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Identification and Quantification of Inhibitors for Angiotensin-Converting Enzyme in Hypoallergenic Infant Milk Formulas

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The potential of hypoallergenic (HA) infant milk formulas containing hydrolyzed milk proteins as main constituents to inhibit angiotensin-converting enzyme (ACE) *in vitro* was investigated. Seven commercially available HA products designed for babies up to 4 months showed a potent inhibition of ACE *in vitro*, with IC_{50} values ranging between 3.2 and 68.5 mg of nitrogen/L. For six samples of conventional milk-based infant formulas and three breast milk samples, no inhibition was observed. Inhibitory potential did not correlate with the degree of hydrolysis. Using reversed-phase high-pressure liquid chromatography (RP-HPLC) coupled to electrospray ionization−time of flight−mass spectrometry (ESI−TOF−MS), 15 peptides known to inhibit ACE were identified. Among them, the highly potent ACE inhibitor Ile-Trp ($IC_{50} = 0.7$ μM) was detected and quantified for the first time in the HA samples, representing the most effective ACE-inhibiting peptide that has ever been detected in food items. The overall inhibitory potential of the HA infant milk formulas could partly be explained by Ile-Trp.

KEYWORDS: Angiotensin-converting enzyme; blood pressure; bioactive peptides; HPLC−MS; infant nutrition

INTRODUCTION

Angiotensin-converting enzyme (ACE) is a dipeptidyl-carboxypeptidase (EC 3.4.15.1) containing zinc in its active center. This enzyme is important in blood-pressure regulation, because in the renin angiotensin system, ACE catalyzes the conversion of the inactive decapeptide angiotensin I to the octapeptide angiotensin II, which exhibits a strong vasoconstricting action, resulting in an increase in blood pressure (*1*). Furthermore, ACE inactivates bradykinin, a vasodilatory peptide, within the kinin−kallikrein system (*2*). Selective inhibition of ACE is an important pharmacological strategy for the treatment of hypertension (*3*). It is well-known that also peptides, derived from the hydrolysis of food proteins, may inhibit ACE *in vitro* and *in vivo* (*4*). Fermented sour milk products based on the use of selected starter cultures are commercialized in Japan (Ameal S, from Calpis Co. Ltd., Tokyo, Japan) and Finland (Evolus, from Valio Co. Ltd., Helsinki, Finland). For these products, a hypotensive effect *in vivo* was found in animal experiments as well as in clinical studies (*5, 6*), which could be attributed to defined peptides, namely, the tripeptides Val-Pro-Pro and Ile-Pro-Pro, which are released from casein during fermentation (*7, 8*). However, it should be noted that recent publications did not support the hypothesis of a blood-pressure-lowering effect of these tripeptides (*9, 10*).

In so-called hypoallergenic (HA) infant milk formulas, the allergenic potential of milk proteins is reduced by using more or less intensively hydrolyzed casein or whey proteins as constituent. The degree of hydrolysis and composition of peptides may greatly vary because of the production and methods of preparation (enzymes used for hydrolysis, temperature, etc.) (*11*). Against the background of the enormous number of papers dealing with the formation of ACE-inhibiting peptides during hydrolysis of milk proteins, surprisingly little knowledge is available about a possible ACE-inhibiting potential of HA formulas. A moderate ACE-inhibiting activity, expressed in a relative ACE inhibitory index comparable to ripened cheese, was reported for a single sample of a HA infant milk-based food by Meisel et al. (*8*). Hernández-Ledesma et al. (*12*) evaluated inhibition of ACE *in vitro* by a number of commercial infant milk formulas and found only moderate effects, except for two samples based on extensively hydrolyzed casein or whey protein, respectively, which showed a somewhat more pronounced inhibition of ACE. The ACE inhibitory activity of the nonhydrolyzed formula increased during simulated gastrointestinal digestion, while no significant change was observed in the activity of the hydrolyzed infant milk formula.

