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Original article

Characterization of caseins from Mongolian yak, khainak, and bactrian camel

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Summary — The composition of acid-precipitated caseins from ruminant Mongolian domestic animals was analyzed and a comparative study between camel *(Camelus bactrianus)* and dromedary *(Camelus dromedarius)* was realized. Acid-precipitated whole caseins were analyzed for amino acid composition, separated by anion exchange chromatography and identified by alkaline urea-PAGE. Elution profiles and electrophoretic mobilities of the main components of yak and khainak caseins were nearly identical to their cow counterparts. However, the main part of α_{S1}-casein of yak was eluted in lower molarity in NaCl. Characterization by PAGE, amino acid composition and N-terminal sequence of individual caseins from camel *(Camelus bactrianus)* indicated that milk of this ruminant contains dominantly α_{S1}-, α_{S2}-, and β-casein and small amounts of κ-casein as is the case for the milk of dromedary *(Camelus dromedarius)*.

caseins / yak / khainak / camel

Résumé — Caractérisation des caséines de ruminants de Mongolie: yak, khainak et chameau bactrien. La composition en acides aminés des caséines totales de deux ruminants de Mongolie (yak, khainak) a été déterminée. Les différentes caséines ont été séparées par chromatographie sur échangeur d'ions et analysées par électrophorèse en milieu urée et à pH alcalin. Les caséines des deux espèces étudiées ont un comportement voisin, proche de celui des caséines de vache. La seule différence notable réside dans le fait que chez le yak, la caséine α_{S1} est éluée à une molarité inférieure à celle utilisée lors de la séparation des caséines de vache et de khainak. Une étude comparative a été réalisée entre le chameau *(Camelus bactrianus)* et le dromadaire *(Camelus dromedarius)*. Ces deux espèces renferment principalement les caséines α_{S1}, α_{S2} et β, dont la séquence N-terminale a été déterminée. La caséine κ n'est que faiblement représentée.

caséines / yak / khainak / camélidés

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INTRODUCTION

Many populations of Central Asia, and Mongols in particular, have thousands of years of old traditions of using milk and milk products for nutritional purposes. Sometimes, they use also particular dairy products as curative agents (Kadirova, 1985). The first European written mentions of the use of milk as main staple of nomadic tribes originating from Asia by ancient Greeks are due to Homer (~800 BC), Herodot (~500 BC) and Strabo (~100 BC) in their descriptions of barbarian populations termed γαλακτοφαγοι (galactophagi). Antique Greeks attributed to this type of diet the particular strength and fierceness of described nomads. The earliest conserved written Chinese records of the mare's milk use for preparation of koumys can be traced to almost 2000 BC, to the descriptions of northern nomadic tribes by Han historian Ssu-ma Ch'ien (summarizing all preexisting information around the 1st century BC) and to conserved runic and Ouigur petroglyphs on still existing stellae. Nowadays, dairy products derived from milk of animals herded on the Mongolian steps and deserts constitute still a major element of indigenous diet. In 1995, Mongolia, counting about 2.5 millions inhabitants, had 416 000 cows, 708 000 yaks and 58 000 khainaks (hybrid between cow and yak), and had also 367 000 bactrian camels. Unfortunately, despite the studies of Grosclaude et al (1982), despite long alimentary practice, information about milk proteins of Mongolian domestic animals is rather fragmentary. Moreover, available information concerning dromedary milk (Farah and Farah-Riesen, 1985; Beg et al, 1984, 1986a and b, 1987; Abdel Rahim, 1987; Mehaia, 1987a and b; Mohammed and Larsson-Raznikiewicz, 1989, 1991; Farah, 1993) is related mainly to the Arabian dromedary *Camelus dromedarius* species, and very scarce for the camel *Camelus bactrianus* species. The present work has been carried out in order to present a more systematic description of the major milk proteins of principal Mongol stock animals and to compensate for this gap. This paper describes the separation of the caseins from Mongolian cow, yak, khainak, and *Camelus bactrianus* by different ion exchange chromatographies and their further purification by reversed-phase HPLC. For comparison, the caseins from *Camelus dromedarius* (Arabian dromedary) were separated using the same set of purification methods. The major casein components were characterized by urea and SDS polyacrylamide gel electrophoresis, and by determination of their amino acid composition and N-terminal sequence.

MATERIALS AND METHODS

Preparation of milk samples

Milk was collected from well identified single Mongolian domestic animals. The milk was lyophilized in the Food Industry Research Institute of Ulan Bator, Mongolia. The lyophilized samples were kept frozen at –4 °C until further processing. The dromedary milk was a kind gift of Professor Mahmoud Sitohy and was also prepared from the single dromedary from Egyptian desert animal stock.

