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Short communication: Bovine κ-casein variants result in different angiotensin I converting enzyme (ACE) inhibitory peptides

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ABSTRACT

Proteins in bovine milk are a common source of bioactive peptides. The peptides are released by the digestion of caseins and whey proteins. Peptides derived from the different genetic variants A, B, C, E, F1, F2, G1, G2, H, I, and J of bovine κ-casein (CSN3) were investigated for their inhibitory activities against angiotensin I converting enzyme (ACE). Amino acid sequences of the CSN3 variants were analyzed in silico to detect potential ACE inhibitory peptides. Besides known biologically active peptides, exclusive peptides were identified in some CSN3 variants and their biological activity was determined: within CSN3*B and CSN3*C, the ACE inhibitory peptide ASP (IC_{50} = 242.3; the IC_{50} value is equivalent to the micromolar concentration of peptide mediating a 50% inhibition of ACE activity) and within CSN3*C the peptide AHHP (IC_{50} = 847.6) was detected. Furthermore, the peptides VSP (IC_{50} = 21.8) and ACHP (IC_{50} = 360.7) were identified in CSN3*F1 and CSN3*G2, respectively.
Key words: bovine κ-casein, angiotensin I converting enzyme inhibitory peptide

Many food proteins, including milk and dairy products, contain biofunctional peptide sequences within their primary structures, which may have essential functions within physiological processes such as the regulation of blood pressure (reviewed by Murray and FitzGerald, 2007). These peptides are inactive within the sequence of the protein and are released during gastrointestinal digestion or food processing, or are produced by the body itself. Proteolysis during milk fermentation and cheese ripening also leads to the formation of various bioactive peptides (see e.g., Meisel 1997; Gobbetti et al., 2002; Gomez-Ruiz et al., 2006). Bioactive peptides show a wide range of effects including antimicrobial properties, blood pressure lowering effects, cholesterol-lowering ability, antithrombotic and antioxidant activities, enhancement of mineral absorption, cyto- or immunomodulatory effects, and opioid activities. Some peptides are multifunctional and can exert more than one of the effects mentioned (Meisel, 2004).

Hypertension is a major risk factor for cardiovascular diseases and can affect people of all ages. Antihypertensive peptides may have the ability to lower blood pressure through the inhibition of vasoactive enzymes such as angiotensin I converting enzyme (**ACE**, EC 3.4.15.1). Angiotensin I converting enzyme is a key enzyme in the regulation of peripheral blood pressure and electrolyte homeostasis. It is classically associated with the rennin-angiotensin system converting angiotensin I to the highly potent vasoconstrictor octapeptide angiotensin II (Skeegs et al., 1956). Inhibition of ACE is a widely used principle for the treatment of hypertension (reviewed by Conlin, 2001)

Angiotensin I converting enzyme inhibitory peptides have been identified from vegetable and animal proteins (Ariyoshi, 1993), but milk proteins are currently the main source. Bovine milk is one of the most important components of the nutrition of young mammals and newborn infants. Moreover, milk and dairy products are popular foods and a common source of proteins for adults. In 2006, the per capita consumption of milk was 64.8 kg in Germany (Milch und Markt, 2008). Oligopeptides derived from bovine milk and milk products were reported to inhibit ACE activity (Nakamura et al., 1995; Meisel et al., 2006). Reducing blood pressure with the help of oligopeptides derived from bovine milk proteins in human subjects was demonstrated in different studies (Hata et al., 1996; Seppo et al., 2003; Cadée et al., 2007) but all of these studies were carried out without considering the genetic milk protein variants and the different peptides resultant.

Milk proteins consist of 2 major protein groups, whey proteins and caseins, with caseins representing the main fraction (80%) of bovine milk proteins (Groenen and van der Poel, 1994). The group of caseins contains α_{S1}-casein, α_{S2}-casein, β-casein (**CSN2**), and κ-casein

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(**CSN3**); the 2 major whey proteins are α-lactalbumin and β-lactoglobulin. Each of these proteins occurs in different allelic forms, controlled by codominant genes (for reviews, see Ng-Kwai-Hang and Grosclaude, 1992; Farrell et al., 2004).

