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Plasmin-digest of β -lactoglobulin with antibacterial properties

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The objective of the present study was to identify the antibacterial properties of plasmin digest of β -lactoglobulin (PD β). After enzymatic treatment, PD β was fractionated using reversed-phase high-performance liquid chromatography. The antibacterial activity of plasmin, β -lactoglobulin, PD β , fractionated peptides and nisin as a control was tested against pathogenic (*Escherichia coli* and *Staphylococcus aureus*) and probiotic (*Lactobacillus casei* and *Lactobacillus acidophilus*) bacteria. Four antibacterial peptides, β L1, β L2, β L3 and β L5, PD β and nisin as a control revealed antibacterial activity against pathogenic and probiotic bacteria tested. Finally, the identification of the antibacterial peptides was carried out using LC-Mass. Four antibacterial peptides showed molecular masses of 673.116, 819.320, 916.407 and 2410.515 Da, respectively. Two identified antibacterial peptides were β L1 and β L3, which corresponded to residues 78–83 and 83–91 of bovine β -lactoglobulin. The results showed that PD β as a source of antibacterial peptides can be considered for controlling bacterial contamination.

Keywords: bovine; β -lactoglobulin; antibacterial; peptide; plasmin

Introduction

For the neonate, milk is known to provide not only excellent nutrition but also protection against infection. A variety of protective factors such as natural antimicrobial proteins thought to be responsible for antibacterial properties of milk (Gobbetti, Minervini, & Rizzello, 2007; Goldman & Goldblum, 1990; Meisel, 1998, 2005). The total antibacterial effect in milk is more than the aggregate of the individual contributions of immunoglobulin and non-immunoglobulin defence proteins. This is most likely due to the synergistic effects of them and another contributing factor may be the presence of naturally occurring antibacterial peptides (Clare & Swaisgood, 2000; Pakkanen & Aalto, 1997; Schanbacher, Talhouk, & Murray, 1997). Antibacterial peptides are in a latent or inactive state within protein molecules but can be released during enzymatic digestion (Korhonen & Pihlanto-Leppala, 2003, 2004; Salami et al., 2010).

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β -lactoglobulin is the major whey protein in the milk of ruminants and monogastrics. It represents approximately 10% of the total milk protein and 58% of the whey protein. It is an anionic protein with a pI of 4.8 and a molecular weight (Mw) of 18.280 Da and contains normally 162 residues (Creamer & Sawyer, 2003; Guo, Fox, Flynn, & Kindstedt, 1995; Hambling, McAlpine, & Sawyer, 1992). β -lactoglobulin has a variety of useful functional characteristics such as viscosity, gelation, foaming, solubility and emulsification that have made this protein a proper choice in the formulation of modern foods and beverages (Chatterton, Smithers, Roupas, & Brodkorb, 2006). β -lactoglobulin and its peptide fragments have various bioactivities such as antimicrobial, immunomodulating, opioid and hypocholesterolemic activities (Hernández-Ledesma, Recio, & Amigo, 2008).

A wide variety of antibacterial peptides have been derived from bovine milk proteins. Some of them are, lactoferrin (Bellamy et al., 1992), α_{S1} -casein (Lahov & Regelson, 1996; McCann et al., 2006) α_{S2} -casein (McCann et al., 2005; Recio & Visser, 1999; Zucht, Raida, Adermann, Magert, & Forssman, 1995), k -casein (Liepke, Zucht, Forsmann, & Standker, 2001; López-Expósito, Minervini, Amigo, & Recio, 2006a; Malkoski et al., 2001; Matin, Monnai, & Otani, 2000), α -lactalbumin (Pellegrini, Thomas, Bramaz, Hunziker, & Von Fellenberg, 1999). There are also reports of antibacterial peptides liberated from bovine β -lactoglobulin (El-Zahar et al., 2004; Pellegrini, Dettling, Thomas, & Hunziker, 2001; Pihlanto-Leppala et al., 1999).

Dalgaard, Heegaard, and Larsen (2008) also reported that plasmin mediated the hydrolysis of native β -lactoglobulin. They found five varying peptides that could be identified in the plasmin digestion of β -lactoglobulin.

To our knowledge there is no report that isolates and characterises antibacterial peptides from plasmin-digests of bovine β -lactoglobulin (PD β) against pathogenic and probiotic bacteria.

