscles present a common ultrastructural pattern with multinucleated cells that contain several cylindrical myofibrils. The myofibrils consist of repeating sarcomers formed by thick and thin filaments. The arthropod thick filaments consist mainly of myosin, similar to vertebrate striated muscles. The insect myosin has the same basic structure as the vertebrate myosin (two heavy chains to which the light chains are attached). But the myosin filaments are thicker and contain more myosin per unit length in insects compared to vertebrates (*47*). In addition, invertebrate thick filaments contain a central core of paramyosin, which is absent in vertebrate muscles. The function of paramyosin is still unclear. Winkelman (*48*) suggests that paramyosin mechanically provides structural stability during tension development, while others suggest that paramyosin influences the ATP-ase activity of the contractile proteins (*49*).

The thin filaments in arthropods are formed by actin molecules, in a double helical conformation. Moreover, actin is associated with tropomyosin and troponin, as in vertebrate striated muscle. The thin/thick filament ratio is 2/1 in vertebrates, while in arthropods it reaches higher values, like 3/1 in insect flight muscle. The interaction between insect actin and myosin is also explained by the Sliding Filament Theory (*50*).

In insects, the exoskeleton is the attachment site for muscles. Specialized myocuticular attachments, like epidermal tendon cells, are found among arthropod skeletal fibers. Collagen plays only a very minor role in the anchorage of fibers to the exoskeleton (*47*).

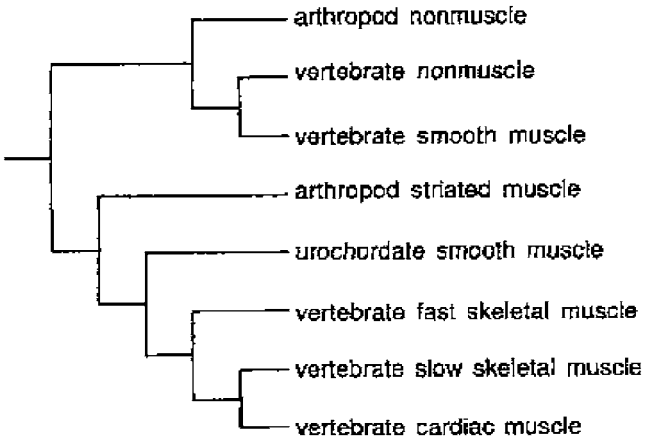

Figure 4. Evolutionary relationship of muscle tissues. The most probable relationship for vertebrate and invertebrate muscle tissues (*54*).

ACE INHIBITORY PEPTIDES DERIVED FROM INVERTEBRATES

In contrast to the many ACE inhibitory peptides derived from vertebrate muscle, very few studies on ACE inhibitory peptides from invertebrate muscles have been conducted. Two studies report on ACE inhibitory peptides detected in hydrolysates of oyster. Among hydrolysis of oyster proteins with 11 proteases, hydrolysis with denazyme AP resulted in the most preferable extract after sensory tests (*51*). The hydrolysate showed an inhibitory activity against ACE with an IC_{50}-value of 550 μg/mL. After separation by column chromatography, the most active fraction had an IC_{50}-value of 85 μg/mL. The peptide isolated from this fraction was identified as LF (IC_{50} = 126 μM). Katano et al. (*52*) hydrolyzed pearl oyster meat with alkaline protease. The hydrolysate was orally administrated to SHR and resulted in a significant decrease of systolic blood pressure. Four active peptides were isolated and identified as FY, AW, VW, and GW.

Furthermore, sequential hydrolysis of defatted Antarctic krill muscle by pepsin and trypsin resulted in an ACE inhibitory extract. The active peptide isolated from the extract was found to be KLKFV, showing an IC_{50}-value of 30 μM (*53*).