Preparation and separation of caseins

The whole casein was obtained from skim milk by precipitation at the isoelectric point (pH 4.6) using 1 N hydrochloric acid. The precipitate was washed with distilled water, solubilised at pH 7 by addition of sodium hydroxide, precipitated again at pH 4.6 and washed three times with distilled water. Finally, the whole casein was solubilised at pH 7, freeze-dried and stored at –20 °C. The individual caseins were separated by ion exchange chromatography on QAE-Sepharose (Pharmacia, Orsay, France), by applying a gradient from buffer A (0.02 mol/L imidazole, pH 7.0, 3.3 mol/L urea, 0.3% (v/v) 2-mercaptoethanol) to buffer B (0.02 mol/L imidazole, pH 7.0, 3.3 mol/L urea, 0.3% (v/v) 2-mercaptoethanol, 1 mol/L NaCl), at room temperature,

with a flow rate of 200 mL/h. The ion exchange chromatography was performed on an Econo system (Bio-Rad, Ivry sur Seine, France) equipment.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was performed in a vertical mini slab gel apparatus Protean II (Bio-Rad). The acrylamide gel (10% in 4 mol/L urea, Tris-HCl, pH 8.8 buffer solution) was prepared according to Davis (1962). Electrophoresis was performed at constant amperage (15 mA during 7 min in the stacking gel, and 30 mA for about 30 min in the running gel). The SDS-PAGE was carried out according to the method of Laemmli (1970). The running gel (8.0 × 6.0 × 0.075 cm) contained 15% and the stacking gel 4% acrylamide. Casein ratios were calculated by scanning the electrophoretic gel, by using a Bioprofil station Program.

Chymosin test

Chymosin of calf stomach (EC 3.4.23.4.), 23.6 U/mg of protein (Sigma Chemical Co), was dissolved in distilled water (1 mg/mL). An aliquot (5 μg) of the enzyme was supplied to 1 mg of bovine κ-, and β-casein, and to 10 mg of camel and dromedary caseins issued from fraction 3 of ion exchange chromatography (see *Results*), dissolved in 1 mL of a 50 mmol/L imidazole/ HCl buffer (pH 6.5). The resulting solution was incubated at 37 °C for 20 min. The reaction was stopped by adding solid urea until 3 mol/L. Then, the solution was submitted to alkaline urea PAGE, as described above.

Reversed-phase high performance liquid chromatography (RP-HPLC)

RP-HPLC preparative purification (LiChroCART 100 C_{18} column, 10 mm id × 250 mm) was carried out on a Waters instrument (Waters Associates, Millford, MA, USA) equipped with an interface module system assisted by a chromatography work station Maxima 820. The column was equilibrated with solvent A (0.1% TFA in H_2O, pH 2.5). The separation of caseins was achieved by applying a linear gradient from 60% solvent A/40% solvent B (80% acetonitrile/20% H_2O/0.085% TFA) to 30% solvent A/70% solvent B during 40 min. The flow rate was 1 mL/min and absorbency was recorded at 214 nm.

Amino acid analysis

The proteins were hydrolyzed under vacuum in the presence of constant boiling 6 N HCl for 24 h at 110°C in a Pico-Tag station (Waters). After acid hydrolysis, the amino acids were derivatised with phenyl isothiocyanate (PITC) according to Bidlingmeyer et al (1984) and quantified by RP-HPLC on a Pico-Tag C_{18} column (3.9 mm id × 15 cm). Speed-vac dried samples were dissolved in 95% 2 mmol/L Na_2HPO_4, pH 7.4, 5% acetonitrile. The column was equilibrated in solvent A (94% 0.14 mol/L CH_3COONa, 0.5 mL TEA/L, pH 6.4, 6% acetonitrile); the elution was performed with a gradient from solvent A to solvent B (40% H_2O / 60% acetonitrile) according to Dalgalarrondo et al (1990). Both the column and solvent were maintained at 38 °C. The flow rate was 1.0 mL /min and absorbency was recorded at 254 nm.

N-terminal sequencing

The N-terminal amino acid sequence analysis was performed on an Applied Biosystems model 477A sequencer with an 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 at the NCBI using the BLAST network service.

RESULTS AND DISCUSSION

Acid-precipitated whole caseins from cow, yak, khainak, camel *(Camelus bactrianus)* and dromedary *(Camelus dromedarius)* were separated by anion-exchange chromatography on QAE-Sepharose column (fig 1). As already known, classical anion exchange chromatography on DEAE-cellulose column resolved whole bovine casein into different fractions containing γ-, κ-,

β-, α_{s2}- + α_{s1}-, and α_{s1}-casein, respectively (Mercier et al, 1968). As shown in figure 1B and C, the separation of khainak and yak caseins gave an elution pattern similar to that obtained from cow casein (fig 1A). Eight peaks were obtained for yak and khainak caseins, and nine for cow casein. Under the conditions used, caseins of

