Plasmin Hydrolysis of β-Casein: Foaming and Emulsifying Properties of the Fractionated Hydrolysate

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Bovine β-casein (βCN) was hydrolyzed by plasmin. The hydrolysate was fractionated by ultrafiltration and selective precipitation, which resulted in several peptide fractions of which the peptide composition was monitored by reversed-phase high-performance liquid chromatography. Poorly soluble, hydrophobic peptide fractions, containing peptides from the C-terminal half of the βCN sequence, possessed improved foam-forming and -stabilizing properties compared to those of intact βCN, especially at pH 4.0. Soluble peptide fractions, containing a variety of peptides from the "middle" part of the βCN sequence in different proportions, possessed improved emulsion-forming capacity at pH 6.7, compared to that of intact βCN, and showed large variations in emulsion stability. The fraction containing the hydrophilic N-terminal part of βCN showed inferior foam, emulsion, and surface-active properties, especially at pH 6.7. The differences in functionality found between the various peptide fractions may be attributed either to synergistic effects between peptides or to a specific functionality of some individual peptides.

Keywords: *β-Casein; peptides; plasmin; foam; emulsions*

INTRODUCTION

The strongly amphipathic nature of proteins enables them to adsorb onto interfaces, and this functionality is exploited by food and pharmaceutical industries to stabilize foams and emulsions (Mulvihill and Fox, 1989). The functionality of proteins can be altered by chemical, physical, enzymatic, and genetic modification (Phillips et al., 1994). Enzymatic modifications have the advantage of mild reaction conditions (Arai and Fujimaki, 1991; Phillips et al., 1994) and can be used to enhance some functionalities of food proteins (Panyam and Kilara, 1996). Enzymatic hydrolysis of proteins has several consequences: decrease in molecular weight (MW); increase in the number of ionizable groups; exposure of hydrophobic groups; etc. (Panyam and Kilara, 1996). This implies that the solubility and the surface activity of the material will be altered, and, consequently, the propensity of the hydrolyzed protein to form and stabilize foams and emulsions may be different from that of the intact protein.

Plasmin is a proteolytic enzyme with a preference for Lys−X and Arg−X bonds. In milk the enzyme is associated with casein micelles in which α_{s2}-casein and β-casein (βCN) are the most susceptible to plasmin degradation [for a review see Bastian and Brown (1996)]. In fresh milk βCN is converted by plasmin into several large, intermediate degradation products, the γ-caseins, and to the complementary parts called proteose peptones (Andrews, 1978a,b; Eigel et al., 1984). Wilson et al. (1989) modified isolated βCN by plasmin hydrolysis to yield γ-caseins and proteose peptones. The surface pressure at the air/water (A/W) interface induced by these hydrolysis products appeared to be different from that of the intact protein. Considering the expected relation between surface-active properties and foam and emulsion properties (Mulvihill and Fox, 1989), it can be expected that the foam and emulsion properties of βCN are altered by plasmin hydrolysis.

Shimizu et al. (1984) found that the α_{s1}CN(f 1−23) peptide fraction showed an emulsion activity index similar to that of intact α_{s1}CN at concentrations >2% (w/v). However, in a later paper by the same authors (Shimizu et al., 1986), a synergistic effect in emulsion activity between the studied α_{s1}CN(f 1−23) and traces of coexisting peptides was demonstrated. Such a synergistic effect was also mentioned by Lee et al. (1987b). The objective of the present study was to produce, by means of plasmin hydrolysis, well-defined peptide mixtures from βCN with altered foam, emulsion, and surface-active properties. To that end, we fractionated the hydrolysate stepwise to produce special peptide fractions, so that the influence of removing certain peptides could be tested.

MATERIALS AND METHODS

Materials. Bovine βCN (90% βCN based on dry weight, 95% βCN based on 100% nitrogen, w/w) was purchased from Eurial (Rennes, France). Bovine plasmin (EC 3.4.21.7) and aprotinin were obtained from Sigma (St. Louis, MO; articles P-7911 and A-6012, respectively). Unless stated otherwise, all other chemicals were of analytical grade and were purchased from Merck (Darmstadt, Germany) or BDH (Poole, U.K.).

***β*CN Hydrolysis and Fractionation of the Hydrolysate.** Figure 1 shows an outline of the βCN hydrolysis and the fractionation of the hydrolysate. Bovine βCN was hydrolyzed by plasmin (3% w/v βCN; enzyme/substrate = 1/2300 w/w; pH 6.8; 40 °C) using a pH-stat method (Adler-Nissen, 1986). During the hydrolysis a precipitate formed (this precipitate disappeared at temperatures lower than approximately 4 °C). The precipitate was removed by centrifugation (1500*g*, 10 min, 30 °C), and the resulting supernatant was incubated, yielding

