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Characterizing the *in vitro* **Digestion** and **ACE inhibitory** Peptides of Bovine **β-casein Variants**

Bjørn Petrat-Melin, Thao T. Le, Hanne S. Møller, Lotte B. Larsen, Jette F. Young
Department of Food Science
Aarhus University, DK-8830 Tjele Denmark

bjornpetratmelin@food.au.dk
+45 87 15 74 28

Background

Bovine β-casein is a well-known source of bioactive peptides. These peptides are encrypted within the primary structure of β-casein, and may be released by the action of digestive enzymes, either *in vitro* or in the gastrointestinal tract. The specificity of proteases is determined largely by the amino acids surrounding the scissile bond. Hence, changes in the sequence of proteins may change their susceptibility to certain proteases. Furthermore, the bioactivity of a peptide depends primarily on its amino acid composition and sequence. Therefore, amino acid substitutions in genetic variants of β-casein may affect their bioactive potential.

Aims

- Characterize the extent and pattern of the *in vitro* digestion of purified β-casein variants.
- Identify peptides of interest by tandem MS
- Assess antioxidant and angiotensin-1 converting enzyme (ACE) inhibitory capacity of β-casein derived synthesized peptides
- Assess ACE inhibition by synthesized peptides before and after incubation with intestinal cells

Conclusions

- Peptide pattern after simulated gastrointestinal digestion of β-casein variants is affected by amino acid substitutions
- Bioactive peptides with different ACE inhibitory capacities are generated by simulated gastrointestinal digestion of β-casein variants
- ACE inhibitory capacity of the bioactive peptides VYPFPGPIHN, VYPFPGPIPN, and TER is affected differently by the intestinal brush-border

Methods

Purification

β-casein (A^1, A^2, B, I)

- Thawing and storage at 4 deg. C for 48 h
- Ultracentrifugation at 150.000 g
- Acid precipitation

κ-casein (A, B, E)

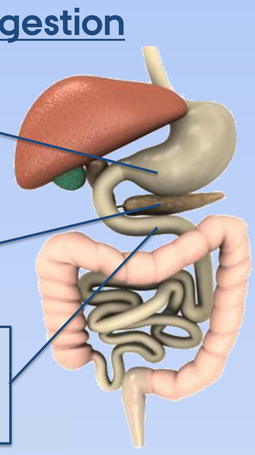
- Acid precipitation of all caseins
- Calcium-precipitation of α- and β-caseins
- Ion-exchange FPLC

In Vitro Digestion

1. Gastric step (pepsin)
pH = 2.0, T = 37°C
t = 60 min
E:CN = 1:200

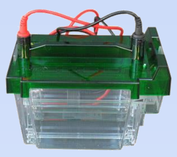
2. Pancreas
+ 50 mM HCO_3^-
+ pancreatic enzymes

3. Duodenal step (pancreatic enzymes)
pH = 6.5, T = 37°C
t = 5 or 120 min., E:CN=1:200

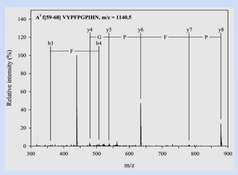


Characterization and activity

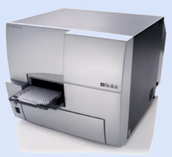
SDS-PAGE



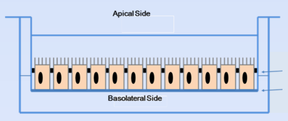
LC-MS/MS



Fluorometric ACE inhibition assay



Caco-2 cells



Results

- Simulated gastrointestinal digestion of β-casein variants A^1, A^2, B, and I resulted in different peptide fragments, assessed by SDS-PAGE (**Figure 1**) and tandem MS (**Table 1**)
- The Ser_{122} → Arg_{122} substitution in the B variant resulted in a novel trypsin cleavage site, and tandem MS indicated the presence of the tripeptide TER (B f[120-122]) (italics in **Table 1**)
- TER and two β-casomorphin-like decapeptides derived from variants A^1/B and A^2/I (bold in **Table 1**) were synthesized for determination of ACE inhibition
- IC_{50} of ACE inhibition is lower for TER and VYPFPGPIHN, compared to VYPFPGPIPN (**Table 2**)
- Incubation with differentiated intestinal cells significantly changed ACE inhibition by VYPFPGPIPN and TER (**Figure 2**)