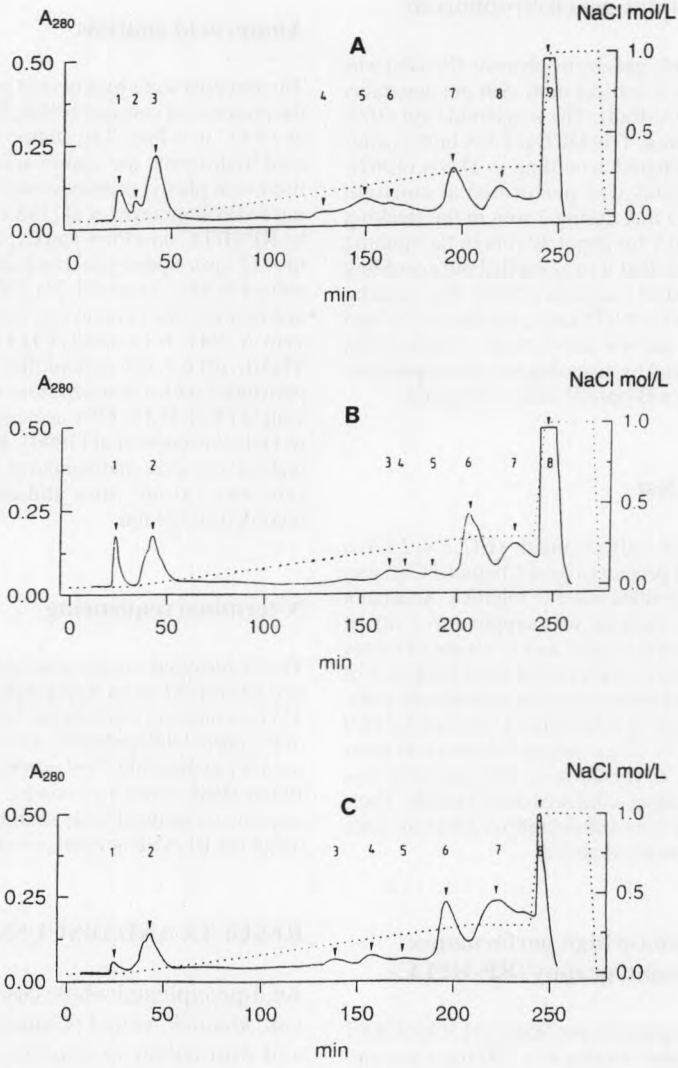

Fig 1. Fractionation of acid precipitated total caseins of cow (**A**), khainak (**B**), yak (**C**), *Camelus dromedarius* (**D**), and *Camelus bactrianus* (**E**) by ion- exchange chromatography on QAE-Sepharose. 6.8 mL fractions were collected at the flow rate of 200 mL/h.

*Fractionnement des caséines entières de vache (**A**), khainak (**B**), yak (**C**),* Camelus dromedarius *(**D**) et* Camelus bactrianus *(**E**) par chromatographie d'échange d'ions sur colonne de QAE-Sepharose. Le volume de chaque fraction était de 6,8 mL et le débit de 200 mL/h.*

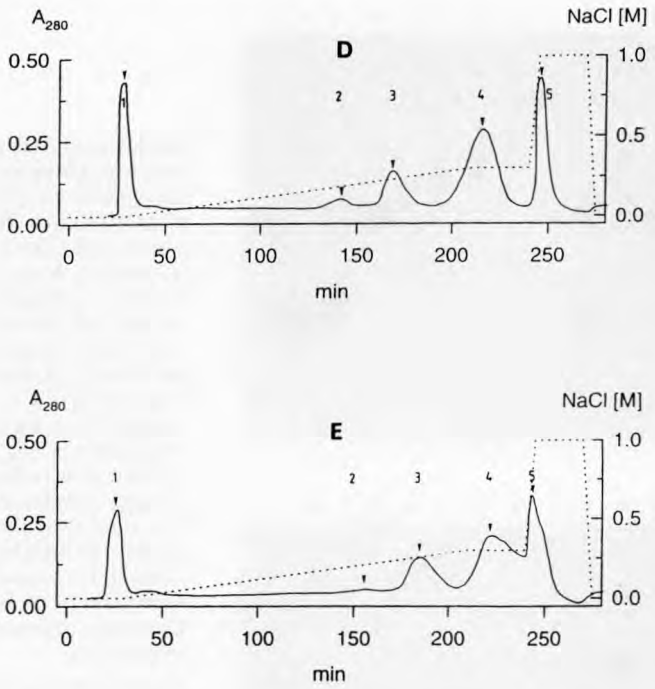

Fig 1. (continued)

Camelus species (fig 1D, E) gave a different elution pattern than those obtained with yak and khainak caseins.

Caseins of yak and khainak

The alkaline urea-PAGE shows (fig 2) that the first two peaks eluted by ion exchange chromatography of yak and khainak whole casein did not contain any protein band. Elution of casein components began at a molarity of 0.12 mol/L in NaCl. The electrophoretic mobilities of proteins issued from peak 3 of yak and 3 and 4 of khainak (eluted at 0.12 mol/L NaCl) were nearly identical with electrophoretic mobility of cow κ-casein. A test with chymosin has confirmed that the major part of yak and khainak κ-casein was contained in fraction 3, although this casein was also found as a contaminant in fractions 4 and 5 of yak, and 5 and 6 of khainak caseins. The main part of the protein eluted at 0.16 mol/L NaCl and corresponding to peaks 4 and 5 of yak, and to peaks 5 and 6 of khainak casein migrated by electrophoresis near the region of migration of cow β-casein. Proteins obtained from fractions 6 and 7 of yak, and 7 and 8 of khainak (elution beginning at 0.26 mol/L NaCl) gave bands with electrophoretic mobilities similar to those of cow α_s-casein. Fraction 8 of yak casein contained a very small amount of protein and, despite several analyses, did not show any visible band. Yak milk predominantly consists of α_{s1}C-casein (Grosclaude et al, 1982), what may explain that almost all α_{s1}-casein was eluted in peak 7, at 0.27 mol/L NaCl. On the contrary, fractions 9 of cow and 8 of khainak,