Within the reference protein sequence of bovine CSN3 protein, amino acid exchanges at positions 10, 97, 104, 135, 136, 155, and 148 lead to the different allelic variants A, B, C, E, F1, F2, G1, G2, H, I, and J (reviewed by Farrell et al., 2004). Rare CSN3 alleles occur specifically in endangered breeds, like the CSN3*G2 in Pinzgauer cattle, first described at the protein level by Erhardt (1996) and some years later at the DNA level by Prinzenberg et al. (1999). Compared with α_{S1}-casein, α_{S2}-casein, and CSN2 there are only a few ACE inhibitory peptides identified within bovine CSN3 (Meisel et al., 2006). On the other hand Gomez-Ruiz et al. (2007) found novel potent ACE inhibitory peptides in ovine CSN3. For that purpose this study should clarify if there are potential ACE inhibitory peptides in bovine CSN3 and if the genetic variants of CSN3 affect the presence of those peptides.

κ-Casein variants (A, B, C, E, F1, F2, G1, G2, H, I, J) known from literature were digested in silico using the "analysis" function of the BIOPEP database held by the University of Warmia and Mazury in Olsztyn, Poland (http://www.uwm.edu.pl/biochemia/). For the analysis of the different protein variants, the following enzymes were chosen: pepsin, trypsin, chymotrypsin A, chymotrypsin C, pancreatic elastase I, pancreatic elastase II, oligopeptidase B, and oligopeptidase II. The selection of putative ACE inhibitory peptides was done in consideration of 2 aspects: the peptide occurs in the region of the protein polymorphism and the peptide carries proline at the C-terminus of the sequence. This precondition was chosen because of the observation that the majority of ACE inhibitory peptides have a Tyr, Phe, Trp, or Pro at the C-terminal end (Meisel et al., 2006). The chosen peptides (Ala-His-His-Pro, Ala-Cys-His-Pro, Val-Ser-Pro and Ala-Ser-Pro) were synthesized (Peptides and Elephants GmbH, Nuthetal, Germany). The ACE inhibitory potency was determined by the IC_{50} value (equivalent to the micromolar concentration of peptide mediating a 50% inhibition of ACE activity) according to the method of Cushman and Cheung (1971) and modified as described by Meisel and Pentzien (2008).

The in silico digestion of CSN3*A with pepsin, trypsin, chymotrypsin A, chymotrypsin C, pancreatic elastase II, and oligopeptidase B resulted in the identification of ACE inhibitory peptides which are not only known from caseins, but also from other sources (Table 1). The IC_{50} values of these peptides demonstrate the potency of milk proteins concerning ACE inhibitory effects. The peptides listed in Table 1 occur in each genetic variant of CSN3 with the exception of Ser-Phe (position 104–105) and Ala-Arg (position 96–97) and are in the same range as the ACE inhibitory peptides derived from other milk proteins (Meisel et al., 2006).

The peptide Ser-Phe is solely present in the CSN3*I allele because of the mutation in this allele at position 104 compared with the reference sequence. This peptide shows an IC_{50} value of 130.2 and is also known from garlic (Meisel et al., 2006). The peptide Ala-Arg ($IC_{50} = 95.5$) was primarily identified in porcine skeletal muscle (Sentandreu and Toldra, 2007) and was found in the present study in CSN3*C and CSN3*G1 at position 96–97 of the amino acid sequence.

The in silico analysis of the protein sequence of CSN3 alleles B, C, E, F1, F2, G1, G2, H, I, and J lead, in the regions of polymorphisms, to different peptides compared with the reference sequence of CSN3*A. From all peptides that were identified in the regions of polymorphisms (data not shown), 4 potential ACE inhibitory peptides were chosen and the IC_{50} value was determined (Table 2). The determining factors for the choice of a peptide for further analyses were the occurrence of the sequence in the region of the protein polymorphism and the sequence of the peptide itself following the observation of Meisel et al. (2006) that the majority of ACE inhibitory peptides have a Tyr, Phe, Trp, or Pro at the C-terminal end.

The protein polymorphism at position 148–150 led to 2 different ACE inhibiting peptides. The peptide Val-Ser-Pro derived from CSN3*F1 showed the highest IC_{50} value (21.8) and is also known from maize (Miyoshi et al., 1991). The peptide Ala-Ser-Pro at the same position of the amino acid sequence but derived from the alleles CSN3*B and CSN3*C is not known for ACE inhibitory effects yet and showed an IC_{50} value of 242.3.

The genetic variation at amino acid position 96–99 produced 2 further ACE inhibitory peptides. In CSN3*C, the peptide Ala-His-His-Pro ($IC_{50} = 847.6$) was identified and at the same position the peptide Ala-Cys-His-Pro ($IC_{50} = 360.7$) was identified in CSN3*G2. These 2 peptides are not known for ACE inhibitory effects yet.