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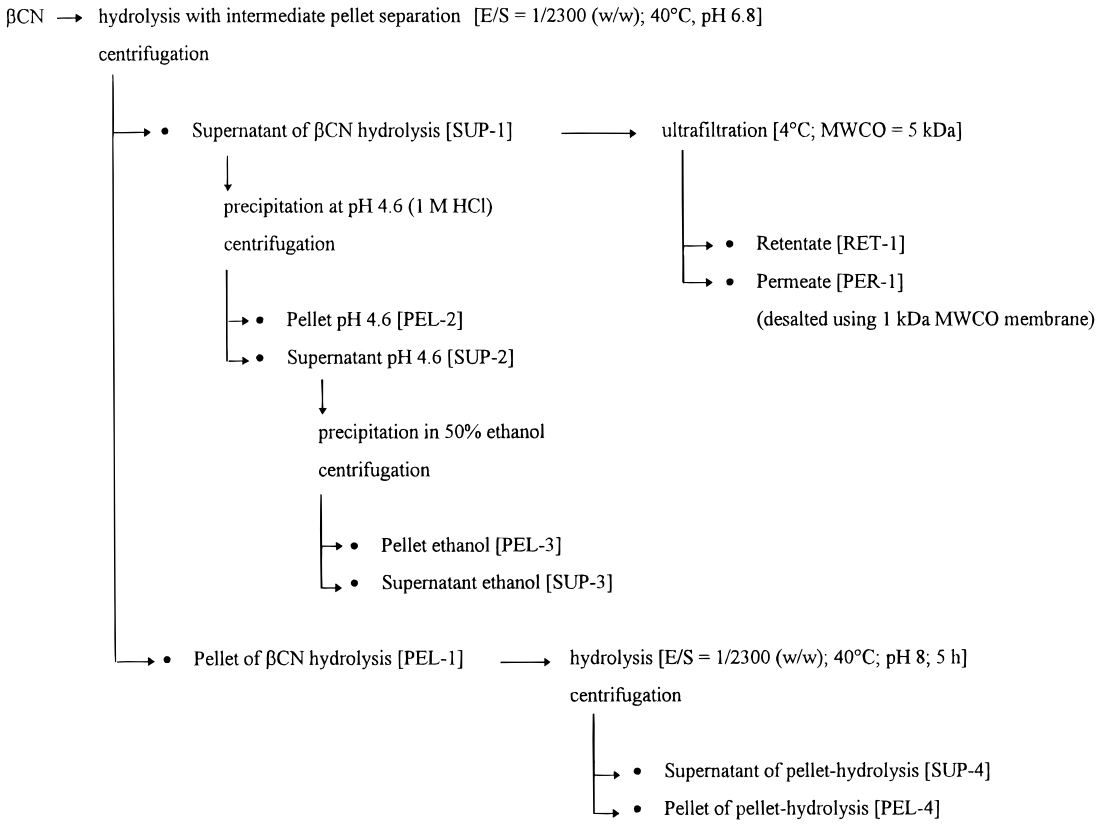

Figure 1. Outline of the βCN hydrolysate fractionation.

further precipitates, which were repeatedly centrifuged off. Centrifugation was after 2, 2.5, 3, 3.75, 4.5, 5.5, and 7 h of hydrolysis (called "hydrolysis with intermediate pellet separation"). The pellets obtained from the several centrifugation steps were combined, and the total resulting pellet (PEL-1) was dissolved in the original volume of water, adjusted to pH 10, after which part of it was hydrolyzed further with plasmin (enzyme/substrate = 1/2300 w/w; pH 8.0; 40 °C; pH-stat method; 5 h). The hydrolysis was terminated by adding aprotinin to the reaction mixture (ratio 1/200, v/v). The two resulting hydrolysates (the supernatant of the βCN hydrolysate, SUP-1, and the hydrolysate of PEL-1) were fractionated by means of selective precipitation (SUP-2, SUP-3, PEL-4, and SUP-4) and ultrafiltration (RET-1 and PER-1; Filtron mini-ultra-set, OMEGA membrane, MWCO 5 kDa, Pall Filtron Corp., Northborough, MA) as outlined in Figure 1. Before ultrafiltration (UF), the supernatant of the βCN hydrolysate (SUP-1) was diluted 3-fold with 2 M acetic acid. After 50% UF (50% reduction of the volume), the retentate was diafiltered (DF) with 1.3 M acetic acid (200% DF, which means 2 times the volume of the retentate is used as diawater), after which the retentate was washed with double-distilled water (200% DF) followed by 50% UF. All peptide fractions were lyophilized and stored at 4 °C before further analysis.

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). The peptide composition of the βCN hydrolysate fractions (Figure 1) was analyzed by RP-HPLC. The RP-HPLC equipment used is described by Visser et al. (1991). Solvent A [0.1% trifluoroacetic acid (TFA) in 10% aqueous acetonitrile, v/v] and solvent B (0.07% TFA in 90% aqueous acetonitrile, v/v) formed the eluent in the following linear gradient steps: from 10% to 16% B over 2 min followed by 2 min isocratic elution; to 26% B over 8 min; to 35% B over 4 min; to 38% B in 32 min; then to 70% B over 3 min and finishing with 5 min isocratic elution at 70% B. A flow rate of 0.8 mL/min was applied. Peak detection and quantitation was performed at 220 nm using Turbochrom data acquisition and processing software (Perkin-Elmer, Ueberlingen, Germany).

