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CHARACTERIZATION OF WHEY PROTEINS FROM MONGOLIAN YAK, KHAINAK, AND BACTRIAN CAMEL

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ABSTRACT

*The composition of whey proteins from ruminant Mongolian domestic animals was analyzed and a comparative study between camel (*Camelus bactrianus*) and dromedary (*Camelus dromedarius*) was made. Whey proteins were separated by ion-exchange chromatography and identified by polyacrylamide gel electrophoresis, amino acid composition and N-terminal sequence determination. The main components of wheys of yak and khainak were nearly identical with their bovine counterparts. Three different forms of α-lactalbumin were isolated in the whey of* Camelus bactrianus *and two from* Camelus dromedarius. *As shown by classical biochemical and immunological studies, β-lactoglobulin was absent from whey of both* Camelus. *Camel whey basic protein (CWBP), having no analogy with known milk and nonmilk proteins, was identified in the whey of* Camelus bactrianus *and* Camelus dromedarius *and its N-terminal sequence was determined.*

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INTRODUCTION

Whey is a coproduct of the dairy industry, produced in large amounts (about 130 millions tons per year; Zall 1992), from cheese or casein production. Whey proteins have many important techno-functional properties, including water binding, emulsification, gel formation, and have excellent nutritional quality. Whey proteins are excellent candidates for the development of new products. The growing interest in their functional use (Kim *et al.* 1987) stimulated the production of a wide range of whey protein products. Food products which include whey proteins due to their beneficial functional properties are beverages (solubility), confectionery (whippability), desserts (emulsification, whippability), dairy products (viscosity), meat products (fat-, water-binding), bakery products (heat stability), infant formula (low-allergenicity). Further utilization of whey proteins requires the implementation of more extensive strategies based upon compositional, physico-chemical and functional properties. As is well known, β-lactoglobulin and α-lactalbumin are the two major whey proteins in milk. β-Lactoglobulin is the dominant whey protein in the milk of various species: bovidae (Palmer 1934), pig (Kessler and Brew 1970), horse (Bell *et al.* 1981), red deer (McDougall and Stewart 1976), donkey (Liberatori *et al.* 1979), sheep (Bell and McKenzie 1964), and goat (Préaux *et al.* 1979). In contrast, α-lactalbumin is the dominant protein in rodent (Vilotte and Soulier 1992), llama (Cantisani *et al.* 1990), camel (Beg *et al.* 1985) and human whey (Brignon *et al.* 1985) and the milk of these species is probably devoid of β-lactoglobulin.

Conti *et al.* (1985) investigated *Camelus dromedarius* whey proteins by gel filtration on Sephadex G-100 and determined some of its components by electrophoresis. This study also revealed the presence of immunoglobulins and serum albumin in camel whey. Two variants of α-lactalbumin were isolated and named α-lactalbumin A and B. They show differences in their isoelectric point (5.1 and 5.3, respectively), amino acid composition and N-terminal sequence. Beg *et al.* (1985) published the complete amino acid sequence of *Camelus dromedarius* α-lactalbumin. Camel α-lactalbumin has a molecular mass of 14.6 kDa, contains 123 amino acid residues, and shows SDS-PAGE mobility like bovine α-lactalbumin. Beg *et al.* (1984, 1986, 1987) separated and characterized three new camel whey proteins. One of them has a molecular weight of 14.0 kDa, contains 117 amino acid residues and is rich in cysteine/half cystine (16 half cystine residues). This new protein shows structural similarities with bovine β-casein A2 at its N-terminal region. The second new camel whey protein consists of 64 amino acid residues and is a fragment of camel β-casein, homologous to the C-terminal fragment of bovine β-casein. The third new camel whey protein found by Beg *et al.* (1987) has 112 amino acid residues, a molecular mass of about 15.0 kDa and was named 'novel camel whey protein'.

Cantisani *et al.* (1990) isolated a protein from llama whey with a molecular mass of 30 kDa that was homologous to the 'novel camel whey protein' Sorensen and Petersen (1992) described a protein in the bovine proteose-peptone fraction with a molecular mass of 17.0 kDa, which displays homologous fragments to the 'novel camel whey protein'. The question, does β-lactoglobulin exist in camel whey? is still unsolved since Liberatori *et al.* (1979) observed antibodies with a cross-reactivity to bovine β-lactoglobulin in camel colostrum in higher titers.

The present work aims at characterizing the physico-chemical properties of the major whey proteins from Mongolian domestic livestock animals such as yak (the polymorphism of whey proteins from Mongolian cow and yak has been yet studied by Grosclaude *et al.* (1976, 1982)), khainak (hybrid of yak and cow) and *Camelus bactrianus*. Moreover, these whey proteins were compared with those of cow and *Camelus dromedarius*, which are well known.

MATERIALS AND METHODS

Preparation of Whey Proteins

Milk was collected as previously described (Ochirkhuyag *et al.* 1997). It was defatted by centrifugation at 37C, 4000 rpm for 15 min. Casein was precipitated at its isoelectric point (pH 4.6) by using 1 N HCl. After centrifugation, the resulting supernatant was dialyzed against distilled water and then freeze dried. Samples were kept at -20C until used.

