

RESEARCH ARTICLE

Identification and characterization of cow's milk proteins from the rat intestinal lymph using a proteomic strategy

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Food proteins were considered to be absorbed into the body after being digested to amino acids, dipeptides, and tripeptides. However, there are studies indicating that some proteins can pass through the intestinal epithelium under normal physiological conditions, perhaps not in sufficient quantities to be of nutritional importance, but in quantities that may be antigenically or biologically active. In the present study, rat intestinal lymph samples were collected using a modified lymph fistula rat model in fasting and cow's milk postprandial states. Low molecular weight proteins were enriched by ultrafiltration and differential solubilization, separated by 1D-SDS-PAGE, digested in-gel based on molecular weight, and identified using nano-LC-MS/MS. In the postprandial rat intestinal lymph, nine bovine-specific proteins (false discovery rate $\leq 1\%$) were identified in different molecular weight regions. Most proteins identified in lymph were highly abundant proteins in the milk, such as β -lactoglobulin and caseins. Seven of the nine identified bovine-specific proteins are allergens in milk. This strategy can be used to search for proteins that can enter the intestinal lymph and analyze their common features. Understanding the common features of these proteins might help to develop protein drugs taken orally, so that therapeutic proteins might embody fusion domains for cross-barrier transport or translocation.

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1 Introduction

Proteins are one of the most abundant type of molecules in the body. Food protein is an essential nutrient needed by the human body for growth and maintenance. Traditionally, it is believed that food proteins are digested in the gastrointestinal

tract by enzymes to amino acids, dipeptides, and tripeptides [1] and then absorbed into the enterocyte across the brush-border membrane via multiple amino acid [2, 3] and peptide transport systems [4, 5]. The absorption of peptides is more efficient than free amino acids [6, 7].

However, several studies have suggested that some proteins can cross the mature mucosal barrier under normal physiological conditions [8, 9], perhaps not in sufficient quantities to be of nutritional importance, but in quantities that may be antigenically or biologically active [10]. Many food allergens can also be absorbed into the blood through the gastrointestinal tract, for example ovalbumin in eggs [11–13], β -lactoglobulin in milk [13–15], and tropomyosin in shrimp [16]. In addition to the uptake of food-originated proteins, some

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Abbreviations: DS, differential solubilization; IPI, International Protein Index; LMWPs, low molecular weight proteins/peptides; MLNs, mesenteric lymph nodes

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protein drugs such as urokinase [17] and bromelain [18] have been reported to be absorbed in the intestines in normal human subjects. Recently, it has been reported that bioactive peptides derived from milk proteins might enter the body and play an important role in the prevention and treatment of metabolic syndrome and its complications [19]. Thus, many proteins have been reported to be absorbed in the form of intact proteins or large fragments.

It has been reported that free food allergens can reach the mesenteric lymph nodes (MLNs) through the afferent lymph, especially after administration of high doses of food allergens [20]. Primed T cells leave the MLNs via the efferent lymph and migrate either to the mucosa to induce local immune responses or to the periphery for the induction of systemic immune responses after entering the bloodstream through the thoracic duct. Nevertheless, presentation of food allergens to T cells is more efficient and long lasting in the MLNs than in the peripheral lymphoid organs [21]. As a result, when food allergens enter the intestinal lymph, they are more likely to trigger an immune response.

The lymphatic capillaries are different from blood microvessels in several significant ways. Lymphatic capillaries are $\sim 50\ \mu\text{m}$ in diameter and do not have a basement membrane, continuous interendothelial junctions, pericytes, or smooth muscle cells. Instead, they consist of a single layer of endothelial cells that overlap slightly with “buttonlike” junctional protein expression, creating pores up to $2\ \mu\text{m}$ in size allowing the one-way absorption of cells, fluid, and proteins [22]. The protein concentration of lymph is lower than blood [23] and the lymph flow rate is slow compared to the hepatic portal vein blood, so proteins that initially entered intestinal lymph may be less diluted than in blood. Furthermore, the lymph contains proteins produced locally by the surrounding tissues and also antigens that gain access through the mucosal surface [24].

The lymph fistula rat model has been described and utilized by several investigators. Variations in methodology involve differences in cannulationsites, use of anesthesia, and pre- and postoperative care. Bollman et al. [25] described one of the first reports of thoracic and intestinal lymphatic duct cannulation in the anesthetized rat. Tso and Simmonds [26] provided a detailed description of the lymph fistula procedure and collection in unanesthetized animals but the animals were placed in restraint cages. In the present study, some modifications were made on a previous rat model to establish a new model that enabled us to collect intestinal lymph fluid from conscious, unrestrained, and ad libitum fed rats, that is, under almost fully physiological conditions.

