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IDENTIFICATION OF PEPTIDE LIGANDS GENERATED BY COMBINATORIAL CHEMISTRY THAT BIND α-LACTALBUMIN

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IDENTIFICATION OF PEPTIDE LIGANDS GENERATED BY COMBINATORIAL CHEMISTRY THAT BIND α-LACTALBUMIN

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ABSTRACT

α-Lactalbumin is a whey protein with high digestibility and low potential for causing allergic problems in infants, making it a strong candidate for use in infant formulas. The development of an efficient and scalable process for isolation of α-lactalbumin is necessary to allow its use on a large scale. Affinity chromatography using short peptides as ligands is a promising technique because it allows the recovery of specific proteins without the use of harsh chemicals or problems due to ligand release. In the present paper we describe the identification of the hexapeptide WHWRKR, obtained from a combinatorial library, that shows affinity for α-lactalbumin.

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INTRODUCTION

α-Lactalbumin is a low molecular weight (14.2 kd), globular, whey protein. It participates in the production of lactose in the mammary gland (1), modifying the enzyme galactosyltransferase to form the lactose synthase complex.

α-Lactalbumin has a well-conserved amino acid sequence. However, its concentration varies significantly among species. In bovine milk, where the total protein content is around 33 g/L, the concentration of α-lactalbumin is, on average, 1.2 g/L (or 3.6%) (2,3), while human milk, with a total protein content of 23 g/L, contains 2.2 g/L of α-lactalbumin (9.6%) (4). When the composition of bovine or human milk is compared, the concentration of β-lactoglobulin is noticeably different. Humans have no β-lactoglobulin in milk, but cow's milk has 3.2 g/L of the protein. These differences may cause problems, such as allergic response, low protein digestion, and gas formation, in human infants who consume cow's milk or when whey proteins are used as a protein source for infant formulas.

Because α-lactalbumin is found in human milk, it is less likely to be an allergen to infants. Thus, this protein is a good candidate to be the protein source in infant formulas, "humanizing" milk substitutes, mimicking amino acid contents found in human milk, and eliminating potentially allergenic proteins (5).

Whey proteins are important ingredients for the food industry because they are easily isolated, provide essential amino acids, and have useful properties, such as in the formation of gels and stabilization of emulsions and foams (6,7). However a fractionation of the whey proteins could provide products with superior value. According to Horton (8), α-lactalbumin (with bovine serum albumin and immunoglobulin) can be used advantageously in infant formulas; lactoferrin and lactoperoxidase have pharmaceutical value; growth factor is used to stimulate growth of human tissue cells; immunoglobulins have anticancer and immunity-boosting properties; and β-lactoglobulin is used for its gelling abilities and for solubility and nutrition properties desirable in clear dietetic and sports beverages. Several reports (9,10,11) suggest that the gelling properties of α-lactalbumin and β-lactoglobulin mixture are better than those of individual proteins or more complex mixtures, such as whey protein isolate (WPI) and whey protein concentrate, and that α-lactalbumin and β-lactoglobulin also have a more reliable composition.

Several methods of fractionation of whey proteins have been reported in which researchers utilized a wide range of techniques to accomplish separation. Recent studies include ion-exchange chromatography (12), displacement chromatography (13), and fluidized ion-exchange chromatography (14,15). The separation of α-lactalbumin has been accomplished by aqueous two-phase partitioning (16), anion-exchange chromatography (17), thermal isoelectric precipitation (18), and centrifugation (19). While methods such as partitioning and precipitation of-

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ten do not produce enough recovery and/or purity, some chromatographic methods are too expensive or not suitable for scaled-up operations.

Affinity chromatography is based on the specific interaction between a ligand and the target protein. This technique has the potential of separating a protein from complex mixtures in a single pass, reducing costs dramatically, and increasing recovery yields, making this somewhat expensive technique suitable for practical use on a large scale. Blomkalns and Gomez (5) report the use of affinity chromatography using immobilized metal ions to purify α-lactalbumin from bovine whey protein concentrate, obtaining a purity of 90% and a recovery of 80% in the final product. However, the use of immobilized metal ion columns may not be suitable for the food and pharmaceutical industries because of toxicity related to possible column leakage.

Another means of isolating protein is the use of short peptides as ligands for affinity chromatography. In this technique, peptides generated by combinatorial chemistry are screened for affinity to the target protein. The peptides are generated in a solid support in such a way that each bead of support contains multiple copies of only one peptide.

The use of a solid-phase peptide resin also presents the advantage of not requiring further purification prior to use, when compared to affinity chromatography using monoclonal antibodies. Furthermore, the purification cost for antibodies can be up to 85% of the total production cost, and leakage into the product could elicit an immune response after human consumption.

Solid phase libraries are suitable for generation of affinity chromatographic media because peptides are relatively stable, not likely to provoke an immune response in case of leakage from the column, and can be manufactured in large quantities at relatively low costs (20). Peptide ligands have been used previously for the purification of several proteins, such as von Willebrand factor (20), S-protein (21), α-amylase (22), and α-1 antitrypsin (23,24).

The present work describes the identification of peptide ligands, generated by combinatorial chemistry onto a solid support, that bind α-lactalbumin in a solution of whey protein isolate.