Fractionation of Whey Proteins

The individual whey proteins were purified by ion-exchange chromatography on a DEAE-Sephacel (Pharmacia, Orsay, France) column (26 mm i.d. × 26 cm) by applying a gradient (Fig. 1) from buffer A (50 mM Tris-HCl, pH 8.0, 5 mM $CaCl_2$) to buffer B (50 mM Tris-HCL, pH 8.0, 5mM $CaCl_2$, 1 M NaCl), at room temperature, at a flow rate of 2.5 mL/min. The ion-exchange chromatography was carried out on an Econo system (Bio-Rad, Ivry sur Seine, France).

High Performance Liquid Chromatography (HPLC)

Analytical and preparative reversed-phase HPLC separations were performed on a Waters instrument (Waters Associates, Millford, MA) equipped with an interface module system, assisted by a chromatography work station Maxima 820. Analytical RP-HPLC of whey proteins was carried out on a Nucleosil 5 C_{18} column (4.6 mm i.d. × 25 cm), at a flow rate of 1 mL/min. A LiChroCART 100 C_{18} (10 mm i.d. × 25 cm) column was used for preparative

run, at a flow rate of 2 mL/min. Absorbency was recorded at 214 nm. Buffers and gradients were used according to Pearce (1983).

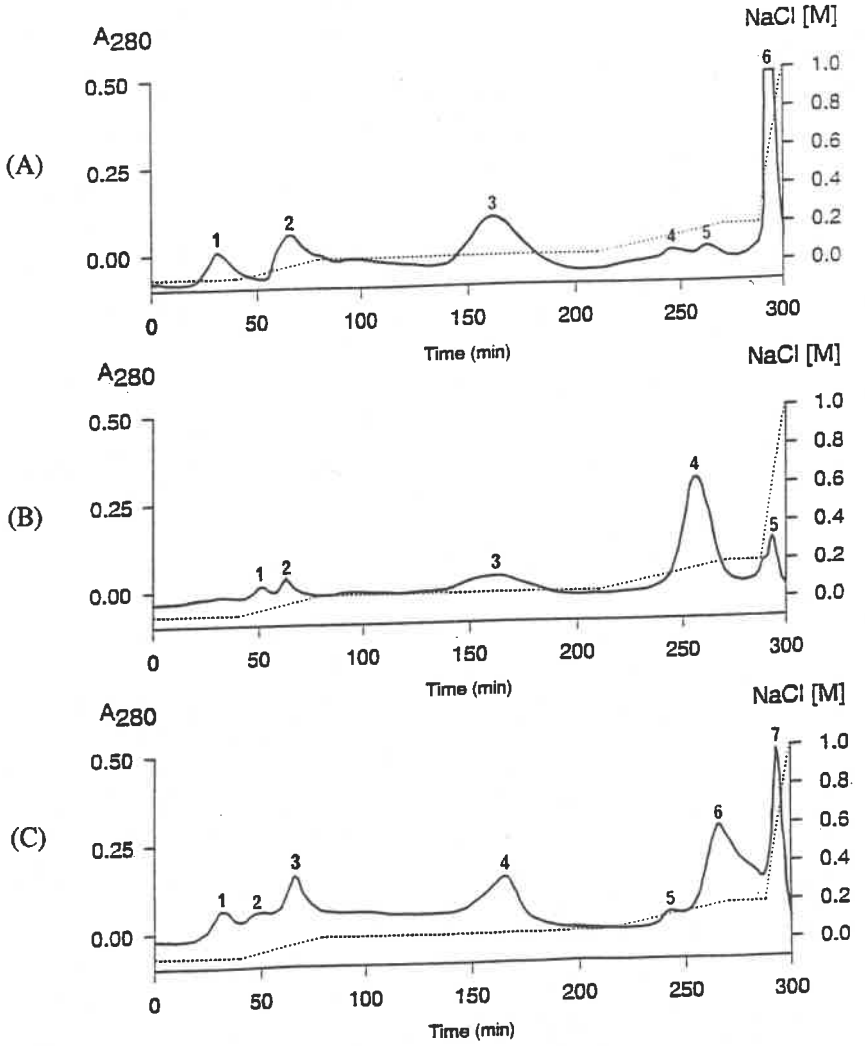

FIG. 1. FRACTIONATION OF THE TOTAL WHEY PROTEIN FRACTIONS OF BOVINE (A), YAK (B), AND KHAINAK (C) MILK BY ION-EXCHANGE CHROMATOGRAPHY ON DEAE-SEPHACEL

Fractions (6.8 mL) were collected at a flow rate of 150 mL/h.

High Perfusion Liquid Chromatography

Ion-exchange perfusion liquid chromatography was carried out on a Biocad Sprint system (PerSeptive Biosystems, Voisins le Bretonneux, France). A HQ Poros 20μ (4.6 mm. i.d. × 100 mm) column was used for anion-exchange purification. After equilibrating the column in buffer A (25 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$), separations were carried out at a flow rate of 5 mL/min, using a linear gradient from 0 to 30% B (25 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$, 1 M NaCl) over 20 column volumes. A HS-Poros 20 μ (4.6 mm. i.d. × 100 mm) cation-exchange column was used to purify lactoferrin and camel whey basic protein. The column was equilibrated with 5 column volumes of buffer C (25 mM Tris-HCl, pH 7.5, 5 mM $CaCl_2$) at a flow rate of 5 mL/min, and separation was performed, using a linear gradient from 0 to 80% of D (25 mM Tris-HCl, pH 7.5, 5 mM $CaCl_2$, 1 M NaCl) within 20 column volumes. Absorbencies were recorded at 280 and 214 nm.

