New Ultrarapid Method for the Separation of Milk Proteins by Capillary Electrophoresis

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A new method for the separation of major milk proteins has been investigated by capillary electrophoresis. The method is based on the micellar electrokinetic chromatography principle, which is a combination of classical capillary zone electrophoresis and hydrophobic interaction chromatography, resulting in the use of surfactant included in the electrophoretic media. These proteins are separated successfully after complete denaturation with sodium dodecyl sulfate and DL-dithiothreitol. The separation of α_s-, β-, and κ-caseins is discriminating but needs improvement with α_{s1}- and α_{s2}-caseins. Separation of major whey proteins, α-lactalbumin and β-lactoglobulin, is also perfectly achieved, and migration times are different from those of the caseins. This capillary electrophoresis technique, combined with the appropriate protocol for sample preparation, gives a separation of each protein in ≤90 s.

Keywords: *Capillary electrophoresis; critical micellar concentration; denaturation; milk proteins; purification efficiency*

INTRODUCTION

New perspectives in terms of milk protein separation have been investigated for fast identification of milk samples and to separate protein solution.

Milk proteins have been extensively characterized using batch or column chromatography (Gordin et al., 1972; Andrews et al., 1985; Manji et al., 1985; Ng-Kwai-Hang and Pélissier, 1989; Hollar et al., 1991) and polyacrylamide gel electrophoresis (Ng-Kwai-Hang and Kroeker, 1984; Basch et al., 1985; Zeece et al., 1985). However, these methods are laborious and time-consuming. Recently, new methods based on the capillary zone electrophoresis (CZE) technique were developed. These methods based on the classical CZE principle were usually carried out using urea (De Jong et al., 1993; Otte et al., 1994; Van Riel et al., 1995; Patterson et al., 1995; Recio et al., 1996). However, these techniques take about half an hour to achieve total protein separation, and the inherent disadvantage was urea crystallization occurring after a few electrophoretic runs.

A new rapid method for the milk protein separation by micellar electrokinetic chromatography (MEKC) was studied. Our method was a combination of classical free zone capillary electrophoresis and hydrophobic interaction chromatography generated by surfactant micelles introduced within the separation buffer. Protein samples were completely denatured before injection to disrupt the casein micelles' ultrastructure and give a net negative charge to protein monomers.

MATERIALS AND METHODS

Raw Materials. Skimmed milk was obtained from the Triballat dairy (composition of milk is given in Table 1).

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Table 1. Composition of the Nitrogen Fractions of both Fresh and Powdered Milk[a]

	fresh milk	milk powder
TN	34.65	365.4
NCN	7.43	80.12
NPN	1.92	21.04
dry extract	90.48	949.86

[a] TN is the total nitrogen value, NCN is the noncaseinic nitrogen value, and NPN is the nonprotein nitrogen value. All results are given in g/kg.

To avoid any heat treatment (such as sterilization or pasteurization), the milk was microbiologically purified by microfiltration on a mineral membrane as described by Trouvé et al. (1991): milk was filtered at 50 °C on a mineral membrane of 1.4 μm pore diameter and 1.4 m^2 surface. Milk was evaporated and low-heat-dried on a multistage dryer tower (Niro atomiser MSD, Rueil Malmaison, France). Inlet and outlet temperatures were 250 and 88 °C with a feed rate of 250 L/h. Air temperature in the integrated fluid bed was 70 °C; exhaust air temperature in the second part of the vibrofluidizer was 35 °C.

The powder was stored at 4 °C in sealed plastic bags under an N_2 atmosphere to prevent any oxidation of protein sulfhydryl groups (composition of powder is given in Table 1).

Protein Preparation. *Preparation of Reconstituted Milk.* Skimmed milk powder was reconstituted to initial milk dry matter content (9.05% in mass) with deionized water and allowed to equilibrate for 1 h with stirring.

Isolation of Micellar Caseins. Casein micelles were obtained from milk heated at 100 °C for 4 min. They were obtained by ultracentrifugation of the heated milk at 45000*g* for 45 min at a temperature of 20 °C on a Kontron centrifuge (Kontron Instruments). The micelles were resuspended in a milk simulated ultrafiltrate (Jenness and Koops, 1962) to prevent any structural alteration of casein micelles. This medium possesses the same pH and mineral composition of a milk ultrafiltrate (Table 2). For its preparation, each compound is pounded and successively solubilized in distilled water, at room temperature.

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Table 2. Composition of the Jenness−Koops Media (Jenness and Koops, 1962)

name of compound	formula	g/L	*n* (mmol)
potassium dihydrogenophosphate	KH_2PO_4	1.58	10.253
potassium citrate, monohydrated	$K_3C_6H_5O_7 \cdot H_2O$	0.508	1.566
sodium citrate, dihydrated	$Na_3C_6H_5O_7 \cdot 2H_2O$	1.791	6.089
potassium sulfate	K_2SO_4	0.18	1.034
calcium chloride, dihydrated	$CaCl_2 \cdot 2H_2O$	1.32	8.978
magnesium citrate, tetradecahydrated	$Mg_3C_{12}H_{10}O_{14} \cdot 14H_2O$	0.752	1.069
potassium carbonate	K_2CO_3	0.3	2.17
potassium chloride	KCl	1.078	14.458

Purified Proteins. α_s-, β-, κ-caseins were purified from the skimmed milk powder, as previously described by Cayot et al. (1993).

