Effect of Protein Concentration on Rates of Thermal Denaturation of Whey Proteins in Milk

Andrew J. R. Law* and Jeffrey Leaver

Molecular Recognition Group, Hannah Research Institute, Ayr KA6 5HL, Scotland, U.K.

The effects of varying the concentrations of total protein, whey protein, and casein on levels of thermal denaturation of four main whey protein fractions in skim milk, namely immunoglobulins, serum albumin/lactoferrin, β-lactoglobulin, and α-lactalbumin, were examined by gel permeation fast protein liquid chromatography. On heating at 80 °C, the rates of denaturation of the individual whey proteins in skim milk increased with total protein concentration and, to a lesser extent, with increasing whey protein concentration. The levels of denaturation of the whey proteins were not affected by increasing micellar casein concentration but decreased markedly when the casein concentration was reduced below that in skim milk. In milks from different cows, rates of denaturation of individual whey proteins were not affected by β-lactoglobulin phenotype but were higher in milk from cows in late lactation.

Keywords: *Whey protein; denaturation; aggregation*

INTRODUCTION

Controlled thermal denaturation of whey proteins is commonly carried out in order to modify the processing characteristics of milk in the manufacture of various dairy products. On heating milk above 60 °C, the whey proteins undergo denaturation which involves an initial loss of their compact globular conformation, followed by aggregation (De Wit, 1990). On mild heating, the conformational changes may be reversible, but on more severe heating, the whey proteins tend to become associated through hydrophobic interaction (Parnell-Clunies *et al*., 1988; Regester *et al*., 1992) or disulfide linkage with one another or with the casein micelles (Sawyer, 1969; Noh and Richardson, 1989).

In the manufacture of yogurt, an initial heat treatment (typically 80−85 °C for 15−30 min) is carried out which causes extensive denaturation of the whey proteins. The association of the denatured whey proteins with the casein micelles gives improved texture and viscosity of the product (Davies *et al*., 1978; Robinson and Tamime, 1986). Similarly, forewarming milk (85 °C for 30 min), which causes almost complete denaturation of the whey proteins, improves the heat stability of concentrated milks and changes the viscosity of products such as sweetened condensed milk (Singh and Creamer, 1992). Also, in the manufacture of cheese, yields can be increased by carrying out an initial heat treatment which leads to incorporation of denatured whey proteins into the rennet curd (Banks *et al*., 1993).

Previous studies have shown that denatured whey proteins precipitate together with the caseins when heat-treated milk is subsequently adjusted to pH 4.6 (Rowland, 1937). The rates of denaturation of the whey proteins, on the basis of this decreased solubility at pH 4.6, have been determined by various methods as summarized previously (Law *et al*., 1994). These studies have provided useful information about conditions that cause denaturation of the whey proteins in milk and whey products, but comparatively little work has been carried out on the effect of protein concentration on the rates of denaturation of the individual whey proteins in milk. In the manufacture of some products such as yogurts, condensed milks, and hard and soft cheeses, an initial concentration step may be carried out by evaporation under vacuum or by ultrafiltration (Chapman *et al*., 1974). The concentrations or ratios of the proteins may also be adjusted by microfiltration or by the addition of skim milk and whey powders of different compositions (Robinson and Tamime, 1986).

In the present work, therefore, we have studied the effects of changing the concentrations of total protein, whey protein, and casein on the rates of denaturation of four main whey protein fractions in milk, while maintaining a constant concentration of nonprotein material. We have also examined the effects of β-lactoglobulin phenotype and stage of lactation of individual cows on the rates of denaturation of the individual whey protein fractions.

MATERIALS AND METHODS

Milk Samples. Bulk milk and individual milk samples were collected from Friesian cows in the Institute herd. The milks were skimmed by centrifugation at 1000*g* for 30 min. The concentrations of total protein, whey protein, and casein were adjusted as described below.

Total Protein Concentration. The total protein concentration of skim milk was increased by ultrafiltration using a stirred cell with a polysulfone membrane having a 10K molecular weight cutoff (Sartorius Limited, Epsom, Surrey, U.K.). Ultrafiltrate from the stirred cell was also added back to the original skim milk to reduce the concentration of total protein. The final concentrations of total protein, determined from the changes in volume, were 25, 50, 75, 125, 150, and 200% of the original.