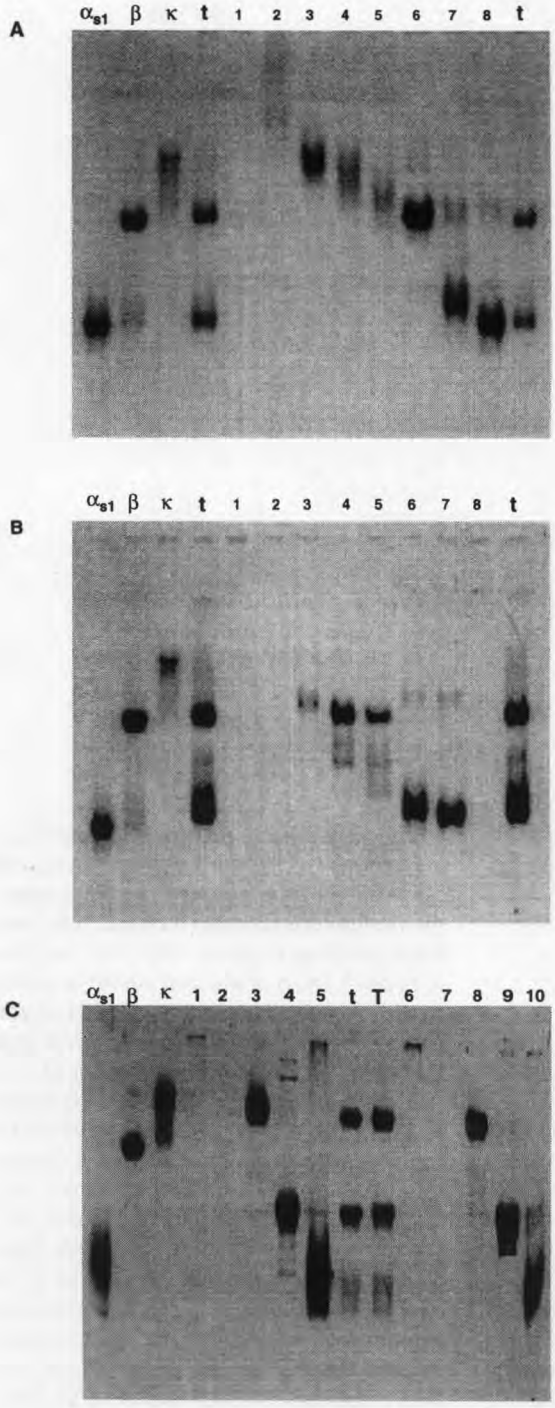

Fig 2. Urea-PAGE patterns of fractions issued from ion exchange chromatography on QAE-Sepharose of khainak (**A**), yak (**B**), *Camelus bactrianus* and *Camelus dromedarius* (**C**) caseins. **A.** α_S, β, and κ are α_S, β, and κ -cow caseins, respectively; t is total yak casein; 1 to 8 are fractions 1 to 8, respectively. **B.** α_S, β, and κ are α_S, β, and κ -cow caseins, respectively; t is total khainak casein; 1 to 8 are fractions 1 to 8, respectively. **C.** α_S, β, and κ are α_S, β, and κ -cow caseins, respectively; t is total *Camelus bactrianus* casein; T is total *Camelus dromedarius* casein; 1 to 5 are fractions 1 to 5 of *Camelus bactrianus* casein, respectively; 6 to 10 are fractions 1 to 5 of *Camelus dromedarius* casein, respectively.

*Électrophorégramme (milieu urée) des fractions issues de la chromatographie d'échange d'ions sur colonne de QAE-Sepharose des caséines de khainak (**A**), yak (**B**),* Camelus bactrianus *et* Camelus dromedarius *(**C**). **A.** α_S, β, et κ: caséines α_S, β, et κ de vache, respectivement; t : caséine entière de yak; 1 à 8 : fractions 1 à 8 respectivement, issues de la chromatographie sur échangeur d'ions. **B.** α_S, β, et κ: caséines α_{S1} β, et κ de vache, respectivement; t : caséine entière de khainak ; 1 à 8 : fractions 1 à 8 respectivement, issues de la chromatographie sur échangeur d'ions. **C.** α_S, β, et κ: caséines α_S, β, et κ de vache, respectivement ; t : caséine entière de* Camelus bactrianus*; T: caséine entière de* Camelus dromedarius *; 1 à 5 : fractions 1 à 5 respectivement, issues de la chromatographie sur échangeur d'ions de la caséine entière de* Camelus bactrianus*; 6 à 10 : fractions 1 à 5 respectivement, issues de la chromatographie sur échangeur d'ions de la caséine entière de* Camelus dromedarius.

which were eluted at the same molarity of NaCl, above 0.3 mol/L, contained significant amounts of α_{s1}-casein. At this molarity (0.3 mol/L NaCl), fraction 8 of yak contained only small quantities of proteins. Urea-PAGE of whole casein from yak and khainak (fig 5) shows a similar composition in individual caseins, ie, 43.2 and 47.0% α_s-casein, 35.4 and 33.8% β-casein, and 16.4 and 14.2% κ-casein, respectively. Based on urea-PAGE and on the amino acid composition of total casein (table I), one can assume that caseins of yak and khainak are nearly identical with those of cow.

Table I. Amino acid composition of total caseins. *Composition en acides aminés des caséines entières.*

	Cow[a1]	*Cow*[b2]	*Yak*[b2]	*Khainak*[b2]
Asx	7.9	7.1	6.2	6.8
Glx	21.8	22.9	22.4	23.2
Ser	5.6	5.5	5.8	5.9
Gly	2.1	1.8	1.8	2.0
His	2.8	2.8	2.6	2.8
Arg	3.7	4.2	3.7	4.0
Thr	5.1	4.3	5.0	4.6
Ala	3.5	3.2	3.1	3.0
Tyr	5.3	5.0	4.9	4.9
Val	6.8	5.9	6.1	6.2
Met	2.7	1.3	1.3	1.3
Cys	0.7	nd	nd	nd
Ile	6.4	4.9	4.9	4.8
Leu	10.4	8.8	9.0	9.2
Phe	5.2	4.9	4.4	3.9
Lys	8.3	7.3	6.5	6.6
Pro	10.0	9.8	11.3	10.0
Trp	1.4	nd	nd	nd

[a] From Renner, 1991. [b] This study. [1] Values are g/100 g total casein. [2] Values are number of residues/100 residues.