Capillary Electrophoresis (CE). RET-1 and SUP-3 (Figure 1) were analyzed by CE, basically following the method of de Jong et al. (1993). The CE equipment used was a Beckman P/ACE System 5500 controlled by Beckman Software Gold System version 8, using a hydrophilically coated Supelco capillary (Bellefonte, PA), 27 cm × 50 μm, and a 5 mM sodium citrate−6 M urea−0.05% (w/v) methylhydroxyethylcellulose (Tylose, Hoechst, Frankfurt am Main, Germany) buffer adjusted to pH 2.5 with citric acid. CE separation was performed at 10 kV and 30 °C for 60 min. Peak detection was performed using UV absorption at 214 nm.

Mass Spectrometry (MS). Some peptide components from PER-1, SUP-1, SUP-3, and PEL-1 (Figure 1) were collected (preparative RP-HPLC) and identified by electrospray ionization MS (ESI-MS). This was performed on a VG Platform quadrupole mass spectrometer (Micromass, Cheshire, U.K.). Samples were dissolved in a mixture of acetonitrile−water−formic acid (500:500:1, v/v/v) and injected into a flow of acetonitrile−water (1:1, v/v, 5 μL/min). The potential at the capillary tip was maintained at 3.2 kV; the cone voltage was 30 V. Nitrogen was used as nebulizing and drying gas. Calibration was performed using horse heart myoglobin. The raw mass spectral data were processed and transformed with the Masslynx software version 2.2 (Micromass, Cheshire, U.K.). Peptide identification was obtained from the molecular masses determined, combined with sequence data of the protein and the known specificity of plasmin. The maximum difference between the mass values measured and the theoretical values was approximately 1.5 Da.

Functional Properties (Foam, Emulsion, Surface Activity). Foam, emulsion, and surface-active properties of the peptide fractions were tested in small-scale screening tests at pH 6.7, which is the pH of milk, and $\mu = 0.075$ (0.02 M HCl−imidazole buffer containing 3.44 g/L NaCl and 0.2 g/L NaN_3) and at pH 4.0, which is representative for acidic foods, and $\mu = 0.075$ (0.03 M citrate buffer containing 3.15 g/L NaCl and 0.2 g/L NaN_3) at 20 °C.

Foam-forming and -stabilizing ability was tested with a whipping method: a volume of 100 mL of a 0.01% (w/v) protein solution was placed in a graduated glass cylinder and whipped for 70 s at 2500 rpm using a small impeller (Figure 2). Foam height was monitored for 1 h (the first measurement at 2 min after starting stirring), and the foam quality (size of the

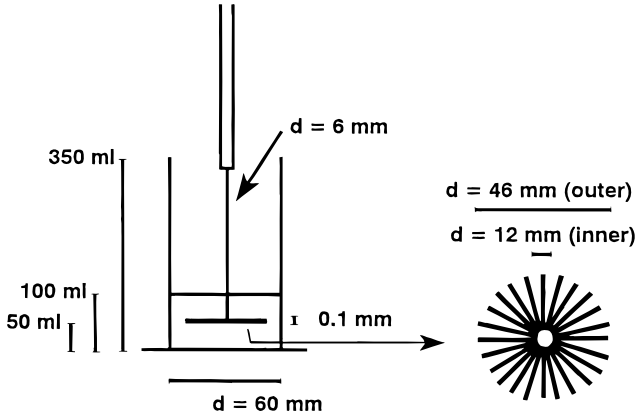

Figure 2. Schematic representation of the experimental setup for the foam screening test with a detail of the small impeller used. d = diameter.

bubbles, coalescence, etc.) was judged visually. The measurements were performed in duplicate.

Emulsions were made by mixing 2 mL of tricaprilin oil (Sigma) and 18 mL of 0.44% (w/v) protein solution for 1 min at 10 000 rpm with a Polytron PT-MR-3000 homogenizer (Kinematica AG, Littau, Switzerland). This pre-emulsion was homogenized in a small-scale high-pressure homogenizer similar to the valve homogenizer described by Tornberg and Lundh (1978), by applying two passages at 60 bar. At several times (t = 0, 1, and 24 h) after homogenizing, emulsion samples were diluted (1:100 v/v) into an SDS solution (0.1% w/v) to delay the instability processes (Pearce and Kinsella, 1978). The emulsion-forming ability was investigated by measuring the particle-size distribution (d_{32}) using the Malvern MasterSizer X (Malvern Instruments Limited, Malvern, U.K.), with optical parameters defined by the manufacterer's standard presentation code, immediately after homogenization (t = 0 h). The instability of the emulsions was estimated by measuring the decrease of the turbidity at 500 nm (Pearce and Kinsella, 1978) for all samples collected. Furthermore, the emulsion samples taken were examined for the presence of flocs and/or aggregates using a Polyvar light microscope (Reichert-Jung, Vienna, Austria) at a magnification of 400.