Polyacrylamide Gel Electrophoresis (PAGE)

SDS-PAGE was performed in a vertical mini slab gel apparatus Protean II (Bio-Rad). The SDS-PAGE was carried out according to the method of Laemmli (1970), at room temperature. The running gel and the stacking gel contained 15% and 4% acrylamide, respectively. Silver staining of the gel was carried out according to Nesterenko *et al.* (1994) while staining with Schiff's reagent was done according to Groves *et al.* (1992).

Amino Acid Composition Analysis

The purified individual whey proteins in their native and oxidized forms (with performic acid) were hydrolyzed with 6 N HCl (Pierce) for 24 h at 110C, in a Pico-Tag station (Waters). The amino acids were derivatized with phenylisothiocyanate (PITC) according to the method of Bidlingmeyer *et al.* (1984) and separated by RP-HPLC on a Pico-Tag C_{18} column (3.9 mm i.d. × 15 cm). The column was equilibrated with solvent A (94% 0.14 M CH_3COONa, 0.5 mL TEA/L, pH 6.4/6% acetonitrile) and elution was performed by using a gradient from solvent A to solvent B (40% H_2O/60% acetonitrile) as described in Table 1. Both the column and solvents were maintained at 38C. The flow rate was 1.0 mL/min and absorbency was recorded at 254 nm.

N-terminal Sequencing

The N-terminal amino acid sequence was determined using an Applied Biosystems model 477A sequencer with on-line identification of the phenyl thiohydantoin derivatives. Reagents used for sequencing were purchased from

Perkin Elmer (Paris Nord II, France). The amino acid sequences obtained were searched in the PCGen database and the NCBI, using the Blast network service.

TABLE 1.
ELUTION GRADIENT USED FOR
PHENYLTHIOCARBAMYL AMINO ACIDS SEPARATION

time (min)	Flow rate (mL/min)	solvent A (%)	solvent B (%)
0	1	100	0
1	1	100	0
11	1	54	46
11.5	1	0	100
12.5	1	0	100
13	1.5	0	100
13.5	1.5	100	0
21	1.5	100	0

Isoelectric Focusing

Isoelectric focusing was performed on ready-to-use gels (Serva, Gagny, France) in the pH range 3-10. Isoelectric focusing was achieved at 4C on a 2117 Multiphor II apparatus (LKB, Bromma, Sweden), at a constant current of 7 mA.

Peptic Hydrolysis of Whey Proteins

Since β-lactoglobulin is particularly resistant to peptic hydrolysis (Reddy *et al.* 1988), to determine its presence, hydrolysis of whey proteins was carried out essentially as described by Kinekawa and Kitabatake (1996). The pH of a whey protein solution (70 mg/mL) was adjusted to 2.0 by addition of 1 N HCl, and the solution was preincubated at 37C for 10 mm. The enzymatic reaction was carried out at 37C, during 1 h, by using an enzyme (pepsin A from porcine stomach EC. 3.4.23.1, 3400 units per mg protein, Sigma) / substrate ratio of 1 / 200 (w/w). The reaction was stopped by adjusting the pH to 8.0 with 1 N NaOH. The reaction mixture was analyzed by size-exclusion chromatography on a TSKgel G 2000 SWXL column (7.8 mm i.d. $\times$ 30 cm). The column was equilibrated, and the sample eluted with a 50 mM Tris/HCl, pH 8.0 buffer at room temperature. The flow rate was 0.3 mL/min, and the absorbency was measured at 280 nm. The column was calibrated with low molecular mass markers (Sigma, Saint Quentin Fallavier, France).

RESULTS AND DISCUSSION

Separation of Whey Proteins of Species *Bovidae*

Whey proteins of cow, yak and khainak were separated by anion exchange chromatography on DEAE-Sephacel using a stepwise NaCl gradient (Fig. 1). The elution profiles obtained with whey of yak and khainak were similar to that obtained with bovine whey. The different fractions collected were analyzed by SDS-PAGE. As shown in Fig. 2, α-lactalbumin was eluted at 0.1 M NaCl for cow, yak and khainak whey. β-Lactoglobulin was eluted at 0.15 M NaCl for cow, yak and khainak. As can be observed in Fig. 2, β-lactoglobulin of yak and khainak co-eluted with a protein of higher molecular mass. Serum albumins were collected in fractions 2, 2 and 3 for cow, yak and khainak, respectively. The major fractions were purified further by RP-HPLC and identified by their amino acid composition, determined after acid hydrolysis (Table 2). The obtained data for α-lactalbumin and β-lactoglobulin of yak and khainak agree well with those of their bovine counterpart.