The aim of the present study, therefore, was to investigate the ACE-inhibiting potential of commercially available HA infant milk formula in comparison to conventional products used for infant feeding. After the determination of IC_{50} values, representing the minimum concentration of peptides needed for a 50% inhibition of ACE *in vitro*, for a number of commercially

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available products, individual peptides being responsible for the inhibition were identified by reversed-phase high-pressure liquid chromatography (RP-HPLC) and mass and ultraviolet (UV) spectrometry. Studies simulating gastrointestinal digestion followed to derive conclusions about possible physiological consequences.

MATERIALS AND METHODS

Reagents. ACE from rabbit lung, hippuryl-L-histidyl-L-leucine (HHL), *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES), pepsin (EC 3.4.4.1), and the dipeptides Ile-Trp (IW), Leu-Trp (LW), and Trp-Leu (WL) were purchased from Sigma (Deisenhofen, Germany). The dipeptide Trp-Ile (WI) was from BioLux (Stuttgart, Germany). Corolase PP, a proteolytic enzyme preparation from pig pancreas glands that besides trypsin and chymotrypsin contains numerous amino- and carboxylpeptidase activities was obtained from Röhm (Darmstadt, Germany). All other chemicals used were of analytical grade.

Samples. A total of 13 samples of infant milk formulas, designed for babies up to 4 months, were purchased from local retail stores and included six samples of conventional infant milk formula and seven HA infant formula based on hydrolyzed milk proteins. Three breast milk samples were obtained as a gift from breastfeeding mothers.

Sample Preparation. Samples of infant milk formulas (100 mg) were dissolved in 1 mL bidistilled water. The nitrogen content of the solutions was determined using the Kjeldahl method. Solutions were further diluted with bidistilled water to the appropriate nitrogen concentration used in the inhibitory assay. For further investigations via RP-HPLC and liquid chromatography−electrospray ionization−time of flight−mass spectrometry (LC−ESI−TOF−MS) analysis, HA infant milk formulas were defatted by centrifugation (20 min at 10 000 rpm for 2 times). After filtration (5 μm pore size, regenerated cellulose), samples were freeze-dried. Then, remaining intact proteins were precipitated by suspending 100 mg of each dried sample in 1 mL of a mixture of cold (4 °C) 70% (v/v) acetonitrile in water. After incubation for 12 h at 4 °C, the filtrate was evaporated to dryness and the complete dry residue was dissolved in 1 mL of bidistilled water for further analysis of the content of Ile-Trp. The nitrogen of these solutions was determined by the Kjeldahl method.

Determination of ACE Inhibition. ACE activity was measured using the method by Cushman and Cheng (*13*) with some modifications. To a mixture of 100 μL of a solution of 5 mM HHL [dissolved in a buffer containing 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) and 300 mM NaCl at pH 8.3] and 25 μL of inhibitor solution, which had been preincubated for 10 min at 37 °C, was added 20 μL of ACE (1.25 mU), dissolved in bidistilled water. The mixture was incubated for 120 min at 37 °C. The reaction was terminated by adding 125 μL of 1 N HCl. The amount of hippuric acid formed was measured via RP-HPLC (see below) after filtration through 0.2 μm syringe membrane filters (regenerated cellulose). A total of 20 μL of the sample was injected. Blank samples were prepared by replacing the inhibitor solution by 25 μL of water. All assays were performed in triplicate. The results were expressed as a percentage of the residual activity measured in the presence of an inhibitor compared to the ACE activity measured for a blank sample. The concentration of nitrogen originating from the solutions of infant milk formulas needed to inhibit 50% of ACE activity under the described conditions (IC_{50}) was calculated after plotting ACE inhibition (%) versus the nitrogen concentration and nonlinear regression analysis, using a three-parameter logistic model. For data analysis, the software SigmaPlot 5.0 (SPSS, Richmond, VA) was used.