MATERIALS AND METHODS

Resins

During the experiments several resins were used. The original resin used was a polyhydroxylated methacrylate polymer (TosoHaas AF Chelate 650, TosoHaas, Montgomeryville, Pa, USA) modified to generate a free amino group using 4,7,10-trioxa-1,13-tridecanediamine (25). The resin is highly hydrophilic and has bead diameters varying from 50 to 200 μm. The resulting 1000 Å diameter pores

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are suitable for isolating proteins up to 5 MDa. The aminated resin was used as a solid support for the synthesis of the peptides. The amino groups of the resin were acetylated, generating the "acetylated resin."

Primary Screening

For primary screening, a hexamer peptide library was used. The library was generated by chemical synthesis on a solid phase (the aminated resin) through the divide-couple-recombine technique. Eighteen out of the 20 natural L-amino acids were used (methionine and cysteine were excluded) to produce the library, which could potentially generate over 34 million (or 18^6) different peptides. The peptide density was approximately 100 μmoles of peptide per gram of resin. α-Lactalbumin was labeled using ^{14}C-formaldehyde as in the procedure described in (26).

Aliquots of 125 mg of the library were equilibrated with 50 mmol/L phosphate buffer, pH 7.0, for at least 1 h. The resin was rinsed with 5 mL of phosphate buffer, followed by incubation with a 1% casein solution for 1 h. The casein solution was drained, and the resin was incubated with 5 mL of 1 μmol/L α-lactalbumin (Sigma, Saint Louis, Mo, USA) in phosphate buffer, pH 7.0, followed by incubation with a solution of 2 mol/L NaCl in 50 mmol/L phosphate buffer, pH 7.0, for 1 h. This step intended to block the peptides that would require over 2 mol/L NaCl to elute the target protein. The solution was drained, and 5 mL of a 1 μmol/L α-lactalbumin labeled with ^{14}C and 6.6 g/L WPI solution was added and allowed to incubate for 1 h. The final solution was drained, and the resin was rinsed with 200 mL of phosphate buffer.

The beads were mixed with a solution of 1% low-melt agarose and poured onto the hydrophilic side of a plastic film containing a previously made high-melt agarose frame. The gel was allowed to air-dry overnight in a fume hood. After the gel was completely dry, it was put in contact with autoradiographic film for at least 5 days and reexposed for at least 15 days to confirm the results (26). Peptide-retaining beads were removed from the gel and sent for amino acid sequencing to the Protein Chemistry Laboratory, Texas A & M University (College Station, Tex, USA).

Secondary Screening

About 1 g of each positive sequence was synthesized for the secondary screening. The resins were made using a Gilson AMS Multiple Peptide Synthesizer, as in the procedure described by Buettner et al. (25), at a peptide density of about 100 μmoles of peptide per 1 g of resin. Four aliquots of 20 mg of each resin were transferred to an Ultrafree-MC (Millipore, Bedford, Mass, USA) centrifugal

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filtration cartridge (pore size 0.45 μm) and equilibrated for at least 1 h with 0.5 mL of 50 mmol/L phosphate buffer, pH 7.0. After draining by centrifugation, three out of the four cartridges had 0.5 mL of 1.0 μmol/L α-lactalbumin in phosphate buffer added, while the fourth had only phosphate buffer added. The cartridges were incubated for 1 h at room temperature and then drained. The filtrate was collected for determination of protein concentration. Subsequent incubations were made with 0.5 mol/L NaCl, 1.0 mol/L NaCl, and 2% acetic acid in each cartridge for 1 h each. After the filtrates were collected, protein concentrations were measured using a micro bicinchoninic acid assay (Pierce, Rockford, Ill, USA) in a microplate format.

Percentage of binding was determined from the difference between the values obtained for the treatments with and without protein. Resins that bound over 60% of the α-lactalbumin were considered positives.

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Chromatographic Assays

In addition to an aminated resin, an acetylated resin, and a peptide resin with no affinity to α-lactalbumin, the positive resins were packed in 0.6-mL high-performance liquid chromatography (HPLC) columns and equilibrated with phosphate buffer for at least 1 day at a flow rate of 0.1 mL/min at room temperature. A solution of 5.55 g/L WPI in phosphate buffer was used to further verify the binding ability of the resins. A 0.5-mL aliquot of the WPI solution was injected and eluted using a salt gradient (0–0.5 mol/L NaCl in phosphate buffer in 50 min with a flow rate of 0.15 mL/min) followed by a 2% acetic acid elution. All peaks, monitored by absorbance at 280 nm, were collected and their composition determined by Sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

RESULTS AND DISCUSSION

Primary Screening

A total of 800 mg of beads from the library was screened for binding to α-lactalbumin. From these experiments, 14 positive beads were selected and the peptides sequenced. Eighteen sequences were generated from the sequencing results (14 hexapeptides and 4 pentapeptides) (Table 1).