[a] Données Renner, 1991. [b] Cette étude. [1] Valeurs en g/100 g de caséine totale. [2] Nombre de résidus/100 résidus.

Caseins of camel (Camelus bactrianus) *and dromedary* (Camelus dromedarius)

Samples of caseins of *Camelus bactrianus* and *Camelus dromedarius*, as well as their fractions obtained by ion-exchange chromatography, were examined in order to determine whether they present differences or have a similar composition as caseins from *Bovidae* (see above). The separation of whole caseins of *Camelus* species by anion-exchange chromatography on QAE-Sepharose column gave elution profiles different from those obtained with bovine casein. Caseins from each species of camels were eluted in five peaks (fig 1D, E). The electrophoretic pattern of acid-precipitated whole caseins from bactrian and dromedary camels (fig 2C: t, *Camelus bactrianus*, T, *Camelus dromedarius*) shows that they contained two well defined sharp bands and a diffuse one. The electrophoretic pattern of each peak obtained by anion exchange chromatography indicated that peak 1 contained a protein with a low mobility which has a molecular mass of about 63 000 (as determined by SDS-PAGE) and that peak 2 was deprived of any protein. Casein components were obtained in peaks 3, 4 and 5, which were eluted at 0.21, 0.26 and above 0.30 mol/L NaCl, respectively. After urea-PAGE (fig 5), casein ratios were calculated by scanning the electrophoretic gel, by using a Bioprofil station Program. The whole camel casein contained about 38% α_{s1}-, 21%, α_{s2}- and 41% β-casein, respectively. Among the β-casein fraction, 12% were constituted by κ-casein which was co-eluted with β-casein (see below).

The different fractions issued from ion exchange chromatography of both *Camelus* species have been further purified by RP-HPLC (data not shown), before determining their amino acid composition (table II) and N-terminal sequence (table III). A 20–24 amino acid long N-terminal sequence of fractions 4, 5 and 3 of *Camelus dromedarius* and *Camelus bactrianus* has been deter-

Table II. Amino acid composition of fractions 3, 4 and 5 of *Camelus dromedarius* and *Camelus bactrianus*. Comparison with cow α_{s1}-, α_{s2}-, β- and κ-casein (values are given in number of residues/100 residues).

Composition en acides aminés des fractions 3, 4 et 5 de Camelus dromedarius *et* Camelus bactrianus. *Comparaison avec les caséines* α_{s1}, α_{s2}, β *et* κ *de vache (nombre de résidus/100 résidus).*

	fraction 5		α_{s2} *cam*[a]	α_{s2} *cow*[b]	*fraction 4*		α_{s1} *cam*[a]	α_{s1} *cow*[c]	*fraction 3*		β *cam*[a]	β *cow*[d]	κ *cam*[a]	κ *cow*[e]
	Drom	*Bact*			*Drom*	*Bact*			*Drom*	*Bact*				
ASX	6.8	7.2	6.5	8.7	9.4	9.0	9.1	7.5	3.7	3.6	3.8	4.3	6.2	7.1
GLX	24.5	23.4	21.8	19.3	21.0	22.0	20.9	19.6	20.8	20.8	19.5	18.7	17.7	16.0
SER	5.8	6.3	6.7	8.2	6.6	6.9	8.0	8.0	5.6	5.7	6.1	7.7	6.3	7.7
GLY	1.9	2.2	1.9	1.0	3.2	3.1	2.3	4.5	1.0	1.1	1.2	2.4	2.2	1.2
HIS	2.7	2.5	2.7	2.7	2.5	2.6	2.3	2.5	1.8	1.8	1.8	2.4	1.9	1.8
ARG	2.0	2.8	1.8	2.9	5.3	4.8	4.9	3.0	1.9	1.6	1.9	1.9	2.7	3.0
THR	8.6	7.3	8.0	7.2	4.0	4.8	4.9	2.5	5.0	5.1	5.0	4.3	7.1	8.9
ALA	2.1	3.8	2.9	2.9	3.6	2.9	3.0	4.5	3.0	3.8	2.9	2.4	4.8	8.3
PRO	5.0	6.3	5.1	5.1	9.0	9.0	8.4	8.5	17.2	16.5	18.3	16.7	14.4	11.8
TYR	5.1	4.6	5.7	5.8	4.2	4.1	4.6	5.0	2.0	1.6	2.5	1.9	3.6	5.3
VAL	5.9	5.7	6.1	6.8	5.3	5.5	4.8	5.5	8.0	7.7	8.0	9.1	7.1	6.5
MET	1.7	1.6	1.6	1.9	2.1	1.9	1.7	2.5	3.3	3.1	2.9	2.9	1.5	1.2
CYS	n.d	n.d	1.0	1.0	n.d	n.d	0.0	0.0	n.d	n.d	0.0	0.0	0.6	1.0
ILE	5.2	5.2	5.3	5.3	5.2	5.4	6.2	5.5	5.5	5.9	5.7	4.8	6.9	7.1
LEU	5.2	6.3	5.1	6.3	9.2	8.6	8.0	8.5	11.6	11.5	10.8	10.5	7.2	4.7
PHE	5.3	4.5	5.1	2.9	2.6	2.8	2.7	4.0	3.9	3.8	3.8	4.3	3.6	2.4
LYS	11.8	9.9	10.6	11.6	6.7	6.4	7.3	8.0	5.7	6.3	5.9	5.3	5.6	5.3
TRP	n.d	n.d	2.2	1.0	n.d	n.d	1.0	1.0	n.d	n.d	n.d	0.5	0.7	0.6
MW × 10^3	26.3[f]	26.0[f]	25	25.3	35.3[f]	34.3[f]	31.0	23.6	27.5[f]	27.4[f]	27.0	24.0	n.d	19.0

