Interfacial Properties of Tryptic Peptides of β-Lactoglobulin

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β-Lactoglobulin (β-lg) was hydrolyzed with trypsin in an ultrafiltration reactor (PM30). The total hydrolysate (TH) was further fractionated (PM1), resulting in a mixture of polypeptides (MP) and a fraction of amino acids and small peptides (AA). Peptides in each fraction were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) and identified by their amino acid composition. The MP fraction was further purified in six fractions by anion-exchange chromatography. Interfacial properties of β-lg, its TH, MP, and AA fractions, and the purified fractions were measured at pH 4 and 7 at two ionic strengths (μ = 0 and 0.6). Peptides responsible for improved interfacial properties of the MP fraction were identified (β-lg 21-40, β-lg 41-60), and structure-function relationships (molecular weight and hydrophobicity) are discussed. Characteristics essential to good interfacial properties are clustering of hydrophilic and hydrophobic residues in distinct zones and a minimum molecular weight allowing this distribution.

INTRODUCTION

The interfacial properties of proteins are essential to obtain stable food emulsions and foams. Structure-function relationships of proteins have been investigated by many authors (MacRitchie, 1978; Graham and Phillips, 1979a-c; Halling, 1981; Kinsella, 1984; Dickinson and Stainsby, 1982; Kinsella et al., 1989; Leman and Kinsella, 1989; Tornberg et al., 1990). Interfacial properties of proteins are influenced by conformation factors such as molecular flexibility, steric hindrance, amphipathic structure, hydrophobicity, molecular size, secondary structure, and net charge. They can also be influenced by physicochemical properties of the protein solution involved in the diffusion of the molecule to the interface, such as pH and ionic strength.

Research on the functional properties of peptides began a few years ago in a search for new applications but especially to have a better understanding of structure-function relationships of proteins (Shimizu et al., 1984). Jost and Monti (1982) have found a positive correlation between surface activity and peptide chain length, and it has since been generally accepted that a peptide should have a minimum length (>20 residues) to possess good emulsifying and interfacial properties (Lee et al., 1987a; Chaplin and Andrews, 1989; Turgeon et al., 1991). Chaplin and Andrews (1989) reported a correlation between hydrophobicity and peptide functionality. Also, enzyme specificity is important to peptide functionality as it strongly influences the nature of peptides produced (molecular size and hydrophobicity). For example, tryptic peptides from whey proteins have better emulsifying and interfacial properties than chymotryptic peptides (Jost and Monti, 1982; Turgeon et al., 1991). Peptides generally have better emulsifying properties at acidic pH (Shimizu et al., 1984, 1986; Lee et al., 1987a,b; Turgeon et al., 1991), indicating that environmental conditions influence interfacial and emulsifying properties. Finally, some synergistic effects of peptides on emulsifying properties have been noted (Shimizu et al., 1986; Lee et al., 1987b), indicating that peptide behavior is not as simple as was first thought.

The objectives of the present investigation were to study interfacial properties of peptide fractions obtained by tryptic hydrolysis of β-lactoglobulin (the major protein of whey), to determine the physicochemical characteristics of those peptides, and to understand their structure-function relationships.

MATERIALS AND METHODS

Material. β-Lactoglobulin (β-lg, 82% w/w protein) was obtained from Triballat (Noyal-sur-Vilaine France), trypsin (bovine pancreas, type III-S, 12200 BAEE units of specific activity per milligram of protein) from Sigma Chemical Co. (St. Louis, MO), and acetonitrile from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA), phenyl isothiocyanate (PITC), and amino acid standards were from Pierce Chemical Co. (Roissy Ch.-de-Gaulle, France). All other products were of reagent grade. Buffers and mobile phases were prepared with HPLC grade water (Millipore, Velizy, France) and deaerated by helium bubbling.

Preparation of Peptide Fractions. Peptide fractions were obtained by ultrafiltration of tryptic hydrolysates of β-lactoglobulin (β-lg) as previously described by Turgeon and Gauthier (1990). After 45 min of hydrolysis (protein content 3.5% w/w, pH 8.0, 40 °C, enzyme substrate ratio 1:200), the mixture was pumped into the hollow fiber membrane module, and the proteolytic products were removed continuously by ultrafiltration using a membrane with a molecular weight cutoff of 30 000 (HF1-43-PM30, Romicon Inc., Woburn, MA). The permeate and retentate of the first ultrafiltration were named total hydrolysate (TH) and reaction mixture (RM), respectively. After completion of the first ultrafiltration, the TH was further fractionated by a second ultrafiltration with a smaller cutoff membrane of 1000 (HF1-43-PM1, Romicon) to remove amino acids and small peptides. The retentate of the second ultrafiltration was named mixture of polypeptides (MP) and represented about 40% of the nitrogen content of β-lg. The permeate fraction was composed of amino acids and small peptides (AA).

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). The HPLC equipment consisted of an autosampling injector Model 232 (Gilson, Villiers le Bel, France), a ternary HPLC pump Model 8800 (Spectra Physics, Darmstadt), a temperature control system (Prolabo, Paris), and a two-way variable UV detector Model 8490 (Spectra Physics). The chromatographic apparatus was fitted with Nelson 2600 chromatography analytical software by means of a 900 Series intelligent interface (Nelson, CA).