The peptides GNTDFL and GNTKFL were generated from just one sequence, but the amino acid in the fourth position was not positively identified. AFWVI was obtained from a bead with a pentapeptide probably due to lack of one reaction cycle during the library synthesis. FRIEQ, RLYVY, and HWRKR are

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Table 1. Sequences Obtained After Primary Screening

SLRKPA	RRLYVY	YLSVHG
GNTDFL	WHWRKR	NFNVIG
GNTKFL	NVLTKD	AFWVI
RHPLNT	GEIYNI	FRIEQ
HFRIEQ	ELVIPK	RLYVY
KQWYLR	INHIQN	HWRKR

pentapeptides obtained by omitting the first amino acid residue of HFRIEQ, RRLYVY, and WHWRKR, respectively. These peptides were synthesized because the sequencing results for the first amino acid position was unclear.

The most prevalent amino acid residues found in the positive beads were arginine (R), asparagine (N), isoleucine (I), and leucine (L), with 8 positions each, followed by valine (V) with 6, histidine (H), lysine (K), and tyrosine (Y) with 5, glycine (G), phenylalanine (F), and tryptophan (W) with 4, glutamine (Q), glutamic acid (E), proline (P), and threonine (T) with 3, alanine (A), and serine (S) with 2, and aspartic acid (D) with 1 position. The prevalence of each amino acid in each position is presented in Table 2. No obvious trend was noted on the distri-

Table 2. Prevalence of Amino Acids in Each Position After Primary Screening

	1	**2**	**3**	**4**	**5**	**6**
A	1	0	0	0	0	1
D	0	0	0	1	0	1
E	1	1	0	0	1	0
F	0	3	0	0	**2**	0
G	**3**	0	0	0	0	2
H	1	2	1	0	1	0
I	1	0	1	3	2	1
K	1	0	0	2	2	1
L	0	3	2	1	1	**2**
N	2	**3**	1	0	2	1
P	0	0	1	0	2	0
Q	0	1	0	0	1	1
R	2	1	2	1	0	2
S	0	0	1	0	0	0
T	1	0	**2**	1	0	1
V	0	1	1	3	1	0
W	1	0	3	0	0	0
Y	1	0	0	3	0	1

Position 1 corresponds to a terminal amine, position 6 is the closest to the matrix. Bold numbers refer to degenerate sequences.

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bution of individual amino acids in the peptides based on their hydrophobic or charge properties: hydrophobic (I, L), hydrophilic (R, K), charged (R, K), and uncharged residues (N, I, L) were all prevalent. This result is similar to that reported regarding peptide specificity for von Willebrand factor (20) and for α-1-proteinase inhibitor (24).

Although Mondorf, Kaufman, and Carbonell (26) reported a measurable relationship between the concentration of labeled protein and the signal obtained in radiography, comparisons of signals obtained from different particles in different gels were difficult to obtain due to small differences in particle diameter, development conditions, and gel coverage. Thus, during the primary screening, all beads that showed signals above background were sequenced, synthesized, and tested in the secondary screening.

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Secondary Screening

Results for the secondary screening are summarized in Table 3. From the binding obtained, 6 resins were considered strong binders of α-lactalbumin: KQWYLR, RRLYVY, WHWRKR, AFWVI, RLYVY, and HWRKR.

Analysis of the prevalence of amino acid residues in those peptides reveals a high number of arginine (5) and tryptophan (4), followed by tyrosine (3), valine, lysine, and leucine (2), and glutamine, histidine, isoleucine, and phenylalanine (1). The prevalence of each amino acid in each position after the second screening is shown in Table 4.

Figure 1 shows the hydropathy of each peptide. Because of their redundant sequences with longer peptides, RLYVY and HWRKR were not included in Fig. 1. The results were calculated using the values for individual amino acid residues given by Eisenberg et al. (27) and grouping 3 contiguous residues for each value. WHWRKR is the only peptide that shows a primarily hydrophilic character, indicated by negative values for free energy transfer in Fig. 1. AFWVI is a hydrophobic peptide, and RRLYVY has a strongly hydrophilic side (RR) and a strong hydrophobic one (LYVY). The peptide KQWYLR displays a hydrophilic character in both extremities and has a hydrophobic midsection. Although WHWRKR has an end with two tryptophan residues, histidine and the following arginine, both hydrophilic residues govern the overall character of this region.

Calcium-binding proteins are known to bind short peptides exhibiting clusters of basic amino acids in close conjunction with hydrophobic residues (28). This has been demonstrated for calmodulin (29), troponin C (30), and myosin light chain (31), as well as for the interaction of α-lactalbumin with melittin, a bee venom peptide (28). Three of the original peptides contain a combination of basic and hydrophobic residues, KQWYLR, RRLYVY, and WHWRKR, suggesting that the binding of α-lactalbumin to these peptides may be through the calcium-

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Table 3. Percentage of α-Lactalbumin Binding to Each Hexapeptide Resin During Secondary Screening

Peptide	% Bound
SLRKPA	32.0
GNTDFL	4.1
GNTKFL	11.2
RHPLNT	13.6
HFRIEQ	12.6
KQWYLR	**65.8**
RRLYVY	**70.7**
WHWRKR	**86.6**
NVLTKD	5.8
GEIYNI	4.1
ELVIPK	8.2
INHIQN	10.6
YLSVHG	22.0
NFNVIG	4.8
AFWVI	**61.7**
FRIEQ	14.8
RLYVY	**77.8**
HWRKR	**74.6**

100% = 7.1 μg α-lactalbumin. Resins binding 60% or more α-lactalbumin were considered strong binders.