Separation of Camel Whey Proteins

By chromatography on HQ-Poros, whey proteins from *Camelus bactrianus* and *Camelus dromedarius* were separated into 7 fractions (Fig. 3 A and B). As observed by PAGE (Fig. 4), serum albumin was eluted in fraction 5 for both *Camelus bactrianus* and *Camelus dromedarius*. Analysis by SDS-PAGE shows that α-lactalbumin was eluted in fraction 2 for *Camelus bactrianus* and *Camelus dromedarius*. It was further fractionated by FPLC, using a linear gradient of NaCl from 0 to 1 M, prior to a final purification by RP-HPLC. Two resulting fractions were analyzed by SDS-PAGE and IEF (Fig. 5). Their amino acid composition was determined (Table 3) and the first 23 N-terminal amino acid residues were sequenced (Table 4). These results confirmed existence in milk of *Camelus bactrianus* of two forms of α-lactalbumin, as seen previously in milk of *Camelus dromedarius* (Conti *et al.* 1985), with a slight difference in their amino acid composition and isoelectric point (5.1 and 5.3, respectively), though identical molecular weights were observed according to their mobility in SDS-PAGE at pH 8.0. Conti *et al.* (1985) reported that the two forms of α-lactalbumin in *Camelus dromedarius* whey differed at the first N-terminal position. According to our sequence data, the two forms of α-lactalbumin of *Camelus bactrianus* and *Camelus dromedarius* show no differences in the first 23 positions and their sequences were identical to α-lactalbumin of *Camelus dromedarius* (Beg *et al.* 1985) and of llama (Cantisani *et al.* 1990). The presence of a small amount of a third α-lactalbumin in fractions eluted at a higher molarity of NaCl (more than 0.2 M) on anion-exchange chromatography of *Camelus bactrianus* whey was also observed. The 23 N-terminal amino acid

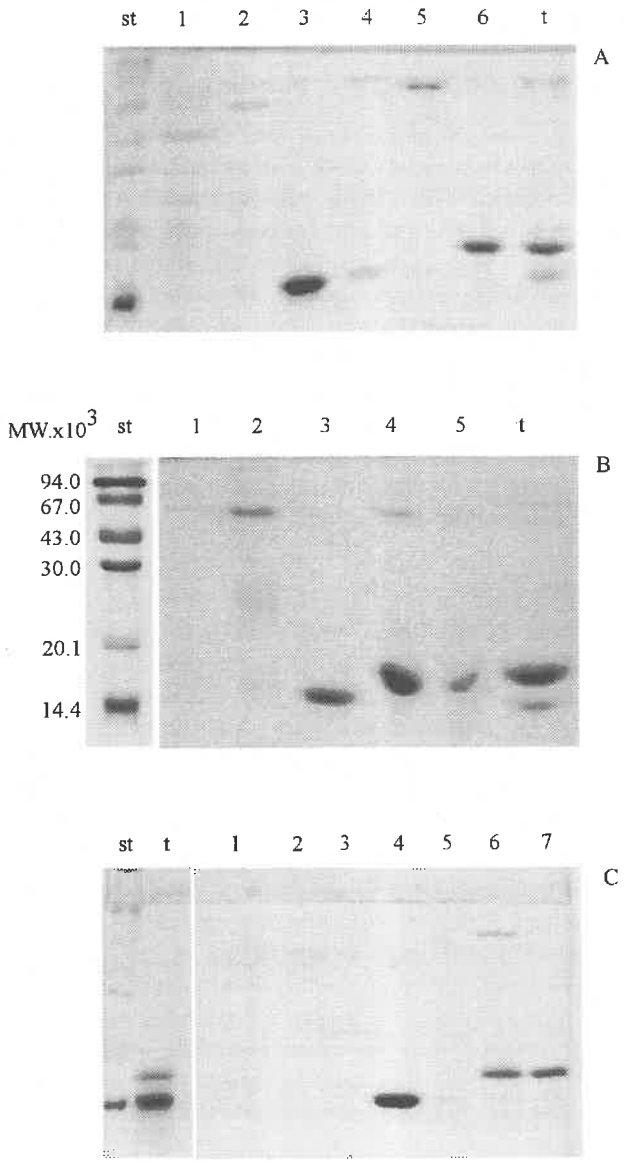

FIG. 2. SDS-PAGE OF BOVINE (A), YAK (B), AND KHAINAK (C) WHEY PROTEIN FRACTIONS OBTAINED BY DEAE-SEPHACEL CHROMATOGRAPHY

(A) 1 to 6 are fractions 1 to 6, respectively. (B) 1 to 5 are fractions 1 to 5, respectively. (C) 1 to 7 are fractions 1 to 7, respectively. t is total cow, yak and khainak whey proteins. st. is molecular mass standards. All gels were stained with Coomassie Blue.

TABLE 2.
AMINO ACID COMPOSITION OF α-LACTALBUMIN AND β-LACTOGLOBULIN OF SPECIES *BOVIDAE*
(values are residue/100 residues)

	α-lactalbumin				β-lactoglobulin			
	Cow[a]	Cow	Khainak	Yak	Cow[b]	Cow	Khainak	Yak
ASX	21	18.8	19.0	17.1	16.0	15.1	15.3	13.4
GLX	13	13.8	14.2	14.5	25.0	25.3	25.6	24.1
SER	7	6.6	6.6	7.0	7.0	6.8	6.9	7.0
GLY	6	6.9	6.4	6.6	3.0	3.3	4.0	5.3
HIS	3	3.8	3.6	3.6	2.0	2.2	2.0	2.3
ARG	1	1.1	1.1	1.3	3.0	2.9	3.0	3.0
THR	7	7.7	7.4	7.9	8.0	8.4	8.3	8.8
ALA	3	3.3	3.4	3.6	14.0	14.6	14.0	15.0
PRO	2	2.4	2.4	3.2	8.0	8.6	8.8	8.8
TYR	4	3.7	3.8	3.9	4.0	3.7	3.7	3.7
VAL	6	5.8	5.8	6.0	10.0	9.9	9.8	9.0
MET	1	1.5	1.1	1.5	4.0	4.4	4.4	4.3
ILE	8	7.3	7.1	7.1	10.0	8.7	8.7	9.0
LEU	13	13.9	13.8	13.4	22.0	23.1	22.9	22.9
PHE	4	4.2	4.1	4.2	4.0	4.0	4.1	4.2
LYS	12	10.5	11.3	10.0	15.0	14.0	13.8	14.4
Cys/2	8	n.d.	n.d.	n.d.	5	n.d.	n.d.	n.d.
TRP	4	n.d.	n.d.	n.d.	2	n.d.	n.d.	n.d.

a - sequence data from Harley and Schuler (1987).
b - sequence data from Alexander et al (1989).
n.d. - non determined.