The dominant genetic variant of CSN3 in German cattle breeds is CSN3*A, whereas the B allele shows a high frequency in German Brown (0.479; Erhardt, 1989). Compared with CSN3*A, the B allele shows association with higher milk protein content and positive effects on coagulation properties and cheese yield (NgKwai-Hang and Grosclaude, 1992). In addition to these positive attributes, the present study shows an-

Table 1. Peptide sequences identified after in silico analyses from CSN3*A[1] with angiotensin I converting enzyme (ACE) inhibitory activity

Peptide sequence	Location within CSN3*A	IC_{50} value[2]	Additional information	Reference
Ile-Arg	9–10	695.0	β-Lactokinin from β-lactoglobulin	FitzGerald and Meisel (1999)
Arg-Phe	16–17	93.0	ACE inhibitor from sake lees	Saito et al. (1994)
Tyr-Ile-Pro-Ile-Glh-Tyr-Val-Leu-Ser-Arg	25–34	132.5	Casoxin; Opioid antagonist; Contracting and immunomodulatory; Antihypertensive	Meisel (1998); Takahashi et al. (1997); Maruyama et al. (1987)
Ala-Ile-Pro	107–109	670.0	ACE inhibitor from CSN3	Kohmura et al. (1990)
Val-Thr-Ser-Thr-Ala-Val	164–169	52.0	Antihypertensive peptide from CSN3	FitzGerald et al. (2004)
Pro-Ser-Tyr	36–38	16.0	ACE inhibitor from buckwheat	Wang and Gonzalez de Mejia (2005)
Ile-Pro	26–27; 108–109; 119–120	130.0		Cushman (1981)
Ile-Ala-Lys	22–24	15.7		Gomez-Ruiz et al. (2007)
Asn-Tyr	41–42	32.6	ACE inhibitor from garlic	Meisel et al. (2006)
Ser-Tyr	37–38	66.3	ACE inhibitor from garlic	Meisel et al. (2006)
Ser-Phe	104–105	130.2	ACE inhibitor from garlic	Meisel et al. (2006)
Ala-Arg	96–97	95.5		Sentandreu and Toldra (2007)

[1]CSN3*A = κ-casein allele variant A.

[2]The IC_{50} value is the micromolar concentration of peptide mediating a 50% inhibition of ACE activity.

Table 2. Putative angiotensin I converting enzyme (ACE) inhibitory peptides identified in different bovine CSN3[1] alleles

Peptide sequence	CSN3 allele	Location within the protein	IC_{50} value[2]
Ala-Ser-Pro	B, C	148–150	242.3
Val-Ser-Pro	F1	148–150	21.8
Ala-His-His-Pro	C	96–99	847.6
Ala-Cys-His-Pro	G2	96–99	360.7

[1]CSN3 = κ-casein.

[2]The IC_{50} value is the micromolar concentration of peptide mediating a 50% inhibition of ACE activity.

other reason for preferring milk with the κ-casein allele B, because of the appearance of a new ACE inhibitory peptide at position 148–150 of the protein.

The CSN3*C allele, however, occurs in some European cattle breeds only; for example, in German Simmental with an allele frequency of 4.5% (Prinzenberg et al., 1999). At protein position 148–150, peptides derived from other CSN3 alleles show no ACE inhibitory effects. The rare CSN3 allele F1 was identified by Sulimova et al. (1992) in Zebu and Black and White hybrid cattle.

Several studies demonstrate the blood-pressure-reducing effect of ACE inhibitory peptides derived from milk or milk products in humans (Seppo et al., 2003; Cadée et al., 2007). These studies ignore the occurrence of different genetic variants in the bovine milk. Only Kaminski et al. (2007) considered the different casein alleles within their study, describing the potential effects of β-casomorphins derived from different alleles of the bovine CSN2 on human health. The authors analyze the effect of peptides originating from different CSN2 alleles on human diseases such as diabetes mellitus or sudden infant death syndrome. The present study shows that different CSN3 variants are the source of diverse ACE inhibitory peptides and demonstrates the additional potential of bovine milk protein variants for human health. Moreover, this study shows that, compared with the identified peptides from monomorphic ovine CSN3 (Gomez-Ruiz et al., 2007), the bovine polymorphic CSN3 protein is a more potent source of ACE inhibitory peptides.

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