RP-HPLC of β-lg tryptic fractions was performed on a C_{18} Vydac column (218TP54, Touzart & Matignon, Vitry-sur-Seine,

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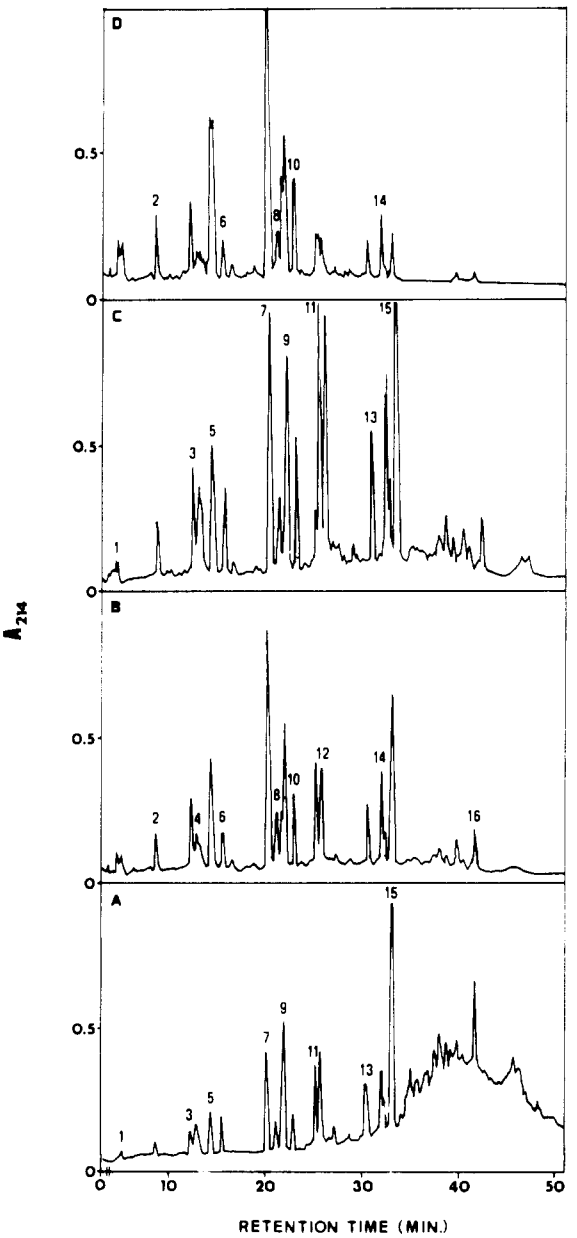

Figure 1. RP-HPLC chromatograms of peptide fractions obtained by tryptic hydrolysis of β-lactoglobulin: (A) reaction mixture (RM); (B) total hydrolysate (TH); (C) mixture of polypeptides (MP); (D) amino acids and small peptides fraction (AA). Conditions: C_{18} Vydac column; column temperature, 40 °C; solvent A, 0.115% (v/v) TFA in water; solvent B, 60% acetonitrile/40% water in 0.1% (v/v) TFA; gradient, 10–80% solvent B in 50 min; flow rate 1 mL/min.

France) under the following conditions: column temperature, 40 °C; flow rate, 1 mL/min; solvent A, 0.115% (v/v) TFA in water; solvent B, 60% acetonitrile/40% water with 0.1% (v/v) TFA. Elution was performed by applying a linear gradient (10–80%) of solvent B over 50 min. Absorbance was recorded at 214 and 280 nm. For peptide identification, fractions were collected and dried in a Speed-Vac concentrator (Bioblock, Paris). Each fraction was further purified on a Nucleosil C_{18} column (4.6 mm i.d. × 25 cm, SFCC, Gagny, France), using the same solvents but with varying gradients. The same conditions were used: 40 °C, 1 mL/min, and absorbance recorded at 214 and 280 nm. The fractions were collected and dried in a Speed-Vac concentrator.

Peptide Identification. After acid hydrolysis under vacuum in the presence of 6 N HCl for 24 h at 110 °C in a Pico-Tag Station (Waters), amino acids were derivatized with PITC according to the method of Bidlingmeyer et al. (1984), and quantified by RP-HPLC on a Pico-Tag C_{18} column (3.9 mm i.d. × 15 cm, Waters). Dried samples were dissolved in 95% 2 mM Na_2HPO_4, pH 7.4/5% acetonitrile; the column was equilibrated in solvent A (94% 0.14 M CH_3COONa plus 0.5 mL of TFA/L, pH 6.4/6% acetonitrile) at 38 °C. Elution was performed according to the method of Bidlingmeyer et al. (1984). The flow rate was 1.0 mL/min, and the absorbance was recorded at 269 nm. The results of amino acid analyses were sufficient for assignment of the tryptic peptides in the known sequence of β-lg.

Fractionation of the Mixture of Polypeptides by Anion-Exchange Chromatography. The mixture of polypeptides (MP) was fractionated on an anion-exchange column (Q Sepharose Fast Flow, 1000 mL) using a BioPilot system (Pharmacia, Les Ulis, France). The column was equilibrated with sodium phosphate buffer (10 mM, pH 7.0) at a flow rate of 30 mL/min. Elution was performed with a linear gradient of NaCl (0–30%)

Table I. Physicochemical Characteristics of Peptides Obtained by Tryptic Hydrolysis of β-Lactoglobulin

peak[a]	sequence	mol wt	isoelectric point[b]	$H_{\phi av}$,[c] kcal/mol
5	1–14	1572	8.88	1.26
7	15–20	678	5.77	1.61
14	21–40	2013	4.52	1.05
16	15–40	2691	4.52	1.18
13	25–40	1610	4.52	1.03
15	41–60	2297	5.40	1.37
12	61–69 +[d] 149–162	4330	5.75	0.94
11	61–70 +[d] 149–162	4458	4.00	0.96
4	61–69	1046	6.24	0.79
4	61–70	1174	8.52	0.87
2	71–75	555	9.36	1.63
9	76–83	885	10.79	1.76
3	84–91	898	4.54	0.95
10	92–100	1047	4.52	1.41
8	92–101	1175	6.79	1.42
5	125–135	900	4.52	0.85
1	136–138	390	6.23	1.38
1	139–141	312	9.36	1.55
7	142–148	819	10.22	1.54
15	149–162	1642	4.55	1.03
missing peptides				
	102–124	1329	5.04	1.13
	76–77	229	9.36	0.98
	78–83	656	9.36	2.03

[a] Peptides separated by RP-HPLC and identified by amino acids analysis. [b] Calculated from dissociation constants of ionic groups according to the method of Tanford (1962). [c] Average hydrophobicity ($H_{\phi av}$) was calculated according to the method of Bigelow (1967). [d] + represents the disulfide bond.

