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Angiotensin converting enzyme inhibitory peptides in Finnish cereals: a database survey

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Angiotensin converting enzyme (ACE) regulates blood pressure (BP) by hydrolytic actions. ACE-inhibitors are widely used in the pharmacological treatment of hypertension. Certain food-derived peptides can also inhibit the activity of ACE. This study shows the occurrences of known ACE-inhibitory peptides in cereal storage protein structures. A literature search yielded thirty-nine candidate peptides. Of these, twenty-two peptides were found to occur in the cereal storage proteins. For instance, of the tripeptides (isoleucine-proline-proline or valine-proline-proline) that lower BP in fermented milk products either one appears in cereal prolamins. In addition, oat globulins possess seven of the candidate peptides in their structures, whereas tripeptides leucine-glutamine-proline (LQP) and valine-serine-proline (VSP) occur repeatedly in C-hordeins and ω-secalins (LQP), and D-hordeins (VSP). Cereal storage proteins, thus, appeared as potential sources of ACE-inhibitory peptides. Novel cereal products with BP-lowering effects may be developed by liberation of the target peptides.

Key words: angiotensin converting enzyme inhibitor, blood pressure, cereal proteins, peptides, primary structure, proteolysis

Introduction

Angiotensin converting enzyme (ACE) is a proteolytic enzyme that regulates blood pressure (BP) by its hydrolytic actions (Fig. 1). ACE converts angiotensin-I (that has no direct effect on BP) to angiotensin-II, which constricts blood vessels (vasoconstrictor) and thereby elevates BP (Fig. 1, left side). In addition, ACE (also called kininase II) cleaves bradykinin (vasodilator) to metabolites that have no blood vessel dilating activity (Fig. 1, right side).

Since ACE is an enzyme, it can be inactivated by blocking its active site with selective enzyme inhibitors. Synthetic ACE-inhibitors are widely used in the pharmacological treatment of hypertension. Certain peptides with suitable structures are also able to inhibit the activity of ACE by binding to its active site. Ferreira et al.

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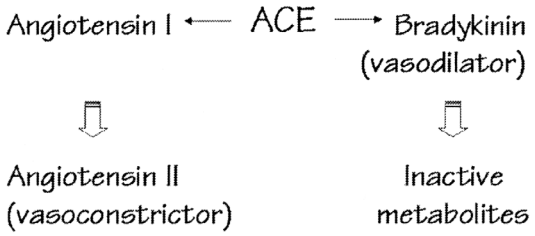

Fig. 1. Hydrolytic effects of angiotensin converting enzyme (ACE) on angiotensin I and bradykinin.

(1970) were the first to report studies in which they isolated ACE-inhibitors from snake venom. At that time, the development of synthetic ACE-inhibitors also began (for a review see Cushman and Ondetti 1999).

ACE-inhibitory peptides can originate also from food proteins. Degradation of food proteins during gastrointestinal digestion or during food processing may produce ACE-inhibitory peptides. In food process the microbial cultures or the added enzymes, as well as the endogenous proteolytic enzymes present in the raw material, may have proteolytic activity that produces active peptides. In order to retain its activity, an orally administered ACE-inhibitory peptide should resist digestive proteases and it should be absorbed into the blood. Small peptides, such as di or tripeptides, are generally considered more able to absorb intact into blood than larger polypeptides.

Calpis and Evolus, commercially available acidified milk products in Japan and in Finland, respectively, lowered BP in hypertensive patients in placebo-controlled studies (Hata et al. 1996, Seppo et al. 2003). The efficacy of the products is based on the same two tripeptides, isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), which are formed from milk caseins during microbial fermentation by certain *Lactobacillus helveticus* strains. In addition, in Japan, a thermolysin-digest of fish protein, with BP-lowering activity, has the status of "Food for specified health use" (Fujita and Yoshikawa 1999). The BP lowering activity of the digest is attributed to a pentapeptide and a tripeptide, leucine-lysine-proline-asparagine-methionine and leucine-lysine-proline (LKP), which possess a long-lasting BP-lowering activity and effective ACE-inhibition *in vitro*, respectively. However, no such cereal-based products are available, and only a few studies relating to cereal proteins as sources of ACE-inhibitors exist (Miyoshi et al. 1991, Kawakami et al. 1995, Gobbetti et al. 1997, Matsui et al. 1999, Suh et al. 2003).