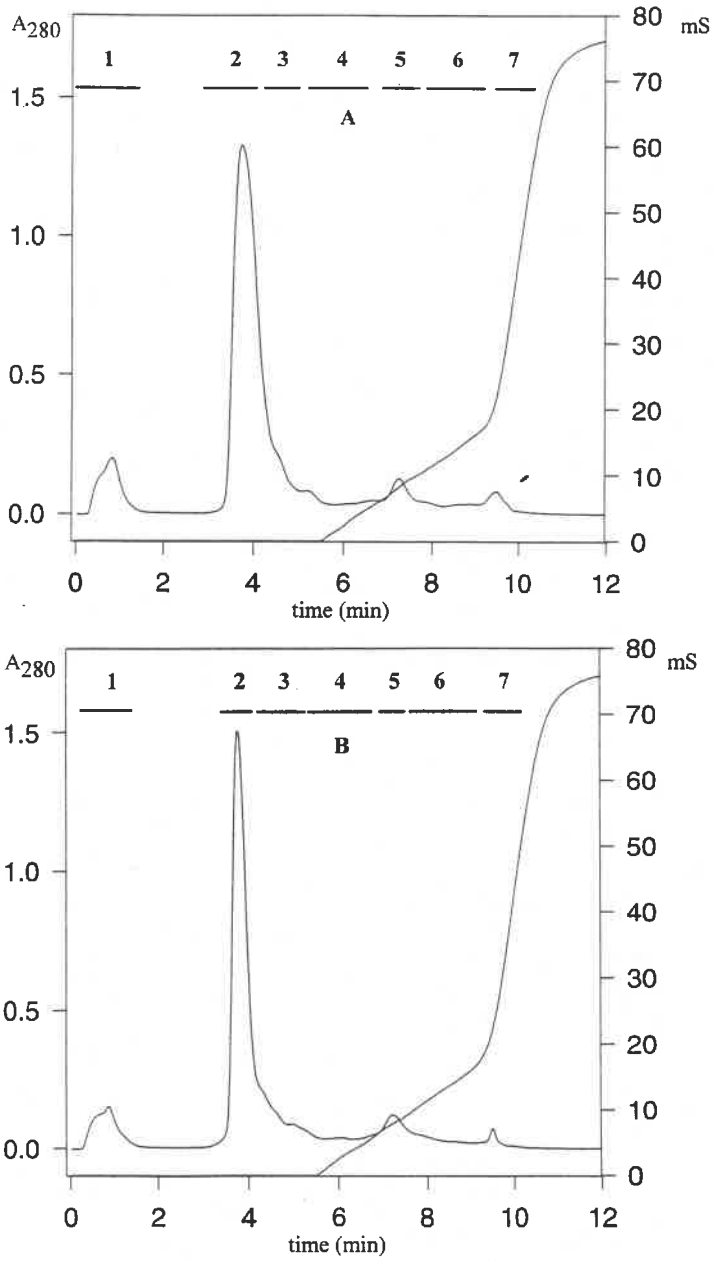

FIG. 3. SEPARATION OF TOTAL WHEY PROTEINS OF *CAMELUS BACTRIANUS* (A) AND *CAMELUS DROMEDARIUS* (B) BY HIGH PERFUSION ION-EXCHANGE CHROMATOGRAPHY ON HQ POROS (20 μ) COLUMN
1 to 7 are collection zones. mS, conductivity.