The purity of each fraction was checked by polyacrylamide gel electrophoresis (PAGE). PAGE was carried out at pH 6.3 with a 7.5% (w/v) acrylamide gel in the presence of 6 M urea and DL-dithiothreitol, according to the method of Ng-Kwai-Hang and Kroeker (1984). The gel was stained with purified Coomassie brilliant blue, as previously described by Kundu et al. (1996). Kundu's method is based on purification on silica gel with three steps of elution [elution solvent is a mix of $CHCl_3$/MeOH used with a ratio of 7:1 (v/v), 9:1, and 0:10].

α-Lactalbumin was from Sigma Chemical Co. (St. Louis, MO).

β-Lactoglobulin was purified using both the method described by Fox et al. (1967) as modified by Ebeler et al. (1990) and the method described by Kinekawa and Kitabatake (1996). The method described by Fox et al. (1967) is commonly used in our laboratory, and we wanted to test the new method of Kinekawa and Kitabatake (1996) by comparing the two β-lactoglobulins. Ebeler's method was based on the selective precipitation of proteins by trichloroacetic acid, and Kinekawa's method consisted of pepsinic hydrolysis of whey.

Assessment of Whey Protein Purity. Purity of β-lactoglobulin was checked by SDS−PAGE (Laemmli, 1970). Electrophoresis was carried out at pH 8.9 on 14% acrylamide minigels (Novex, San Diego, CA). After migration, gels was stained with purified Coomassie brilliant blue (Kundu et al., 1996).

Sample Preparation. All of the reagents were purchased from Merck (Darmstadt, Germany) and are of the highest purity available (for analysis grade).

Proteins purified in urea media were primarily dialyzed against deionized water before electrophoresis specific treatment. Samples were then prepared in total denaturing conditions, resulting in the use of an anionic surfactant, sodium dodecyl sulfate, at the concentration of 10% (w/w) combined with a strong mercaptan (DL-dithiothreitol) at the concentration of 7% (w/w). To accelerate disulfide bond reduction, samples were heated at 100 °C for 3 min. This protocol induced the destruction of hydrophobic links (Låås, 1989) and disulfide bonds of milk proteins (Wolf, 1993), giving a net negative charge to proteins monomers (An der Lan et al., 1983) (Figure 1).

Separation Buffer Preparation. A 3 mM sodium borate (Fisher Scientific) was used as separation buffer; the pH of this buffer is 9.5. To generate a hydrophobic micellar phase, sodium dodecyl sulfate at concentrations above critical micellar concentration (cmc = 8.2 mM) was added (James and Lord, 1992). Six concentrations were tested from cmc to cmc × 6.

Capillary Electrophoresis. Capillary electrophoresis was performed on a Beckman P/ACE 5000 system (Beckman Instruments, Palo Alto, CA) controlled by a computer equipped with System Gold software in its GEM version (Beckman). The protein separation was performed in uncoated fused silica capillaries (20 μm i.d., 27 cm length) purchased from Beckman. Electrophoresis was conducted at 20 °C and voltage of 30 kV, toward cathode. The UV detector was set at 214 nm and applied at 7 cm of the cathode capillary extremity (collection rate of the detector was 10 Hz). The injection time was 2 s.

The capillary was rinsed sequentially between successive electrophoretic runs with 0.1 M sodium hydroxide (1 min) and 3 mM sodium borate buffer (2 min). Separations were performed in separation buffers described under Separation Buffer Preparation.

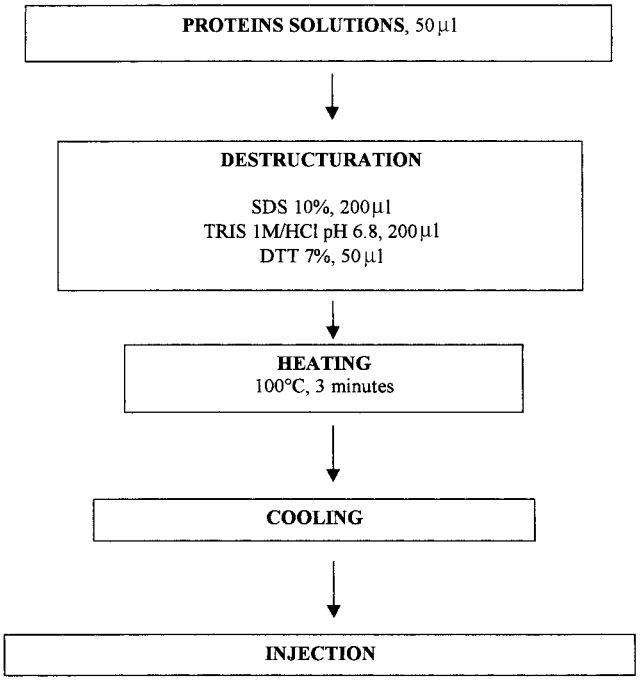

Figure 1. Flow sheet for sample preparation. SDS, sodium dodecyl sulfate; DTT, DL-dithiothreitol.