The present study collects data on the ACE-inhibitory peptides, and explores the primary structures of the main storage proteins of Finnish cereals (rye, wheat, barley, oat) for these candidate peptides. Structural similarities between the candidate peptides and also requirements for the effective proteolysis of cereal proteins are discussed.

Material and methods

Selection of the candidate peptides

Literature concerning the ACE-inhibitory peptides was thoroughly searched for ACE-inhibitory peptide sequences. Candidate peptides were selected based on following criteria:

1. the candidate peptide has an IC50-concentration (by which 50% of the activity of ACE is inhibited) of 10 μM or less, and
2. the candidate peptide consists of two to four amino acids, since larger peptides were considered too sensitive to digestive proteases.

Database exploration

The amino acid sequences of the main storage proteins of wheat, rye, barley, and oats were explored in a protein structure database environment (*i*ProClass) for candidate peptides. Single-letter amino acid codes of the candidate peptides were entered and submitted to database, after which the search engine returned a set of proteins that contained the particular amino acid

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sequence. After this, the search was limited by organism to include wheat, barley, oats, and rye proteins; each organism was searched separately. The sequence identification codes of the resulted cereal storage proteins were collected and saved (data not shown). The searched storage proteins included avenins and globulins for oats, hordeins for barley, secalins for rye, and glutenins and gliadins for wheat.

Results

Thirty-nine ACE-inhibitory candidate peptides, which comprised of eight dipeptides, twenty-seven tripeptides, and four tetrapeptides, were found in the literature. The IC50-concentrations (for ACE inhibition) of the selected peptides varied from 0.21 to 10 μM (Table 1). Surveys made in *i*ProClass database resulted in twenty-two candidate peptides that occur in the structures of the cereal storage proteins (Table 1). For instance, either one of the tripeptides (IPP and VPP) that lowers the blood pressure in hypertensive patients (Hata et al 1996; Seppo et al. 2003) occurs in the searched cereal prolamins (Table 1). In addition, two tripeptides, LKP and leucine-arginine-proline (LRP) that have strong ACE-inhibitory activities *in vitro* appear in the structures of oat globulins (LKP), gamma-hordeins (LRP), and gamma-gliadins (LRP) (Table 1). It is also noteworthy that two of the candidate peptides, valine-serine-proline (VSP) and leucine-glutamine-proline (LQP), occur repeatedly (up to sixteen repeats per single protein) in the structures of barley D-hordeins (VSP) and rye omega-secalins and barley C-hordeins (LQP).

Discussion

The storage proteins of Finnish cereals possess most (22/39) of the known ACE-inhibitory peptides. The findings, thus, indicate good possibilities to use cereal proteins as sources of BP-lowering peptides. This study focused on the main storage proteins of cereals, as they comprise the majority of cereal proteins. In addition, semi-manufactured products of cereal storage proteins are commercially available (such as wheat gluten). Results presented in this study are based on database surveys that were performed in the protein structure database environment (*i*ProClass), which consists of reported protein structures. Therefore some variation in primary structures of certain proteins may exist within a species; for instance, at least three slightly different primary structures are reported in the database for 12S oat globulins (Shotwell et al. 1988, 1990; Schubert et al. 1990; Tanchak et al. 1995).

Certain structural characteristics were evident. For instance, proline, tyrosine, or tryptophan most commonly appeared in the carboxyl-terminal of the candidate peptides, whereas hydrophobic amino acids with aliphatic side chains, such as glycine, isoleucine, leucine, and valine, occurred predominantly in the amino-ends of the peptides (Table 1). Tripeptides that have this kind of terminal structure seem predominantly to have basic amino acid (lysine or arginine) or proline as central amino acid (Table 1). These observations are in good agreement with previous findings, which indicated that the most potent C-terminal amino acids for dipeptides binding to ACE were tryptophan, proline, tyrosine, and phenylalanine (Cheung et al. 1980). Similarly, valine and isoleucine appeared potential among N-terminal amino acids for dipeptides (Cheung et al. 1980). Among tripeptides isolated previously, very similar characteristics were observable as hydrophobic-aliphatic amino acids appeared in N-terminal, basic amino acids in centre, and proline as C-terminal amino acid (Matsumura et al. 1993). These kinds of observations on the structural characteristics of peptides may help to predict new active peptide sequences.