Milk and milk-derived foods are very good sources of protein. However, in the Western world, approximately 2.5% of infants/children and 0.3% of the adults are allergic to milk [27]. Consequently, in this study we used a proteomic approach to identify which cow's milk proteins can be absorbed into the rat intestinal lymph under normal physiological condition. The rat intestinal lymph samples were collected using a modified lymph fistula rat model, and low molecu-

lar weight proteins of the lymph were enriched. They were separated by 1D-SDS-PAGE, digested in-gel based on molecular weight, and identified using a nano-LC-MS/MS system. Furthermore, we characterized the milk proteins that can enter the intestinal lymph and examined the shared features of these proteins. This is the first attempt, to our knowledge, to identify food proteins absorbed into the intestinal lymph using proteomic strategy.

2 Materials and methods

2.1 Animals

This study was approved by the Institute of Basic Medical Sciences Animal Ethics Committee at the Peking Union Medical College (Animal Welfare Assurance number: #A5518–04). Specific pathogen-free male Sprague-Dawley rats (250–300 g) were purchased from the Institute Of Laboratory Animal Science, Chinese Academy Of Medical Science & Peking Union Medical College. They were given a standard laboratory diet and free access to tap water, and kept in a room with controlled temperature ($22 \pm 1^\circ\text{C}$) and humidity (65–70%), and a 12:12 h light:dark cycle.

2.2 The modified intestinal lymph fistula rat model

Details of the surgical procedure have been described previously [25, 28, 29] with some modifications. The rats were fasted for 24 h before surgery but had free access to tap water throughout. Under pentobarbital anesthesia, a laparotomy was performed, and the small and large intestines were exteriorized, placed on the left side of the animal, and covered with gauze, which was kept moist with warm saline. The liver was retracted toward the diaphragm and kept out of the way with a piece of wet gauze. The superior mesenteric lymph duct was cannulated with a tubing (medical grade; 0.50-mm id and 0.80-mm od; Tyco Electronics, Castle Hill, Australia) filled with undiluted heparin [30]. The lymphatic tube was secured in place with a drop of cyanoacrylate glue and was passed through the abdominal wall and exteriorized at the dorsum of the neck [31]. The intestines were rinsed with warm saline and replaced into the abdominal cavity. Abdominal skin and muscle were closed with resorbable sutures. An Eppendorf tube preloaded with protease inhibitors (final concentrations, $16.7\ \mu\text{M}$ bestatin, $8.3\ \mu\text{M}$ pepstatin, and $5\ \text{mM}$ EGTA; Sigma-Aldrich, Australia) was fixed to the neck skin. The Eppendorf tube had two holes drilled in the cap, one for the lymphatic tube insertion and the other for lymph collection using a 1 mL syringe. After surgery, the rat was kept independently and had free access to tap water.

2.3 Collection of mesenteric lymph

Fasted lymph samples were collected after the surgery every hour using a 1 mL syringe. Four hours later, the rat was

given cow's milk diet with a bottle of 200 mL milk (Meng Niu Dairy Company Limited, China) for the rats to eat freely. Postprandial lymph samples were collected every half hour until 16 h after surgery. The collecting Eppendorf tubes were preloaded with protease inhibitors to prevent any ex vivo protein modification of the intestinal lymph samples. At the end of the experiment, the lymph samples were centrifuged ($1700 \times g$, 4°C , 10 min) to remove any cellular materials [32] and immediately stored at -80°C until analysis.

2.4 Enrichment of low molecular weight proteins/peptides

To enrich low molecular weight proteins/peptides (LMWPs), two different approaches were used: ultrafiltration and differential solubilization (DS). The procedures are described elsewhere [33, 34] with some modifications given below.

2.4.1 Centrifugal lymph ultrafiltration

The centrifugal filter membranes were rinsed and used according to the manufacturer's specifications. A 3 mL aliquot of lymph sample was diluted with 15 mL of 25 mM NH_4HCO_3 (pH 8.2), 20% v/v ACN and applied onto a Centrplus centrifugal concentrator membrane (MWCO 30 000, Millipore, Billerica, MA, USA). The sample was centrifuged at $3000 \times g$ until 90% of the input sample had passed through the membrane. The filtrate was lyophilized to dryness and resuspended in 30 μL of 25 mM NH_4HCO_3 (pH 8.2) and stored at -80°C until use.

2.4.2 DS

A 50 μL lymph sample was diluted 1:2 with 100 μL of denaturing solution (7 M urea, 2 M thiourea, and 20 mM DTT), slowly dropped into 4 mL precooled acetone (-20°C), and immediately stirred by vortex mixing at 4°C for 12 h, followed by centrifugation at $19\,000 \times g$ for 30 min at 4°C . The precipitate was brought up in 500 μL of 70% ACN containing 12 mM HCl and mixed at 4°C for 2 h, centrifuged again at $19\,000 \times g$ for 15 min at 4°C . The LMWPs were extracted into the supernatant, which was lyophilized and stored at -80°C until it was analyzed by tricine-SDS-PAGE.