NEW POSSIBLE SOURCES OF ACE INHIBITORY PEPTIDES

As ACE inhibitory peptides have already been discovered in various vertebrate muscles, and as the structure of vertebrate and invertebrate muscle is very similar, invertebrates/insects may be new sources of ACE inhibitory peptides. The basic proteins of vertebrate and invertebrate muscle are actin, myosin, and collagen. The phylogenetic relationship of muscle tissues was studied by Oota and Saitou (*54*). By superimposition of deduced tissue trees from structural gene trees, valid information on the developmental pattern of muscle tissues was provided. **Figure 4** shows that arthropod striated muscle and vertebrate skeletal and cardiac muscle share a common ancestor, so they did not evolve independently. Moreover, arthropod nonmuscle and vertebrate smooth muscle and nonmuscle share a common ancestor. The emergence of skeletal and cardiac muscle type tissues preceded the vertebrate/arthropod divergence.

Protein databases contain the amino acid sequence of actin, myosin, and collagen of a number of species. Multiple alignments are used to evaluate the degree of identity of various amino acid sequences. In the Swiss-Prot database at the Expasy

```
1      MCDEDETTALVCDNGSGLVKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEA 60
2      MCDDDETTALVCDNGSGLVKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEA 60
3      MCDDD-VRALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGLMVGMGQKDSYVGDEA 59
4      MGDED-IAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRPRHQGVMVGMGQKDSYVGDEA 59
       * *:*   *** *****: ************************:***************

1      QSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAPLNPKANREK 120
2      QSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPTLLTEAPLNPKANREK 120
3      QSKRGILTLKYPIEHGIITNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREK 119
4      QSKRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPEEHPVLLTEAPLNPKANREK 119
       *****************:*************************.***************

1      MTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGYALPHAIMRL 180
2      MTQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLDSGDGVTHNVPIYEGYALPHAIMRL 180
3      MTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAILRL 179
4      MTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVLDSGDGVSHTVPIYEGYALPHAIMRL 179
       ********** ******************************:*.*************:**

1      DLAGRDLTDYLMKILTERGYSFVTTAEREIVRDIKEKLCYVALDFENEMATAASSSSLEK 240
2      DLAGRDLTDYLMKILTERGYSFVTTAEREIVRDIKEKLCYVALDFENEMATAASSSSLEK 240
3      DLAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMATAAASTSLEK 239
4      DLAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMTTAASSSSLEK 239
       **********************.************************:**:***:*:****

1      SYELPDGQVITIGNERFRCPETLFQPSFIGMESAGIHETTYNSIMKCDIDIRKDLYANNV 300
2      SYELPDGQVITIGNERFRCPETLFQPSFIGMESAGIHETAYNSIMKCDIDIRKDLYANNV 300
3      SYELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETVYNSIMKCDVDIRKDLYANTV 299
4      SYELPDGQVITIGNERFRCPEAMFQPSFLGMESSGIHETSYNSIMKCDVDIRKDLYANIV 299
       **********************::*****:****.***** ********:********* *

1      MSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQQMWIT 360
2      LSGGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVWIGGSILASLSTFQAMWIT 360
3      MSGGTTMYPGIADRMQKEITALAPSTIKIKIIAPPERKYSVWIGGSILASLSTFQQMWIS 359
4      LSGGTTMFPGIADRMQKEVTALAPPTMKIKVIAPPERKYSVWIGGSILASLSTFQQMWIS 359
       :******:**********:*****.*:***:************************ ***:

1      KQEYDEAGPSIVHRKCF 377
2      KQEYDEAGPSIVHRKCF 377
3      KEEYDESGPGIVHRKCF 376
4      KQEYDESGPSIVHRKCF 376
       *:****:**.*******
```

Figure 5. Multiple alignment of the actin sequences of *Sus scrofa* (pig, 1), *Oncorhynchus keta* (chum salmon, 2), *Bombyx mori* (silkworm, 3), and *Crassostrea gigas* (pacific oyster, 4) (http://www.ebi.ac.uk/clustalw/). "*" indicates that the residues in that column are identical in all sequences in the alignment; ':' means that conserved substitutions have been observed; '.' means that semi-conserved substitutions are observed. In bold, five ACE inhibitory peptides isolated from the actin of bonito. Framed, six ACE inhibitory sequences only present in the actin sequence of *B. mori* or *C. gigas*.