RP-HPLC. For the determination of the ACE inhibition, a HPLC system from Knauer (Berlin, Germany) consisting of a K-1001 pump with an online degasser and a K-1500 solvent organizer, a column oven, and a photodiode array detector DAD-2700 and K-2001 was used. The column was a C18-Eurosphere 100, 5 μm (125 × 4.6 mm; Knauer, Berlin, Germany). The injection volume was 20 μL, and the temperature was set at 25 °C. Elution was achieved by a gradient of a buffer containing 10 mM KH_2PO_4 at pH 3.0 (solvent A) and methanol (solvent B) at a flow rate of 1.0 mL/min. The gradient was as follows: 15−40% solvent B in 5 min, 40−80% solvent B in 2 min, 2 min at 80% solvent B, 80−15% solvent B in 1 min, and equilibration for 1 min at 0% solvent B. Hippuric acid was detected at 228 nm and was quantified after calibration with external standard solutions.

For identification and quantification of Ile-Trp, a HPLC system from Amersham Pharmacia Biotech (Uppsala, Sweden), consisting of a pump P-900 with an online degasser, a column oven, and a UV detector UV-900 was used. After the eluate flowed through the UV detector, it was monitored using a fluorescence detector F 1050 (Merck, Darmstadt, Germany). The column was a C18-Eurosphere 100, 5 μm (125 × 4.6 mm, Knauer, Berlin, Germany). The injection volume was 20 μL, and the temperature was set at 30 °C. Elution was achieved by a gradient of 0.15% phosphoric acid in water (solvent A) and methanol (solvent B) at a flow rate of 0.7 mL/min. The gradient was as follows: 4 min at 3% solvent B, 3−42% solvent B in 18 min, 42−56% solvent B in 10 min, 56−100% solvent B in 8 min, 3 min at 100% solvent B, 100−3% solvent B in 4 min, and equilibration for 3 min at 3% solvent B.

LC−ESI−TOF−MS. A PerSeptive Biosystems Mariner time-of-flight mass spectrometer (TOF−MS) equipped with an electrospray ionization source (ESI) working in the positive mode was used (Applied Biosystems, Stafford, CA). An Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA), consisting of a high-pressure gradient pump system, column oven, automatic injector, and diode array detector, was coupled to the ESI−TOF−MS instrument. The column used was a C18-Eurosphere 100, 5 μm (250 × 4.6 mm; Knauer, Berlin, Germany). A gradient was applied with 5 mM ammonium acetate at pH 5.5 (solvent A) and a mixture of acetonitrile and 5 mM ammonium acetate [84:16 (v/v); solvent B]. The gradient was as follows: 0% solvent B for 2 min, 0% solvent B to 25% solvent B in 9 min, 25% solvent B to 50% in 14 min, 50−75% solvent B in 15 min, 75−100% solvent B in 1 min, 8 min at 100% solvent B, 100−0% solvent B in 4 min, and equilibration for 18 min at 0% solvent B. The injection volume was 20 μL; the flow rate was 0.7 mL/min; and the temperature was set at 35 °C. Peptides were detected at 220 and 280 nm. Mass spectrometric analysis was performed with a PerSeptive Biosystems Mariner TOF−MS instrument equipped with an ESI in general in the positive (Applied Biosystems, Stafford, CA). Calibration of the mass scale was established using a mixture of bradykinin, angiotensin I, and neurotensin. The monoisotopic molecular masses were determined using the peak with the lowest *m*/*z* ratio (monoisotopic peak) from prominent multiple-charged ions and the equation $M_r = zM_z - 1.0078z$, where M_r is the monoisotopic molecular mass, M_z is the *m*/*z* ratio, *z* is the number of charges, and 1.0078 is the mass of a proton.

Simulated Gastrointestinal Digestion. Two infant milk formulas (samples 3 and 8) were subjected to simulated gastrointestinal digestion according to a method described recently (*12*). Sample 3 was a HA infant milk formula containing hydrolyzed whey protein, and sample 8 was a conventional infant milk formula with intact protein. From the infant milk formula samples, aqueous solutions were prepared (corresponding to a nitrogen content of 0.12% as determined using the Kjeldahl method). The pH value was adjusted to 4.3 followed by centrifugation (10 000 rpm for 20 min) and filtration (5 μm pore size, regenerated cellulose). Filtrates were hydrolyzed with pepsin (42.5 kU/g nitrogen) for 90 min at 37 °C. After this, the pH was adjusted to 7.5 with 1 N NaOH, followed by hydrolysis with CorolasePP (177 U/g of nitrogen) at 37 °C for 240 min. The reaction was terminated by heating to 95 °C for 10 min. Samples were stored at −20 °C until determination of ACE inhibition.