2.5 Tricine-SDS-PAGE and in-gel digestion

The lyophilized low molecular weight proteins extracted from the lymph were dissolved in PAGE sample buffer (50 mM Tris-HCl, pH 6.8, containing 50 mM DTT, 0.5% SDS, and 10% glycerol) and incubated at 57°C for 5 min. The solution was resolved by tricine-SDS-PAGE [35]. After electrophoresis, each gel band was cut into slices according to the molecular weight. The gel slices were washed twice by incubation in

100 mM ammonium bicarbonate for 15 min at 37°C , followed by 100 mM ammonium bicarbonate/ACN (1:1) for 15 min at 37°C . After cooling to room temperature, the samples were reduced using 20 mM DTT with heating at 56°C for 1 h, followed by alkylation using 55 mM iodoacetamide at room temperature for 1 h in the dark. The gel slices were dried, and sequence grade modified trypsin was added at a protein-to-enzyme ratio of 30:1, followed by incubation overnight at 37°C . The resulting peptides were extracted from the gel slices by incubation once in 50 mM ammonium bicarbonate for 1 h at 37°C and twice in 5% formic acid/ACN (1:1) for 1 h at 37°C . The three collected extractions were pooled with the initial digestion supernatant, desalted by SPE (Oasis column; Waters, Inc., Milford, MA, USA), dried in a Speed-Vac (SAVANT RVT4104, Thermo Fisher Scientific, Bremen, Germany), resuspended with 10 μL of 0.1% formic acid, and stored at -80°C until use.

2.6 Nano-LC-MS/MS analysis

The resulting peptides were analyzed by nano-LC-MS/MS using an Agilent 1200 HPLC system coupled to an LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Each sample was loaded with the maximal volume of 8 μL onto a Michrom Peptide Captrap column (MW 0.5–50 kDa, 0.5×2 mm; Michrom Bioresources, Billerica, MA, USA) with a flow rate of 20 $\mu\text{L}/\text{min}$ in 0.1% formic acid 99.9% water. The trap column effluent was transferred to an RP microcapillary column (0.1×150 mm, packed with Magic C18, 3 μm , 200 Å; Michrom Bioresources) in an Agilent 1200 HPLC system. The elution gradient for the reverse column changed from 95% mobile phase A (0.1% formic acid, 99.9% water) to 40% mobile phase B (0.1% formic acid, 99.9% ACN) within 120 min at a flow rate of 500 nL/min. The LTQ-Orbitrap Velos mass spectrometer was operated in data-dependent acquisition mode. Survey scan MS was acquired in the Orbitrap in the 300–2000 m/z range with the resolution set to a value of 60 000. The 20 most intense ions per survey scan were selected for CID fragmentation, and the resulting fragments were analyzed by the LTQ-Orbitrap Velos mass spectrometer. Dynamic exclusion was employed with a 60-s window to prevent repetitive selection of the same peptide.

2.7 Protein sequence database searching and data validation

The MASCOT Daemon software (version 2.4.0, Matrix Science, London) was used to search the MS/MS data against a combined database (70 328 sequences) made up of the International Protein Index (IPI) Bovine database v3.73 and IPI rat database v3.87. Individual MASCOT search was performed for each gel slice. Carbamidomethylation of cysteines was set as a fixed modification; oxidation of methionine and protein N-terminal acetylation were set as a variable modification. The specificity of trypsin digestion was set for cleavage after

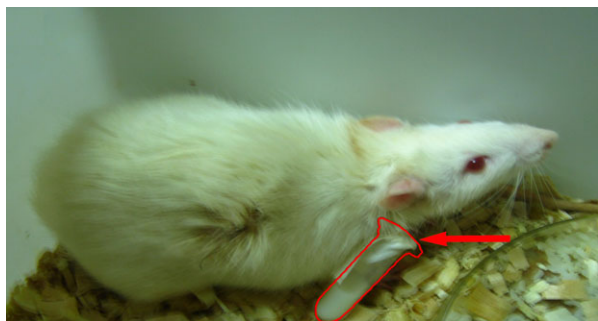


Figure 1. The modified lymph fistula rat model. The arrow indicates the collection tube.

K (lysine) or R (arginine), and two missed trypsin cleavage sites were allowed. The mass tolerances in MS and MS/MS were set to 5 ppm and 0.6 Da, respectively, and the instrument setting was specified as “ESI-Trap.” After the MASCOT search, the significance threshold and ion score cutoff were set to 0.05 using MudPIT (Matrix Science, London, UK) protein scoring. The false discovery rate was adjusted to 1% when the search result was exported. Only proteins that presented at least two significant sequences (ion scores >26) were reported using the report builder.

2.8 Data analysis

The potential bovine proteins were singled out from the MASCOT results. To identify bovine-specific proteins from the rat intestinal lymph, all the peptides of potential bovine proteins were searched against the IPI rat database v3.87 manually. Only the peptides that were not found in the IPI rat database were considered to be the bovine-specific peptides. Proteins identified with at least one bovine-specific peptide were considered to be bovine-specific proteins.