This study was aimed at exploring the antibacterial effects of bovine plasmin, β -lactoglobulin, PD β and fractions liberated from PD β on a number of pathogenic and probiotic bacteria. Then the antibacterial peptides were identified and compared with nisin in terms of their antibacterial potential.

Materials and methods

Materials

Bovine β -lactoglobulin, bovine plasmin (EC Number 3.4.21.7) and nisin (produced by *Lactococcus lactis*) were supplied by Sigma-Aldrich Chemie GmbH (Munich, Germany). Sodium dihydrogen phosphate and sodium monohydrogen phosphate, used to prepare the buffer solution, were obtained from Merck (Darmstadt, Germany). The reversed phase-high performance liquid chromatography (RP-HPLC) solvents were trifluoroacetic acid (TFA) and acetonitrile (grade A), and both purchased from Merck. For microbiological tests, Brain Heart Infusion Agar (BHIA), Brain Heart Infusion Broth (BHIB), de Man, Rogosa and Sharpe Agar (MRS Agar) and de Man, Rogosa and Sharpe Broth (MRS Broth) were obtained from Merck. Cultures of *Escherichia coli* (PTCC 1399) and *Staphylococcus aureus* (PTCC 1431) came from the Iranian Research Organization for Science and Technology (IROST) in Tehran. Cultures of *Lactobacillus acidophilus* (DSMZ 1643) and *Lactobacillus casei* (DSMZ 1608), as probiotic bacteria, were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures) in Germany.

Methods

Enzymatic proteolysis

Similar to Dalasgaard et al. (2008), to produce plasmin digest of β -lactoglobulin (PD β), 3 mg of bovine β -lactoglobulin was solved in 1 mL of a 10 mM phosphate buffer pH 6.8. Then, bovine plasmin was added to the solution at an enzyme/substrate ratio of 1:150 (v/v). The resulting sample was placed in a shaking incubator at 30°C for 44 h.

Fractionation of the plasmin digest of β -lactoglobulin

Following Dalasgaard et al. (2008), the PD β was fractionated using Unicam crystal 200 series RP-HPLC (Cambridge, UK) which consisted of a G1315B diode-array absorption detector and a G1312A binary pump equipped with an automatic injector. Aliquots of the PD β were injected on a C18 reversed-phase column (15 $\times$ 2.1 mm, 5- μ m particle size). Two solvents were used: (1) 0.1% TFA in water and (2) 80% acetonitrile and 0.1% TFA. The flow rate of the liquid samples was 0.250 mL per min. The samples were eluted in the 20°C thermostated column. We applied a linear gradient of solvent B, with the time schedule being as follows: 2–10 min: 40%, 15 min: 50%, and 45–50 min: 100%. In the end, the column was equilibrated with solvent A for 10 min. The UV detector recorded results at 241 nm. The RP-HPLC separation was repeated three times, and the peptide fractions were collected automatically.

Measurement of concentration of peptide fractions

The concentration (mg/mL) of peptide fractions at 241 nm was determined by comparing the percentage of the area under their peaks with that of the standard peptide (following Josan et al., 2008). Specifically, the percentage of the area under each peptide peak was multiplied by the concentration of the standard peptide divided by the percentage of the area under the peak of the standard peptide.

Determination of antibacterial activity

Plasmin, β -lactoglobulin, the PD β and fractionated peptides were tested to see if they had antibacterial effects on *E. coli*, *S. aureus*, *L. acidophilus*, and *L. casei*.

For the effect of peptide fractions, first the overnight culture of each bacterium was prepared. Then, the colony-forming unit (CFU) of each culture was determined, and the proportion of CFU to optical density was calculated. After that, each culture was diluted to a concentration of approximately 10^6 cells/mL.

Finally, for each sterile Eppendorf vial, around 50 μ L of the 0.45 mg/mL peptide fraction and 10 μ L of the bacterial culture were added to 450 μ L of the BHI broth or the MRS broth. The plasmin, β -lactoglobulin and PD β samples contained 50 μ L of the respective solution.

The control sample contained 50 μ L of the 20 mM phosphate buffer in place of the experimental solutions. In order to make sure the phosphate buffer does not affect bacterial growth, we prepared a blank sample in which the phosphate buffer replaced both experimental solutions and bacterial cultures.