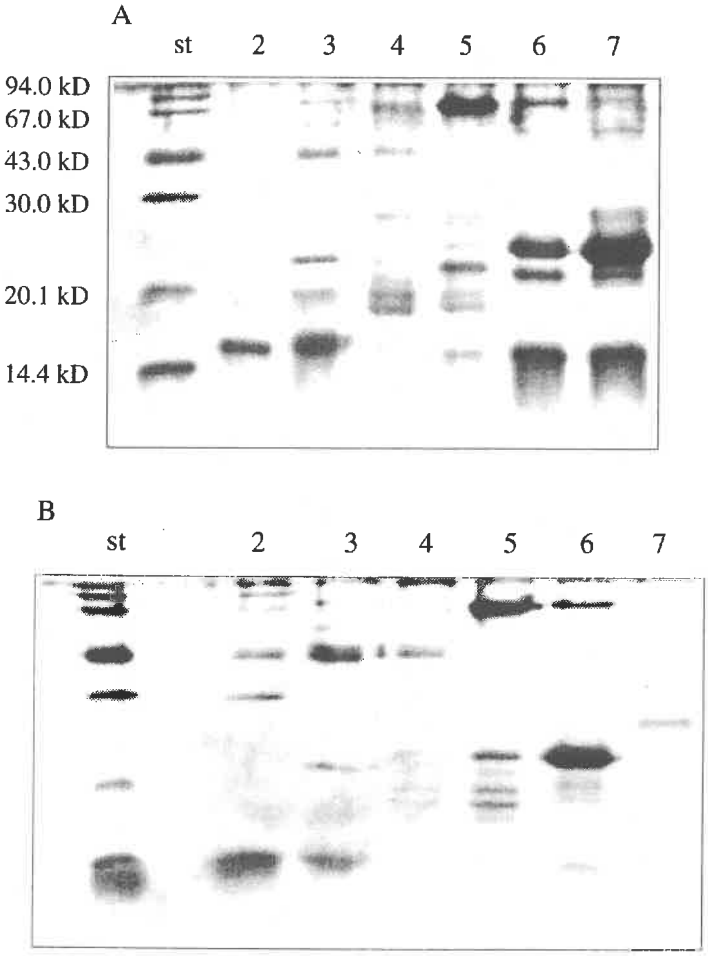

FIG. 4. SDS-PAGE PATTERNS OF *CAMELUS BACTRIANUS* (A) AND *CAMELUS DROMEDARIUS* (B) WHEY PROTEIN FRACTIONS OBTAINED BY HIGH PERFUSION ION-EXCHANGE CHROMATOGRAPHY ON HQ-POROS
st. is molecular mass standards. 2 to 7 are fractions 2 to 7, respectively.
Gels were silver stained.

residues of this α-lactalbumin had an identical sequence to two other forms. It is likely that milk of *Camelus bactrianus* contains glycosylated forms of α-lactalbumin, as has been observed in cow (about 5% of total quantity of α-lactalbumin) and in llama whey (Cantisani *et al.* 1990). To confirm this

suggestion, the double-staining technique for glycoproteins (with Schiff's reagent) and protein was applied to the SDS-PAGE (data not shown). Unfortunately, the three bands corresponding to the three different forms of *Camelus bactrianus* and *Camelus dromedarius* α-lactalbumin gave no visible band with Schiff's reagent. This could be due to the selectivity of the Schiff's reagent for reducing sugars.

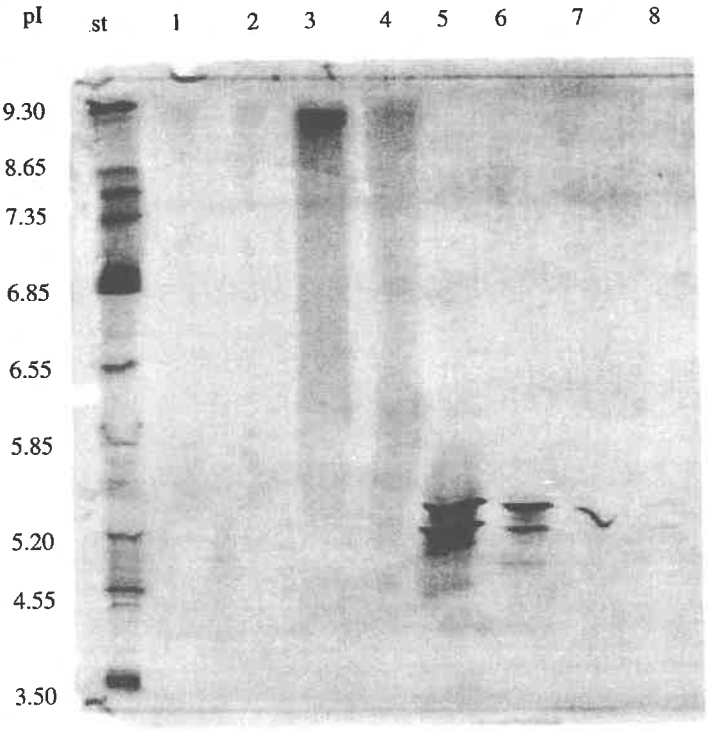

FIG. 5. ISOELECTRIC FOCUSING OF WHEY PROTEINS OBTAINED BY HIGH PERFUSION ION-EXCHANGE CHROMATOGRAPHY
1 and 4: whey basic protein (fractions 2 and 3, respectively) from *Camelus dromedarius*,
2 and 3: whey basic protein (fractions 2 and 3, respectively) from *Camelus bactrianus*;
5 and 6: α-lactalbumin A and B, respectively, from *Camelus dromedarius*;
7 and 8: α-lactalbumin A and B, respectively, from *Camelus bactrianus*.

All the fractions obtained by anion-exchange chromatography on HQ-Poros of *Camelus bactrianus* and *Camelus dromedarius* whey proteins were evaluated by double immunodiffusion against anti-bovine β-lactoglobulin antiserum. Only

the first fraction (eluted without NaCl) gave a positive reaction with antiserum to bovine β-lactoglobulin. This fraction was purified further by cation-exchange chromatography on a HS-Poros 20 μ column, at pH 7.5, and 4 peaks were

TABLE 3.
AMINO ACID COMPOSITION OF CAMEL α-LACTALBUMIN A AND B
(values are residue/100 residues)