2.9 Analysis of PTM

The PEAKS DB was developed by incorporating the de novo sequencing results into the database search, which makes

it a particularly useful tool for identifying peptides with PTMs [36]. The MS/MS data were searched against IPI Bovine database v3.73 using the PEAKS 6 software according to the manufacturer's instructions.

3 Results

3.1 The modified lymph fistula rat model and collection of intestinal lymph samples

Several animal models for continuous collection of intestinal lymph fluid have been described [37]. The commonly employed procedures, however, used anesthetized and/or restrained animals. As a result, animals were not able to eat and drink freely. In the present study, some modifications were made on a previous rat model to establish a new model that enabled us to collect intestinal lymph fluid from conscious, unrestrained, and ad libitum fed rats, that is, under almost fully physiological conditions (Fig. 1).

Using this rat model, the intestinal lymph samples were collected from four rats in fasted and postprandial states. Although the lymph flow rates of the four rats were different from each other, the general trend was similar (Fig. 2). After surgery, the flow rate gradually increased and reached 700 $\mu\text{L/h}$ approximately 4 h later, an effect that may be due to the rats' recovered consciousness from anesthesia. When the rats were given cow's milk diet, the flow rate increased sharply, achieved its peak approximately 1 h later, and lasted nearly an hour, then dropped gradually to the baseline. These patterns are similar to previous studies [37, 38].

3.2 Enrichment of LMWPs, 1D-SDS-PAGE separation and in-gel digestion

The LMWPs were enriched by two different methods, ultrafiltration and DS and separated by tricine-SDS-PAGE. The resulting SDS-PAGE bands were different between these two methods (Fig. 3).

After electrophoresis, the ultrafiltration gel band was cut into four slices (Fig. 3B), and the F and P gel bands were cut into five slices (Fig. 3C) according to the molecular weight.

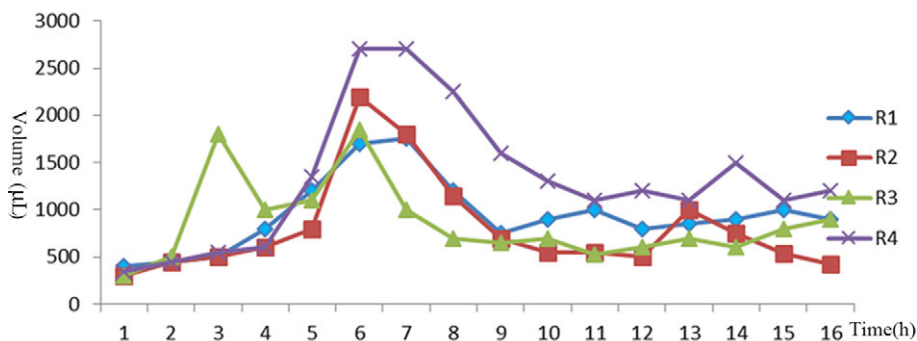


Figure 2. Volume of lymph collected at various sampling times. Lines of different colors represent individual rats, plotted by the time (h) on the horizontal axis and yield of lymph (μL) on the vertical axis.

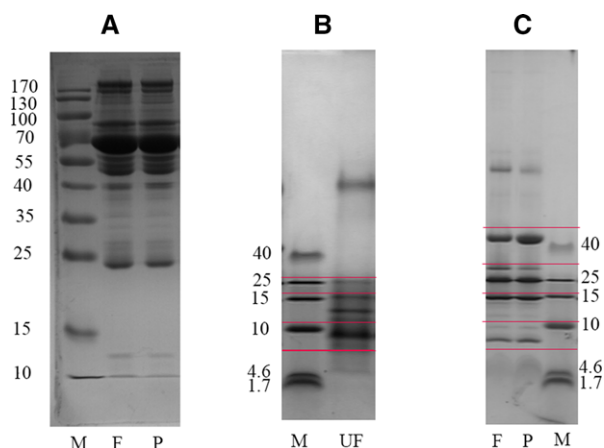


Figure 3. (A) SDS-PAGE analysis of the lymph samples; M, molecular weight marker; F, fasted lymph; P, postprandial lymph. (B) SDS-PAGE analysis of LMWPs of postprandial lymph enriched by ultrafiltration (UF). (C) SDS-PAGE analysis of LMWPs enriched by DS; F, fasted lymph; P, postprandial lymph; molecular weight unit, kDa.

The resulting peptides were analyzed by nano-LC-MS/MS after in-gel digestion. In this way, the approximate experimental molecular weight of the identified proteins can be obtained.