Whey Protein Concentration. The concentration of whey protein was increased by the addition of freeze-dried whey protein to skim milk. The whey protein was prepared beforehand by precipitating casein from skim milk at pH 4.6 and 20 °C with addition of 1 M HCl, and passing the supernatant through Whatman No. 42 filter paper. Whey protein was obtained by dialyzing the acid filtrate against deionized water for 72 h, and freeze-drying. The final concentrations of whey protein in skim milk, determined by gel permeation FPLC as described below, were 123, 146, 178, and 259% of the original.

* Author to whom correspondence should be addressed [fax +44 (0)1292 671 052; e-mail lawa@main.hri.sari.ac.uk].

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Micellar Casein Concentration. The casein content of skim milk was increased by resuspending casein micelles in skim milk. Casein micelles were first sedimented by centrifuging skim milk at 50000*g* for 1 h at 20 °C in a fixed angle SS-34 rotor in a Sorvall centrifuge (Du Pont U.K. Ltd, Stevenage, Herts). The supernatant was decanted, and the casein micelles resuspended in appropriate volumes of skim milk to give casein concentrations 150 and 200% of that in the original skim milk.

The concentration of casein in skim milk was also reduced by adding acid whey that had been adjusted to pH 6.7 and dialyzed against milk. Acid whey was first prepared by precipitating casein from skim milk at pH 4.6 and 20 °C with addition of 1 M HCl, and the supernatant passed through Whatman No. 42 filter paper. The filtrate was adjusted to pH 6.7, and 50 mL was dialyzed against 2 × 3 L of milk for a total of 21 h, the milk being changed after 5 h. The dialysate was added to skim milk in various proportions to give concentrations of casein 9.1, 20.0, 33.3, and 50.0% of that in the original skim milk.

Individual-Cow Samples. To examine the effects of stage of lactation and β-lactoglobulin phenotype on rates of denaturation of the whey proteins, 23 milk samples were collected from 19 cows at different stages of lactation. The β-lactoglobulin phenotypes were determined by anion-exchange FPLC as described by Andrews *et al.* (1985).

Heat Treatment. Skim milk samples (10 mL) were placed in thin-walled glass test tubes 15 mm in diameter and 150 mm in length. The tubes were placed in a waterbath at 80 °C, allowed 1 min to warm to temperature, and maintained at this temperature. Samples (1.0 mL) were removed at intervals between 0.5 and 20 min, and rapidly cooled in ice.

Gel Permeation FPLC. Skim milk samples (1.0 mL) from raw and heat-treated milks were diluted with 1.0 mL water and adjusted to pH 4.6 by the addition of 0.5 mL of 0.83 M acetic acid and 0.5 mL of 0.2 M sodium acetate. The solutions were stirred for 20 min and centrifuged at 1000*g* for 5 min. Each supernatant was passed through a 0.22 μm filter. Chromatography of acid filtrates from raw and heated milks was carried out on a Superdex 75 HR 10/30 column (Pharmacia Biotech, St Albans, U.K.), and initial concentrations of whey proteins and their levels of thermal denaturation were determined as described previously (Law *et al.*, 1993).

RESULTS

Total Protein Concentration. The concentration of total protein was increased to twice that in skim milk by ultrafiltration and reduced to one quarter by the addition of ultrafiltrate to skim milk. The ratio of whey protein to casein and the concentrations of nonprotein material were kept constant. Levels of denaturation of total whey protein, determined on the basis of loss of solubility at pH 4.6, increased as the total protein concentration was increased, and decreased as the concentration of total protein was reduced (Figure 1a). As found in previous studies, the susceptibility of the whey proteins to denaturation was immunoglobulins > serum albumin/lactoferrin > β-lactoglobulin > α-lactalbumin. On heating at 80 °C, denaturation of the immunoglobulins occurred rapidly, and the effect of concentration on the rate could not be determined. The levels of denaturation of each of the other three main whey protein fractions, serum albumin/lactoferrin (results not shown), β-lactoglobulin (Figure 1b) and α-lactalbumin (Figure 1c), increased with total protein concentration.