Prolamins, the alcohol-soluble proteins of cereals, evidently have numerous proline rich candidate peptides in their structures (Table 1). For instance, of the tripeptides (IPP or VPP) ei-

Table 1. Occurrences of the candidate peptides in the primary structures of the main storage proteins of wheat, rye, barley, and oats.

Candidate peptide	IC50 μM	Occurence in cereal storage proteins (*i*ProClass)	References for peptide candidates and corresponding IC50-values
AW	10	–	Cheung et al. (1980)
FY	3.7	D-hordeins, rye high molecular weight secalins (rye HMW), wheat high molecular weight glutenins (wheat HMW)	Suetsuna (1998)
IW	2.0	avenins, C-hordeins, γ-gliadins	Cheung et al. (1980)
IY	2.1–3.7	oat globulins, rye HMW, wheat HMW, wheat low molecular weight glutenins (wheat LMW), γ-gliadins	Cheung et al. (1980), Saito et al. (1994), Fujita and Yoshikawa (1999), Matsui et al. (1999), Marczak et al. (2003),
PR	4.1	oat globulins, C-hordeins, rye HMW, γ-secalins, wheat HMW, wheat LMW, γ-gliadins,	Saito et al. (1994)
VF	9.2	avenins, oat globulins, B-hordeins, wheat LMW, γ-gliadins	Matsui et al. (1999)
VW	1.4–1.6	–	Cheung et al. (1980), Saito et al. (1994), Marczak et al. (2003)
VY	7.1	avenins, oat globulins, B-hordeins, γ-secalins, wheat HMW, wheat LMW, α/β, γ-gliadins	Saito et al. (1994)
DLP	4.8	γ-secalins, wheat LMW	Wu and Ding (2002)
FAP	3.8	–	Yamamoto (1997)
GGY	1.3	wheat LMW	Saito et al. (1994)
GPL	2.6	–	Byun and Kim (2001)
GPV	4.7	oat globulins, wheat HMW	Kim et al. (2001)
HHL	4.9	γ-secalins	Shin et al. (2001) (calculated on results)
IKP	2.5–6.9	–	Matsumura et al. (1993), Fujita and Yoshikawa (1999)
IKW	0.21	–	Fujita and Yoshikawa (1999)
IPP	5	wheat LMW, α/β-gliadins	Nakamura and Yamamoto (1995)
IRA	6.4	B-hordeins, wheat LMW, γ-gliadins	Miyoshi et al. (1991)
IRP	1.8	–	Matsumura et al. (1993)
IVY	0.48	B-hordeins, wheat LMW	Matsui et al. (1999)
IWH	3.5	–	Fujita and Yoshikawa (1999)
LAY	3.9	–	Miyoshi et al. (1991)
LKP	0.32	oat globulins	Fujita and Yoshikawa (1999)
LPP	9.6	wheat LMW, γ-gliadins	Yamamoto (1997)
LQP	2.0	avenins, B, C, D, γ-hordeins, rye HMW, ω-secalins, wheat HMW, wheat LMW, α/β, γ, ω -gliadins,	Miyoshi et al. (1991)
LRP	0.29-1.0	γ-hordeins, γ-gliadins	Miyoshi et al. (1991), Matsumura et al. (1993)
LSP	1.7	oat globulins	Miyoshi et al. (1991)
LYP	6.6	–	Yamamoto (1997)
PRY	2.5	rye HMW, wheat HMW	Saito et al. (1994)
VAP	2	–	Yamamoto (1997)

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cont.

Candidate peptide	IC50 μM	Occurence in cereal storage proteins (*i*ProClass)	References for peptide candidates and corresponding IC50-values
VPP	9	avenins, γ-hordeins, rye HMW, wheat HMW, γ-gliadins	Nakamura and Yamamoto (1995)
VRP	2.2	γ-gliadins	Matsumura et al. (1993)
VSP	10	D-hordeins, wheat HMW	Miyoshi et al. (1991)
VWY	9.4	–	Saito et al. (1994)
YQY	4	–	Li et al. (2002)
FVAP	10	–	Yamamoto (1997)
IYPR	10	–	Saito et al. (1994)
VFPS	0.46	–	Matsui et al. (1999)
YGGY	3.4	–	Saito et al. (1994)

ther one occurs in the structures of cereal prolamins (Table 1). In addition, the tripeptide LQP occurs repeatedly in barley C-hordeins and rye omega-secalins. The multiple incidence (up to sixteen repeats) of this particular tripeptide in prolamin structures makes it an interesting target peptide. The amino acid composition of LQP is somewhat typical for cereal prolamins; glutamic acid (including its amine glutamine) and proline normally cover approximately half (mol %) of all amino acids present in cereal prolamins (of wheat, barley, oats, and rye) (Shewry and Tatham 1999).