3.3 The bovine-specific proteins of intestinal lymph

Using nano-LC-MS/MS, nine bovine-specific proteins were identified in the rat intestinal lymph after cow's milk diet was given, and no bovine-specific proteins were found in the fasted lymph samples. There were four bovine-specific proteins in the postprandial lymph samples enriched by DS (Table 1). In the ultrafiltration samples, nine bovine-specific proteins were identified, six of which were found in two different rats (Table 2). The mass spectra for the bovine-specific proteins in the Supporting Information. All the bovine-specific proteins identified using the DS method were found in the sample enriched by ultrafiltration.

Most of the bovine-specific proteins were identified in different molecular weight regions (gel slices), implying that these proteins might exist in the intestinal lymph in various forms (Supporting Information Tables 1 and 2). Many protein fragments are identified in our study, especially BSA.

The molecular weight of BSA is about 69 kDa, but they were identified in the gel slices with the molecular weight <50 kDa. Thus, the BSA identified in our study is in the form of fragments. However, the molecular weights of β -lactoglobulin and caseins are 20 and 25 kDa, and they were identified in the gel slices with molecular weight 10–30 kDa. As a result, these two proteins were identified in the forms of intact proteins and fragments. The protein fragments may be generated by digestive enzymes in the intestine or proteolytic enzymes in the lymph. Moreover, proteins that were identified in a higher molecular weight region may have PTM.

The main bovine-specific proteins identified in the rat intestinal lymph were β -lactoglobulin, serum albumin, and caseins (Tables 1 and 2). These proteins are also the most abundant proteins in milk; caseins make up 80% of the total milk protein, β -lactoglobulin is approximately 10%, and serum albumin is about 1% [39, 40]. α -Lactalbumin is also a major protein in cow's milk, and it makes up approximately 3.5% of the total protein [39]. In summary, most of the identified bovine-specific proteins are highly abundant protein in the milk.

Furthermore, the isoelectric point of most bovine-specific proteins identified in lymph was <7, which may due to that the acidic proteins may be absorbed into the lymph easily (Supporting Information Tables 1 and 2) or were present in the milk with greater levels as compared to basic proteins. Other physicochemical properties of these proteins were also examined, however, nothing in common was found.

4 Discussion

During the collection of lymph samples, the rats were injected antibiotics after surgery to prevent infection and after 4 h recovery from surgery, rats were given a milk diet and the lymph samples were collected. As the intestinal lymph flowed out continuously, and if the rats were give too much time for recovery, the loss of intestinal lymph may influence the rat's health condition. Although the rat could survive for >1 wk after surgery, but the lymph flow rates declined gradually (data not shown). After about 3 days, the rates were too low, so the lymph was likely to clot. To balance between the postsurgical influences and loss of intestinal lymph, we started to collect the lymph samples after 4 h recovery. During the collection of lymph samples, the feces of the rats were normal-looking and there were no symptoms of postsurgical diarrhea.

Table 1. Bovine-specific proteins identified in the LMWPs of postprandial lymph enriched by DS

Accession number	Leading protein name	MS/MS ion scores	Unique peptides	Bovine-specific peptides	Total spectra counting	Protein coverage	Protein mass (Da)
P02769	Serum albumin	67	8	6	25	0.103	1521
P02754	β -Lactoglobulin	53	5	5	16	0.135	20 269
P02666	β -Casein	36	2	2	3	0.036	25 148
P02662	α -S1-casein	29	2	2	2	0.117	24 570

The ion scores of all the peptides are $\geq 95\%$ confidence (ion scores >26).

Table 2. Bovine-specific proteins identified in the LMWPs of postprandial lymph enriched by ultrafiltration

Accession number	Leading protein name	MS/MS ion scores	Unique peptides	Bovine-specific peptides	Total spectra counting	Protein coverage	Protein mass (Da)
P02754	β -Lactoglobulin ^{a,b)}	57	9	9	57	0.298	20 269
P02769	Serum albumin ^{a,b)}	89	8	6	32	0.100	71 521
P02662	α -S1-casein ^{b)}	47	2	2	20	0.122	24 570
P02663	α -S2-casein ^{a,b)}	35	6	6	19	0.126	26 173
P02668	k-Casein ^{b)}	32	3	3	11	0.100	21 370
P02070	Hemoglobin subunit β	28	2	2	7	0.166	16 001
P02666	β -Casein ^{a,b)}	31	2	2	4	0.042	25 148
P00711	α -Lactalbumin	41	2	2	4	0.074	16 235
IPI00712994 ^{c)}	Uncharacterized protein	27	2	2	3	0.093	18 321

a) Identification by two methods.

b) Identification in two rats.

c) No matched Swiss-Prot ID in the databases. The ion scores of all the peptides are $\geq 95\%$ confidence.

The low molecular weight fractions of the lymph were enriched for any proteinaceous material therein and peptides were identified using nano-LC-MS/MS, so whether the high molecular weight proteins, such as the intact BSA, could be absorbed into the intestinal lymph remains unknown. However, in the high molecular weight regions of lymph, there are vast amounts of rat proteins (Fig. 3A), which makes it difficult to identify the extraneous proteins.