The influence on the surface pressure at the A/W interface by the various protein solutions (20 mg/L) was examined by measuring the rate of lowering the surface tension using a Langmuir trough setup as described by Van Aken and Merks (1996).

RESULTS

***β*CN Hydrolysis and Fractionation.** Plasmin hydrolysis of βCN and fractionation of the hydrolysate resulted in eight peptide fractions as outlined in Figure 1. Figure 3 shows RP-HPLC patterns of these fractions. By using the RP-HPLC gradient described, we were best able to separate the genetic variants of βCN and some of the hydrophobic βCN peptides formed after plasmin hydrolysis (Figure 3), although the gradient caused rather broad peaks at retention times between approximately 40 and 60 min. Although not shown in Figure 3, PEL-2 and PEL-3 were analyzed by RP-HPLC as well: PEL-2 consisted mainly of group V peptides (see below), thereby resembling PEL-1; the ethanol precipitation was not very selective and, consequently, resulted in a rather nonspecific peptide composition of PEL-3 (results not shown). The hydrolysis and fractionation have been repeated several times, and the peptide composition (as analyzed by RP-HPLC) in the several fractions obtained was very reproducible.

The peptides were categorized into five groups (I−V), indicated in Figure 3, according to the guidelines of Visser et al. (1989). Peptides in group II were identified

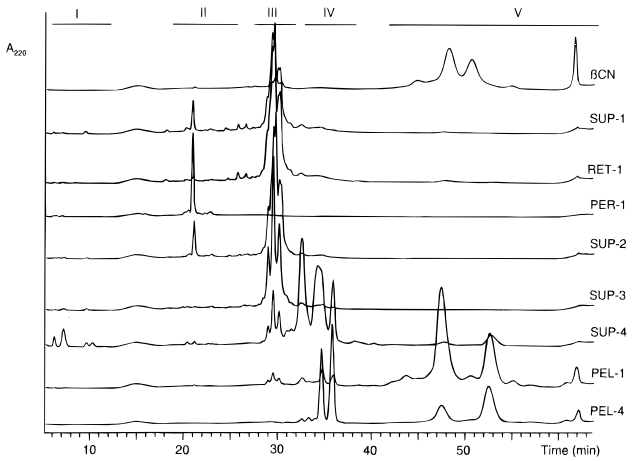

Figure 3. RP-HPLC chromatograms of the βCN hydrolysate fractions: for abbreviations, see Figure 1; for conditions used, see Materials and Methods. Peptide composition of the fractions is categorized in groups I−V (Visser et al., 1989).

Table 1. Yield of the *β*CN Hydrolysate Fractions (Figure 1) and Peptide Composition of These Fractions Categorized in Groups I−V (Visser et al., 1989) in the RP-HPLC Chromatogram

fraction[a]	yield[b] (% w/w)	group I	II	III	IV	V
SUP-1	53	−[c]	+[c]	+	(±)[c]	(±)
ultrafiltration:						
RET-1	46	−	−	+	−	(±)
PER-1	1.5	−	+	−	−	−
precipitation:						
PEL-2	6.8	−	−	±	(±)	+
SUP-2	49	−	+	+	−	−
PEL-3	23	−	+	+	(±)	−
SUP-3	20	−	−	+	−	−
PEL-1	38	−	−	±	±	+
hydrolysis:						
SUP-4	32	+	−	±	+	±
PEL-4	6.5	−	−	−	+	+

[a] For abbreviations, see Figure 1. [b] Based on the βCN starting material. [c] +, present; −, absent; ±, traces.

as part of the hydrophilic N terminus of βCN (sequence 1/2−25/28); peak material present in group III appeared to originate from the middle part of the βCN sequence (f 33/49−97/99); peak material present in group V was identified as peptide material from the C-terminal half of βCN (f 106/114−209); and peaks present in groups I and IV were hydrolysis products of peptides from group V (Visser et al.,1989). Table 1 shows the occurrence of these groups in the various βCN hydrolysate fractions mentioned in Figure 1, as well as the yield of the several βCN hydrolysate fractions.

The hydrolysate fractions SUP-1, SUP-2, and RET-1, on the one hand, and SUP-3, on the other hand, seemed to vary in the composition of peptides belonging to group III (see Figure 3). Therefore, RET-1 and SUP-3 were also analyzed with CE as shown in Figure 4. It appeared that RET-1 contained seven main peaks, whereas SUP-3 contained four main peaks.