	α-lac A Bac	α-lac B Bac	α-lac A Dr	α-lac B Dr	α-lac A Dr*	α-lac B Dr*
ASX	21.7	21.1	21.0	20.6	23.1	22.6
GLX	13.6	14.0	13.9	14.2	14.7	15.3
SER	5.6	6.3	6.1	5.7	5.5	6.4
GLY	7.3	7.3	7.5	7.0	7.8	7.6
HIS	2.6	2.8	3.1	2.9	2.6	2.7
ARG	2.8	3.0	3.0	2.8	2.8	2.3
THR	4.2	4.7	4.3	4.5	5.3	5.9
ALA	2.9	3.6	2.8	3.0	3.4	3.9
PRO	1.3	2.1	1.3	1.6	1.4	1.6
TYR	1.0	0.9	0.9	1.1	1.5	0.6
VAL	1.8	2.0	1.8	1.9	1.5	2.6
MET	2.1	1.3	1.8	1.8	2.4	2.3
ILE	6.0	5.7	5.9	6.1	9.6	8.8
LEU	10.4	9.9	10.0	10.1	11.8	14.5
PHE	3.2	3.2	3.3	3.4	4.3	3.9
LYS	13.3	13.6	13.2	13.5	12.9	12.0
CYS	n.d.	n.d.	n.d.	n.d.	4.8	5.2
TRP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* - data from Conti et al (1985).
n.d.- non determined amino acid.
Bac - *Camelus bactrianus.*
Dr - *Camelus dromedarius*

TABLE 4.
N-TERMINAL SEQUENCES OF *CAMELUS BACTRIANUS* α-LACTALBUMIN A, B AND C. COMPARISON WITH *CAMELUS DROMEDARIUS A* (BEG *ET AL.* 1985) AND *LLAMA GLAMA* α-LACTALBUMINS A, B AND C
(Cantisani *et al.* 1990)

Camelus bactrianus (A, B and C)	KQFTK XKLSD ELKGM NGNGG ITL
Camelus dromedarius A	KQFTK CKLSD ELKGM NGNGG ITL
Llama glama L (A, B and C)	KQFTK CKLSD ELKGM NGNGG ITL
Camelus dromedarius α-lac A*	KQF
Camelus dromedarius α-lac B*	EQF

* - from sequence data Conti et al (1985).
X - non determined.

collected (data not shown). As observed by SDS-PAGE (Fig. 6), the first peak contained several proteins with molecular masses of about 14.0 kDa, 16.0 kDa, 43.0 kDa and 54.0 kDa. The second and the third peak contained a single protein with molecular mass of approximately 20 kDa. Determination of its amino acid composition (Table 5) and its isoelectric focusing (Fig. 5) showed that it is a basic protein. The N-terminal sequence of this camel whey basic

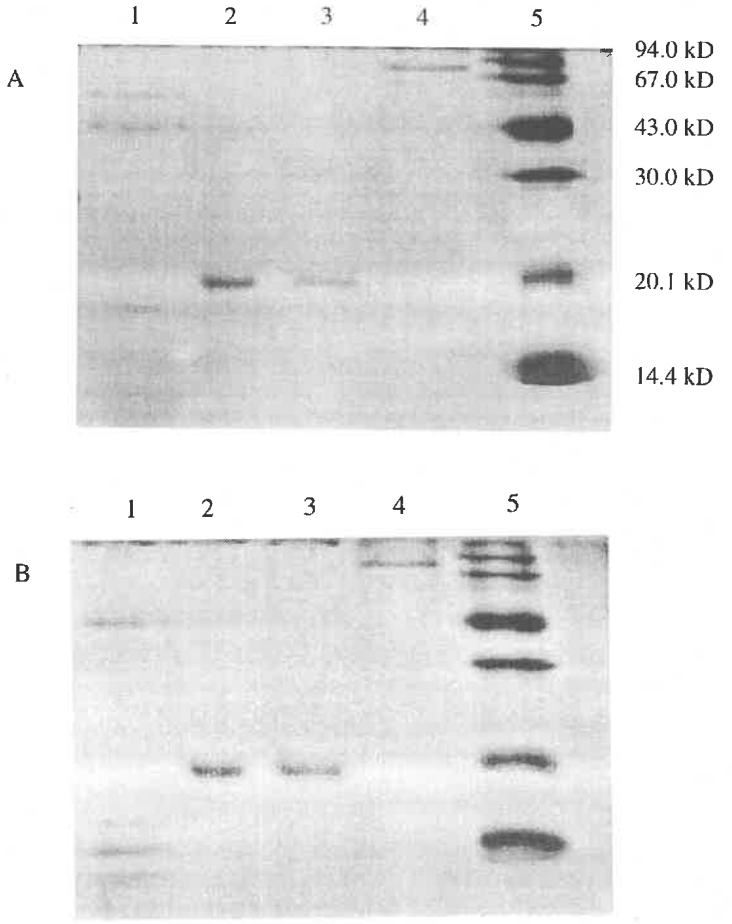

FIG. 6. SDS-PAGE OF FRACTIONS 1 TO 4 ISSUED FROM CHROMATOGRAPHY ON HS-POROS OF FRACTION 1 OBTAINED AFTER SEPARATION ON HQ-POROS CHROMATOGRAPHY

Camelus bactrianus (A) and *Camelus dromedarius* (B). 5 is molecular mass standards. Gels were silver stained.