All nine bovine-specific proteins were found for the first time in the lymph after the rats were given cow's milk diet. β -Lactoglobulin is a food allergen in milk, and it has been identified in serum by ELISA in many previous studies [13, 15]. The specific antibodies were found in the plasma after BSA was ingested orally [9, 41], which indirectly indicates that this protein could be absorbed into circulation. The remaining seven proteins are the newly identified proteins. Using these methods, it is simple and efficient to identify food proteins from lymph.

β -Lactoglobulin and caseins are the major allergens of cow's milk [42]. β -Lactoglobulin possesses the common features of food allergens, such as heat-, enzyme-, and low pH-resistance and water-solubility [43]. In addition, β -lactoglobulin is a member of lipocalin family, has the typical lipocalin structure [44], and exhibits significant fatty acid binding [45]. One of the major functions of the intestinal lymph is lipid absorption and translation, and cow's milk contains vast amounts of fat. As a result, when bound to fatty acids, β -lactoglobulin may be transferred to the lymph more easily. Casein is relatively hydrophobic, making it poorly soluble in water. It is found in milk as a suspension of particles called "casein micelles," which show only limited resemblance to surfactant-type micelles in the sense that the hydrophilic parts reside at the surface, and they are spherical [46]. Caseins can bind to lipoproteins in the chylomicrons [47], which can promote intestinal absorption and systemic dissemination of dietary antigens [48], and we think this may be one of the mechanisms the caseins enter lymph.

We do not know if PTM plays a role in protein entering the lymph. Identifying PTMs without enrichment adds no

extra steps to conventional identification procedures. The only difference is that this method requires software that can conduct unrestricted identifications of PTMs. The PEAKS DB is a powerful software for the identification of PTMs [36]. We used PEAKS to search the MS/MS data of postprandial lymph samples, but there were no shared or similar PTMs among the bovine-specific proteins (data was not shown), and this may be due to that it was not the comprehensive PTM information as the sequence coverage was not very high. However, PTMs of these bovine-specific proteins should be studied more closely in further studies, as PTM may be a factor of protein entering the lymph.

There are several routes that have been reported to allow the passage of exogenous proteins interintestinally, including transport through the specialized microfold (M) cells of Peyer's patches and isolated lymphoid follicles or across the epithelium, either via the cells (transcellular pathway) or between the cells (paracellular pathways) [49]. Most absorbed food proteins will be degraded by the enzymes present in the endocytic vesicles and lysosomes. However, a small proportion of intact food proteins could reach the lamina propria and intestinal lymph [50]. Then, the antigen-presenting cells, especially the dendritic cells, can capture these proteins and migrate via draining lymph to regional lymph nodes where they become active antigen-presenting cells, which stimulate T cells and B cells for productive or downregulatory (suppressive) immune responses [51]. In the present study, seven of the nine bovine-specific proteins identified from the lymph are known milk allergens [42]. Therefore, the remaining two proteins (hemoglobin subunit β and uncharacterized protein (IPI00712994)) may be new milk allergens. However, further studies will be needed to clarify whether they could cause food allergies. Consequently, our strategy may be used as an experimental method to search for novel food allergens.

This strategy can be used to search for proteins that can enter the intestinal lymph and analyze their common features. Understanding the common features of proteins that can enter the lymph might help to develop protein drugs taken

orally, so that therapeutic proteins might embody fusion domains for cross-barrier transport or translocation. Furthermore, approaches to reduce the absorption of dietary proteins that trigger food allergies may be explored.

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The authors have declared no conflict of interest.

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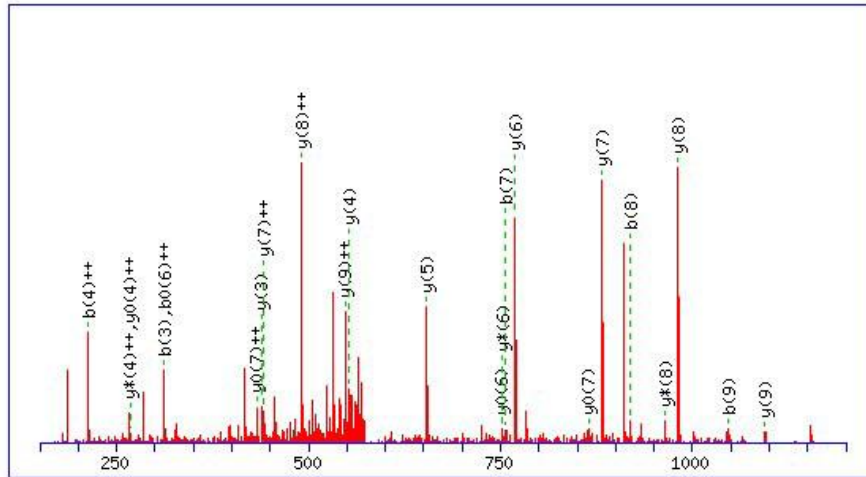
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The mass spectra for the bovine-specific proteins