RESULTS AND DISCUSSION

Separation of Caseins. Electropherograms obtained with electrophoretic buffer supplemented with SDS at cmc × 2 to cmc × 6 (data not shown) are bad, mainly due to the excessive amount of hydrophobic phase. It generates a noisy electropherogram; our hypothesis for these results is that the hydrophobic phase is too abundant above the cmc to allow a good separation of the protein, which is then "imprisoned" in the micellar phase (Figure 2).

Results obtained with electrophoretic media supplemented with SDS at the cmc were better than those obtained only with sodium borate buffer without SDS. Our hypothesis is that the complex formed by the SDS and the protein gets dissociated during its migration within the capillary (Figure 2).

Figure 3 shows the electropherograms obtained with purified β-casein (a) and α_s-casein (b). Following capillary electrophoresis purified β-casein was resolved into one major peak and three minor peaks (Figure 3a). α_s-Casein was resolved in one major peak and three minor peaks (Figure 3b).

This capillary electrophoresis is consistent with the PAGE (urea denaturing conditions) pattern of these enriched fractions (Figure 4). In fact, the PAGE profile shows that the β-casein collected fraction contains traces of α_s-casein and that the α_s-casein collected fraction

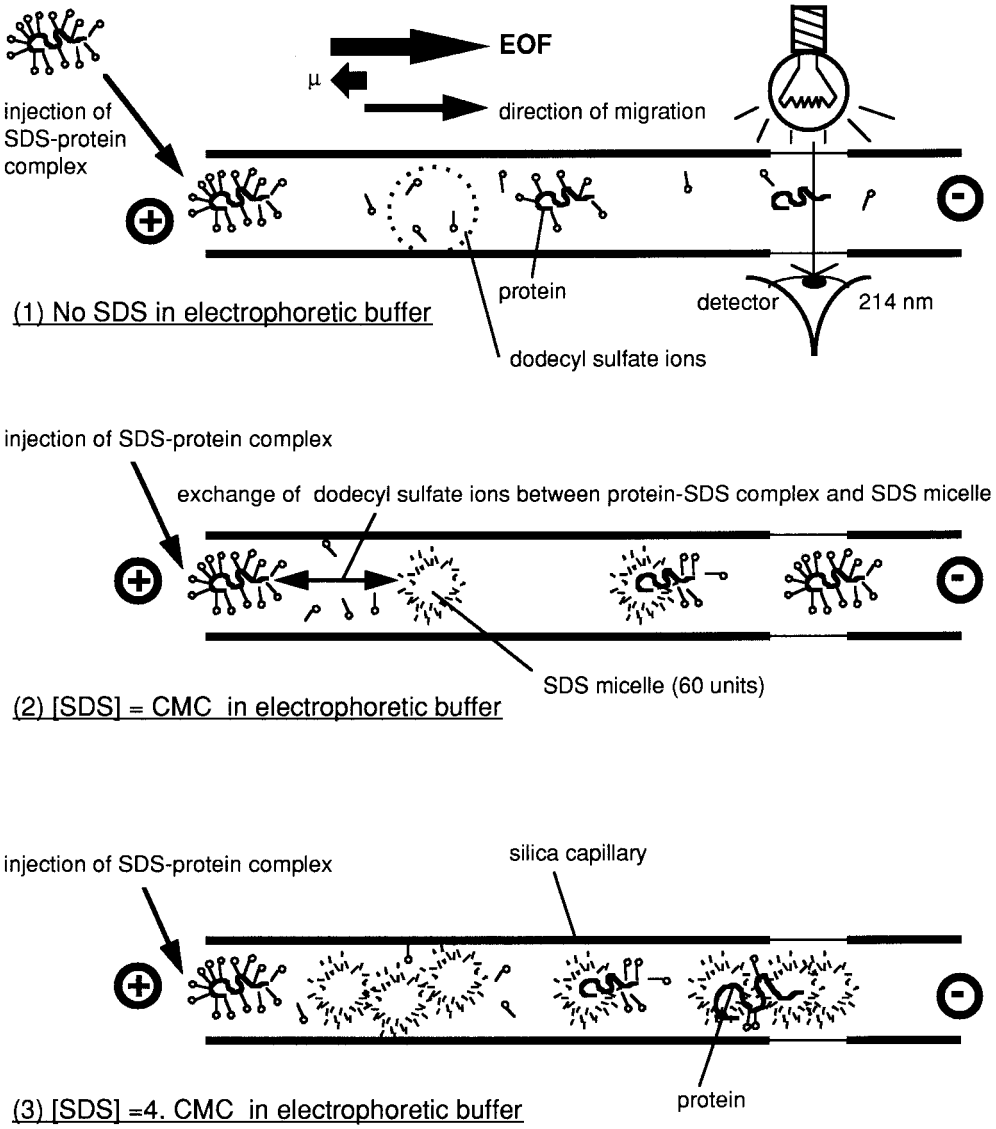

Figure 2. Hypothetical scheme on the relation between the SDS concentration in electrophoretic buffer and its interaction with the SDS/protein complex during the electrophoretic run. EOF, electro-osmotic flow; μ, electrophoretic mobility of the protein or protein/SDS complex; cmc, critical micellar concentration of SDS (8.2 mM). The electrophoretic buffer is 3 mM sodium borate (pH 9.5) supplemented or not with SDS [according to case 1, 2, or 3: (1) buffer contains no SDS, so the complex SDS/protein could be perturbed (result is an alteration of the electrophoretic mobility within the capillary as the separation occurs; (2) SDS at its cmc within the buffer, separation is efficient, and the main criterion is SDS/protein complex hydrophobicity; (3) SDS is in excess, the amount of micellar phase is two high, and SDS/protein complex gets imprisoned between the SDS micelles, generating a poor separation].