TABLE 5.
AMINO ACID COMPOSITION OF *CAMELUS BACTRIANUS* (BWBP) AND *CAMELUS DROMEDARIUS* (DWBP) WHEY BASIC PROTEIN
(values are residue/100 residues)

	BWBP	DWBP		BWBP	DWBP
ASX	8.2	7.9	TYR	1.3	1.6
GLX	10.5	10.7	VAL	7.5	7.5
SER	7.6	7.1	MET	0.5	0.6
GLY	11.3	10.8	ILE	4.0	4.0
HIS	4.0	3.9	LEU	8.2	7.9
ARG	11.1	11.2	PHE	1.7	1.5
THR	4.2	4.5	LYS	1.8	1.9
ALA	10.2	9.8	CYS	n.d.	n.d.
PRO	8.0	7.6	TRP	n.d.	n.d.

n.d.- non determined.

protein (CWBP) is shown in Table 6. Compared with known sequences of camel and llama milk proteins, CWBP showed no homology. The presence of a "heterogeneous camel milk whey noncasein protein" and a "llama whey protein with unknown function" was already reported (Beg *et al.* 1987; Cantisani *et al.* 1990). CWBP may belong to this category of proteins. The fourth peak, according to SDS-PAGE (Fig. 6; MW: 78.0 kDa) and N-terminal sequence (Table 7) contained lactoferrin. The first 20 N-terminal amino acid sequence of *Camelus bactrianus* and *Camelus dromedarius* lactoferrin showed 56% homology with lactoferrin of sheep and pig.

In order to detect the presence of β-lactoglobulin in the whey of *Camelus bactrianus* and *Camelus dromedarius*, total whey was submitted to a proteolysis by pepsin. Since bovine β-lactoglobulin is not susceptible to pepsin, even after 40 h hydrolysis (Dalgalarrondo *et al.* 1995), if this protein exists in milk of *Camelus bactrianus* and *Camelus dromedarius* a peak should be obtained by size-exclusion chromatography in the molecular weight range 16-20 kDa. Its absence (data not shown) after peptic action confirms that milk from *Camelus bactrianus* and *Camelus dromedarius* is devoid of β-lactoglobulin. The observed cross-reactivity of the proteins contained in the first peak eluted by DEAE-Sephacel chromatography with antiserum of bovine β-lactoglobulin, can be explained by the presence of lactoferrin in this peak. Brignon *et al.* (1985) have reported that the bovine lactoferrin may give positive cross-reactivity with antiserum to β-lactoglobulin.

TABLE 6.

N-TERMINAL SEQUENCES OF WHEY BASIC PROTEIN

CWBP[1]	S-E-D-P-P-A-X-G-S-I-V-P-R-R-E-W-W-A-L-A-	
Camel[2]	S-L-N-E-P-K-D-I-M-Y-M-E-P-S-I-S-R-E-D-L~S-A-R-R-H-Q-N-Q-N-P-K-L-L-H-P-V-P-Q-E-S-	
	29	49
Llama[3]	S-L-V-S-L-N-E-P-K-D-E-I-Y-M-E-S-Q-P-	
Cow[4]	S-S-X-Q-P-Q-S-Q-N-P-K-L-P-L-S-I-L-K-E-K-	

1. *Camelus bactrianus* and *Camelus dromedarius* whey basic protein (this study).
2. Heterogeneous *Camelus dromedarius* milk whey non-casein protein. (Beg et al, 1987).
3. Unknown *Lama glama* L. protein (Cantisani et al, 1990).
4. Bovine 17 kDa protein (Sorensen and Petersen, 1992).

X: non determined.

TABLE 7.
N-TERMINAL SEQUENCES OF *CAMELUS BACTRIANUS* LACTOFERRIN. COMPARISON WITH COW AND PIG LACTOFERRIN

Camelus bactrianus	**A**-X-K-**K**-X-**V**-**R**-**W**-X-**T**-T-**S**-P-A-**E**-S-X-**K**-X-A-
Bos taurus	**A**-P-R-**K**-N-**V**-**R**-**W**-C-**T**-I-**S**-Q-P-**E**-W-F-**K**-C-R-
Sus crofa L.	**A**-P-R-**K**-G-**V**-**R**-**W**-C-**T**-I-**S**-T-A-**E**-Y-S-**K**-C-R-

X - non determined.

CONCLUSION

Results of this study indicate that the whey of *Camelus bactrianus* contains a major protein, α-lactalbumin, existing in three different forms, as in llama whey. These three different forms of α-lactalbumin have identical sequences of the first 23 N-terminal amino acids. The first two forms eluted together (at 0.1M NaCl) during DEAE-Sephacel chromatography and migrated in the same region on SDS-PAGE. They could be separated by isoelectric focusing and/or by anion exchange chromatography, using columns with high resolving power. The third form eluted at a higher NaCl concentration (about 0.15 M) and during SDS-PAGE showed lower mobility (higher apparent molecular weight) than the other two forms. An unknown protein, named camel whey basic protein (CWBP) is present in whey of *Camelus bactrianus*. It showed no obvious structural similarities with other well characterized milk and nonmilk proteins.

Whey proteins display excellent nutritional properties and are used as protein supplement in the formulation of an enteral dietary product for patients with pulmonary disease or renal insufficiency (Trimbo 1992). As it is known, the presence of β-lactoglobulin in milk may cause a number of problems both in food processing and nutrition. Particularly, the compact globular structure of β-lactoglobulin makes it difficult to digest (Chobert *et al.* 1997). β-Lactoglobulin is responsible for some of the observed allergies to cow's milk (Asselin *et al.* 1988). Since camel milk is devoid of β-lactoglobulin (or contains a very small amount of it), it could be interesting as a new raw material for infant formula and for nutrition in countries where these animals thrive.

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