Table 4. Prevalence of Amino Acids in Each Position for the Peptides Considered Strong Binders After Secondary Screening

	1	2	3	4	5	6
A	1	0	0	0	0	1
F	0	1	0	0	0	0
H	0	1	0	0	0	0
I	0	0	0	0	1	0
K	0	0	0	1	1	0
L	0	1	1	0	0	0
P	0	0	0	0	1	0
R	1	1	1	1	0	1
S	1	0	0	0	0	0
V	0	0	0	1	1	0
W	1	0	2	0	0	0
Y	0	0	0	1	0	1

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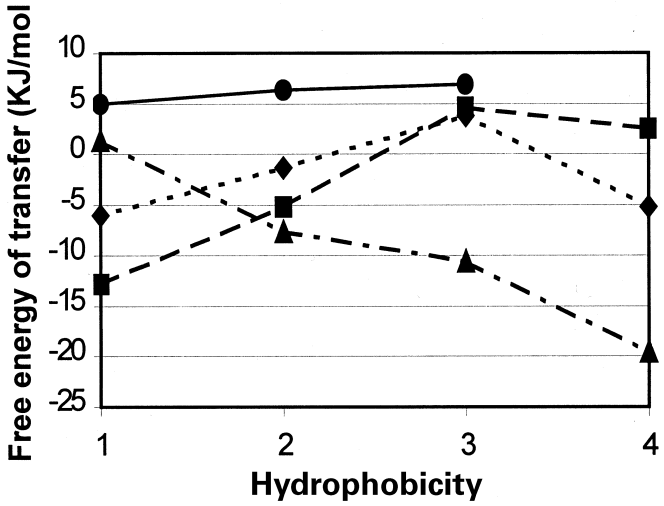

Figure 1. Hydropathic character of peptides KQWYLR (◆), RRLYVY (■), WHWRKR (▲), and AFWVI (●) calculated using values from Eisenberg et al. (27). Point 1 was obtained by the sum of the hydrophobicities of the three first contiguous amino acid residues in each peptide. The subsequent data points were obtained by shifting one amino acid position and recalculating the hydrophobicity.

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binding site. Peptide AFWVI is strongly hydrophobic but does not include a basic residue in its structure.

The high number of low-binding and false positives is believed to be due to the way the primary screening was conducted. In this work, beads containing the peptides were incubated with the radiolabeled target protein, which produces a mark on radiographic film. If two or more beads are close to each other, it is difficult to determine which one is responsible for the positive signal. Comparing the results obtained in this work with those reported in the literature is difficult because most selections for peptides that display affinity to proteins have been done using biopanning (23,32,33) in which short peptides are expressed on the surface of viruses. In biopanning, short DNA fragments are cloned into viral particles and incubated with the target protein, the mixture is subjected to progressively more severe conditions that select for stronger binders. In the end, the viral particles containing the peptides that show high affinity to the target protein are amplified, and the DNA sequence for the peptide is determined, thus allowing the identification of the original peptide. In this way, the low-binding peptides are excluded during the repeated washes, and the probability of finding a low binder or false positive at the end is very low.

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Mondorf, Kaufman, and Carbonell (26), using a screening technique similar to the one used in this work, screened a peptide library against ^{14}C-labeled fibrinogen and found five beads with signals significantly stronger than background signals. From the five beads, four produced complete sequences (the other had a truncated sequence), and three showed affinity to fibrinogen during later experiments.

The long exposure times required to obtain signals also contributed to the identification of low-binding positives. Longer exposure times allow weak binders, containing small amounts of proteins to accumulate enough energy to produce a signal on the radiographic films.

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Chromatographic Assays

Resins containing the peptides KQWYLR, RRLYVY, WHWRKR, and AFWVI were selected for HPLC assays. Again, RLYVY and HWRKR were not tested because their amino acids were identical to those in longer peptides.

Figure 2a shows the chromatographic profile obtained using KQWYLR. α-Lactalbumin eluted before the gradient was applied and was present throughout the initial plateau (fractions 3 and 6), as well as in the β-lactoglobulin-rich fractions (13 and 14 in Fig. 2b). The last peak shows that the fraction (27) eluted with 2% acetic acid was composed of a mixture of the proteins contained in WPI (Fig. 2b). Chromatograms for the resins containing the peptides RRLYVY (Fig. 3a) and AFWVI (Fig. 4a) showed similar behavior, with α-lactalbumin being eluted early in the run (fraction 5 in Fig. 3b, and fractions 3, 4, and 5 in Fig. 4b) and with low concentrations of this protein being eluted in the β-lactoglobulin-rich fraction (fraction 12 in Fig. 3b and fractions 13 and 14 in Fig. 4b). The SDS-PAGE profiles (Figs. 2b, 3b, and 4b) show that these columns are capable of binding α-lactalbumin, but they are not capable of purifying it because the fractions appear to be contaminated with other whey proteins. The use of phosphate buffer at pH values of 5.0 and 4.0 during application of the sample and elution did not improve the separation profile for any of the resins (data not shown).