Table II. Rates of Adsorption (mN m^{-1} s^{-1}) of β-Lactoglobulin (β-lg) and Its Peptide Fractions (TH, MP, AA) Obtained by Tryptic Hydrolysis of β-Lactoglobulin[a]

	pH 4		pH 7	
sample	$\mu = 0$[b]	$\mu = 0.6$[c]	$\mu = 0$	$\mu = 0.6$
β-lg	0.37[b]	0.85[a]	0.27[a]	0.82[a]
TH	0.38[b]	0.57[b]	0.11[ab]	0.16[bc]
MP	0.78[a]	0.92[a]	0.23[ab]	0.25[b]
AA	0.10[c]	0.09[c]	0.09[b]	0.06[c]

[a] Means in row with same lower case letter are not significantly differents ($p > 0.05$). [b] Prepared in water. [c] Prepared in McIlvaine's phosphate–citrate buffer.

Table III. Molecular Weight Distribution of Peptides Contained in Fractions TH, MP, and AA

	surface area,[a] %		
mol wt	TH	MP	AA
<1000	28	22	42
1000–2000	37	34	45
2000–4000	21	24	5
>4000	14	20	6

[a] Percentage of peaks containing peptides of a molecular range, calculated from the integration of total surface area of RP-HPLC chromatograms.

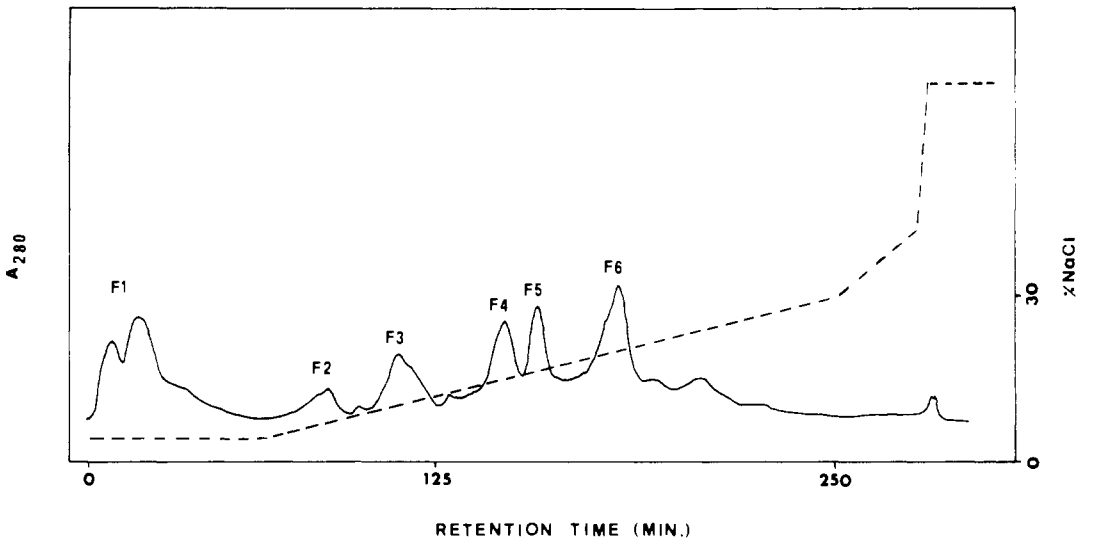

Figure 2. Anionic exchange chromatogram of MP fraction. Conditions: column (1 L), Q-Sepharose Fast Flow; 10 mM sodium phosphate buffer, pH 7.0; gradient, 0–30% NaCl in 180 min; flow rate 30 mL/min.

in the same buffer over a period of 180 min. Absorbance was recorded at 280 nm. Six fractions were collected, dialyzed, lyophilized, and characterized by RP-HPLC.

Interfacial Properties. The surface tension measurements of peptide fractions obtained by ultrafiltration (HT, MP, AA) and those obtained after fractionation by anion exchange of MP were made using the Wilhelmy plate method (Adamson, 1982; Turgeon et al., 1991). The surface tension was determined with a balance Model Q11 coupled to a measuring apparatus Model KWS 73.D7 (HBM, Darmstadt) and a glass lamella. The glass lamella (22 × 22 × 0.5 mm) and all glassware were carefully cleaned with a commercial cleaning solution (No-Chromix, Godax Inc.) and rinsed with deionized water (Millipore). Protein solutions (0.005% W/V) were prepared in water at pH 4 or 7 (adjusted with HCl or NaOH, 0.01 N) or in McIlvaine's (1921) phosphate–citrate buffer (pH 4 or 7, $\mu = 0.6$) containing 0.01% sodium azide. The ionic strength of 0.6 was specifically chosen to simulate a food system. Rates of adsorption of protein components (mN m^{-1} s^{-1}) were calculated from surface tension curves as a function of time and corresponded to the slope of the tangent at a surface pressure depression of 5 mN m^{-1}.

Statistical Analysis. Results presented in Tables II and V were treated by analysis of variance followed by Duncan's multiple range test.