[a] From amino acid composition of camel milk caseins (Larsson-Raznikiewicz and Mohamed, 1986). [b] From sequence data for cow α_{s2}-casein (Brignon et al, 1977). [c] From sequence data for cow α_{s1}-casein B (Mercier et al, 1971). [d] From sequence data for cow β-casein A2 (Ribadeau Dumas et al, 1972). [e] From sequence data for cow κ-casein A (Mercier et al, 1973). [f] Estimated from SDS-PAGE (see fig 4).

[a] *Composition en acides aminés des caséines de lait de chameau (Larsson-Raznikiewicz et Mohamed, 1986).* [b] *Série de données sur la caséine de vache* α_{s2} *(Brignon et al, 1977).* [c] *Série de données sur la caséine* α_{s1} *(Mercier et al, 1971).* [d] *Série de données sur la caséine* β *de vache A2 (Ribadeau Dumas et al, 1972).* [e] *Données sur la caséine de vache* κ*A (Mercier et al, 1973).* [f] *Estimation SDS-PAGE (voir fig 4).*

Table III. N-terminal sequence of α_{s1}-, α_{s2}- and β-caseins of *Camelus bactrianus* and *Camelus dromedarius*; comparison with α_{s1}-, α_{s2} and β-caseins of cow, goat, mouse and pig.

Séquence N-terminale des caséines α_{s1}, α_{s2} et β de Camelus bactrianus *et* Camelus dromedarius; *comparaison avec les caséines α_{s1}, α_{s2} et β de vache, chèvre, souris et porc.*

	5 10 15 20
α_{S1}	
Cow	**R** - **P**-**K**-H-**P**-I-K-H-Q-G-L-P-**Q**-E-V-L-N-E-N-L-
Goat	**R** - **P**-**K**-H-**P**-I-N-H-R-G-L-S-P-E-V-**P**-N-E-N-L-
Mouse	**R** - L-H-S-R-N-A-V-S-S-Q-T-**Q**-Q-Q-H-S-**S**-S-**E**-
Pig	**R** - **P**-**K**-P-**P**-**L**-**R**-H-Q-**E**-H-L-**Q**-**N**-**E**-**P**-**D**-**S**-R-**E**-
Camel B	**R**/K-**P**-**K**-Y-**P**-**L**-**R**-Y-P-**E**-V-F-**Q**-**N**-**E**-**P**-**D**-**S**-**I**-**E**-
Camel D	**R**/K-**P**-**K**-Y-**P**-**L**-**R**-Y-P-**E**-V-F-**Q**-**N**-**E**-**P**-**D**-**S**-I-**E**-
α_{S2}	
Cow	**K**-N-T-**M**-E-H-V-S-S-S-**E**-**E**-S-I-**I**-S-Q-E-T-Y-**K**-
Goat	**K**-**H**-K-**M**-E-H-V-S-S-S-**E**-**E**-P-I-N-I-F-**Q**-E-I-Y-
Mouse	**K**-Q-R-**M**-E-**Q**-Y-I-S-S-**E**-**E**-S-M-D-N-S-**Q**-E-N-F-
Pig	**K**-**H**-**E**-**M**-E-H-V-S-S-S-**E**-**E**-S-I-N-I-S-**Q**-E-K-Y-
Camel B	**K**-**H**-**E**-**M**-D-**Q**-G-X-X-X-**E**-**E**-Q-N-**I**-V-P-**Q**-K-X-**K**-
Camel D	**K**-**H**-**E**-**M**-D-**Q**-G-X-X-X-**E**-**E**-Q-N-**I**-V-P-**Q**-K-X-**K**-
β	
Cow	**R**-**E**-L-**E**-**E**-L-N-V-P-**G**-**E**-I-V-**E**-**S**-L-S-S-S-**E**-**E**-S-I-T-
Goat	**R**-**E**-Q-**E**-**E**-L-N-V-V-**G**-**E**-T-V-**E**-**S**-L-S-S-S-**E**-**E**-S-I-T-
Mouse	**R**-**E**-T-T-F-T-V-S-S-E-T-D-S-I-**S**-S-E-E-S-V-**E**-H-I-N-
Pig	**R**-A-**K**-**E**-**E**-L-N-A-S-**G**-**E**-T-V-**E**-**S**-L-S-S-S-**E**-**E**-S-I-T-
Camel B	**R**-**E**-**K**-**E**-**E**-F-K-T-A-**G**-**E**-A-L-**E**-X-I-X-X-X-X-X-X-X-X-
Camel D	**R**-**E**-**K**-**E**-**E**-F-K-T-A-**G**-**E**-A-L-**E**-**S**-I-X-X-X-**E**-**E**-Q-T-H-