The vials containing pathogenic bacteria (*E. coli* and *S. aureus*) were placed in a normal incubator at 37°C for 18 h, while the vials with probiotic bacteria (*L. acidophilus* and *L. casei*) were placed in a CO₂ incubator under the same conditions. The optical density of each test sample was measured at 620 nm using Cecil 7400 UV-Visible

Spectrophotometer (Cambridge, UK). The difference between mean values of surviving bacteria was statistically determined using Duncan's New Multiple Range Test (MRT), p value < 0.05, on SPSS 19 (2010).

Determination of minimum inhibitory concentration

Minimum inhibitory concentration (MIC) was determined only for antibacterial compound and nisin as a control. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation at an absorbance of 620 nm (McCann et al., 2006).

Different concentrations of the peptide solution and around 10 μ L of the bacterial culture (approximately 10^6 cells/mL) were added to 450 μ L of the BHI or the MRS broth.

The control sample contained different concentrations of the phosphate buffer in place of the peptide solution, and the blank sample contained the phosphate buffer instead of both peptide solutions and bacterial cultures.

The vials containing pathogenic bacteria were incubated in a normal incubator at 37°C for 18 h, whereas the vials having probiotic bacteria were placed in a CO₂ incubator under the same conditions. The optical density of all samples was measured at 620 nm using Cecil Spectrophotometer. The experiments were repeated three times for each sample.

Lastly, the results were compared with the MIC of nisin (an antibacterial peptide produced by *L. lactis*). Nisin was chosen because it is an approved bacteriocin used in food industry (Jay, 2000). Antibacterial test of nisin was performed by adding nisin powder to 0.02 M hydrochloric acid and centrifuging it at 7000 g for 10 min at 4°C. This solution was filtered through a 0.2 μ m filter for sterilisation purposes.

Identification of antibacterial peptides

Antibacterial peptides were identified according to molecular mass. Purified antibacterial peptides were mixed with a solution containing 80% (v/v) acetonitrile and 20% (v/v) water before they were analysed for their molecular mass using an Agilent LC-MS 6410 QQQHPLC Series 1200 (Santa Clara, USA), which consisted of a model G1312 D binary pump, a model G1329A auto sampler and a model G1315 D diode-array detector. The detector was equipped with an electrospray ionisation source (capillary voltage at 3000 V).

The MS spectra of samples were recorded in Full SCAN (from mass/charge 50 to 2000) in positive mode (ESI+), with a fragmentation voltage of 120 V and with a collision voltage of zero. Nitrogen was used as the drying gas at a flow rate of 12 L per min, a nebulising gas pressure of 60 psig (413.7 kPa), and a temperature of 300°C. The Mass Hunter software (Agilent, Santa Clara, USA) was used to calculate the mass of the antibacterial peptides.

The determined molecular masses of the antibacterial peptides were compared with those of peptides generated by theoretical enzymatic digests of the original milk proteins using the ExPASy database (<http://us.expasy.org>). As this database does not contain any data for plasmin, we decided to use the trypsin results instead because the two enzymes function similarly.

Results and discussion

Purification and determination of antimicrobial activity

Proteolytic digestion of β -lactoglobulin by plasmin yielded plasmin digest of β -lactoglobulin (PD β). This compound contained several peptide fragments which could be partially

separated by reversed-phase chromatography. The chromatogram was divided into six peaks numbered from 1 to 6 (Figure 1). The fractions associated with these peaks were named β L1, β L2, β L3, β L4, β L5 and β L6. These fractions had retention times of 10.4, 12.6, 13.8, 14.4, 15.9 and 16.6 min and peptide concentrations of 0.51, 0.72, 0.48, 0.33, 0.57 and 0.27 mg in every 200 μ L.

This research fractionated β -lactoglobulin into β L1, β L2, β L3, β L4, β L5 and β L6 through plasmin hydrolysis. This contrasts with the conclusion of Chen and Ledford (1971) which found β -lactoglobulin is almost non-digestible by plasmin. Like our research, Dalasgaard et al. (2008) were able to fractionate PD β . They came up with five fractionated peptides (named E1, E2, E3, E4 and E5) using a relatively long incubation period (44 h). However, they did not work on the antibacterial properties of the obtained peptides.

Plasmin, β -lactoglobulin, PD β and fractionated peptides were studied for their antibacterial activity against pathogenic (*E.coli* and *S.aureus*) and probiotic bacteria (*L.casei* and *L.acidophilus*). The number of bacteria which survived exposure to fractionated peptides is given in Tables 1 and 2. The β L4 and β L6 were not antibacterial against both pathogenic and probiotic bacteria, but β L1, β L2, β L3 and β L5 showed antibacterial activity against the tested bacteria. These latter fractions are hereafter called antibacterial peptides. It should be noted that these peptides did not stop bacterial growth but only exhibited antibacterial inhibition (at a concentration of 0.5 mg/mL). However, the β L1 peptide was the only peptide that could stop the growth of probiotic bacteria at this concentration.