contains traces of β-casein. This statement could be explained by the ionic strength of the buffer used for β-casein (0.2 M NaCl) elution that may be too high, causing the coelution of α_s-caseins. Futhermore, the increase of ionic strength (from 0.2 to 0.3 M NaCl) inducing elution of α_s-caseins may be quite untimely, occurring with the coelution of residual β-casein. The three peaks present in Figure 3a (minor) and in Figure 3b (major) could be assimilated as the band of α_s-casein that occurred slightly in the PAGE pattern of β-casein enriched fraction and strongly in the pattern of the α_s-casein collected fraction. In the same way, the major peak of Figure 3a and the minor peak of Figure 3b could be assimilated as β-casein.

Therefore, our electrophoretic technique allows a separation of these two major casein in 0.56 min for α_s-caseins and in 0.71 min for β-casein. Futhermore, high-performance capillary electrophoresis (HPCE) seems to enhance the detection of impurities in collected fractions compared to purity assessment done both by urea−PAGE and by fast protein liquid chromatography (Table 3). The performance of these techniques will be discussed later.

Separation of Major Whey Proteins. *Separation of α-Lactalbumin.* For α-lactalbumin, electropherograms obtained with unsupplemented buffer and buffer supplemented with SDS at cmc × 2 to cmc × 6 (data not shown) give bad results. The best results were obtained with electrophoretic media supplemented with SDS at its cmc. Separation of sample was achieved in 0.87 min. The electropherogram shows only one major peak, which could then be identified as α-lactalbumin (Figure 5).

Separation of β-Lactoglobulins. *(a) Enzymically Purified β-Lactoglobulin.* Best results were obtained with electrophoretic media supplemented with SDS at its cmc. Following capillary electrophoresis, sample separation gives three peaks (Figure 6a). The electro-

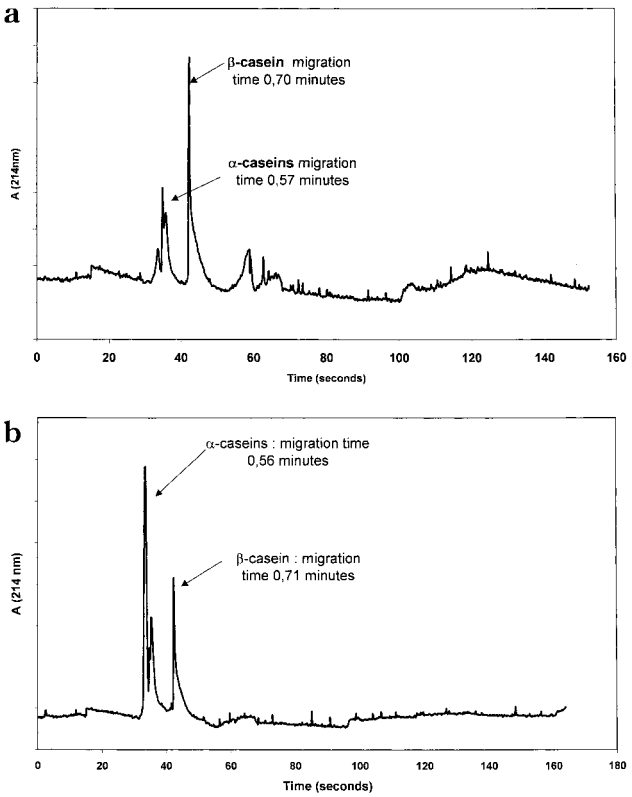

Figure 3. (a) Electropherograms of β-casein purified fraction. Sample was totally denatured and 2 s pressure injected; electrophoresis was performed at 30 kV toward cathode in cmc SDS-supplemented buffer. (b) Electropherogram of α-casein purified fraction. Sample was totally denatured and 2 s pressure injected into the capillary; electrophoresis was performed at 30 kV toward cathode in cmc SDS-supplemented buffer.