The MS results indicated that SUP-1 and SUP-3 contained a mixture of peptides (group III), of which four peptides could be identified: βCN(f 29−105) of genetic variants A^1 and A^2 (mass = 8757.2 and 8717.3, respectively) and βCN(f 29−107) of the genetic variants A^1 and A^2 (mass = 9023.0 and 8981.7, respectively). The MS results of PEL-1 indicated that the first, earlier eluting, main peak in group V (RP-HPLC) was βCN(f 106−209) (mass = 11823.2), and the second main peak of group

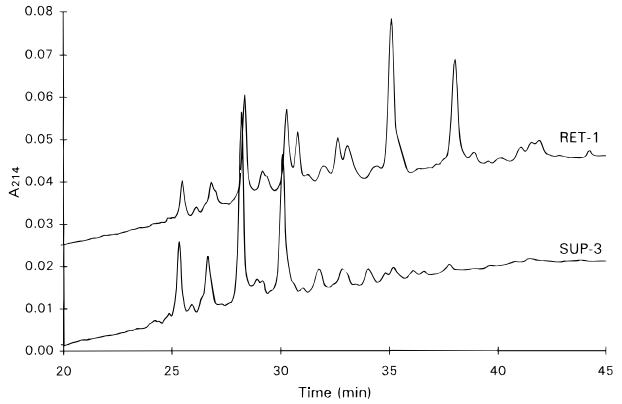

Figure 4. CE electropherogram of the βCN hydrolysate fractions RET-1 and SUP-3: for abbreviations, see Figure 1; for conditions used see Materials and Methods.

V was a mixture of βCN(f 108−209) and βCN(f 114−209) (mass = 11559.0 and 10829.0, respectively). The MS results of PER-1 showed that the only peak present (in group II; RP-HPLC) was βCN(f 1−28) (mass = 3478.0, including four phosphoserines). These results are in agreement with the literature (Visser et al., 1989).

Functional Properties. Most of the fractions dissolved well in the buffers used. Only the hydrophobic fractions (PEL-1, SUP-4, and PEL-4) were poorly soluble at both pH values (as was βCN at pH 4.0). The reproducibility of the screening tests was investigated with the intact protein. The maximal difference in foam height was obtained for the initial amount of foam produced with the whipping method (maximal standard deviation ~ 5 au). The standard deviation of the particle-size distribution (d_{32}) in the emulsion test was approximately 0.1 μm. The surface pressure curves, obtained with the Langmuir trough setup, were almost identical for the duplicate measurements. The screening tests used were reproducible enough to detect differences in functionality between the peptide fractions and the intact protein.

Figure 5 shows the results of the foam screening test. Differences were found in foam-forming ability: PEL-1 formed the highest amount of foam at pH 6.7, whereas at pH 4.0 SUP-3 and SUP-4 formed the highest amount of foam. At pH 4.0 βCN did not form a foam at all. The various peptide fractions produced foams with a clear variability in foam stability: PEL-1 (and βCN) formed the most stable foam at pH 6.7, while SUP-4 and PEL-4 were found to result in the highest foam stability at pH 4.0. The foams formed with PER-1 at pH 6.7, and with PER-1 and SUP-3 at pH 4.0, showed very rapid coalescence (PER-1 at pH 6.7 within 2 min). Furthermore, some visual characteristics of the several foams showed clear differences. The hydrophobic fractions (PEL-1, SUP-4, and PEL-4) formed "flocculated" foams at pH 6.7. At this pH, PEL-1 and βCN showed no coalescence, while at pH 4.0 PEL-1, SUP-4, and PEL-4 showed no coalescence. After approximately 20 min the foams of these hydrophobic fractions had a rather dry appearance.

Table 2 shows the results of the emulsion screening test at pH 6.7. The emulsifying properties of the eight fractions tested were diverse: the relatively hydrophilic fractions (SUP-1, RET-1, PER-1, SUP-2, and SUP-3) formed smaller emulsion particles than intact βCN, whereas the more hydrophobic fractions (PEL-1, SUP-4, and PEL-4) formed flocculated emulsions. Because of this flocculation, the measurement of the particle-size distribution was rather inaccurate and was, therefore, not taken into account. By microscopic observation, however, it was shown that these hydrophobic, poorly soluble peptides were able to form rather small emulsion droplets (diameter approximately 2−3 μm), most of which were flocculated (Figure 6). The stability of the emulsions formed with SUP-1, SUP-2, and RET-1, which all mainly contained peptides from group III (Figure 3), was slightly lower than that of the βCN emulsions. Fraction SUP-3, containing only peptides