In addition to prolamins, potential candidate peptides also occurred in oat globulins, the unique salt-soluble storage proteins of oats. Tripeptides, LKP and leucine-serine-proline (LSP), with low IC50-concentrations (0.32 μM and 1.7 μM, respectively), appeared only in oat globulins. The oat globulins also possess a third candidate tripeptide (glycine-proline-valine) and four candidate dipeptides (isoleucine-tyrosine, proline-arginine, valine-phenylalanine, and valine-tyrosine) in their structures. These findings along with the relatively good solubility properties of oat globulins raise them as potential sources of ACE-inhibitory peptides. Avenins, the prolamins of oats, also contain five of the candidate peptides in their structures (Table 1).

The cleavage of the desired peptide from the structures of cereal proteins, however, may be complicated. Specific difficulties in the liberation of the target peptides arise from the tight structures of cereal storage proteins. Cereal storage proteins compared to for example milk caseins have relatively compact protein structures. The tightly compacted structures of cereal proteins must therefore partly be opened before effective hydrolysis can occur. Opening of the structures can include mild denaturation, reduction of disulfide bonds between and inside proteins, partial proteolysis etc. It also is obvious that proteins are easier substrates for enzymes when they are in a soluble and open form. Protein solubility, however, is strongly dependent on solvent, pH, and temperature, as certainly is the activity of proteolytic enzymes. Cleavage of a known peptide, with a known sequence and location, apart from the protein structure with a desired enzyme, thus, requires conditions that are beneficial not just for protein availability but also for the enzyme activity.

This database survey shows that wheat, rye, barley, and oats possess most of the known ACE-inhibitory peptide sequences in their storage protein structures. Cereals, thus, appear to be potential sources of BP-lowering peptides, if the active peptides can be liberated. Liberation of peptides in food processes, however, requires safe enzymes and good understanding of the substrate protein characteristics. The development of cereal foods that contain bioactive peptides

may also require novel product concepts, such as liquid-based cereal products with high protein contents.

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SELOSTUS

Angiotensiini I -muuntavaa entsyymiä estävien peptidien aminohapposekvenssien esiintyminen viljan varastoproteiinien rakenteessa

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Angiotensiini I:stä verenpainetta kohottavaa angiotensiini II:a vapauttava entsyymi (ACE) on keskeinen verenpaineen säätelyssä. Tietyt elintarvikeproteiineista peräisin olevat peptidit estävät sen toimintaa. Tässä tutkimuksessa selvitettiin jo tunnettujen ACE:a estävien peptidien aminohapposekvenssien esiintymistä kotimaisten viljojen varastoproteiinien rakenteissa.

Kirjallisuustutkimuksen perusteella tarkasteluun otettiin 39 peptidiä. Peptidien esiintymistä viljojen varastoproteiineissa tutkittiin *i*ProClass proteiinien rakennetietokannan avulla.

Viljojen varastoproteiinit sisälsivät yhteensä 22 haetuista peptideistä. Verenpainetta alentavien maitotuotteiden tripeptideistä jompikumpi (valiini-proliini-proliini tai isoleusiini-proliini-proliini) esiintyi poikkeuksetta kaikkien viljojen prolamiineissa. Kauran globuliinien rakenteessa oli puolestaan kaikkiaan seitsemän haetuista peptideistä. Mielenkiintoinen havainto oli kahden tripeptidin toistuva esiintyminen (jopa 16 kertaa proteiinia kohti) rukiin omega-sekaliineissa, ohran C-hordeiineissa (leusiini-glutamiini-proliini) ja ohran D-hordeiineissa (valiini-seriini-proliini). Tutkimustulosten perusteella viljaproteiineissa on lupaavasti verenpainetta alentavia peptidejä. Uudenlaisten verenpainetta alentavien viljatuotteiden kehittäminen vaatii peptidien tehokasta vapauttamista proteiinirakenteesta, mikä puolestaan edellyttää elintarvikekäyttöön soveltuvia turvallisia proteolyyttisiä entsyymejä sekä substraattiproteiinien ominaisuuksien tuntemista.