The profile obtained with the peptide WHWRKR is presented in Fig. 5a. Compared to the elution sequences in the other resins, α-lactalbumin was eluted at higher concentrations of gradient, and according to SDS-PAGE, the fractions containing this protein had a low degree of contamination (Fig. 5b). The α-lactalbumin-rich fractions eluted in 0.25 mol/L NaCl, while the β-lactoglobulin-rich fraction was eluted in 0.5 mol/L NaCl.

Because nearly all proteins present in WPI bound to the resins, the specificity of the peptides for α-lactalbumin was questionable. The HPLC profile shown in Fig. 6a was obtained by running WPI through the aminated resin. This resin contains free amino groups and bound most of the major WPI proteins, but

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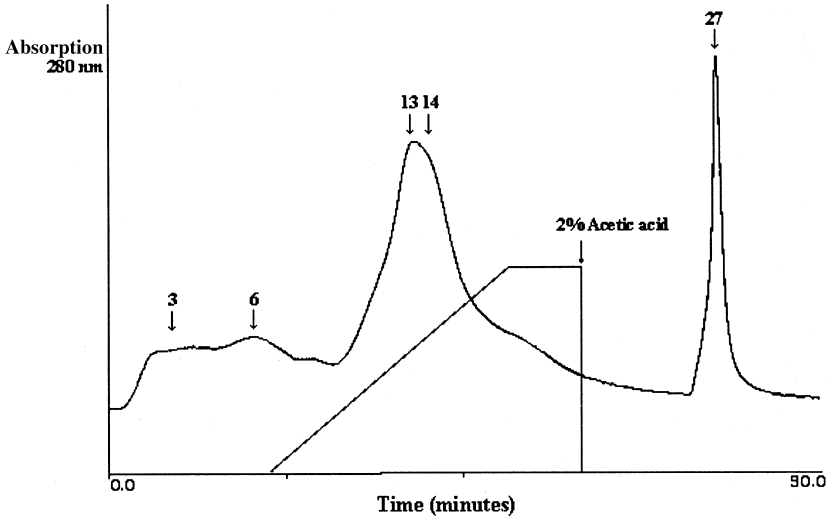
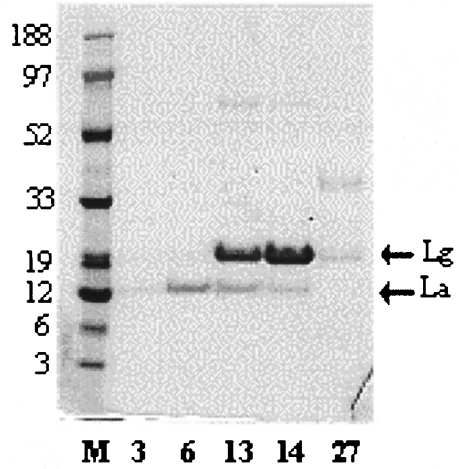


(a)



(b)

Figure 2. (a) Chromatogram of 500 μL of a 5.55 g/L solution of WPI in 50 mmol/L phosphate buffer, pH 7.0, with the peptide KQWYLR. Elutions were made with a linear salt gradient (0 to 0.5 mol/L NaCl in phosphate buffer) and a final wash with 2% acetic acid. Numbers refer to the corresponding fraction analyzed through SDS-PAGE. Absorbance is reported in arbitrary units. (b) SDS-PAGE of the selected fractions from the chromatography run of the peptide KQWYLR. M denotes the molecular weight markers. Alpha-lactalbumin (La) and β-lactoglobulin (Lg) positions are indicated by the arrows. Molecular weights of the markers are indicated beside each band. The same markers were loaded in all gels.

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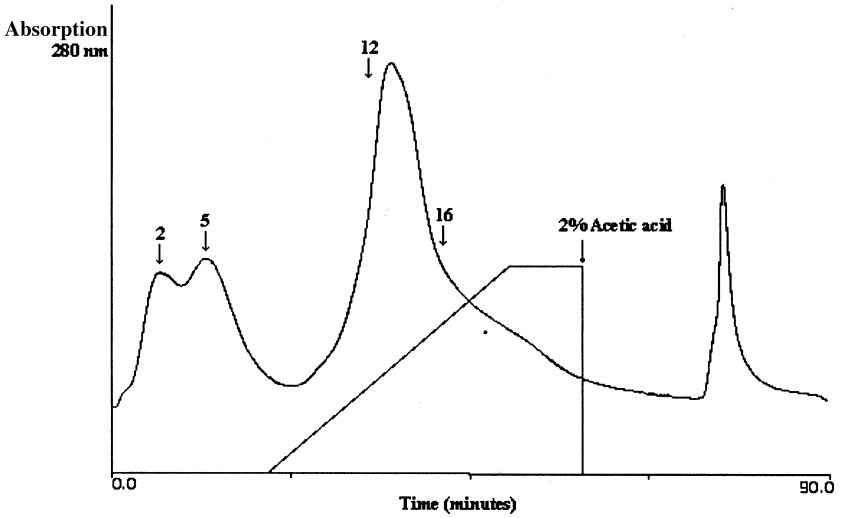

(a)

(b)

Figure 3. (a) Chromatogram of 500 μL of a 5.55 g/L solution of WPI in 50 mmol/L phosphate buffer, pH 7.0, with the peptide RRLYVY. Elutions were made with a linear salt gradient (0 to 0.5 mol/L NaCl in phosphate buffer) and a final wash with 2% acetic acid. Numbers refer to the corresponding fraction analyzed through SDS-PAGE. Absorbance is reported in arbitrary units. (b) SDS-PAGE of the selected fractions from the chromatography run of the peptide RRLYVY. M denotes the molecular weight markers.