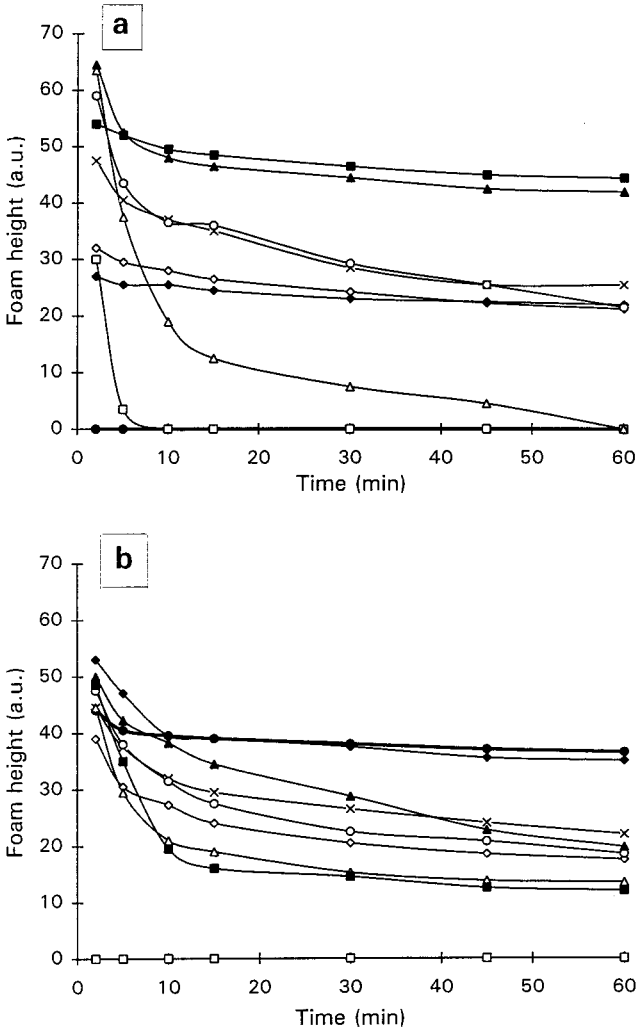

Figure 5. Foam height as produced with the various βCN hydrolysate fractions, as a function of time after whipping (means of duplicate measurements): for conditions used, see Materials and Methods; pH 4.0 (a) and 6.7 (b). (●) βCN; (◇) SUP-1; (◆) PEL-1; (×) RET-1; (□) PER-1; (○) SUP-2; (△) SUP-3; (▲) SUP-4; (■) PEL-4; for abbreviations, see Figure 1.

Table 2. Screening-Test Results of Emulsions Made with βCN Hydrolysate Fractions[a]

fraction[b]	d_{32}[c] (μm)	stability
βCN	2.25	+++[d]
SUP-1	1.53	+
PEL-1	nd[e]	flocculation
RET-1	1.44	++
PER-1	1.49	coalescence
SUP-2	1.86	+
SUP-3	1.72	coalescence
SUP-4	nd	flocculation
PEL-4	nd	flocculation

[a] For conditions used, see Materials and Methods. [b] For abbreviations, see Figure 1. [c] d_{32} is the particle-size distribution of the emulsion droplets. [d] +, ++, and +++ indicate the extent of stability. [e] nd means not determined.

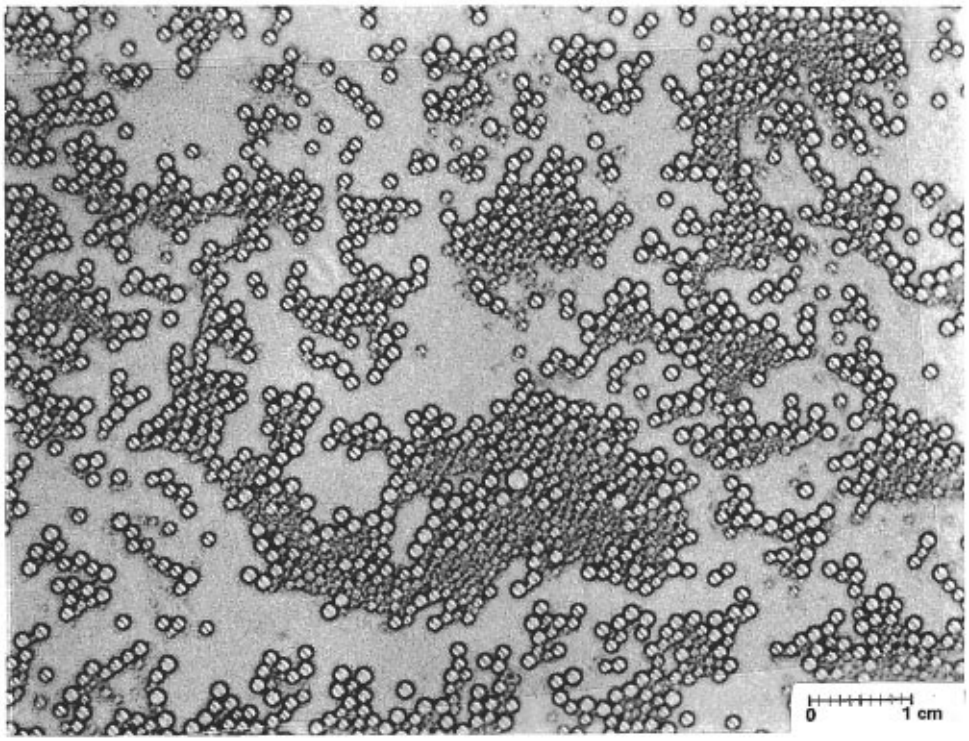

Figure 6. Microscopic observation of flocculated emulsion droplets of emulsions made with PEL-1; magnification 400×. (The figure is reproduced here at 67% of the original.)