RESULTS

Peptides present in the four peptide fractions obtained by tryptic hydrolysis of β-lg (RM, HT, MP, AA) were identified by RP-HPLC analysis (Figure 1). After separation on the Vydac column and repurification on Nucleosil, about 20 different peptides were found in the peptidic fractions. Table I represents sequences of amino acids corresponding to peaks in RP-HPLC profiles (Figure 1).

β-Lg possesses 17 potential tryptic cleavage sites (3 Arg and 14 Lys) in its primary sequence (Braunitzer et al., 1973). Lys47–Pro48 was not cleaved, due to steric hindrance (Hirs et al., 1956). Two other bonds were rarely or never cut (Lys8–Gly9 and Lys77–Ile78), and resulting peptides (1–8, 9–14, and 76–77, 78–83) were not identified on chromatograms, whereas peptides 1–14 and 76–83 were present. This poor degree of cleavage could be related to a relatively short hydrolysis time (45 min, enzyme to substrate ratio 0.5%). After 2 h of hydrolysis in similar conditions (enzyme to substrate ratio 0.5%), Dalgalarrondo et al. (1990) also reported the absence of peptides 76–77 and 78–83 and found only small quantities of peptides 1–8 and 9–14. Carles (1983) also reported cleavage of a Lys–Ile bond with a yield of 40% during tryptic hydrolysis of β-casein. Two nonspecific cleavages were observed, Tyr20–Ser21 bond hydrolysis as already obtained by Dalgalarrondo et al. (1990) and Met24–Ala25 hydrolysis hitherto unknown.

The peptide 102–124 was not assigned to any of the peaks identified. Dalgalarrondo et al. (1990) have also failed to isolate it by RP-HPLC. This peptide of 23 residues possesses a disulfide bridge and a free thiol group. Hydrolysis conditions (pH 8) may have increased the reactivity of the free thiol group and have led to intra- and intermolecular rearrangement, resulting in the formation of new disulfide bridges (Andersson, 1970). Peptide aggregates of higher molecular weight could have been confined in the retentate of the first ultrafiltration and may be among the unidentified peaks at the end of the chromatogram of the RM fraction (Figure 1A). Performic oxidation of this fraction shifted these peaks, indicating the presence of disulfide bridges (results not shown).

The rates of adsorption of β-lg and its peptide fractions TH, MP, and AA are reported in Table II. Rate of adsorption of β-lg in water ($\mu = 0$) was higher at pH 4 than at pH 7 but doubled in the presence of salts ($\mu = 0.6$). Waniska and Kinsella (1985) reported optimum adsorption rates of β-lg at pH 4.9, corresponding to the isoelectric point region (pI = 5,4; Eigel et al., 1984). At this pH, electrostatic repulsions are minimized and more protein molecules can pack at the interface, resulting in a more rapid decrease of interfacial tension (Waniska and Kinsella, 1988; MacRitchie, 1978; Halling, 1981). Salts are known to enhance hydrophobic interactions. Ions interact with charged groups of proteins, decreasing electrostatic attractions between opposite charges on neighboring groups (Cheftel et al., 1985). This could allow hydrophobic interactions to become more predominant, making adsorption to the interface easier.

Hydrolysis of β-lg resulted in TH fractions having adsorption rates inferior or equal to that of β-lg (Table II). According to Lee et al. (1987a), peptides should be more than 20 residues long to possess good emulsifying properties. Computation of peak areas of RP-HPLC for the TH fraction (Table III) showed that 65% of its peptides were less than or equal to 2000 daltons, which might explain the low value of adsorption rates. After a molecular weight fractionation of the TH fraction by ultrafiltration, two fractions having different interfacial properties were