There was a statistically significant difference in mean number between the bacteria surviving in the presence of antibacterial peptides and those present in the control samples ($P < 0.05$).

In this study, we exposed the ability of plasmin to produce antibacterial peptides (β L1, β L2, β L3 and β L5) from β -lactoglobulin. These findings were similar to those of Pellegrini et al. (2001) who reported that proteolytic digestion of bovine β -lactoglobulin by trypsin yielded four peptide fragments with bactericidal activity.

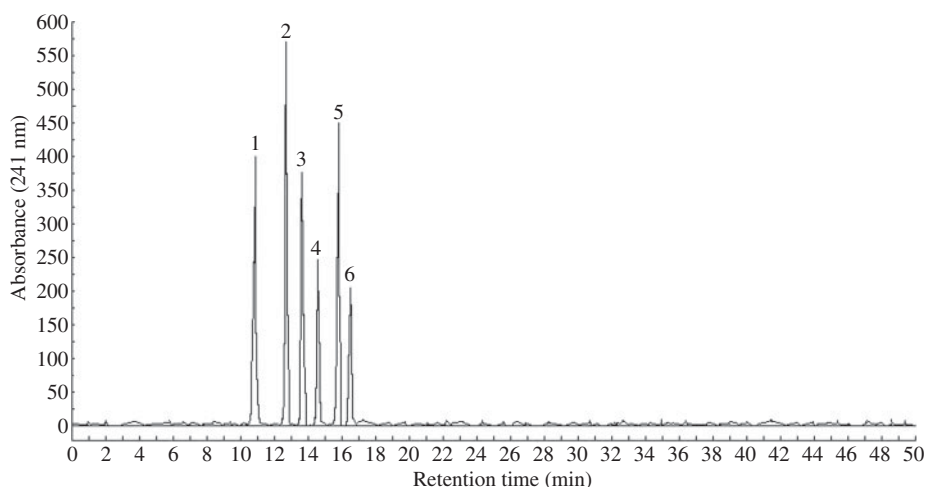


Figure 1. Chromatograms obtained by RP-HPLC with UV detection at 241 nm of peptides after plasmin digestion of β -lactoglobulin.

Table 1. Numbers (log 10 cfu/mL) of surviving pathogenic bacteria following exposure to fractionated peptides.

Bacterial isolate		
Pathogenic bacteria		
Peptides	<i>E. coli</i>	<i>S. aureus</i>
β L1	8.200 $\pm$ 0.16 ^c	7.722 $\pm$ 0.09 ^a
β L2	8.344 $\pm$ 0.11 ^d	8.124 $\pm$ 0.25 ^b
β L3	8.352 $\pm$ 0.11 ^d	8.059 $\pm$ 0.25 ^b
β L4	8.885 $\pm$ 0.14 ^f	8.949 $\pm$ 0.1 ^f
β L5	8.524 $\pm$ 0.2 ^e	8.316 $\pm$ 0.14 ^d
β L6	8.887 $\pm$ 0.2 ^f	8.95 $\pm$ 0.2 ^f
Control	8.885 $\pm$ 0.16 ^f	8.95 $\pm$ 0.14 ^f

Note: Means with different superscripts are significantly different ($P < 0.05$).

β -lactoglobulin similar to β L4 and β L6 peptides could not liberate antibacterial activity against tested bacteria. These results are consistent with the findings of Pihlanto-Leppala et al. (1999), who reported that undigested β -lactoglobulin had no inhibitory activity against *E. coli* JM103 at a high concentration, while proteolytic digested with β -lactoglobulin inhibited the activity of the tested bacteria. In addition to β -lactoglobulin, plasmin had no antibacterial activity on bacteria tested at a maximum concentration of 200 unit/mL. Although Farouk (1982) reported antibacterial activity of trypsin and chymotrypsin, they used higher enzyme concentrations (up to 10,000 unit/mL).