from group III, and PER-1, containing only peptides from group II, formed emulsions that completely separated by coalescence within 1 h. At pH 4.0 all fractions, except SUP-4 and PER-1, formed flocculated emulsions, and the particle-size distributions could therefore not be measured with the Malvern MasterSizer X. The emulsion made with PER-1 formed emulsion droplets of about 3 μm, and this emulsion creamed within 1 h. Fraction SUP-4 formed emulsion droplets of approximately 1.8 μm. The emulsion of SUP-4 flocculated when dispersed in the SDS solution; consequently, the turbidity results could not be meaningfully interpreted. However, this emulsion was rather stable (no visible oil separation, and no increased particle size after 2 days, measured with the Malvern MasterSizer X).

Figure 7 shows the increase of surface pressure of the buffered peptide solutions. At pH 6.7 all peptide fractions were soluble at the low concentrations used. The rate by which the surface pressure increased varied among fractions: SUP-4 increased the surface pressure very rapidly (even faster than βCN), while PEL-1 and PEL-4 were slower than βCN. The surface pressures of the hydrophobic fractions (PEL-1, SUP-4, and PEL-4) and intact βCN were almost identical after approximately 1 h; the surface pressure of PER-1 was low, and the surface pressures of SUP-1, SUP-2, SUP-3, and RET-1 reached approximately half the value of βCN. At pH 4.0 both βCN and PEL-1 were not completely soluble (even at the low concentrations used), and the adsorption curves of these fractions only give an indication of their surface-active behavior under these conditions. It appeared that the initial surface pressure of the βCN solution increased very rapidly (possibly due to the presence of traces of surface-active material other than βCN), after which the increase of the surface pressure delayed. At this acidic pH the hydrophobic fractions (PEL-1, SUP-4, and PEL-4) reached the highest surface pressure. βCN and PER-1 reached almost the same final surface pressure as SUP-1, SUP-2, SUP-3, and RET-1, and this value was similar to the final surface pressure of these fractions at pH 6.7.

DISCUSSION

Under the conditions used for hydrolysis and fractionation it is possible to produce several fractions from βCN that distinctly differ in peptide composition. With these mild hydrolysis conditions βCN could be degraded into mainly three parts: more hydrophobic fractions (PEL-1, SUP-4, and PEL-4), amphipathic fractions (SUP-1, SUP-2, SUP-3, and RET-1), and a strongly hydrophilic fraction (PER-1).

βCN is an amphipathic protein with a fairly distinct segregation of charged and hydrophobic residues (Swaisgood, 1982). The C-terminal half of the sequence, corresponding to some γCNs, is very hydrophobic, which will be the reason for its precipitation at higher temperatures. This feature was utilized for the hydrolysis with intermediate pellet separation. The hydrophobicity of these γCNs may also result in a high surface pressure induced by these peptides, which has already been reported in the literature (Wilson et al., 1989). Our results also demonstrate an important influence on the surface pressure of the hydrophobic fractions (PEL-1, SUP-4, and PEL-4; Figure 7), at both pH 6.7 and 4.0. At pH 6.7 these fractions formed flocculated foams and emulsions, which may be caused by strong interactions between the hydrophobic peptides present. However, when made at 4 °C (at which temperature the solubility of SUP-4 was increased, and PEL-1 and PEL-4 were still poorly soluble), the emulsions of the hydrophobic fractions were also flocculated. Apparently, the hydrophobic interactions are not the only driving force for flocculation. Another possible explanation for flocculation is that solid particles of the hydrophobic fractions, which were observed at pH 6.7, cause bridging flocculation of the droplets or bubbles (Walstra, 1987). A further possibility is that the peptides, present in these frac-