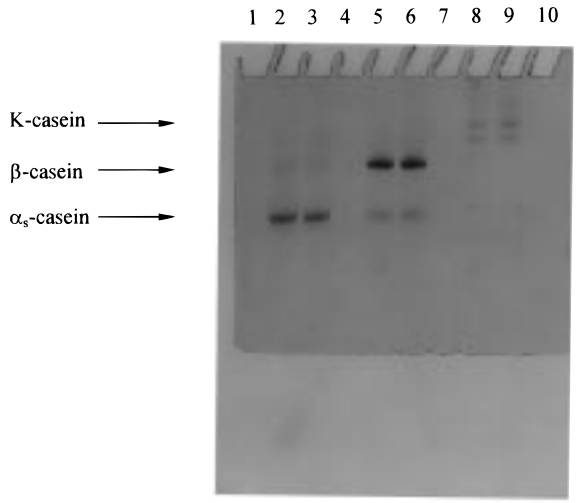

Figure 4. Visualization of purity of batch-collected fractions by urea−PAGE using a 7% (w/v) polyacrylamide gel: (lanes 2 and 3) α_s-casein purified fraction; (lanes 5 and 6) β-casein purified fraction; (lanes 8 and 9) κ-casein purified fraction.

pherogram obtained after injection of pepsin alone was superimposed on the electropherogram obtained with purified β-lactoglobulin. The β-lactoglobulin could then be identified at a migration time of 1.16 min.

(b) β-Lactoglobulin Purified with TCA. Best results are obtained with electrophoretic media supplemented with SDS at its cmc. Following capillary electrophoresis, sample separation gives one group of peaks. In comparison with the SDS−PAGE pattern, which shows only one protein species, these peaks could be considered as β-lactoglobulin (Figure 6b). In this case, the migration time is 0.53 min.

Table 3. Average Purities of Separated Casein Fractions As Measured by Fast Protein Liquid Chromatography Analysis (FPLC) and Densitometric Analysis of the Urea PAGE Pattern (Cayot et al., 1993) and High-Performance Capillary Electrophoresis (HPCE) (Peak Areas Have Been Taken into Account)[a]

casein fraction / casein component	whole casein	κ-casein	β-casein	α_s-casein
	FPLC			
κ	11.4	100	2.9	2.3
β	42.7	0	91	2.3
α_s	45.9	0	6.1	95.4
	Densitometry of the Urea PAGE Pattern			
κ	18.1	100	4.6	0
β	38.1	0	95	0.9
α_s	43.8	0	0.4	99.1
	HPCE			
κ	ND[b]	ND	ND	ND
β	ND	ND	78	26
α_s	ND	ND	22	74

[a] All results given in % purity. [b] ND, not determined.

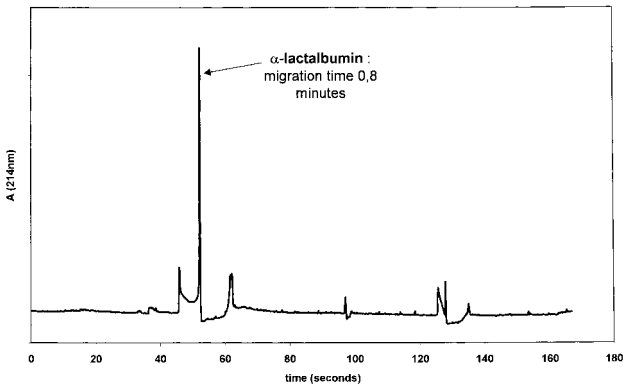

Figure 5. Electropherogram obtained with purified α-lactalbumin. Sample was totally denatured and 2 s injected into the capillary; electrophoresis was performed at 30 kV toward cathode in cmc SDS-supplemented buffer.

(c) Discussion. This analysis is not consistent with the SDS−PAGE pattern of purified (Figure 7) β-lactoglobulins. In fact, the PAGE outlines no differences between TCA and pepsin purification processes even though sample preparation is similar for PAGE and HPCE. This suggested that HPCE includes another separation criterion (see Separation of Micellar Caseins).

It became clear that HPCE is more sensitive than PAGE to the conformational structure of proteins. The TCA purification method modifies the structure of β-lactoglobulin, whereas pepsin treatment does not affect protein structure.

Furthermore, the affinity for the micellar phase could change depending on the hydrophobic region exposed. According to this hypothesis and according to our experimental results, we could assume that β-lactoglobulin purified with TCA possesses less accessible hydrophobic regions than enzymically purified β-lactoglobulin.

It became clear that HPCE is more sensitive than PAGE to the conformational structure of proteins. TCA affects protein structure, whereas pepsin does not alter protein conformation. The TCA method should denature the protein; this was already assessed by immunochemical methods (D. Lorient, personal communication).