PD β that contained β -lactoglobulin, plasmin, antibacterial peptides and peptides without antibacterial properties proved to be stronger than any of antibacterial peptides in terms of antibacterial potential (data not shown). The PD β inhibited *E. coli* growth (more successfully than the antibacterial peptides) and completely stopped the growth of the other three bacteria. β -lactoglobulin and plasmin had no antibacterial properties, so the high antibacterial potential of PD β might be related to antibacterial peptides and synergism between them. The high antibacterial potential of PD β for controlling bacterial contamination was proved in medium. As a result, further researches are necessary to

Table 2. Numbers (log 10 cfu/mL) of surviving probiotic bacteria following exposure to fractionated peptides.

Bacterial isolate		
Probiotic bacteria		
Peptides	<i>L. casei</i>	<i>L. acidophilus</i>
β L1	ND ^a	ND ^a
β L2	8.599 $\pm$ 0.27 ^b	8.993 $\pm$ 0.37 ^d
β L3	8.588 $\pm$ 0.27 ^b	8.913 $\pm$ 0.37 ^c
β L4	9.610 $\pm$ 0.12 ^f	10.006 $\pm$ 0.3 ^g
β L5	8.903 $\pm$ 0.15 ^c	9.274 $\pm$ 0.26 ^e
β L6	9.610 $\pm$ 0.14 ^f	10.006 $\pm$ 0.14 ^g
Control	9.610 $\pm$ 0.14 ^f	10.006 $\pm$ 0.14 ^g

Note: ND: not determined as no growth was observed following incubation. Means with different superscripts are significantly different ($P < 0.05$).

investigate the ability of PD β in food chain as a natural preservative. β L1, β L2, β L3, β L5 peptides and PD β with antibacterial activity were selected for MIC evaluation in the next step.

Determination of minimum inhibitory concentration

The MIC of the antibacterial peptides (β L1, β L2, β L3, β L5) and PD β were determined against target bacteria, and the results were compared with the MIC of nisin (Table 3). As can be seen, the MIC of PD β and β L1 peptide ranged from 15 to 30 μ g/mL against the Gram-positive bacteria (*S. aureus*, *L. casei* and *Lactobacillus acidophilus*) and was around 50 μ g/mL against Gram-negative bacterium (*Escherichia coli*), indicating that PD β and β L1 are active against all tested bacteria. β L2 and β L3 had MIC ranging from 25 to 40 μ g/mL against the Gram-positive bacteria and higher MIC (70 and 80 μ g/mL) against the Gram-negative bacterium, suggesting that these are generally more destructive of the Gram-positive bacteria. The MIC of β L5 was 50 μ g/mL against the Gram-positive bacteria and 110 μ g/mL against the Gram-negative bacterium, revealing that this peptide was active against the Gram-positive bacteria, but not so against the Gram-negative bacterium. In contrast, nisin showed a relatively low MIC (ranging from 2 to 3 μ g/mL) against Gram-positive bacteria, but a very high MIC (550 μ g/mL) against the Gram-negative bacterium, indicating that nisin was highly destructive of Gram-positive bacteria, but not so against the Gram-negative bacterium.

The β L1, β L2, β L3 and β L5 peptides and the PD β liberating antibacterial activity were against Gram-negative and Gram-positive bacteria. Gram-positive bacteria were more sensitive than the Gram-negative bacterium to the action of the β L1, β L2, β L3 and β L5 peptides and the PD β . The higher resistance of Gram-negative bacteria might relate to the structural complexity of their cell membrane since their cell wall contains, in addition to the cytoplasmic membrane, an outer membrane consisting of lipopolysaccharide, phospholipid, lipoprotein and protein, all supporting the bacterial cell (Hancock & Lehrer, 1998). This is consistent with the findings of López-Expósito, Gomez-Ruiz, Amigo, and Recio (2006b) who reported that Gram-positive bacteria were more susceptible than Gram-negative bacteria to the action of antibacterial peptides.

Nisin proved to be stronger than antibacterial peptides and the PD β in terms of antibacterial activity against Gram-positive bacteria. The inactivity of nisin against the Gram-negative bacterium can be attributed to its relatively large size, which prevents it from penetrating the outer membrane of the Gram-negative cell wall (Heike & Sahl, 2000).

Table 3. Minimum inhibitory concentration (MIC) of antibacterial compounds against tested bacteria (μ g/mL unite).