MS/MS Fragmentation of **VLVLDTYKK**

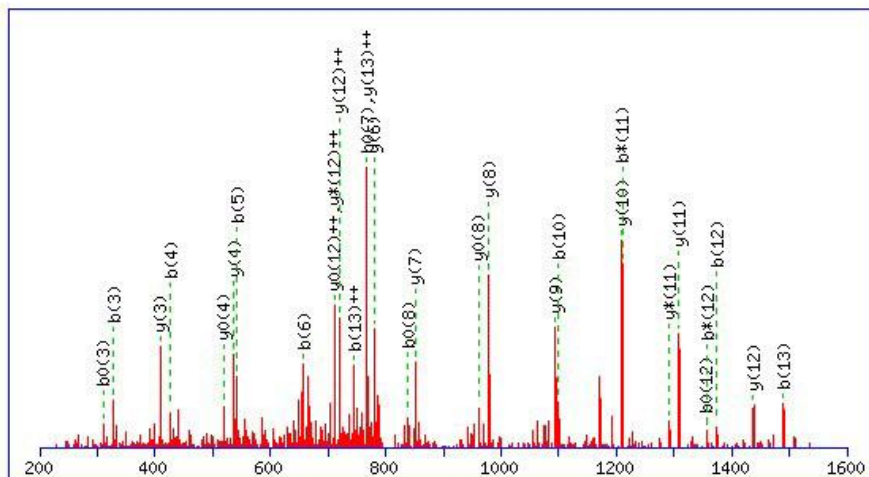
Found in **IP100699698**, Tax_Id=9913 Gene_Symbol=PAEP Beta-lactoglobulin



Monoisotopic mass of neutral peptide Mr(calc): 1192.6703
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 26 Expect: 0.0025
Matches : 24/78 fragment ions using 60 most intense peaks

MS/MS Fragmentation of **TPEVDDEALEKFDK**

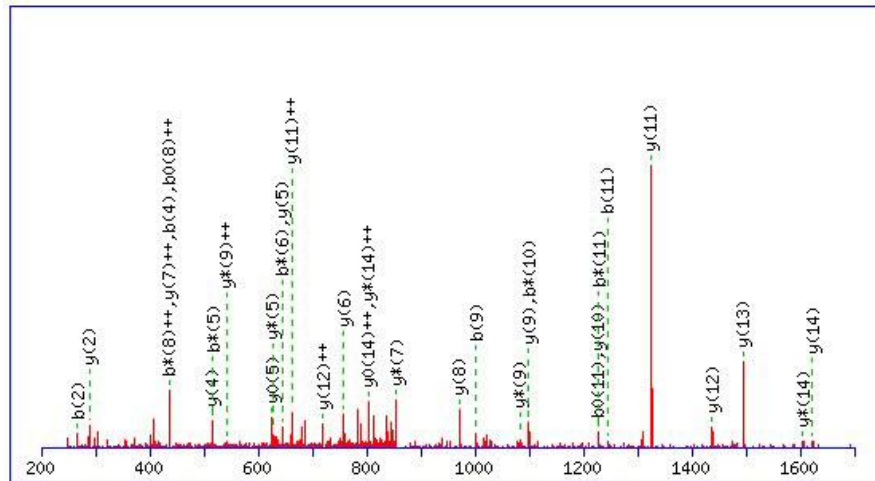
Found in **IP100699698**, Tax_Id=9913 Gene_Symbol=PAEP Beta-lactoglobulin



Monoisotopic mass of neutral peptide Mr(calc): 1634.7675
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Ions Score: 155 Expect: 3.5e-016
Matches : 32/134 fragment ions using 40 most intense peaks