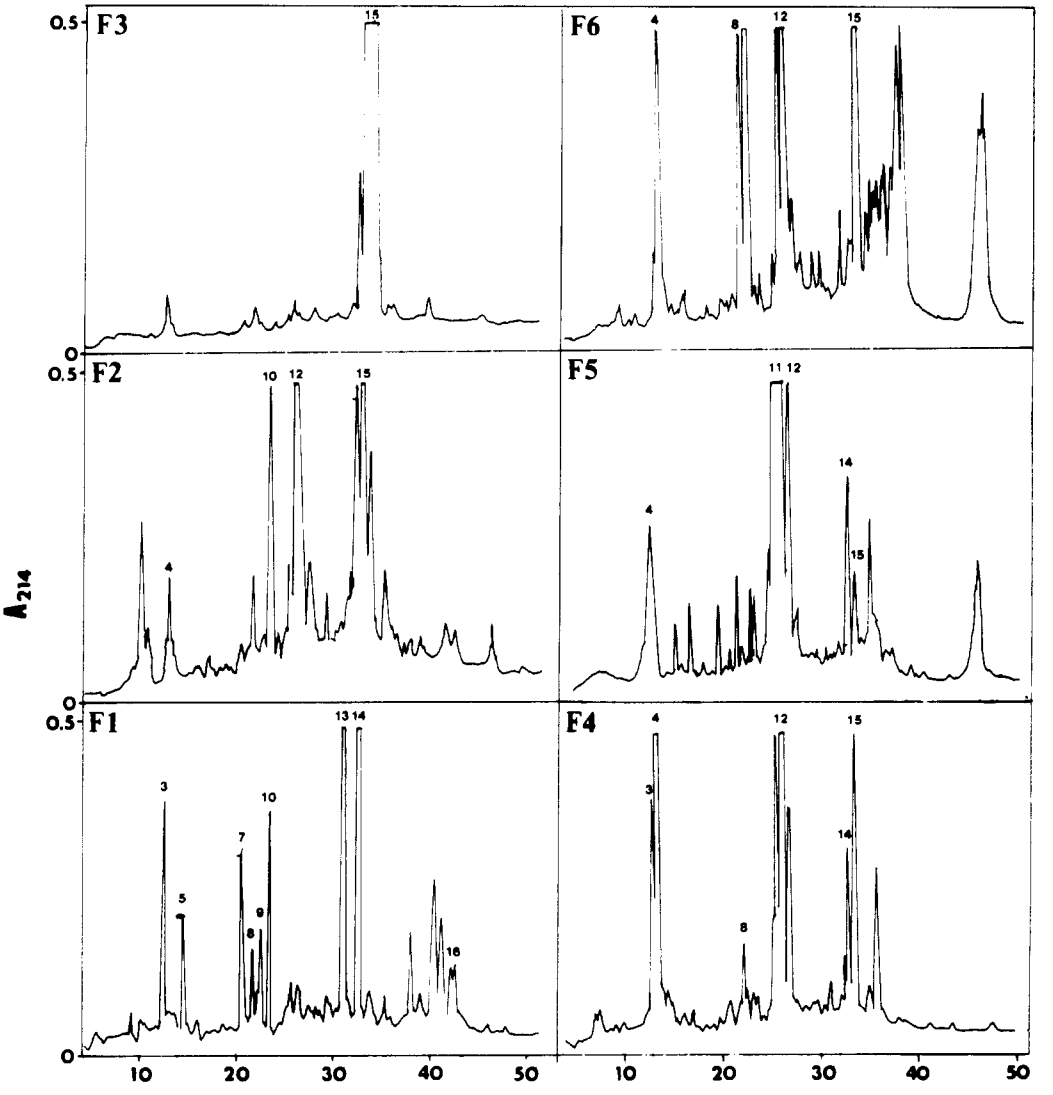

Figure 3. RP-HPLC chromatograms of peptide fractions (F1–6) obtained by anionic exchange chromatography of MP fraction. Conditions: C_{18} Vydac column; column temperature, 40 °C; solvent A, 0.115% (v/v) TFA in water; solvent B, 60% acetonitrile/40% water in 0.1% (v/v) TFA; gradient; 10–80% solvent B in 50 min; flow rate 1 mL/min.

obtained. The AA fraction was composed of about 87% of peptides with molecular weight <2000 (Table III) and showed very poor interfacial properties (Table II). The MP fractions, enriched in higher molecular weight peptides (Table III), had higher adsorption rates at pH 4 than TH fractions, as previously observed by Turgeon et al. (1991) with whey peptide fractions.

Interfacial properties of MP were better than those of β-lg at pH 4 but equal at pH 7 in water (Table II). Contrary to β-lg, ionic strength had little influence on peptide fractions (MP, HT, AA), probably because peptides lack the three-dimensional structures of globular proteins and their hydrophobic regions are therefore more accessible. On the other hand, pH has an influence on the adsorption rate of peptide fractions. Higher adsorption rates at pH 4 corresponded to the isoelectric point of most of the peptides and especially of the larger peptides (Table I). In this region, repulsion is minimum and peptides can adsorb rapidly and effectively.

A simple molecular weight fractionation gave improved interfacial properties, but the behavior of peptides in mixtures is complex and influenced by environmental conditions. To obtain a better understanding of peptide interactions and to try to establish a structure–function relationship, the MP fraction (having the best interfacial properties) was further fractionated by anion exchange. Six fractions (F) were obtained (Figure 2), representing about 39% (F1), 4% (F2), 21% (F3), 10% (F4), 8% (F5), and 18% (F6) of the MP fraction after dialysis. Peptides of these fractions were separated by RP-HPLC (Figure 3) for assignment and their interfacial properties measured (Table V).

F1 was composed of the peptides (Table IV) that were not retained by the column. Theoretically, at pH 7 peptides with isoelectric points (p*I*) lower than 6 are negatively charged and are retained on the MonoQ gel. But as already reported in literature (Regnier, 1984), some molecules can deviate from this rule as found in the case of peptides 84–91 (p*I* = 4.54; Table I), 125–135 (p*I* = 4.52), 92–100 (p*I* = 4.52), and 21–40, 25–40 (p*I* = 4.52). The two major peaks of F1 were composed of peptides 21–40 (28.7%) and 25–40 (21.4%).

F2 (Figure 3; Table IV) was composed of larger molecular weight peptides (>2000) such as peptide 61–69 +

Table IV. Peptidic Composition[a] of Fractions Obtained after Anion Exchange Separation of the Fraction MP

peak	sequence	fraction 1	2	3	4	5	6
3	84–91	7.2					
4	61–69				32.2	7.9	9.8
4	61–70						
5	1–14						
5	125–135	5.1					
7	15–20	6.8					
7	142–148						
9	76–83						18.0
	125–138						
10	92–100	6.8	15.0				
11	61–70[b] + 149–162				10.3	46.8	7.4
12	61–69[b] + 149–162		39.0		45.0	31.8	17.0
13	25–40	21.4					
14	21–40	28.7					
15	41–60		43.0	>95	9.5	1.7	13.5
15	149–162						

[a] Percentage of each peak, calculated from the integrated surface of RP-HPLC chromatograms. Only peaks representing more than 5% of the total surface were included. [b] Peptides linked by a disulfide bond.