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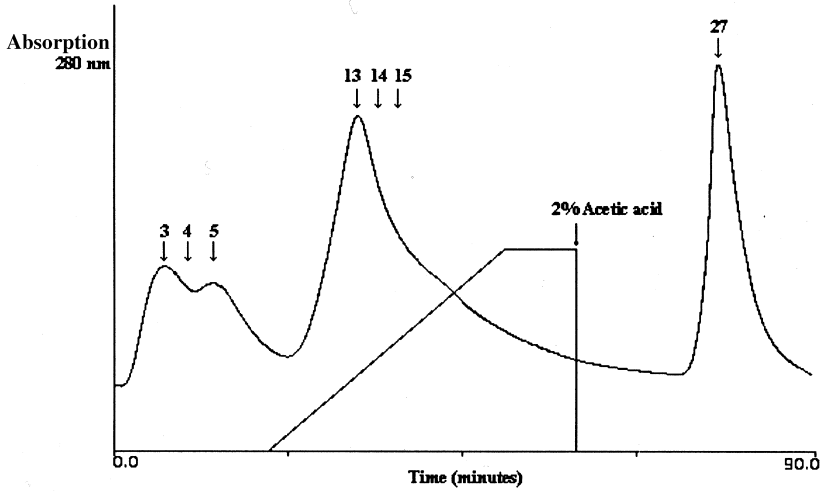
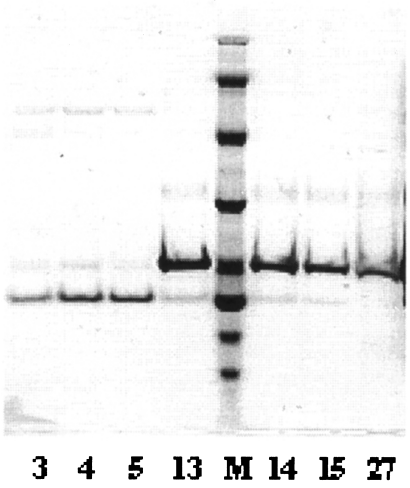


(a)



(b)

Figure 4. (a) Chromatogram of 500 μL of a 5.55 g/L solution of WPI in 50 mmol/L phosphate buffer, pH 7.0, with the peptide AFWVI. Elutions were made with a linear salt gradient (0 to 0.5 mol/L NaCl in phosphate buffer) and a final wash with 2% acetic acid. Numbers refer to the corresponding fraction analyzed through SDS-PAGE. Absorbance is reported in arbitrary units. (b) SDS-PAGE of the selected fractions from the chromatography run of the peptide AFWVI. M denotes the molecular weight markers.

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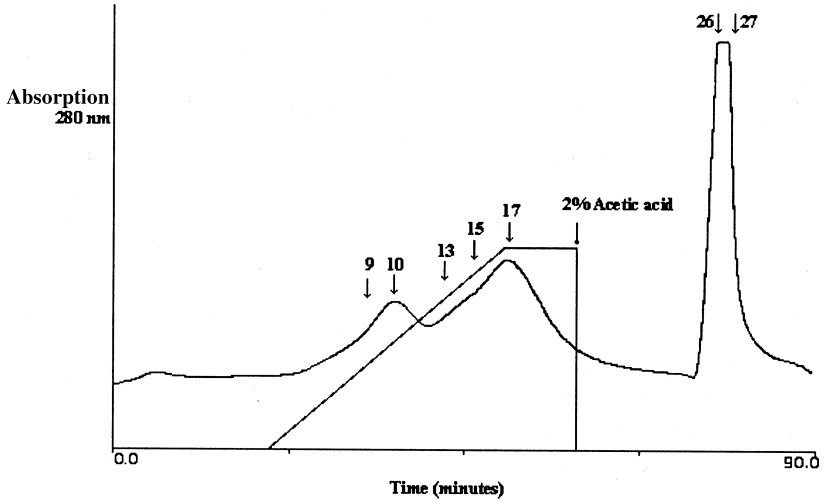
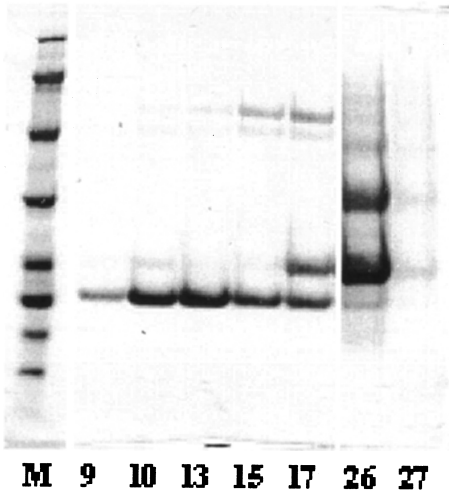