Peptides	MIC (μ g/mL)			
	<i>E. coli</i>	<i>S. aureus</i>	<i>L. casei</i>	<i>L. acidophilus</i>
β L1	55	30	22	22
β L2	80	40	35	35
β L3	70	35	25	25
β L5	110	50	50	50
PD β	50	20	15	15
Nisin	550	3	2	2

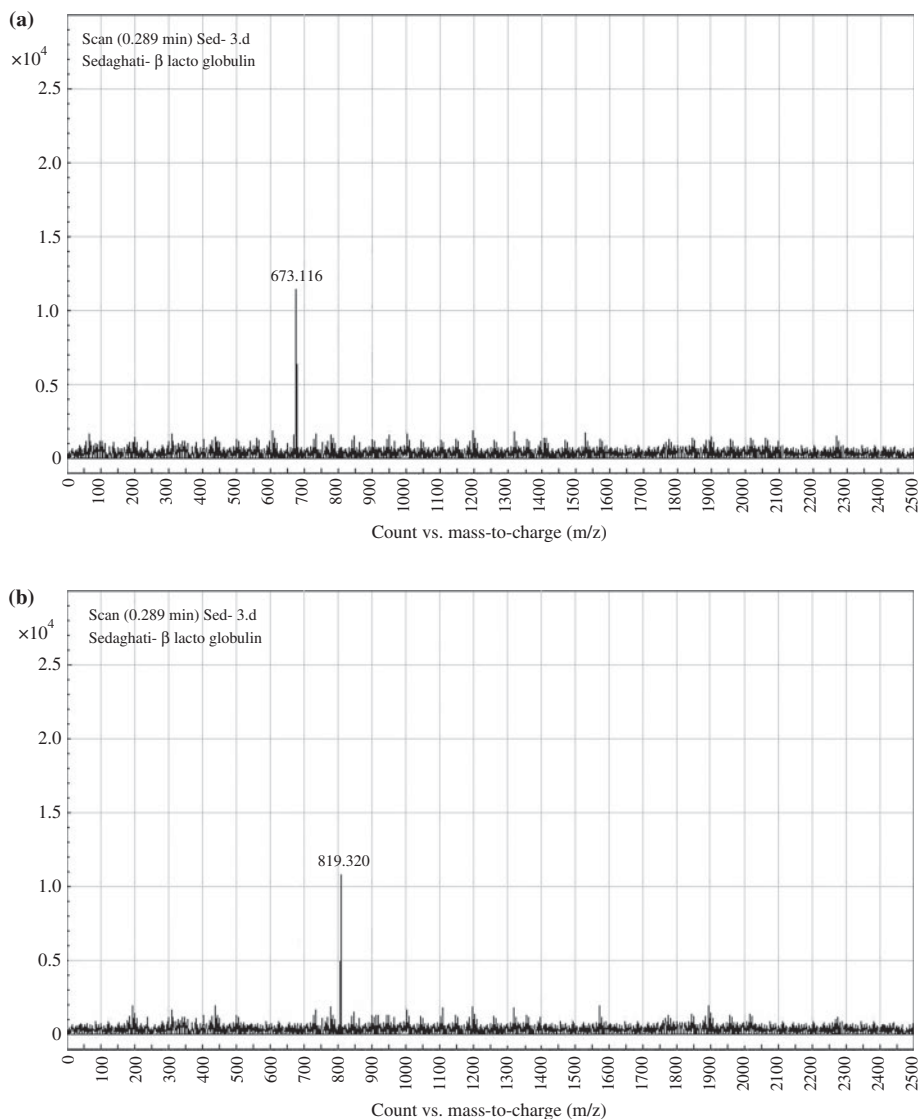


Figure 2. (a) Molecular mass diagram of β L1 peptide; (b) Molecular mass diagram of β L2 peptide.

Identification of antibacterial peptides

The antibacterial peptides were subjected to LC-Mass for determination of molecular mass. Figure 2(a) shows that β L1 has a molecular mass of 673.116 Da, which is close to the theoretical peptide mass (673.850 Da). The molecular mass of this compound exactly corresponds to the f(78–83) of bovine β -lactoglobulin, with an amino acid sequence of IPAVFK and a theoretical pI of 10.1 (83.34% hydrophobic residues). The cleavage of β -lactoglobulin at Lys83-Ile84 was essential for the formation of cationic β L1 peptide. Our findings were similar to those of Dalasgaard et al. (2008) who introduced the