Molecular Biology server (http://www.expasy.org), the actin amino acid sequence of four different species was selected. Two vertebrate species, *Sus scrofa* (pig) and *Oncorhynchus keta* (chum salmon), and two invertebrate species, *Bombyx mori* (silkworm) and *Crassostrea gigas* (pacific oyster), were chosen. Both vertebrate species and the pacific oyster are known sources of ACE inhibitory peptides. The multiple alignment between the actin sequences was conducted with Clustal W (http://www.ebi.ac.uk/clustalw/) (**Figure 5**). A high homology in amino acid sequence is observed between the actin sequences. So there is a high possibility that an active peptide present in one sequence will also be present in the other three. For example, the ACE inhibitory peptides IVGRPRHQG, IWHHT, ALPHA, FQP, IY, and IWH that have been found in the actin of bonito by Yokoyama et al. (*20*) are also present in the actin sequences of the four species chosen for the alignment. Next to these ACE inhibitory peptides, the actin sequence of *B. mori* contains ACE inhibitory peptides that are not present in the other actin sequences: GL, TVY, and GI (**Figure 5**). As exemplified in **Figure 5**, for the invertebrates *B. mori* and *C. gigas* together six unique ACE inhibitory peptides were found different from pig and salmon actin. This raises the research objective to evaluate invertebrate species, such as mollusc and insects, as potential sources of ACE inhibitory peptides.

In hydrolysates of the protein of the cotton leafworm, *Spodoptera littoralis*, hydrolyzed with various enzymes (gastrointestinal digestion, alcalase, and thermolysin), ACE inhibitory activity was observed. A gastrointestinal digestion resulted in the highest ACE inhibitory activity (*55*). Furthermore, an in silico analysis of the actin, myosin, and collagen sequences of the silkmoth *B. mori* reveals the presence of ACE inhibitory peptides in these sequences and in vitro tests conducted on hydrolysates of the protein of these insect larvae reveal ACE inhibitory activity (*56*).

Table 2. Enzymes Used To Produce ACE Inhibitory Peptides from Muscle Protein

protease	origin	specificity
	Digestive Enzymes	
pepsin	porcine stomach	N-terminus of Phe, Leu, Tyr
trypsin	bovine pancreas	C-terminus of Arg and Lys
α-chymotrypsin	bovine pancreas	C-terminus of Tyr, Trp, Phe, Leu
pancreatin	bovine pancreas	endo- and exopeptidases
	Other Enzymes	
thermolysin	*Bacillus thermoproteolyticus*	N-terminus of Leu and Phe
collagenase	*Clostridium histolyticum*	N-terminus of Gly in Pro-X-Gly-Pro
pronase E	*Streptomyces griseus*	broad, endoprotease
proteinase K	*Tritirachium album*	C-terminus of aromatic or aliphatic (hydrophobic) amino acids
alcalase = subtilisin A	*Bacillus licheniformis*	broad, preference for large, uncharged residue in P1
ficin	fig tree latex	broad, endoprotease
papain	*Carica papaya*	broad, endoprotease
endoproteinase Glu-C	*Staphylococcus*	N-terminus of Asp and Glu
endoproteinase Lys-C	*Myxobacter*	N-terminus of Lys
endoproteinase Asp-N	*Pseudomonas fragi*	N-terminus of Asp, Glu, and cysteic acid bonds

FACTORS AFFECTING IN VIVO ACTIVITY OF ACE INHIBITORY PEPTIDES

As ACE inhibitory peptides are rarely present as such in foods, they must be released from the parent protein by hydrolysis with enzymes, heat, base, or acid. Various enzymes have been used to release peptides from muscle proteins. **Table 2** states the enzymes and their specific cleavage sites. Yokoyama et al. (*20*) hydrolyzed bonito muscle by various proteases and the thermolysin digest showed the most potent ACE inhibitory activity. Katayama et al. (*26*) used eight different enzymes (pepsin, trypsin, α-chymotrypsin, endoproteinase Glu-C, endoproteinase Lys-C, endoproteinase Asp-N, pancreatin, and modified trypsin) to hydrolyze porcine skeletal muscle proteins. Among the digests, the pepsin hydrolysate showed the strongest activity. In another study conducted on porcine skeletal muscle protein, ACE inhibitory activity was measured of hydrolysates produced by pepsin, trypsin, α-chymotrypsin, thermolysin, proteinase K, pronase E, ficin, and papain (*25*). The thermolysin digest demonstrated the highest activity.