Table V. Rates of Adsorption (mN m^{-1} s^{-1}) of Peptide Fractions Obtained by Anion Exchange[a]

fraction	pH 4 $\mu = 0$[b]	pH 4 $\mu = 0.6$[c]	pH 7 $\mu = 0$	pH 7 $\mu = 0.6$
F1	1.24[b]	1.38[b]	1.31[a]	0.76[a]
F2	1.30[b]	1.03[c]	0.20[b]	0.13[bc]
F3	4.50[a]	2.86[a]	1.27[a]	0.15[b]
F4	0.14[c]	0.13[e]	0.02[b]	0.02[c]
F5	0.22[c]	0.11[e]	0.02[b]	0.03[c]
F6	0.38[c]	0.37[d]	0.30[b]	0.17[b]

[a] Means in row with same lower case letter are not significantly different ($p > 0.05$). [b] Prepared in water. [c] Prepared in McIlvaine's phosphate–citrate buffer.

149–162 (39%) and peptide 41–60 (43%). F3 was made up of more than 95% peptide 41–60 (Table IV). Fractions 4–6 were mixtures of peptides in different proportions (Table IV).

The rates of adsorption of the six fractions obtained by anion-exchange chromatography are shown in Table V. At pH 4, F3 possessed the best interfacial properties with adsorption rates of 4.5 mN m^{-1} s^{-1} in water and 2.86 mN m^{-1} s^{-1} at $\mu = 0.6$, corresponding to an increase over β-lg adsorption rates (Table II) by factors of 12 and 3, respectively. The peptide of this fraction (41–60) had a molecular weight of 2300 and an average hydrophobicity ($H_{\phi av}$) of 1.37 kcal/mol (Table I).

F1 and F2 also possessed better interfacial properties at pH 4 than β-lg. F1 is made up of 50% peptides 21–40 and 25–40 (Table IV) with molecular weights higher than 1500. Good interfacial properties of fraction 2 probably resulted from its 43% content of peptide 41–60, since a 45% content of peptide 61–69 + 149–162 gave poor interfacial properties to fraction 4. Fractions 4–6 had poor interfacial properties despite the presence of large molecular weight peptides, for example, peptides 61–69 + 149–162 and 61–70 + 149–162 constituted more than 75% of fraction 5.

At pH 7 in water, F1 and F3 had the best interfacial properties, being 4 times superior to β-lg under the same conditions. Other fractions had low interfacial properties. Under these conditions, rates of adsorption of F1 and F3 were equivalent (Table V).

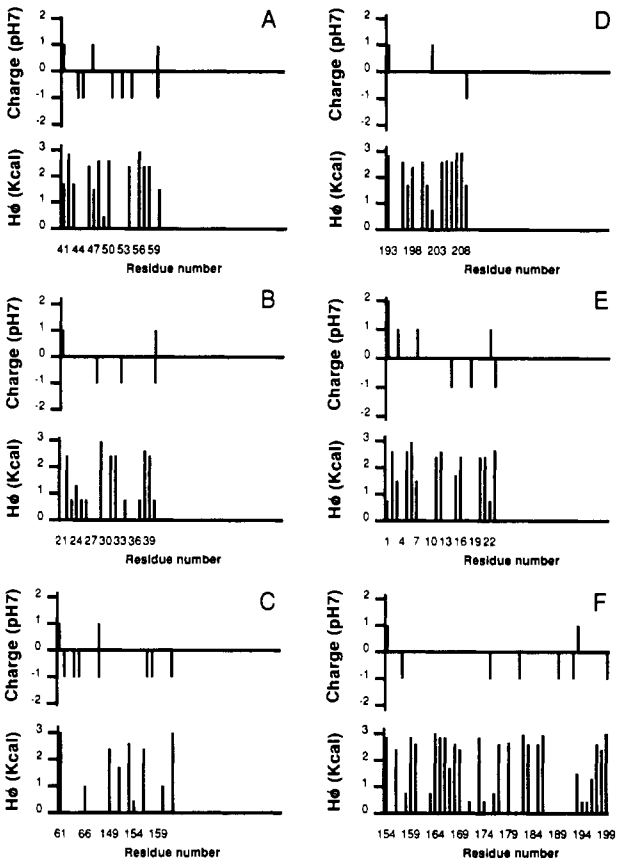

Figure 4. Schematic illustration of the linear chain distribution of charged and hydrophobic amino acid residues of some peptides from β-lactoglobulin and caseins. (A) β-lg 41–60; (B) β-lg 21–40; (C) β-lg 61–70 + 149–162; (D) β-cn 193–209; (E) α-cn 1–23; (F) α-cn 154–199.

At pH 7, the addition of salts ($\mu = 0.6$) decreased rates of adsorption of peptide fractions. This effect was more pronounced for fractions 1 and 3 and is the opposite of the behavior of proteins in the presence of salts (Graham and Phillips, 1979b; Waniska and Kinsella, 1985; MacRitchie, 1978).

DISCUSSION

A minimum molecular weight of 2000 has already been recognized as essential to good emulsifying and interfacial properties (Jost and Monti, 1982; Lee et al., 1987a; Chaplin and Andrews, 1989; Turgeon et al., 1991). Many peptides obtained from tryptic hydrolysis of β-lg meet this condition (Table I), peptides 61–69 + 149–162 and 61–70 + 149–162 being the largest with molecular weights of 4330 and 4458, respectively. However, these peptides had very poor interfacial properties, showing that molecular weight is not the only important factor in determining the ability to have good interfacial properties.

Fractions with good interfacial properties (F1–F3) are composed of the peptides 21–40, 25–40, and 41–60. These peptides are characterized by a distribution of hydrophobicity in discrete regions (three to five residues) separated by polar residues (two or three residues) (Figure 4A,B). In the same way, poor interfacial properties of peptides 61–69 + 149–162 and 61–70 + 149–162 could be related to uniform distribution of hydrophobic and hydrophilic amino acids (Figure 4C) and to rigidity provided by disulfide bond.