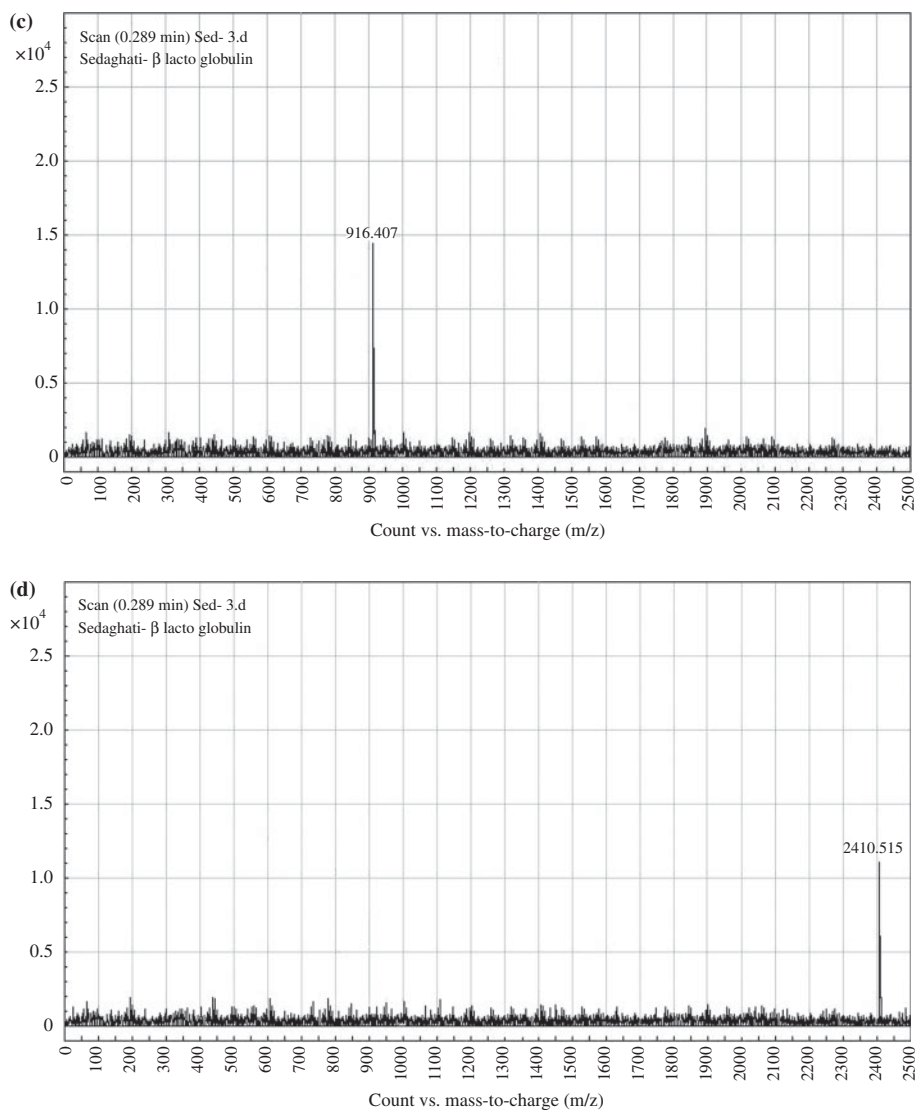


Figure 2. (c) Molecular mass diagram of β L3 peptide. (d) Molecular mass diagram of β L5 peptide.

E1 peptide with similar molecular mass and amino acid sequence (IPAVFK) from β -lactoglobulin.

The mass spectrum (Full Scan ESI) for β L2 shows that this compound has a molecular mass of 819.320 Da (Figure 2(b)). The molecular mass of β L2 does not correspond to the mass of any peptide generated by the theoretical digests. This molecular mass might relate to a peptide with around seven amino acids.

The β L3 peptide has a molecular mass of 916.407 Da (Figure 2(c)), which corresponds to the theoretical peptide mass (916 Da). This compound's molecular mass fully matches the f(83–91) of bovine β -lactoglobulin, with its amino acid sequence being

IDALNENK and its theoretical pI being 4.07 (37.5% hydrophobic residues). The formation of cationic and hydrophobic β L3 required the cleavage of β -lactoglobulin at Lys91-Val92.

The Full Scan ESI mass spectrum of β L5 exposes that this is a compound with a molecular mass of 2410.515 Da (Figure 2(d)). The molecular mass of β L5 corresponds with no masses of peptides produced by the theoretical digests. This molecular mass could relate to a peptide with around 21 amino acids.