When administrating these active peptides to SHR, it is possible that a discrepancy is observed between the results of the in vitro and in vivo techniques or between an oral and intravenous administration. To exert an antihypertensive effect in vivo after oral ingestion, the ACE inhibitory peptides have to be resistant against gastrointestinal digestion; they have to be absorbed from the intestine in active form and have to reach the cardiovascular system. A number of potential barriers in the human body, such as intestinal absorption, can either activate or inactivate the bioactive peptides. The bioavailability of ACE inhibitory peptides has been reviewed by Vermeirssen et al. (*57*).

CONCLUSION

Nowadays, hypertension is in most cases treated by medicines containing synthetic ACE inhibitors, which can cause serious side effects. Diet-related preventive measures for hypertension are of interest, as this could lead to a decrease in the requirement for antihypertensive medicines, which are known to exert strong side effects. Naturally occurring ACE inhibitory peptides derived from food proteins that are daily consumed can be used as components for functional foods or nutraceuticals. In this review article, more particularly, meat as a source of ACE inhibitory peptides was considered. Meat itself can act as a biologically active food through digestion with appropriate enzymes. The review of Jimenez-Colmenero et al. (*58*) describes meat and meat products and their role as functional foods. However, meat should be seen in a broader concept because in numerous countries in Latin America, Africa and Asia, insects are a part of the daily diet (*59*). This suggests that next to vertebrate muscle protein (from domestic animals and fish) also invertebrates can be interesting sources of ACE inhibitory peptides in the treatment of hypertension.

ABBREVIATIONS USED

AA, amino acid; ACE, angiotensin converting enzyme; SHR, spontaneously hypertensive rat.

LITERATURE CITED

(1) Friedman, M. Nutritional value of proteins from different food sources. A review. *J. Agric. Food Chem.* **1996**, *44*, 6−29.
(2) Korhonen, H.; Pihlanto, A. Food-derived bioactive peptides − Opportunities for designing future foods. *Curr. Pharm. Des.* **2003**, *9*, 1297−1308.
(3) Meisel, H. Biochemical properties of bioactive peptides derived from milk proteins: Potential nutraceuticals for food and pharmaceutical applications. *Livest. Prod. Sci.* **1997**, *50*, 125−138.
(4) Clare, D. A.; Swaisgood, H. E. Bioactive milk peptides: A prospectus. *J. Dairy Sci.* **2000**, *83*, 1187−1195.
(5) Dziuba, J.; Minkiewicz, P.; Nalecz, D. Biologically active peptides from plant and animal proteins. *Pol. J. Food Nutr. Sci.* **1999**, *8*, 3−16.
(6) Ariyoshi, Y. Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends Food Sci. Technol.* **1993**, *4*, 139−144.
(7) Yamamoto, N.; Ejiri, M.; Mizuno, S. Biogenic peptides and their potential use. *Curr. Pharm. Des.* **2003**, *9*, 1345−1355.
(8) Turner, A. J.; Hooper, N. M. The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends Pharmacol. Sci.* **2002**, *23*, 177−183.
(9) Coates, D. The angiotensin converting enzyme (ACE). *Int. J. Biochem. Cell Biol.* **2003**, *35*, 769−773.
(10) Cohen, M. L. Synthetic and fermentation-derived angiotensin converting enzyme inhibitors. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 307−323.
(11) Antonios, T. F. T.; Macgregor, G. A. Angiotensin-converting enzyme−inhibitors in hypertension − potential problems. *J. Hypertens.* **1995**, *13*, S11−S16.
(12) FitzGerald, R. J.; Meisel, H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. *Br. J. Nutr.* **2000**, *84*, S33−S37.
(13) Meisel, H. Multifunctional peptides encrypted in milk proteins. *Biofactors* **2004**, *21*, 55−61.