RESULTS AND DISCUSSION

With regard to the numerous reports dealing with milk proteins as precursors for peptides acting as efficient inhibitors for ACE, we hypothesized that HA infant milk formulas containing more or less intensively hydrolyzed casein or whey proteins as a sole protein source to minimize the allergenic risk for infants may also show pronounced inhibitory effects on ACE when compared to conventional infant milk formula containing intact milk proteins. As a first step of our investigations, an assay for measuring the activity of ACE from rabbit lung *in*

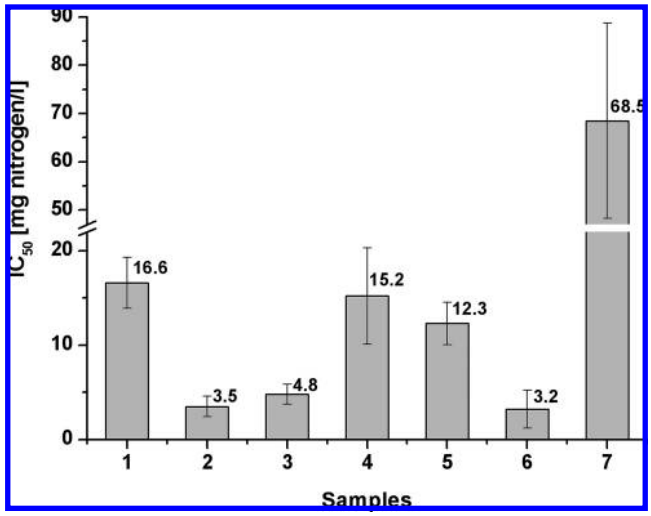

Figure 1. IC_{50} values of commercial HA infant milk formulas. Data are given as means of triplicates ± standard deviation.

vitro in the absence and presence of solutions prepared from various commercially available infant milk formulas was performed. The assay is based on the chromatographic determination of hippuric acid formed during hydrolysis of a synthetic peptide, namely, HHL. Using this well-established assay, IC_{50} values, which is the concentration of inhibitor needed to inhibit 50% of ACE activity, were determined by plotting ACE inhibition in percentage versus the inhibitor concentration, expressed as milligrams of nitrogen per liter. For six conventional infant milk formulas, no inhibition of ACE for concentrations up to 100 mg of nitrogen/L was found. Also for three samples of breast milk, no inhibitory effect could be measured. In contrast to these samples, all HA infant milk formulas investigated showed potent ACE inhibition. Values for IC_{50} ranged from 3.2 to 68.5 mg of nitrogen/L (**Figure 1**). Sample 7, which showed the weakest ACE inhibition of all samples studied, represented a formula based on extensively hydrolyzed milk protein according to the manufacturer. The measured IC_{50} of 68.5 mg of nitrogen/L for sample 7 is in good agreement with inhibition data reported for two samples of intensively hydrolyzed formulas, which showed IC_{50} values of 81 or 101 mg of peptide nitrogen/L (*12*). For all other HA formulas (samples 1−6), however, significant lower IC_{50} values were measured, corresponding to an efficient inhibition of ACE *in vitro*, which has not yet been reported for any food item. No significant differences were found in the peptide profiles of these samples by RP-HPLC (for example, see **Figure 2**), indicating that differences in IC_{50} are not caused by varying degrees of hydrolysis in general but should be due to marginal variations in the peptide profiles and the presence of small amounts of highly potent inhibitors.