Clustering of hydrophobic and hydrophilic residues in distinct regions seems essential to functionality at interfaces. The importance of hydrophobic regions must be considered and could explain some results obtained in the literature. Peptide 193–209 of β-casein (Figure 4D) did not display good emulsifying properties (Lee et al., 1987a), which was attributed to an insufficient molecular weight (1900). We rather suggest a lack of amphipolarity. In this peptide there are not enough hydrophilic residues in the 196–208 region. Another peptide that lacks good emulsifying properties, α_{s1}-casein 1–23 (Shimizu et al., 1986), may not have enough hydrophobic residues (less than three residues) in the hydrophobic regions to adsorb efficiently to the interface (Figure 4E). The peptide α_{s1}-casein 154–199 responsible for improved emulsifying properties of α_{s1}-casein 1–23 in mixture (Shimizu et al., 1986) does possess these distinct hydrophobic zones (162–169, 182–186, 194–199) separated by polar residues (154–157, 188–193) (Figure 4F). This sequence allows many anchoring points at the interface and a better efficacy. Amphipolarity has been used to describe proteins such as α_{s1}-casein (Shimizu et al., 1983) but has not been demonstrated among peptides. β-Lg also has some distinct zones by hydrophobicity and hydrophilicity (Swaisgood, 1982), but its globular structure stabilized by disulfide bonds prevents its spreading at the interface (Waniska and Kinsella, 1985). Nevertheless, this protein is a good source of surface-active peptides.

Environmental conditions such as pH and ionic strength play an important role in interfacial properties of peptides. Mixture of peptides (Turgeon et al., 1991; Lee et al., 1987a,b) or isolated peptides (Shimizu et al., 1984, 1986) already studied had better interfacial and emulsifying properties at acidic pH as found with tryptic peptides from β-lg. Electrostatic repulsion at the interface can explain the different behavior of peptide 41–60 at pH 4 and 7. The peptides isolated in this work and those studied in the literature (Figure 4) have their isoelectric point in the acidic region. In this pH zone, the charges of peptides are reduced, and consequently the repulsion at the interface is minimum. Looking at the charges at pH 7 of the major peptides included in fractions F1–F3 (Figure 4A,B), it can be seen that peptide 41–60 (95% of F3) was more charged (4 Glu, 1 Asp, 2 Lys) than the two major peptides (2 Asp, 2 Lys) of F1 (21–40, 25–40).

The effect of salts on interfacial properties of peptides is complex. Peptide behavior in the presence of salts seems to be the opposite of that of proteins as rates of adsorption of peptides were generally decreased at $\mu = 0.6$. A better knowledge of the effect of pH and salts on peptide conformation and their interactions in solution would surely explain their behavior. For example, some peptides such as melittin, random-coiled in aqueous solutions, will form α-helices in the presence of an interface or at high concentration (Schoch et al., 1980; Terwilliger et al., 1982). In the presence of salts, melittin forms tetramers composed of four α-helical peptides whose hydrophobic moments counterbalance.

Salts had little effect on adsorption rates of the MP fraction, in contrast to a large effect on isolated peptides. Furthermore, rates of adsorption were smaller for peptides in mixtures (Table II) than for purified peptides (Table V). Many hypotheses can be proposed to explain this phenomenom. Peptides in a mixture can compete for the interface (Turgeon et al., 1991). Smaller peptides being less surface-active diffuse faster to the interface than larger peptides, resulting in a smaller decrease in surface tension. If environmental conditions favor association of peptides by electrostatic or hydrophobic interactions, those resulting complexes will migrate more slowly to the interface than unassociated peptides. Various forces are involved because electrostatic associations alone would increase rates of adsorption with the addition of salts and not the opposite (Table V).

CONCLUSION

Structure–function relationships of peptides are complex. Evidence suggests that the following characteristics are essential to good interfacial properties: clustering in distinct zones of hydrophilic and hydrophobic residues and a minimum molecular weight allowing this distribution. To complete understanding of peptide behavior, it would be interesting to select model peptides with potential surface-active properties from proteins of known primary sequence, to produce and purify them for a study of their interfacial properties alone or in mixtures with varying pH and ionic strength. The identification of the peptides responsible for improved interfacial properties in a mixture could allow control of their production and facilitate the separation of these peptides.

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LITERATURE CITED

Adamson, A. W. *Physical chemistry of surfaces*, 4th ed.; Wiley: New York, 1982.

Andersson, L. O. Hydrolysis of disulfide bonds in weakly alkaline media. II. Bovine serum albumin dimer. *Biochim. Biophys. Acta* **1970**, *200*, 363–369.

Bidlingmeyer, B. A.; Cohen, S. A.; Tarvin, T. L. Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.* **1984**, *336*, 93–104.

Bigelow, C. C. Average hydrophobicity of proteins and the relationship between it and protein structure. *J. Theor. Biol.* **1967**, *16*, 187–211.

Braunitzer, G.; Chen, R.; Schrank, B.; Stangl, A. The sequence of β-lactoglobulin. *Hoppe-Seyler's Z. Phys. Chem.* **1973**, *354*, 867–878.

Carles, C. Thèse docteur ingénieur "Sciences agronomiques", Paris, 1983.

Chaplin, L. C.; Andrews, A. T. Functional properties of peptides derived from casein proteolysis. *J. Dairy Res.* **1989**, *56*, 544.

Cheftel, J. C.; Cuq, J. L.; Lorient, D. *Protéines alimentaires*; Technique et documentation-Lavoisier: Paris, 1985.

Dalgalarrondo, M.; Chobert, J. M.; Dufour, E.; Bertrand-Harb, C.; Dumont, J. P.; Haertle, T. Characterization of bovine beta-lactoglobulin B tryptic peptides by reverse phase high performance liquid chromatography. *Milchwissenschaft* **1990**, *45*, 212–216.