(14) Oshima, G.; Shimabukuro, H.; Nagasawa, K. Peptide inhibitors of angiotensin I-converting enzyme in digests of gelatin by bacterial collagenase. *Biochim. Biophys. Acta* **1979**, *566*, 128−137.

(15) Maruyama, S.; Miyoshi, S.; Kaneko, T.; Tanaka, H. Angiotensin-I-converting enzyme inhibitory activities of synthetic peptides related to the tandem repeated sequence of a maize endosperm protein. *Agric. Biol. Chem.* **1989**, *53*, 1077−1081.

(16) Miyoshi, S.; Ishikawa, H.; Kaneko, T.; Fukui, F.; Tanaka, H.; Maruyama, S. Structures and activity of angiotensin-converting enzyme inhibitors in an alpha-zein hydrolysate. *Agric. Biol. Chem.* **1991**, *55*, 1313−1318.

(17) Chen, J. R.; Yang, S. C.; Suetsana, K.; Chao, J. C. J. Soybean protein-derived hydrolysate affects blood pressure in spontaneously hypertensive rats. *J. Food Biochem.* **2004**, *28*, 61−73.

(18) Matsui, T.; Li, C. H.; Osajima, Y. Preparation and characterization of novel bioactive peptides responsible for angiotensin I-converting enzyme inhibition from wheat germ. *J. Pept. Sci.* **1999**, *5*, 289−297.

(19) Seki, E.; Osajima, K.; Matsufuji, H.; Matsui, T.; Osajima, Y. Val-Tyr, an angiotensin-I converting enzyme−inhibitor from sardines that have resistance to gastrointestinal proteases. *J. Jpn. Soc. Biosci. Biotechol. Agrochem.* **1995**, *69*, 1013−1020.

(20) Yokoyama, K. H.; Chiba, H.; Yoshikawa, M. Peptide inhibitors for angiotensin-I-converting enzyme from thermolysin digest of dried bonito. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 6, 1541−1545.

(21) Fujita, H.; Yoshikawa, M. LKPNM: a prodrug type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology* **1999**, *44*, 123−127.

(22) Kohama, Y.; Matsumoto, S.; Oka, H.; Teramoto, T.; Okabe, M.; Mimura, Y. Isolation of angiotensin-converting enzyme−inhibitor from tuna muscle. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 332−337.

(23) Kohama, Y.; Oka, H.; Kayamori, Y.; Tsujikawa, K.; Mimura, T.; Nagase, Y.; Satake, M. Potent synthetic analogs of angiotensin-converting enzyme−inhibitor derived from tuna muscle. *Agric. Biol. Chem.* **1991**, *55*, 2169−2170.

(24) Ono, S.; Hosokawa, M.; Miyashita, K.; Takahashi, K. Isolation of peptides with angiotensin I-converting enzyme inhibitory effect derived from hydrolysate of upstream chum salmon muscle. *J. Food Sci.* **2003**, *68*, 1611−1614.

(25) Arihara, K.; Nakashima, Y.; Mukai, T.; Ishikawa, S.; Itoh, M. Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Sci.* **2001**, *57*, 319−324.

(26) Katayama, K.; Fuchu, H.; Sakata, A.; Kawahara, S.; Yamauchi, K.; Kawamura, Y.; Muguruma, M. Angiotensin I-converting enzyme inhibitory activities of porcine skeletal muscle proteins following enzyme digestion. *Asian-Aust. J. Anim. Sci.* **2003**, *16*, 417−424.

(27) Katayama, K.; Fuchu, H.; Sugiyama, M.; Kawahara, S.; Yamauchi, K.; Kawamura, Y.; Muguruma, M. Peptic hydrolysate of porcine crude myosin has many active fractions inhibiting angiotensin I-converting enzyme. *Asian-Aust. J. Anim. Sci.* **2003**, *16*, 1384−1389.