MS/MS Fragmentation of **HQGLPQEVLENLLR**

Found in **IPI00706094**, Tax_Id=9913 Gene_Symbol=CSN1S1 Alpha-S1-casein



Monoisotopic mass of neutral peptide Mr(calc): 1758.9377

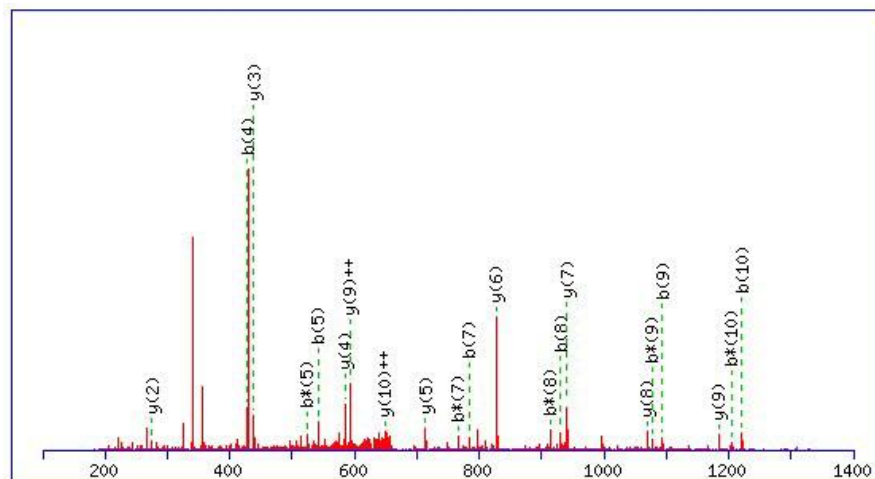
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ions Score: 154 Expect: 3.7e-016

Matches : 33/146 fragment ions using 43 most intense peaks

MS/MS Fragmentation of **ALNEINQFYQK**

Found in **IPI00698843**, Tax_Id=9913 Gene_Symbol=CSN1S2 Alpha-S2-casein



Monoisotopic mass of neutral peptide Mr(calc): 1366.6881

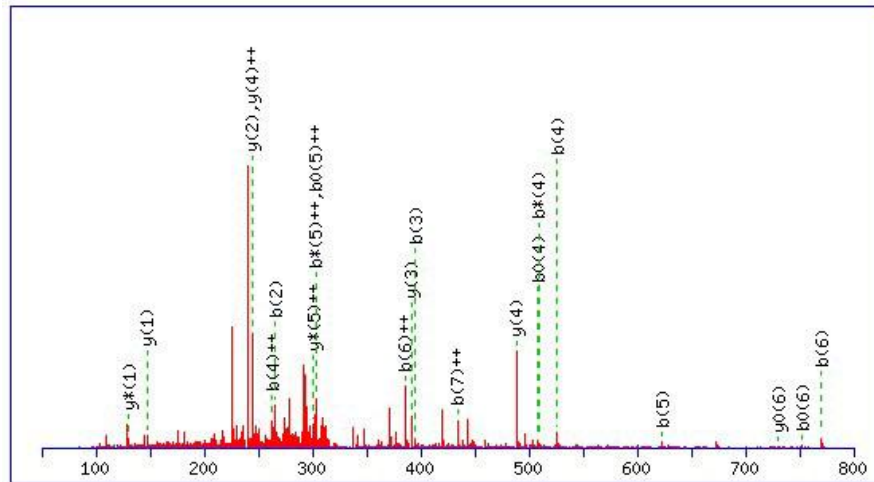
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ions Score: 23 Expect: 0.0046

Matches : 21/96 fragment ions using 48 most intense peaks

MS/MS Fragmentation of **HKEMPFPK**

Found in **IPI00697085**, Tax_Id=9913 Gene_Symbol=CSN2 Beta-casein



Monoisotopic mass of neutral peptide Mr(calc): 1012.5164

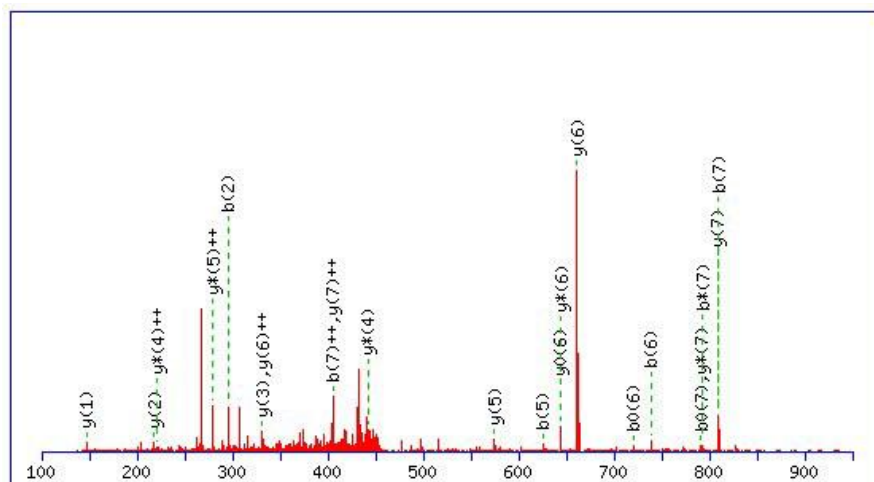
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ions Score: 16 Expect: 0.025

Matches : 21/68 fragment ions using 63 most intense peaks

MS/MS Fragmentation of **FFSDKIAK**

Found in **IPI00707811**, Tax_Id=9913 Gene_Symbol=CSN3 Kappa-casein



Monoisotopic mass of neutral peptide Mr(calc): 954.5175

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Ions Score: 20 Expect: 0.0093

Matches : 22/64 fragment ions using 48 most intense peaks