Further characterization of inhibitory compounds was achieved by RP-HPLC coupled to UV and ESI−TOF−MS detection. On the basis of the theoretical molar mass and the resulting mass spectrometric data calculated for selected peptides resulting from a proteolytic breakdown of whey proteins, which are known as ACE inhibitors (**Table 1**), it was possible to search for the corresponding molecular masses within a chromatographic peptide profile. **Figure 2** shows a complete chromatogram obtained for sample 3 after RP-HPLC with UV detection. As an example for an identified peptide, **Figure 3a** shows the mass spectrum of one peptide, for which a molecular mass ($M + H^+$) of 281.2 Da was detected. To experience if there is only one peak with this mass in the sample, the extracted ion current

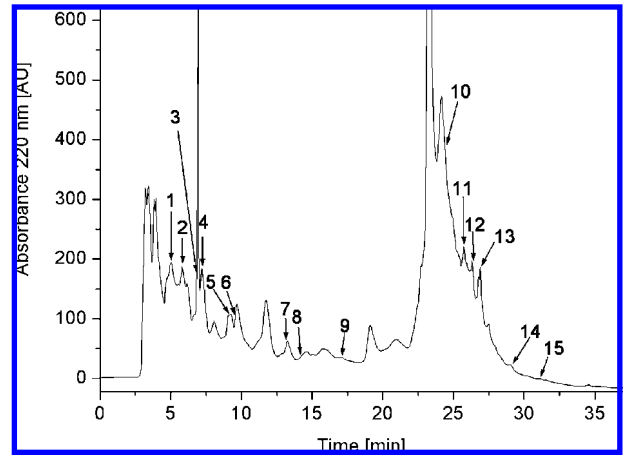

Figure 2. RP-HPLC with UV detection of a HA infant milk formula. Numbers refer to peptides listed in **Table 1**.

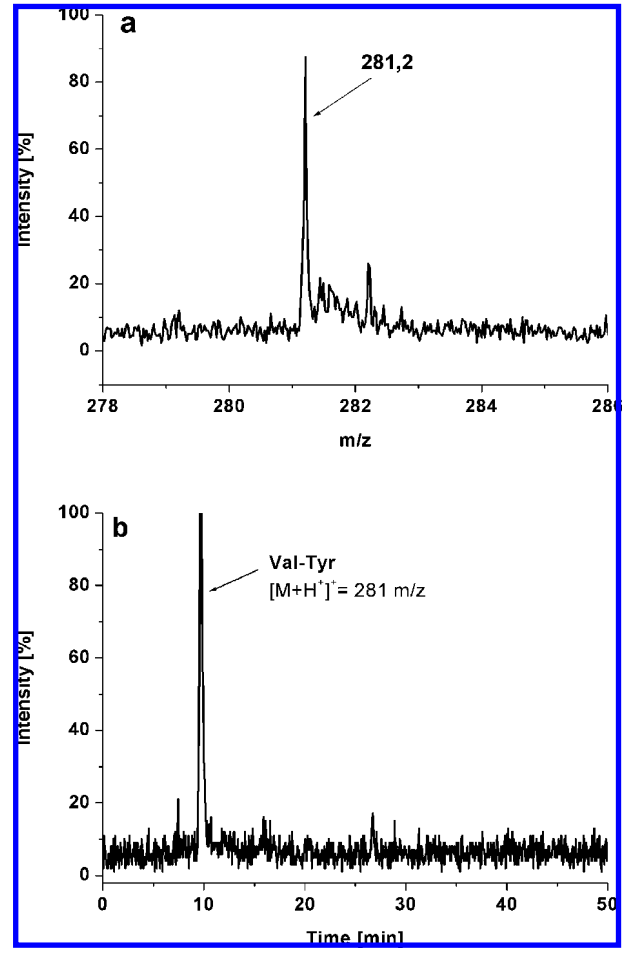

Figure 3. (a) Mass-spectrum of peptide 6 and (b) extracted ion chromatogram for *m*/*z* 281.

(XIC, see **Figure 3b**) of the detected molecular ion was used. Therefore, for this example, unambiguous identification of peptide 6 as the dipeptide Val-Tyr (molecular mass of 280.2 Da) was possible. On the basis of this strategy, a total of 15 peptides were identified (**Table 1**). For the peptides 5 and 6 (Gly-Tyr and Val-Tyr, respectively), which were commercially available as reference material, the corresponding IC_{50} values were measured as 180 and 26 μM, respectively. These data are in good agreement with data published in the literature (see **Table 1**). For the dipeptides 1, 2, and 10, two sequence