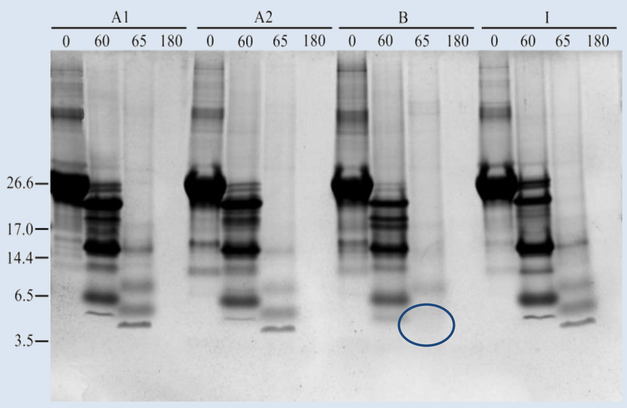


Figure 1. *In vitro* digestion of β-casein variants A^1, A^2, B, and I visualized by SDS-PAGE. 0 = undigested; 60 = 60 min pepsin; 65=60 min pepsin+5 min pancreatic enzymes; 180=60 min pepsin+120 min pancreatic enzymes. The blue circle indicates a band that is "missing" from the B variant at 65 minutes of digestion.

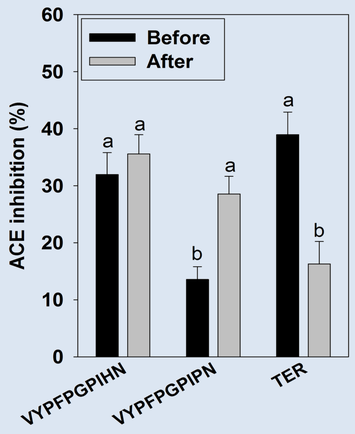


Figure 2. ACE inhibition by β-casein peptides before and after 120 minutes incubation with caco-2 monolayer. Mean values from two experiments performed in triplicate.

Table 1. Selected peptides from regions with amino acid substitutions identified by LC-MS/MS after *in vitro* digestion of bovine β-CN variants A^1, A^2, B, and I

Digested variant	Position	Sequence	Genetic variants
A^1, B	59-68	**VYPFPGPIHN**	A^1, B
A^2, I	59-68	**VYPFPGPIPN**	A^2, I
A^1, B	67-92	HNSLPQNIPPLTQTPVVVPPFLQPEV	A^1, B
I	69-92	SLPQNIPPLTQTPVVVPPFLQPEV	A^1, A^2, B, I
A^1, A^2, B	73-92	NIPPLTQTPVVVPPFLQPEV	A^1, A^2, B, I
I	81-92	PVVVPPFLQPEV	A^1, A^2, B, I
B	114-119	*YPVEPF*	A^1, A^2, B, I
A^1	114-124	YPVEPFTESQS	A^1, A^2, I
A^1, A^2	120-132	TESQSLTLTDVEN	A^1, A^2, I
A^1	120-139	TESQSLTLTDVENLHLPLPL	A^1, A^2, I
B	123-132	*QSLTLTDVEN*	A^1, A^2, B, I

Table 2. ACE inhibition (IC_{50}) of peptides derived from *in vitro* digested β-casein variants

Peptide	Variant	Position	IC_{50} (μM)
TER	B	120 – 122	**90** ± 8.8 a
VYPFPGPIHN	A^1, B	59 – 68	**123** ± 14.2 a
VYPFPGPIPN	A^2, I	59 – 68	**656** ± 7.6 b