Dickinson, E.; Stainsby, G. *Colloids in food*; Applied Science: London, 1982.

Eigel, W. N.; Butler, J. E.; Ernstrom, C. A.; Farrell, H. M.; Harwalkar, V. R.; Jenness, R.; Whitney, R. McL. Nomenclature of proteins of cow's milk: Fifth revision. *J. Dairy Sci.* **1984**, *67*, 1599–1631.

Graham, D. E.; Phillips, M. C. Proteins at liquid interface. I. Kinetics of adsorption and surface denaturation. *J. Colloid Interface Sci.* **1979a**, *70*, 403–414.

Graham, D. E.; Phillips, M. C. Proteins at liquid interface. II. Adsorption isotherms. *J. Colloid Interface Sci.* **1979b**, *70*, 415–426.

Graham, D. E.; Phillips, M. C. Proteins at liquid interface. III. Molecular structures of adsorbed films. *J. Colloid Interface Sci.* **1979c**, *70*, 427–439.

Halling, P. J. Protein-stabilized foam and emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 155–203.

Hirs, C. H. W.; Moore, S.; Stein, W. H. Peptides obtained by tryptic hydrolysis of performic acid-oxidized ribonuclease. *J. Biol. Chem.* **1956**, *219*, 623–642.

Jost, R.; Monti, J. C. *Lait* **1982**, *62*, 521–530.

Kinsella, J. E. Milk proteins: Physicochemical and functional properties. *CRC Crit. Rev. Food Sci. Nutr.* **1984**, *21*, 197–262.

Kinsella, J. E.; Whitehead, D. M.; Brady, J.; Bringe, N. A. Milk proteins: Possible relationships of structure function. In *Developments in dairy chemistry—4. Functional milk proteins*; Fox, P. F., Ed.; Elsevier Applied Sciences: London, 1989; Chapter 2.

Lee, S. W.; Shimizu, M.; Kaminogawa, S.; Yamauchi, K. Emulsifying properties of peptides obtained from the hydrolyzates of β-casein. *Agric. Biol. Chem.* **1987a**, *51*, 161–166.

Lee, S. W.; Shimizu, M.; Kaminogawa, S.; Yamauchi, K. Emulsifying properties of a mixture of peptides derived from the enzymatic hydrolyzates of bovine caseins. *Agric. Biol. Chem.* **1987b**, *52*, 1535–1540.

Leman, J.; Kinsella, J. E. Surface activity, film formation and emulsifying properties of milk proteins. *CRC Crit. Rev. Food Sci. Nutr.* **1989**, *28*, 115–138.

MacRitchie, F. Proteins at interface. *Adv. Protein Chem.* **1978**, *32*, 283–326.

McIlvaine, T. C. A buffer solution for colorimetric comparison. *J. Biol. Chem.* **1921**, *49*, 183–186.

Regnier, F. E. High-performance ion-exchange chromatography. *Methods Enzymol.* **1984**, *104*, 170–189.

Schoch, P.; Sargent, D. F. Quantitative analysis of the binding of melittin to planar lipid bilayers allowing for the discrete-charge effect. *Biochim. Biophys. Acta* **1980**, *602*, 234–247.

Shimizu, M.; Takahashi, T.; Kaminogawa, S.; Yamauchi, K. Adsorption onto an oil surface and emulsifying properties of bovine α_{s1}-casein in relation to its molecular structure. *J. Agric. Food Chem.* **1983**, *31*, 1214–1218.

Shimizu, M.; Lee, S. W.; Kaminogawa, S.; Yamauchi, K. Emulsifying properties of an N-terminal peptide obtained from the peptic hydrolyzates of αs1-casein. *J. Food Sci.* **1984**, *49*, 1117–1120.

Shimizu, M.; Lee, S. W.; Kaminogawa, S.; Yamauchi, K. Functional properties of a peptide of 23 residues purified from the peptic hydrolyzate of αs1 casein: Changes in the emulsifying activity during purification of the peptide. *J. Food Sci.* **1986**, *51*, 1248–1252.

Swaisgood, H. E. Chemistry of milk protein. In *Developments in Dairy Chemistry—1*; Fox, P. F., Ed.; Elsevier Applied Science: London, 1982; Chapter 1.

Tanford, J. The interpretation of hydrogen ion titration curves of protein. *Adv. Protein Chem.* **1962**, *17*, 70–165.

Terwilliger, T. C.; Weissman, L.; Eisenberg, D. The structure of mellittin in the form I crystals and it's implication for melittin's lytic and surface activities. *Biophys. J.* **1982**, *37*, 353–361.

Tornberg, E.; Olsson, A.; Persson, K. The structural and interfacial properties of food proteins in relation to their function in emulsions. In *Food emulsions*, 2nd ed.; Larsson, K., Friberg, S. E., Eds.; Dekker: New York, 1990.

Turgeon, S. L.; Gauthier, S. F. Whey peptide fractions obtained with a two-step ultrafiltration process. Production and characterization. *J. Food Sci.* **1990**, *55*, 106–110, 157.

Turgeon, S. L.; Gauthier, S. F.; Paquin, P. Interfacial and emulsifying properties of whey peptide fractions obtained with a two-step ultrafiltration process. *J. Agric. Food Chem.* **1991**, *39*, 673–676.

Waniska, R. D.; Kinsella, J. E. Surface properties of β-lactoglobulin: Adsorption and rearrangement during film formation. *J. Agric. Food Chem.* **1985**, *33*, 1143–1148.

Waniska, R. D.; Kinsella, J. E. Surface viscosity of glycosylated dervatives of β-lactoglobulin at the air-water interface. *Food Hydrocolloids* **1988**, *2*, 59–67.

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