Table 1. ACE-Inhibiting Peptides Detected in HA Sample 3 and Corresponding IC_{50} Values Known from the Literature[a]

peptide number	time (min)	detected peptide mass $[M + H^+]$ (Da)	theoretical peptide mass $[M + H^+]$ (Da)	sequence	IC_{50} (μM)	reference
1	5.0	189.2	189.22	Gly-Leu or Leu-Gly	2500 8800	*13*
2	6.7	175.2	175.2	Gly-Val or Val-Gly	4600 1100	*13*
3	7.2	279.1	279.13	Tyr-Pro	185	*4*
4	8.8	189.2	189.22	Gly-Ile	647	*4*
5	9.2	239.1	239.08	Gly-Tyr	210 (180 ± 20)	*13*
6	9.8	281.2	281.13	Val-Tyr	35 (26 ± 7)	*14*
7/8	12.8/14.4	288.3	288.34	Ile-Arg or Arg-Leu	695 2439	*4*
9	17.4	295.3	295.31	Tyr-Leu or Leu-Tyr	122 44	*13*
10	25.1	300.2	300.20	Ile-Pro-Ala	141	*16*
11	26.2	318.2	318.17	Ile-Trp	1.5 (0.7 ± 0.3)	*14*
12	26.9	392.2	279.13	Leu-Phe	349	*4*
13	27.3	318.2	318.17	Trp-Leu	(10 ± 1.7)	
14	29.0	392.2	392.25	Leu-Leu-Phe	80	*16*
15	31.4	439.3	439.35	Ala-Leu-Met-Pro	928	*17*

[a] Values in parentheses were determined in the present study (data are given as means of triplicates ± standard deviation).

combinations each are known as inhibitors for ACE, among which it was not possible to distinguish in our study.

This screening for known peptides with ACE-inhibiting properties proved that bioactive peptides are present in HA infant milk formulas, which in combination contribute to the total ACE-inhibiting potential of the food item. Most of the peptides identified had only low inhibitory potential, as expressed by rather high IC_{50} values (**Table 1**). However, in addition to the detection of these peptides of low-inhibition potential, it was possible to identify the dipeptide Ile-Trp (peptide 11 in **Table 1**) for the first time in hydrolyzates of milk proteins. Ile-Trp eluted with a retention time of 26.2 min in the chromatogram (**Figure 3**), as monitored with ESI−TOF−MS. The mass spectrum with the mass peak at an *m/z* of $[M + H]^+ = 318.2$ is shown in **Figure 4a**. Unambiguous characterization of Ile-Trp in the samples was achieved by photodiode array detection, resulting in a UV spectrum with an absorption maximum at $\lambda = 278$ nm (**Figure 4b**). With respect to commercially available reference material for Ile-Trp, Trp-Ile, Leu-Trp, and Trp-Leu, identification of peptide 11 as Ile-Trp was achieved. Retention time and UV and mass spectra of a commercially available reference for Ile-Trp were identical to peptide 11. The phenomenon that the absorption maxima were at 278 nm instead of the theoretically expected value of 280 nm may be due to a slight hypochromic shift caused by the solvent used for chromatography. Ile-Trp (peptide 11) eluted clearly separated from Leu-Trp, Trp-Leu, and Trp-Ile. Trp-Leu (peak 13) was also identified to be present in HA infant milk formulas.

Both peptides proved to be very potent inhibitors for ACE. Whereas for Trp-Leu, an IC_{50} of 10 μM was measured, the IC_{50} for Ile-Trp was 0.7 μM. This is in agreement with the first report on the ACE-inhibiting properties of Ile-Trp, when the dipeptide had been found in enzymatic hydrolyzates of wakame (*Undaria pinnatifida*) and an IC_{50} of 1.5 μM had been reported (*14*).

To the best of our knowledge, Ile-Trp represents the most potent ACE inhibitor that has ever been identified to occur in a commercial food item. The presence of Ile-Trp in hydrolyzates of milk proteins has not been described yet, although the dipeptide is present at positions 59−60 in the sequence of bovine α-lactalbumin. It is noteworthy, that Ile-Trp is not present in the sequence of human α-lactalbumin, which has Leu-Trp at position 59−60.