(a)



(b)

Figure 5. (a) Chromatogram of 500 μL of a 5.55 g/L solution of WPI in 50 mmol/L phosphate buffer, pH 7.0, with the peptide WHWRKR. Elutions were made with a linear salt gradient (0 to 0.5 mol/L NaCl in phosphate buffer) and a final wash with 2% acetic acid. Numbers refer to the corresponding fraction analyzed through SDS-PAGE. Absorbance is reported in arbitrary units. (b) SDS-PAGE of the selected fractions from the chromatography run of the peptide WHWRKR. M denotes the molecular weight markers.

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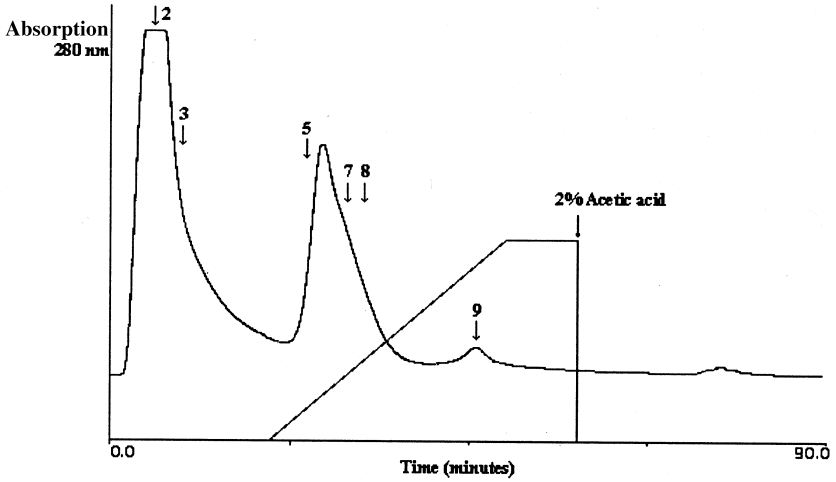

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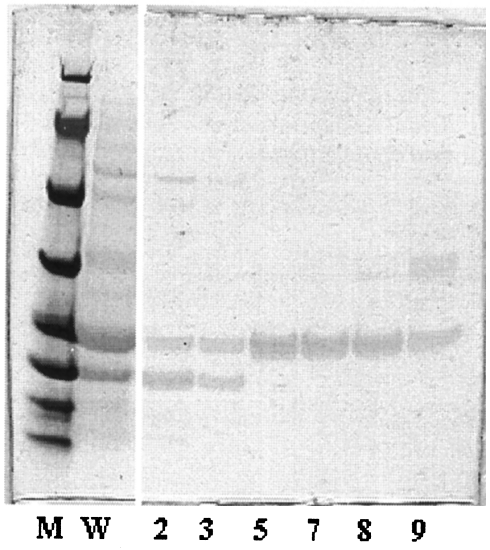

(b)

Figure 6. (a) Chromatogram of 500 μL of a 5.55 g/L solution of WPI in 50 mmol/L phosphate buffer, pH 7.0, with the aminated resin. Elutions were made with a linear salt gradient (0 to 0.5 mol/L NaCl in phosphate buffer) and a final wash with 2% acetic acid. Numbers refer to the corresponding fraction analyzed through SDS-PAGE. Absorbance is reported in arbitrary units. (b) SDS-PAGE of the selected fractions from the chromatography run of the aminated resin. M denotes the molecular weight markers.

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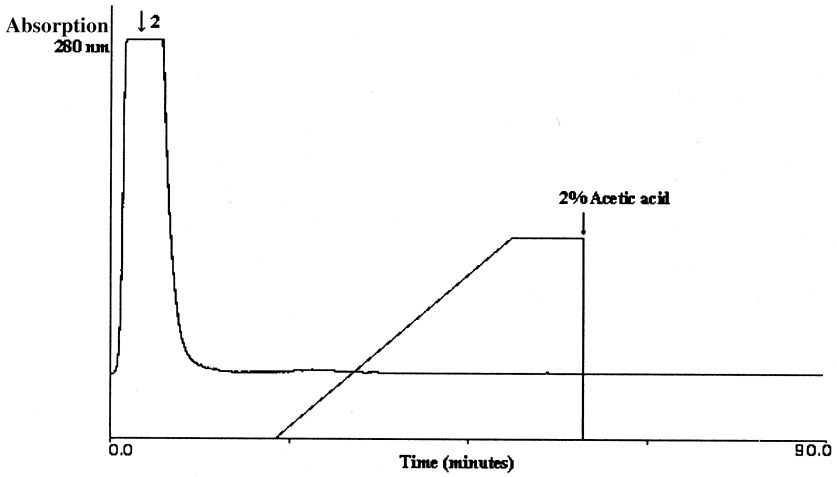