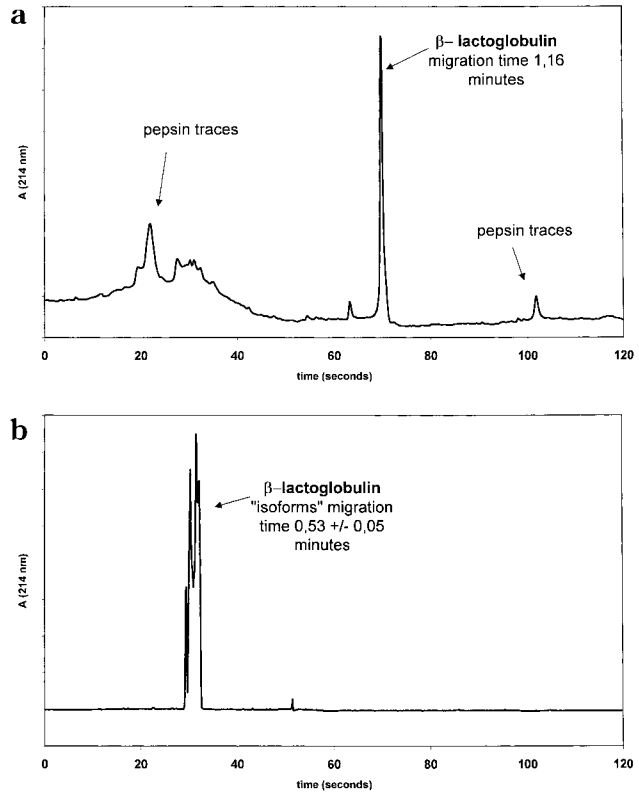

Figure 6. Electropherogram obtained with pepsin-purified β-lactoglobulin (a) or with TCA-purified β-lactoglobulin. (b) Sample was totally denatured and 2 s pressure injected into the capillary; electrophoresis was performed at 30 kV toward cathode in cmc SDS-supplemented buffer.

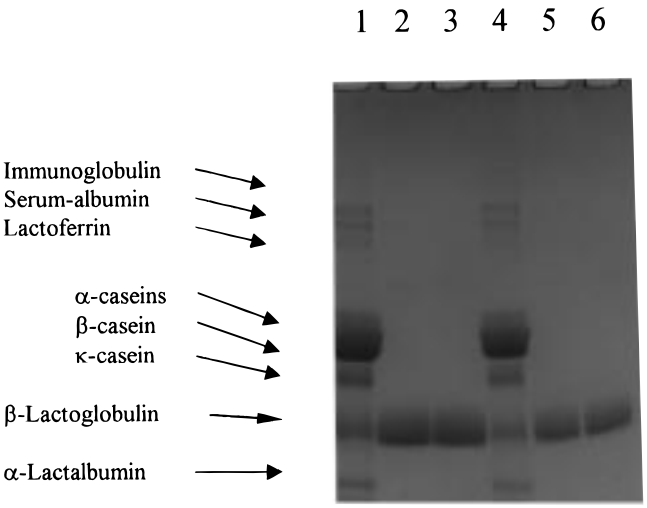

Figure 7. Visualization of purity of isolated β-lactoglobulins by SDS/DTT−PAGE using a 14% (w/v) acrylamide gel: (lanes 1 and 4) whole milk; (lanes 2 and 3) pepsin-purified β-lactoglobulin; (lanes 5 and 6) TCA-purified β-lactoglobulin.

Furthermore, it became clear that our results give indications for the purification method choice. Indeed, the main advantage for the purification with pepsin is that the protein is "native-like" (preservation of its hydrophobic regions, see Separation of Micellar Caseins), but it still has pepsin traces in the samples. The main disadvantage of the TCA method is that you are not perfectly sure that all of the TCA has been eliminated with the dialysis step, and so the structure of the protein is obviously altered.

Separation of Micellar Caseins. Casein micelles were prepared from reconstituted milk and resuspended in a Jenness−Koops medium (Jenness and Koops, 1962). To disrupt the micelle ultrastructure, our technique uses SDS and DTT rather than urea.

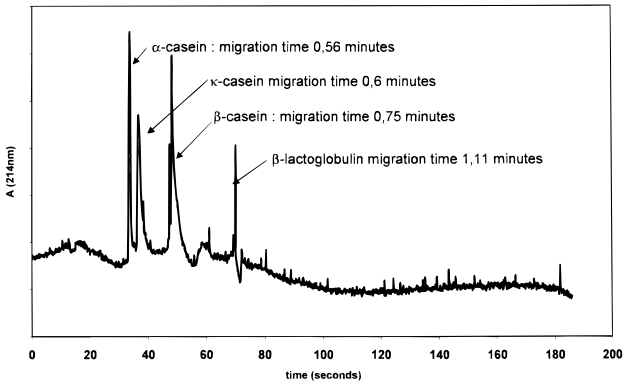

Figure 8. Electropherogram obtained with casein micelle suspension isolated from heated milk. Sample was totally denatured and 2 s pressure injected into the capillary; electrophoresis was performed at 30 kV toward cathode in cmc SDS-supplemented buffer.