(28) Katayama, K.; Tomatsu, M.; Fuchu, H.; Sugiyama, M.; Kawahara, S.; Yamauchi, K.; Kawamura, Y.; Muguruma, M. Purification and characterization of an angiotensin I-converting enzyme inhibitory peptide derived from porcine troponin C. *Anim. Sci. J.* **2003**, *74*, 53−58.

(29) Fujita, H.; Yokoyama, K.; Yoshikawa, M. Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *J. Food Sci.* **2000**, *65*, 564−569.

(30) Li, G.; Le, G.; Shi, Y.; Shrestha, S. Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutr. Res.* **2004**, *24*, 469−486.

(31) Randall, D.; Burggren, W.; French, K. *Eckert Animal Physiology*, edition 4; W. H. Freeman and Company: New York, 1997.

(32) Kinsman, D. M.; Kotula, A. W.; Breidenstein, B. C. *Muscle Foods;* Chapman & Hall: New York, 1994.

(33) Lawrie, R. A. *Meat Science*, edition 5; Pergamon Press: Oxford, 1991.

(34) Suetsuna, K.; Osajima, K. The inhibitory activities against angiotensin I-converting enzyme of basic peptides originating from sardine and hair tail meat. *Bull. Jpn. Soc. Sci. Fish.* **1986**, *52*, 1981−1984.

(35) Matsui, T.; Matsufuji, H.; Seki, E.; Osajima, K.; Nakashima, M.; Osajima, Y. Inhibition of angiotensin I-converting enzyme by *Bacillus licheniformis* alkaline protease hydrolyzates derived from sardine muscle. *Biosci., Biotechnol., Biochem.* **1993**, *57*, 922−925.

(36) Matsufuji, H.; Matsui, T.; Seki, E.; Osajima, K.; Nakashima, M.; Osajima, Y. Angiotensin I-converting enzyme inhibitory peptides in an alkaline protease hydrolyzate derived from sardine muscle. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 2244−2245.

(37) Matsufuji, H.; Matsui, T.; Ohshige, S.; Kawasaki, T.; Osajima, K.; Osajima, T. Antihypertensive effects of angiotensin fragments in SHR. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1398−1401.

(38) Kawasaki, T.; Seki, E.; Osajima, K.; Yoshida, M.; Asada, K.; Matsui, T.; Osajima, Y. Antihypertensive effect of Valyl-Tyrosine, a short chain peptide derived from sardine muscle hydrolyzate, on mild hypertensive subjects. *J. Hum. Hypertens.* **2000**, *14*, 519−523.

(39) Kohama, Y.; Oka, H.; Matsumoto, S.; Nakagawa, T.; Miyamoto, T.; Mimura, T.; Nagase, Y.; Satake, M.; Takane, T.; Fujita, T. Biological properties of angiotensin-converting enzyme−inhibitor derived from tuna muscle. *J. Pharmacobio-Dynam.* **1989**, *12*, 566−571.

(40) Byun, H.; Kim, S. Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska Pollack (*Theragra chalcogramma*) skin. *Process Biochem.* **2001**, *36*, 1155−1162.

(41) Ohba, R.; Deguchi, T.; Kishikawa, M.; Arsyad, F.; Morimura, S.; Kida, K. Physiological functions of enzymatic hydrolysates of collagen or keratin contained in livestock and fish waste. *Food Sci. Technol. Res.* **2003**, *9*, 91−93.

(42) Nakashima, Y.; Arihara, K.; Sasaki, A.; Mio, H.; Ishikaa, S.; Itoh, M. Antihypertensive activities of peptides derived from porcine skeletal muscle myosin in spontaneously hypertensive rats. *J. Food Sci.* **2002**, *67*, 434−437.

(43) Shafiq, S. A. Electron microscopical studies on the indirect flight muscles of *Drosophila melanogaster*. *J. Cell Biol.* **1963**, *17*, 351−362.