Our findings showed that plasmin has an affinity for lysine residue. This is because plasmin attacks Lys-X bonds in two identified antibacterial peptides (β L1 and β L3). The observed affinity of plasmin for lysine residue has also been reported by Bastian and Brown (1996). They found that this enzyme hydrolysed Lys-X and Arg-X bonds and preferentially attacked the former.

Four peptides (β L1, β L2, β L3 and β L5) of the plasmin digest of bovine β -lactoglobulin proved to be antibacterial. The β L1 peptide was the most active antibacterial peptide, corresponding with the f(78–83) sequence of bovine β -lactoglobulin. Although Dalasgaard et al. (2008) reported an E1 peptide of β -lactoglobulin with a similar amino acids sequences, they did not report the antibacterial activity of this peptide. Also the antibacterial peptide with similar amino acid sequence has been reported by Pellegrini et al. (2001). The β L3 peptide was a novel antibacterial domain as its amino acid sequence has not been previously reported. This antibacterial peptide is not similar to the antibacterial domain reported by Pellegrini et al. (2001).

Our results showed that cationic peptide (β L1) and anionic peptide (β L3) have antibacterial activity. These findings were similar to the previous reports that introduced cationic and anionic peptides with antibacterial potential (Epand & Vogel, 1999; McCann et al., 2006; Pellegrini et al. 2001; Zucht et al., 1995).

The current research also explored the relationship between hydrophobicity and antibacterial activity. In addition to β L1 peptide with 83.34% hydrophobicity, hydrophilic β L3 peptide revealed antibacterial activity. This observation disagrees with the finding of Barzyk, Campagna, Wieclaw, Korchowiec, and Rogalska (2009) that 50% or more of residues of an antibacterial peptide should be hydrophobic.

We also investigated the relationship between peptide length and antibacterial activity. The antibacterial peptides, β L1, β L2, β L3 and even β L5 in this work were short (less than 25 amino acid residues). This is consistent with the findings of Rutherford-Markwick and Moughan (2005), who reported that antibacterial peptides should have less than 100 amino acid residues.

Furthermore, an investigation of the amino acid sequences of the antibacterial peptides in this research showed that the C-terminal residue of β L1 and β L3 is a basic amino acid, lysine. This residue may be a deciding factor in antibacterial activity. This finding is in agreement with those of Brown and Hancock (2006), who reported that cationic side chains of arginine, lysine and histidine can mediate in the interaction between peptides and negatively charged bacterial membranes or cell walls, including lipopolysaccharide.

The present research also studied the effects of antibacterial peptides and the PD β on pathogenic and probiotic bacteria in the medium. The future studies may confirm these findings in the food system. The effect of antibacterial peptides on pathogenic bacteria may increase the safety of raw milk and dairy products. However, the problem is that these antibacterial compounds may active against probiotic bacteria. Now, given the fact that the most important qualitative parameter of probiotic microorganisms is their

viability in the final product until the time of consumption (Mortazavian & Sohrabvandi, 2006), it is necessary to evaluate the effect of antibacterial peptides on the viability of probiotic microorganisms in the final product under different conditions (Lahov, Edelsten, Sode-Mogensen, & Sofer, 1971; Lahov & Regelson, 1996).

There is a high interest from the industry in the application of antimicrobial proteins and peptides. Basically, antibacterial peptides can be produced from precursor milk proteins by enzymatic hydrolysis, fermentation, microbial proteolysis and plant mediated proteolysis (Korhonen & Pihlanto-Leppala, 2003). Different methods of antibacterial peptides production suffer from complexity and high cost. The production method of PD β compared with antibacterial peptides seems to be feasible even in large scale without purification and any safety concern. There is also now a current need for more applied research into the use of PD β or antibacterial peptides in the complex food systems, where these peptides are likely to be used.

Conclusions

The present study demonstrated the high potential of PD β as a natural antibacterial agent in the food chain. So there is now a current need for more applied research into the production of PD β in industrial systems and provide interesting opportunities to the dairy industry for expansion of its field of operation. We identified two antibacterial peptides (β L1 and β L3) in the PD β with a potent for inhibiting both Gram-positive and Gram-negative bacteria. Thus these antibacterial peptides might have a potentially valuable role as food additives. Some of the Gram-positive bacteria in our study were probiotic, so the effect of antibacterial peptides on probiotic product should be considered. Antibacterial peptides are effective against Gram-negative bacteria but nisin is more effective against Gram-positive bacteria. Thus the application of a mixture of antibacterial peptides and nisin might improve the quality of the food product.

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