Table 4. Results Obtained in Terms of Migration Time and Comparison with the Values of Total Hydrophobicity (Cheftel et al., 1985) and Molecular Weight (Cayot and Lorient, 1997) of Each Major Milk Protein

type of milk protein	migration time (min)	total hydrophobicity (kJ/residue)	mol wt
α_{s2}-casein	0.55	4.64	25228
α_{s1}-casein	0.56	4.89	23612
β-casein	0.61	5.12	19005
κ-casein	0.71	5.58	23980
α-lactalbumin	0.8	4.68	14174
β-lactoglobulin	1.16	5.03	18362

Sample analysis is achieved in <2 min, and best results are obtained with electrophoretic media supplemented with SDS at its cmc. The electropherogram obtained (Figure 8) gives four major peaks, which could be identified as the four major caseins involved in micelle formation: α_{s1}-, α_{s2}-, β-, and κ-caseins. We also identified thermally grafted β-lactoglobulin, according to our previous results, carried out with SDS−PAGE (Fairise et al., 1997).

In our case, the protein migration rates could be correlated to their total hydrophobicity, although in SDS−PAGE separation is based on protein molecular weight. Therefore, β-lactoglobulin, which has a hydrophobicity higher than the one of α-lactalbumin, possesses the higher migration time (Table 4). At the cmc of SDS, β-lactoglobulin could fix more SDS than α-lactalbumin, as it is more hydrophobic. The electrophoretic mobility (which is against the electro-osmotic flow) is then higher and the migration time increases.

For the four caseins, the same hypothesis could be formulated. Indeed, as β-casein is the most hydrophobic, it fixes more SDS and possesses the higher migration time.

In any case, the differences observed between the migration times of whey proteins and caseins could not be explained by the comparison of the proteins total hydrophobicities, whereas an analysis of the hydrophobic region repartition probably could.

A logarithmic curve showing molecular weight versus electrophoretic migration distance (Weber and Osborn, 1969; Sharpiro et al., 1967) is unappropriate in our case. If it could be, the order of migration should have ben α_{s2}-, β-, α_{s1}-, and κ-casein.

Our migration order is not consistent with this hypothesis, outlining two inversions. These facts have already been reported in the case of glycoproteins (like κ-casein): as the ratio SDS/polypeptide is not sufficient (it needs to be 3:1 at least) or as the percentage of acrylamide tends to be zero (Hames, 1990). Therefore, we could assume that in our micellar electrokinetic chromatography technique, we are in experimental conditions close to those obtained in PAGE with a *T* value of zero.

CONCLUSION

The overall results (Table 4) show that HPCE effectively resolved milk caseins and whey proteins in a very short time (<2 min maximum). Best separations are achieved with 3 mM sodium borate supplemented with SDS at its cmc; however, β-lactoglobulins separated with cmc SDS-supplemented electrophoretic medium give different migration times, depending on the purification process. In fact, our technique has showed that the type of protein obtained possesses different electrophoretic behavior and that β-lactoglobulin purified with TCA possesses less affinity for the hydrophobic phase.

These results allow us to discuss the concept of pure protein. Indeed, what does purity means? Is it the same amino acid sequence or the same conformational structure? According to our electropherograms, it became clear that purity could not be related to the preservation of the conformational structure.

In addition, unlike many other CE techniques used for milk protein separation, our method uses no urea, avoiding de facto all of the problems caused by urea crystallization.

Separations of more complex systems, such as whey powder or whole milk, are now in process.

LITERATURE CITED

An der Lan, B.; Horuk, R.; Sullivan, J. V.; Chrambach, A. An improved and simplified apparatus for protein extraction and concentration from gel slices, using moving boundary electrophoresis. *J. Biochem. Biophys. Methods* **1983**, *7*, 101−113.

Andrews, A. T.; Taylor, M. D.; Owen, A. J. Rapid analysis of bovine milk proteins by fast protein liquid chromatography. *J. Chromatogr.* **1985**, *348*, 177−185.

Basch, J. J.; Douglas, Jr., F. W.; Procino, L. G.; Holsinger, V. H.; Farrell, Jr., H. M. Quantitation of caseins and whey proteins of processed milks and whey protein concentrates, application of gel electrophoresis, and comparison with Harland-Ashworth procedure. *J. Dairy Sci.* **1985**, *68*, 23−31.

Cayot, P.; Lorient, D. *Structures et Technofonctions des Protéines du Lait*; Arilait-Recherches; Lavoisier: Paris, France, 1997.

Cayot, P.; Courthaudon, J.-L.; Lorient, D. Purification of α_s-, β-, and κ-caseins by batchwise ion-exchange separation. *J. Dairy Res.* **1992**, *59*, 551−556.

Cheftel, J. C.; Cuq, J. L.; Lorient, D. Propriétés physicochimiques des acides aminés et des protéines. In *Protéines Alimentaires*; Cheftel, J. C., Cuq, J. L., Lorient, D., Eds.; Lavoisier: Paris, France, 1985.