mined in order to confirm their identification as α_{s1}-, α_{s2}-, and β-caseins. A comparative study (amino acid composition and N-terminal sequence) of the different caseins obtained from the two species of camels (*Camelus dromedarius* and *Camelus bactrianus*), showed that they are identical. Interestingly, the N-terminal sequence of caseins from the two species of *Camelus* showed more homologies to the N-terminal region of caseins of pig than to other animals studied (cow, goat, mouse). However, they preserved the strictly conservative amino acid positions. Two N-terminal amino acid residues (arginine and lysine) have been obtained in equal amount in the sequence of α_{s1}-like casein of the two species of *Camelus*. The animals could be heterozygous for this locus. In the sequence of β-like casein of *Camelus dromedarius* and *Camelus bactrianus*, some amino acids could not be identified with sufficient accuracy. In their bovine counterpart, they correspond to phosphoseryl residues.

Alkaline urea-PAGE (fig 2C), amino acid compositions (table II) and N-terminal sequence (table III) of proteins obtained in peaks 3, 4 and 5 suggested that they were camel β-, α_{s1}- and α_{s2}-like caseins, respectively. The camel α_{s2}-like and α_{s1}-like caseins presented a higher and a lower mobility, respectively, than that of their bovine counterparts. This is probably depending on the degree of their phosphorylation (Mohammed and Larsson-Raznikiewicz, 1991). Camel β-like casein

presented a band with a migration similar to that of cow κ-casein, which agrees well with the result of Larsson-Raznikiewicz and Mohammed (1986).

Larsson-Raznikiewicz and Mohammed (1986) have obtained dromedary κ-like casein, as a little peak between β- and α_{s1}-caseins only once during several separations of dromedary whole casein by anion exchange chromatography on DEAE-cellulose. According to our results, camel κ-like casein co-eluted with camel β-like casein, eg, in fraction 3. As could be seen by alkaline urea-PAGE, camel κ-like casein migrated just below the electrophoretic band of camel β-like casein and it separated in three small bands.

In order to confirm this result, a test with chymosin was performed on fraction 3. The camel κ-like casein was incubated with chymosin and then submitted to alkaline urea-PAGE. The results showed (fig 3) that the three little bands disappeared after chymosin action. Under the same conditions, β-casein was resistant to chymosin action. The concentration of the solution of κ-like camel casein loaded on the gel needed to be high (until 10 mg/mL), otherwise it was impossible to observe its characteristic bands and to see their disappearance upon action of chymosin. Consequently, it can be supposed that camel milk contains very little amounts of κ-casein. Since its cow counterpart contains cysteyl residue, the anion-exchange chromatography fraction containing camel κ-like casein was further passed through an affinity chromatography column containing thiol-activated Affi-Gel 501 (Pharmacia). Unfortunately, this proved to be unsuccessful since no thiol containing protein was retained.

SDS-PAGE patterns of camel whole casein and of its fractions issued from ion-exchange chromatography are presented in figure 4. All the bands observed by SDS-PAGE of whole camel casein were recov-

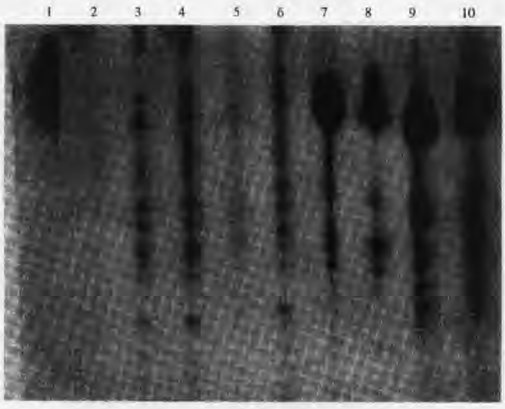

Fig 3. Action of chymosin as evidenced by Urea-PAGE. Lanes: 1, cow κ-casein; 2, cow κ-casein after chymosin action; 3, 4, fraction 2 issued from ion exchange chromatography of *Camelus dromedarius* casein, before and after chymosin action, respectively; 5, 6, fraction 2 issued from ion exchange chromatography of *Camelus bactrianus* casein, before and after chymosin action, respectively; 7, 8, fraction 3 issued from ion exchange chromatography of *Camelus dromedarius* casein, before and after chymosin action, respectively; 9, 10, fraction 3 issued from ion exchange chromatography of *Camelus bactrianus* casein, before and after chymosin action, respectively.