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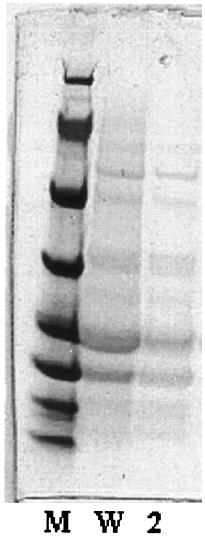

(b)

Figure 7. (a) Chromatogram of 500 μL of a 5.55 g/L solution of WPI in 50 mmol/L phosphate buffer, pH 7.0, with the acetylated resin. Elutions were made with a linear salt gradient (0 to 0.5 mol/L NaCl in phosphate buffer) and a final wash with 2% acetic acid. Numbers refer to the corresponding fraction analyzed through SDS-PAGE. Absorbance is reported in arbitrary units. (b) SDS-PAGE of the selected fractions from the chromatography of the acetylated resin. M denotes the molecular weight markers.

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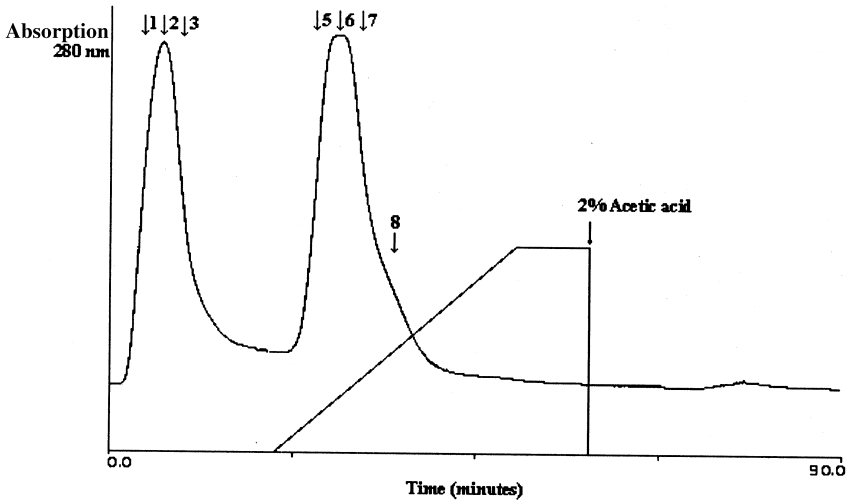

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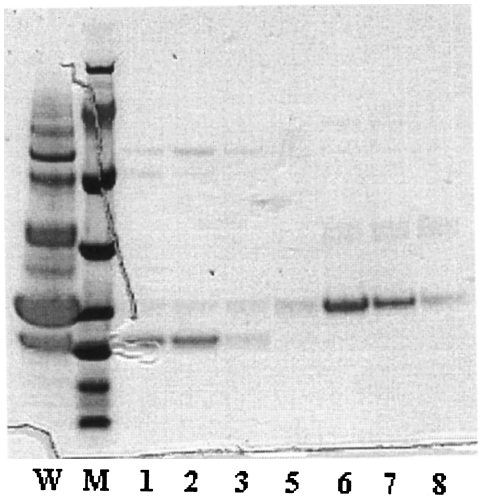

(b)

Figure 8. (a) Chromatogram of 500 μL of a 5.55 g/L solution of WPI in 50 mmol/L phosphate buffer, pH 7.0, with the peptide GNTKFL. Elutions were made with a linear salt gradient (0 to 0.5 mol/L NaCl in phosphate buffer) and a final wash with 2% acetic acid. Numbers refer to the corresponding fraction analyzed through SDS-PAGE. Absorbance is reported in arbitrary units. (b) SDS-PAGE of the selected fractions from the chromatography run of the peptide GNTKFL. M denotes the molecular weight markers.

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it did not bind α-lactalbumin efficiently as indicated by the SDS-PAGE profile (Fig. 6b). Similar results were obtained by Outinen, Tossavainen, and Syväoja (17) who recorded the binding pattern of α-lactalbumin and β-lactoglobulin to polystyrene-divinylbenzene matrices carrying quaternary ammonium groups. They observed that α-lactalbumin was present in the unbound fraction, while β-lactoglobulin bound to the resins.

Chromatographic runs with the acetylated resin (Fig. 7a) show that none of the WPI proteins were bound when the amino groups of the resin were acetylated. The composition of the unbound fraction (fraction 2 in Fig. 7b) was similar to that of the injected sample (fraction W in Fig. 7b) as indicated by similar SDS-PAGE patterns.

Figure 8a shows the elution profile of a resin that contained a peptide with low binding ability for α-lactalbumin (GNTKFL). Little of the target protein bound to this resin, making the unbound fraction the richest in α-lactalbumin (fractions 1, 2, and 3 in Fig. 8b).

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CONCLUSIONS

From these experiments, the peptides isolated during the secondary screening show affinity for α-lactalbumin, while the other proteins present in whey protein isolate bind nonspecifically to the resin or to the available amino groups in the peptides. Thus, we were successful in the identification of a short peptide that binds α-lactalbumin and were able to isolate it from a mixture of proteins. The resin containing the peptide WHWRKR will be further investigated to improve the process and provide better separation profiles.

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