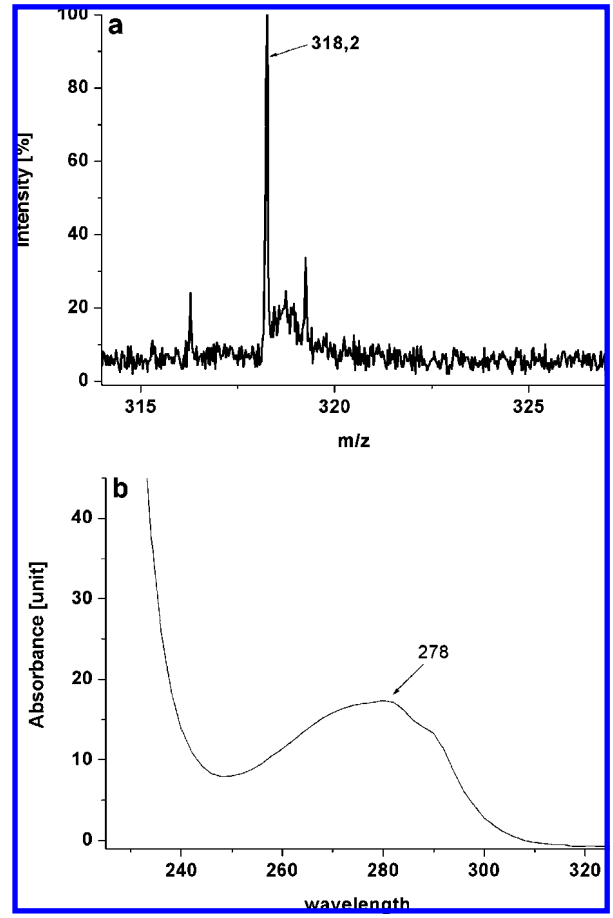

Figure 4. (a) Mass spectrum and (b) UV spectrum of peptide 11 eluting at 26.2 min in the chromatogram, which was identified as Ile-Trp in sample 3.

Further studies focused on the quantification of Ile-Trp in the samples of HA infant milk formulas. On the basis of the spectroscopic properties of the tryptophan moiety, RP-HPLC

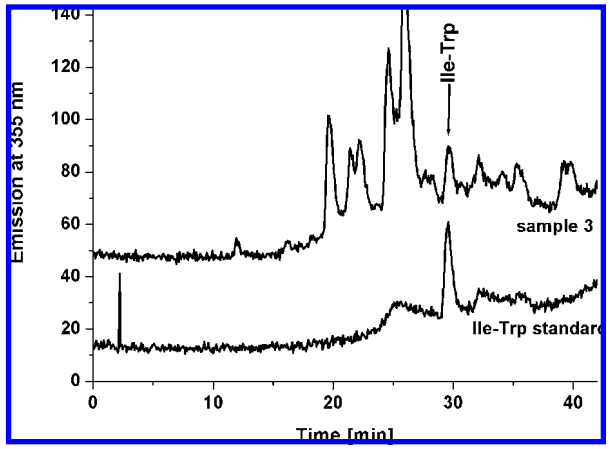

Figure 5. RP-HPLC with fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 355 nm) of a HA infant milk formula (sample 3) and standard peptide Ile-Trp.

Table 2. Amount of Ile-Trp in Samples of Commercial HA Infant Milk Formulas As Measured via RP-HPLC and Fluorescence Detection Compared to IC_{50} Values Measured for the Corresponding HA Sample and Calculated Concentrations of Ile-Trp at the IC_{50} Values[a]

sample	Ile-Trp (mg/g of nitrogen)	IC_{50} of HA sample (mg of nitrogen/L)	Ile-Trp at IC_{50} of HA sample (μM)
1	1.00 ± 0.31	16.6	0.052
2	3.15 ± 0.36	3.5	0.035
3	4.08 ± 0.43	4.8	0.062
4	1.81 ± 0.31	15.2	0.087
5	5.38 ± 0.61	12.3	0.209
6	ca. 0.63	3.2	0.006
7	nd[b]	68.5	