Mise en évidence de l'action de la chymosine par électrophorèse sur gel de polyacrylamide, en présence d'urée. 1 : caséine κ de vache ; 2 : caséine κ de vache après action de la chymosine ; 3 et 4 : fraction 2, issue de la chromatographie sur échangeur d'ions de la caséine entière de Camelus dromedarius, *respectivement avant et après action de la chymosine ; 5 et 6 : fraction 2, issue de la chromatographie sur échangeur d'ions de la caséine entière de* Camelus bactrianus, *respectivement avant et après action de la chymosine ; 7 et 8 : fraction 3, issue de la chromatographie sur échangeur d'ions de la caséine entière de* Camelus dromedarius, *respectivement avant et après action de la chymosine ; 9 et 10 : fraction 3, issue de la chromatographie sur échangeur d'ions de la caséine entière de* Camelus bactrianus, *respectivement avant et après action de la chymosine.*

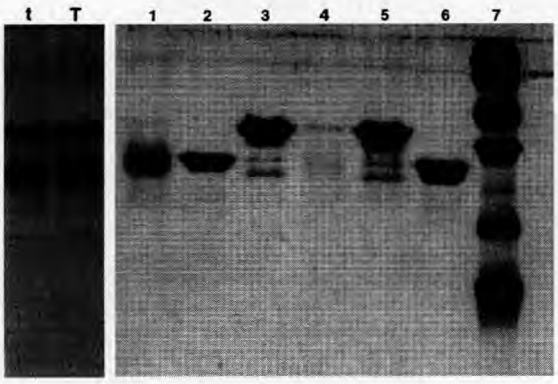

Fig 4. SDS-PAGE of camel caseins. t is whole *Camelus bactrianus* casein; T is whole *Camelus dromedarius* casein; 1, 2, and 3 are fractions 3, 4, and 5 issued from ion exchange chromatography of *Camelus bactrianus* casein, respectively; 4, 5, and 6 are fractions 3, 4, and 5 issued from ion exchange chromatography of *Camelus dromedarius* casein, respectively; 7 is molecular mass standard.

Électrophorèse sur gel de polyacrylamide, en présence de SDS des caséines de Camelus bactrianus *et* Camelus dromedarius. *t : caséine entière de* Camelus bactrianus *; T : caséine entière de* Camelus dromedarius *1, 2 et 3 : fractions 3, 4 et 5 respectivement, issues de la chromatographie sur échangeur d'ions de la caséine entière de* Camelus bactrianus ; *4, 5 et 6 : fractions 3, 4 et 5 respectivement, issues de la chromatographie sur échangeur d'ions de la caséine entière de* Camelus dromedarius ; *7 : marqueurs de poids moléculaires.*

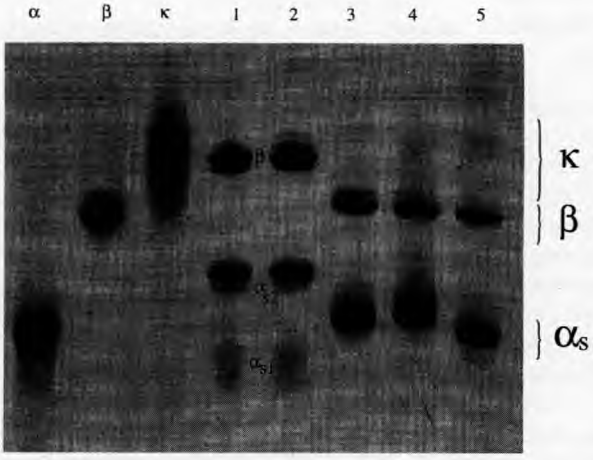

Fig 5. Urea-PAGE patterns of total caseins. 1, *Camelus bactrianus*; 2, *Camelus dromedarius*; 3, yak; 4, khainak; 5, bovine.

Électrophorèse sur gel de polyacrylamide, en présence d'urée des caséines totales de: 1, Camelus bactrianus ; *2,* Camelus dromedarius ; *3, yak; 4, khainak ; 5, vache.*

ered after fractionation by ion-exchange chromatography. The molecular masses of casein components were estimated through calculation with Bioprofil station program. The molecular masses of β-, α_{s1}- and α_{s2}-like casein of dromedary *(Camelus dromedarius)* are 27 500, 35 300, and 26 300, respectively, and those for camel *(Camelus bactrianus)* are 27 400, 34 300 and 26 000, respectively. Some bands with lower molecular masses were present in fraction 4 issued from ion exchange chromatography, possibly resulting from a degradation. The determination of the molecular masses of strongly hydrophilic or hydrophobic proteins results sometimes in under- or

overestimated values (Trieu-Cuot and Gripon, 1981; Creamer and Richardson, 1984). Bovine caseins contain strongly hydrophilic (such as the N-terminal domain of β-casein, as well as 8–12, 56–63, 129–199 residues of α_{s2}-casein) and hydrophobic (1-44, 90-113, 132-199 residues of α_{s1}-casein, as well as C-terminal domain of β-casein) regions (Swaisgood, 1992), and the existence of the similar fragments in camel caseins can be expected too.

CONCLUSIONS

The amino acid compositions of whole caseins from yak and khainak are quite similar and do not differ significantly from the amino acid composition of bovine whole casein. As shown by ion-exchange chromatography and urea-PAGE, yak and khainak caseins present similar chromatographic and electrophoretic patterns.

Camel and dromedary milks contain all four caseins homologous with cow α_{S1}-, α_{S2}-, β-, and κ-caseins. The existence of κ-like casein in camel was demonstrated by alkaline urea-PAGE and chymosin digestion. It is migrating a bit faster than camel β-like casein and it is resolving in three bands. As suggested by the results of anion exchange chromatography on QAE-Sepharose column, which show that camel κ-like casein elutes in higher NaCl molarity (0.21 mol/L) than its bovine counterpart, camel κ-like casein has a higher positive net charge. According to their amino acid composition and their N-terminal sequence, the caseins from milks of two camel species (*Camelus dromedarius* and *Camelus bactrianus*) do not present many dissimilarities. The observed differences of their molecular masses are either due to few variations in their primary sequences or to the divergence of their glycosylation and/or phosphorylation.

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