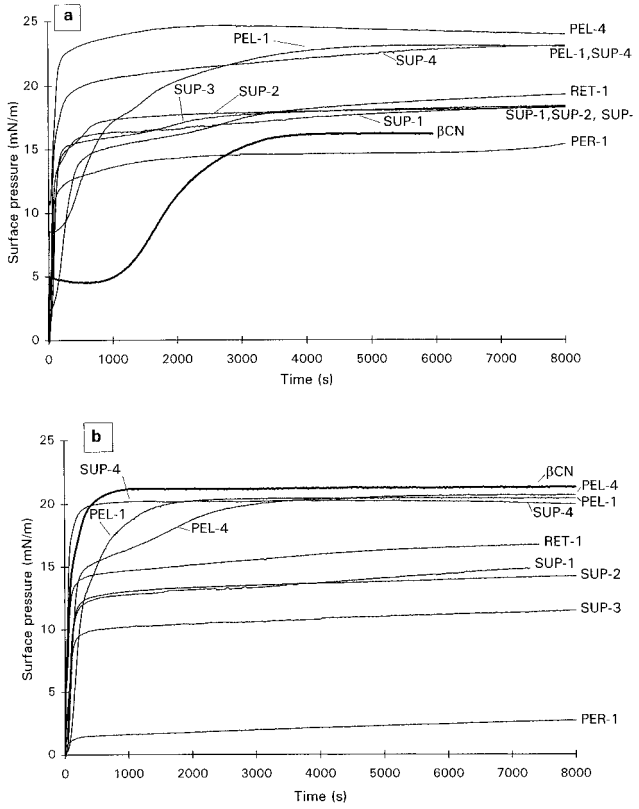

Figure 7. Surface pressure induced by βCN hydrolysate fractions as a function of time at an air/water interface measured using a Langmuir trough: for abbreviations, see Figure 1; for conditions used, see Materials and Methods; pH 4.0 (a) and 6.7 (b).

tions, cannot generate enough repulsion between the emulsion droplets and/or foam bubbles. This would result in thin films between the droplets/bubbles, which may cause flocculated systems.

At pH 4.0 all peptide fractions had enhanced foaming properties as compared to intact βCN. Especially the hydrophobic fractions (PEL-1, SUP-4, and PEL-4) produced stable foams at pH 4.0 (Figure 5), which did not flocculate; this suggests an interesting possibility to apply these kinds of peptides in fluffy, acid foods.

The hydrophilic fraction (PER-1) showed almost no influence on the surface pressure at pH 6.7. At pH 4.0 the surface pressure induced by PER-1 increased dramatically compared to the surface pressure at pH 6.7; similar effects for βCN(f 1−25) have been reported in the literature (Lee et al., 1987a). At this acidic pH, the peptide will be less charged, because it is closer to its isoelectric point [p*I*; calculated p*I* value βCN(f 1−25) = 1.68; Nau et al., 1995]. The inferior foam and emulsion-stabilizing properties of PER-1, especially at pH 6.7, could be related to the low surface pressure induced at this pH. Furthermore, it is generally accepted that peptides must have a certain minimal molecular weight (MW) to retain some functionality (Turgeon et al., 1992), and the small size of the peptide could, therefore, be an additional reason for its poor functional properties at both pH values tested.

The amphipathic fractions (SUP-1, SUP-2, SUP-3, and RET-1), all containing peptides from the central part of the βCN sequence (group III), were expected to possess interesting foam, emulsion, and surface-active properties; this expectation arises from their amphipathic character and because of their rather high MW (about 9000). It appeared that these fractions increased the surface pressure, but not to the extent as observed with βCN at pH 6.7. The fractions were able to form and stabilize a foam, although coalescence occurred: especially the foam made with SUP-3 at pH 4.0 showed rapid coalescence. At pH 6.7 the amphipathic fractions possessed improved emulsion-forming ability, as they formed smaller emulsion droplets than βCN did. The emulsion stability of these fractions varied significantly. As SUP-3 (unstable emulsion) is a more purified fraction than SUP-1, SUP-2, and RET-1 (stable emulsion), these results may suggest a synergistic effect between peptides present in the latter fractions. This has also been reported in the literature (Shimizu et al., 1986; Lee et al., 1987b). However, this functionality can also be produced by one or more particular peptides of group III (RP-HPLC) which were present in RET-1 (and probably also in SUP-1 and SUP-2) but absent in SUP-3 (Figure 4).

At pH 4.0 βCN has poor emulsifying properties. All peptide fractions, except for SUP-4 and PER-1, formed flocculated emulsions at acidic pH. PER-1 led to rather large emulsion droplets, which creamed rapidly; only SUP-4 formed a stable emulsion at this pH. From these results it can therefore be concluded that no general rule exists for good emulsion properties of peptides at acidic pH with respect to hydrophobicity only.

In the present study the influence of plasmin hydrolysis of βCN on the foam, emulsion, and surface-active properties was tested. The methods used to investigate these functional properties are quite suitable to screen for differences in functionality between the various peptide fractions. However, at this moment no information is available yet on the mechanisms responsible for these functional properties.

In conclusion, our results show that by plasmin hydrolysis of βCN and fractionation of the hydrolysate well-defined peptide mixtures can be produced which clearly vary in emulsion, foam, and surface-active properties, as judged by the results of the screening tests. Especially, large variability was found for foam stability at pH 4.0 and for emulsion stability at pH 6.7. Further research will be directed toward purifying the peptides to elucidate the possibility of specific stabilizing properties of peptides. Furthermore, detailed experiments toward foam and emulsion formation and stabilisation of interesting peptide fractions will be performed.

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