(44) North, R. J. The fine structure of the myofibers in the heart of the snail *Helix aspersa*. *J. Ultrastruct. Res.* **1963**, *8*, 206−218.

(45) Royuela, M.; Fraile, B.; Garcia-Anchuelo, R.; Paniagua R. Ultrastructurally different muscle cell types in *Eisenia foetida* (Annelida, Oligochaeta). *J. Morphol.* **1995**, *224*, 87−96.

(46) Hanson, J.; Lowy, J. Structure of smooth muscles. *Nature* **1957**, *180*, 906−909.

(47) Usherwood, P. N. R. *Insect Muscle*; Academic Press: London, 1975.

(48) Winkelman, L. Comparative studies of paramyosins. *Comp. Biochem. Physiol. B* **1976**, *55*, 391−397.

(49) Beinbrech, G.; Ashton, F. T.; Pepe, F. A. The invertebrate myosin filament: subfilament arrangement of the solid filament of insect flight muscle. *Biophys. J.* **1992**, *61*, 1495−1512.

(50) Paniagua, R.; Royuela, M.; Garcia-Anchuelo, R. M.; Fraile, B. Ultrastructure of invertebrate muscle cell types. *Histol. Histopath.* **1996**, *11*, 181−201.

(51) Matsumoto, K.; Ogikubo, A.; Yoshino, T.; Matsui, T.; Osajima, Y. Separation and purification of angiotensin-I converting-enzyme inhibitory peptide in peptic hydrolyzate of oyster. *J. Jpn. Soc. Food Sci. Technol.* **1994**, *41*, 589−594.

(52) Katano, S.; Oki, T.; Matsuo, Y.; Yoshihira, K.; Nara, Y.; Miki, T.; Matsui, T.; Matsumoto, K. Antihypertensive effect of alkaline protease hydrolysate of the pearl oyster Pinctada fucata martencii & separation and identification of angiotensin-I converting enzyme inhibitory peptides. *Nippon Suisan Gakkaishi* **2003**, *69*, 975−980.
(53) Kawamura, Y.; Takane, T.; Stake, M.; Sugimoto, T. Physiologically active peptide motif in proteins − peptide inhibitors of ACE from the hydrolysates of Antarctic krill muscle protein. *Jpn. Agric. Res. Q.* **1992**, *26*, 210−213.
(54) Oota, S.; Saitou, N. Phylogenetic relationship of muscle tissues deduced from superimposition of gene trees. *Mol. Biol. Evol.* **1999**, *16*, 856−867.
(55) Vercruysse, L.; Smagghe, G.; Van Camp, J. ACE inhibitory activity from insects after enzymatic hydrolysis. *Comm. Appl. Biol. Sci.* **2004**, *69/2*, 321−324.
(56) Vercruysse, L.; van der Bent, A.; Smagghe, G.; van Aamerongen, A.; Van Camp, J. Comparison between in vitro and in silico analysis of the ACE inhibitory activity in the silkmoth *Bombyx mori*. In preparation, 2005.
(57) Vermeirssen, V.; Van Camp, J.; Verstraete, W. Bioavailability of angiotensin I converting enzyme inhibitory peptides. *Br. J. Nutr.* **2004**, *92*, 357−366.
(58) Jiménez-Colmenero, F.; Carballo, J.; Cofrades, S. Healthier meat and meat products: their role as functional foods. *Meat Sci.* **2001**, *59*, 5−13.
(59) DeFoliart, G. Insects as food: why the Western attitude is important. *Annu. Rev. Entomol.* **1999**, *44*, 21−50.
(60) Alberts, B.; Bray, D.; Hopkin, K.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Essential cell biology*, edition 2; Garland Science: New York, 2004.

Received for review April 19, 2005. Revised manuscript received July 29, 2005. Accepted August 18, 2005. This research is supported by a Ph.D. grant for L.V. from the Institute for the Promotion of Innovation by Science and Technology in Flanders [IWT] and by Project 01102703 from the Special Research Fund of the Ghent University.

JF0508908