De Jong, N.; Visser, S.; Olieman, C. Determination of milk proteins by capillary electrophoresis. *J. Chromatogr. A* **1993**, *652*, 207−213.

Ebeler, S. E.; Phillips, L. G.; Kinsella, J. E. Purification of β-lactoglobulin: Isolation of genetic variants and influence of purification method on secondary structure. *Milchwissenschaft* **1990**, *45*, 694−698.

Fairise, J. F.; Cayot, P.; Lorient, D. Characterisation of the protein composition of caseins micelles after heating. *Proceedings of Hannah Symposium*, Caseins and Caseinates: Structure Interactions Networks; 1997; in press.

Fox, K. K.; Holsinger, V. H.; Posati, L. P.; Pallansch, M. J. Separation of β-lactoglobulin from other milk serum proteins by trichloracetic acid. *J. Dairy Sci.* **1967**, *50* (9), 1363−1367.

Gordin, S.; Birk, Y.; Volcani, R. Fractionation and characterization of milk proteins by column chromatography and electrophoresis. *J. Dairy Sci.* **1972**, *55*, 1544−1549.

Hollar, C. M.; Law, A. J. R.; Dalgleish, D. G.; Brown, R. J. Separation of Major Casein Fractions using Cation-Exchange Fast Protein Liquid Chromatography. *J. Dairy Sci.* **1991**, *74*, 2403−2409.

Holt, D. L.; Zeece, M. G. Two-Dimensional electrophoresis of bovine milk proteins. *J. Dairy Sci.* **1988**, *71*, 2044−2050.

James, A. M.; Lord, M. P. *MacMillan's Chemical and Physical Data*; MacMillan Press: London, 1992.

Jenness, R.; Koops, J. Preparation and properties of a salt solution which simulates milk ultrafiltrate. *Neth. Milk Dairy J.* **1962**, *16*, 153−164.

Kinekawa, Y. C.; Kitabatake, N. Purification of β-Lactoglobulin from Whey Protein Concentrate by Pepsin Treatment. *J. Dairy Sci.* **1996**, *79* (03), 350−356.

Kundu, S. K.; Robey, W. G.; Nabors, P.; Lopez, M. R.; Buko, A. Purification of commercial Coomassie brilliant blue R-250 and characterization of the chromogenic fractions. *Anal. Biochem.* **1996**, *235*, 134−140.

Laas, T. Electrophoresis in gels. In *Protein Purification*; Janson, J. C., Ryden, L., Eds.; VCH: New York, 1989.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the of bacteriophage T4. *Nature* **1970**, *227*, 680−685.

Manji, B.; Hill, A.; Kakuda, Y.; Irvine, D. M. Rapid separation of milk whey proteins by anion exchange chromatography. *J. Dairy Sci.* **1985**, *68*, 3176−3179.

Ng-Kwai-Hang, K. F.; Kroeker, E. M. Rapid separation and quantification of major caseins and whey proteins of bovine milk by polyacrylamide gel electrophoresis. *J. Dairy Sci.* **1984**, *67*, 3052−3056.

Ng-Kwai-Hang, K. F.; Pélissier, J. P. Rapid separation of bovine caseins by mass ion exchange chromatography. *J. Dairy Res.* **1989**, *56*, 391−397.

Otte, J.; Kristiansen, K. R.; Zakora, M.; Qvist, K. B. Separation of individual whey proteins and measurement of α-lactalbumin and β-lactoglobulin by capillary zone electrophoresis. *Neth. Milk Dairy J.* **1994**, *48*, 81−97.

Paterson, G. R.; Otter, D. E.; Hill, J. P. Application of capillary electrophoresis in the identification of phenotypes containing the β-lactoglobulin C variant. *J. Dairy Sci.* **1995**, *78*, 2637−2644.

Recio, I.; Olieman, C. Determination of denatured serum proteins in the casein fraction of heat treated milk by capillary electrophoresis. *Electrophoresis* **1996**, *17*, 1228−1233.

Trouvé, E.; Maubois, J. L.; Piot, M.; Madec, M. N.; Fauquant, J.; Rouault, A.; Tabard, J.; Brinkman, G. Rétention des différentes espèces microbiennes lors de l'épuration du lait par microfiltration en flux tangentiel. *Lait* **1991**, *71*, 1−13.

Van Riel, J.; Olieman, C. Determination of caseinomacropeptide with capillary zone electrophoresis and its application to the detection and estimation of rennet whey solids in milk and butter milk powder. *Electrtophoresis* **1995**, *16*, 529−533.

Wolf, W. J. Sulfhydryl content of glycinin: effect of reducing agents. *J. Agric. Food Chem.* **1993**, *41*, 168−175.

Zeece, M. G.; Holt, D. L.; Wehling, R. L.; Liewen, M. B.; Bush, L. R. High-resolution two-dimensional electrophoresis of bovine caseins. *J. Agric. Food Chem.* **1989**, *37*, 378−383.

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