[a] Data are given as means of triplicates ± standard deviation. [b] nd = not detectable (<0.5 mg/g of nitrogen).

with fluorescence detection (λ_{ex} = 280 nm, λ_{em} = 355 nm) enabled a direct quantification of the dipeptide even in the complex chromatograms of the hydrolyzed milk samples by comparing retention time and emission with an external standard sample of Ile-Trp (**Figure 5**). Ile-Trp was detected in six of the seven investigated HA formulas, and the amounts could be estimated (**Table 2**). For samples 1−5, amounts of Ile-Trp ranged between 1.00 and 5.38 mg/g of nitrogen (**Table 2**). Assuming that whey protein is the sole protein source used as an ingredient for the HA formulas and, furthermore, assuming that whey proteins contain roughly 20% α-lactalbumin (*15*), which corresponds to 14 μmol of α-lactalbumin/g of whey protein, a maximum theoretical content for Ile-Trp of 28 mg/g of whey nitrogen can be calculated. The values measured for Ile-Trp correspond to 3−19% of this maximum yield, indicating that several other oligopeptides containing the Ile-Trp sequence may be present, which are due to incomplete hydrolysis of the starting material. Further studies are necessary to check whether some of the fluorescent material detected in the chromatograms (**Figure 5**) may be due to such peptides. In this context, it is noteworthy, that no correlation seems to exist between the total ACE-inhibiting potential as expressed by the IC_{50} values and the amount of Ile-Trp measured for the corresponding sample (**Table 2**). For samples 1−4, the concentration of Ile-Trp at the measured IC_{50} of the corresponding HA infant milk formula is between 0.04 and 0.09 μM, which is significantly lower than the IC_{50} value of Ile-Trp, which was determined to be 0.7 μM. For sample 5, the concentration of Ile-Trp at the IC_{50} value of the formula is 0.21 μM, which indicates a substantial contribution of the dipeptide to the ACE inhibition of the complex peptide mixture. The overall inhibitory potential of the HA infant milk formulas, however, could not be explained solely by the known inhibitor Ile-Trp, although this dipeptide is responsible for a significant part of the inhibitory effect. Therefore, it is likely that further inhibiting peptides of currently unknown sequence are present.

Table 3. IC_{50} Values of a HA Infant Milk Formula (Sample 3) and a Sample of a Conventional Infant Milk Formula (Sample 8) before and after Simulated Gastrointestinal Digestion[a]

	IC_{50} of sample 3 (mg of nitrogen/L)	IC_{50} of sample 8 (mg of nitrogen/L)
before digestion	4.8 ± 1	no inhibition
after digestion	4.6 ± 0.8	213.0 ± 35.7

[a] Data are given as means of triplicates ± standard deviation.

In a final experiment, we investigated whether peptides responsible for the ACE inhibition effect of HA infant milk formulas are resistant to simulated gastrointestinal proteolysis (**Table 3**). Inhibition of ACE by one sample of HA formula (sample 3) as well as by one sample of a conventional infant milk formula was measured before and after simulated gastrointestinal digestion according to (*12*). For the conventional milk formula, which contained intact protein, a slight increase in the inhibiting potential as expressed by an IC_{50} value of 213 mg of nitrogen/L after digestion was observed. This confirms results as described by Hernández-Ledesma et al. (*12*), who proved the formation of ACE-inhibiting peptides during simulated gastrointestinal digestion. The low IC_{50} value of the HA sample, however, was not influenced by the enzymatic hydrolysis procedure. This indicates that the peptides, which are present in the HA formula, should survive gastrointestinal hydrolysis and can be resorbed in the intestine. It is therefore likely that HA formulas may also influence the ACE *in vivo*.

In conclusion, with this study, it was proven unambiguously that commercial HA formulas contain peptides that are highly efficient inhibitors for ACE. The potent inhibitor Ile-Trp, a dipeptide originating from the sequence of α-lactalbumin, was identified and quantified for the first time in hydrolyzates of milk proteins. This results place emphasis on the importance for further studies on possible physiological consequences resulting especially for infant nutrition. Corresponding investigations are under way in our laboratory.

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