Denaturation of the immunoglobulins and the serum albumin/lactoferrin fraction did not follow simple kinetics, but the orders of reaction and rate constants for denaturation of β-lactoglobulin and α-lactalbumin were determined by examining the closeness of fit of the denaturation data to two expressions derived from the general rate equation as described previously (Lyster, 1970; Hillier and Lyster, 1979; Dannenberg and Kessler, 1988). For a reaction order, $n \neq 1$, $(C_t/C_0)^{1-n} = 1 + (n - 1)kt$, where C_0 is the initial protein concentration, C_t is the native protein concentration at time t, and k is the rate constant for denaturation. In the special case of a first-order reaction, $\ln(C_t/C_0) = -kt$.

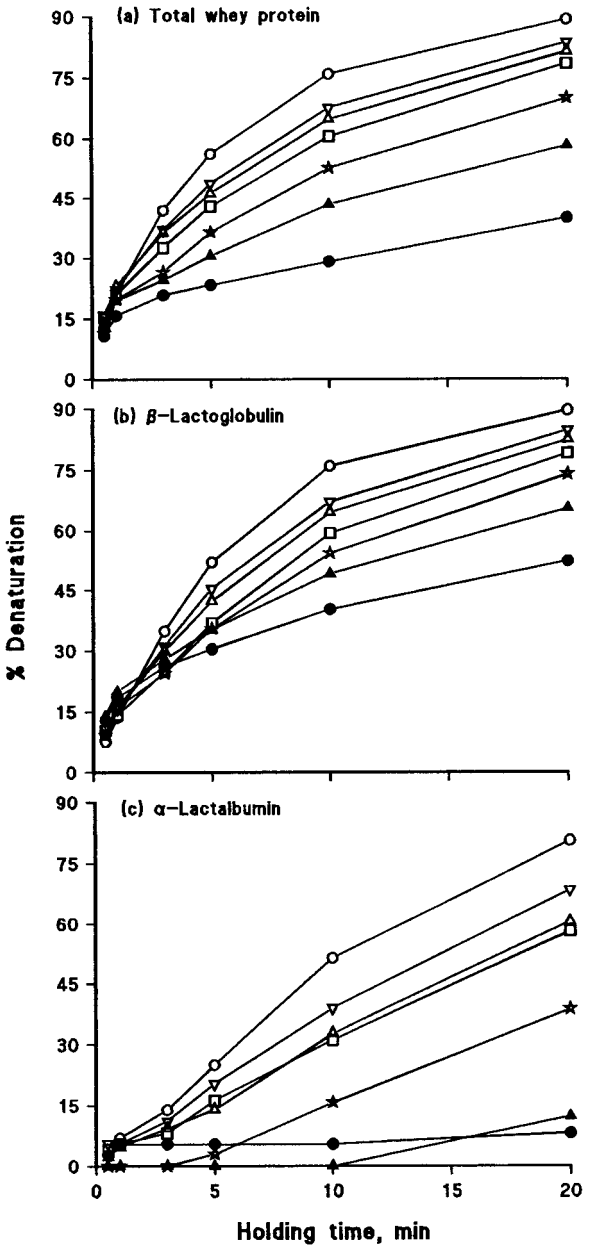

Figure 1. Effect of total protein concentration in skim milk on rates of denaturation of (a) total whey protein, (b) β-lactoglobulin, and (c) α-lactalbumin. (□) Skim milk. Concentrations of total protein as percentages of tthat in the original skim milk: (●) 25%, (▲) 50%, (★) 75%, (△) 125%, (▽) 150%, and (○) 200%.

For β-lactoglobulin, over the entire range of total protein concentration, plots of $(C_0/C_t)^{0.5}$ against holding time (t) were linear, indicating an apparent reaction order (n) of 1.5 (Figure 2a). For α-lactalbumin, over the same total concentration range, plots of $\log_{10}(C_t/C_0)$ against holding time (t) were also linear, indicating an apparent reaction order of 1.0 (Figure 2b). Other workers have obtained values for the reaction orders of β-lactoglobulin and α-lactalbumin close to 1.5 and 1.0, respectively (Dannenberg and Kessler, 1988; Anema and McKenna, 1996). There is, however, considerable variation in literature values for the reaction orders and

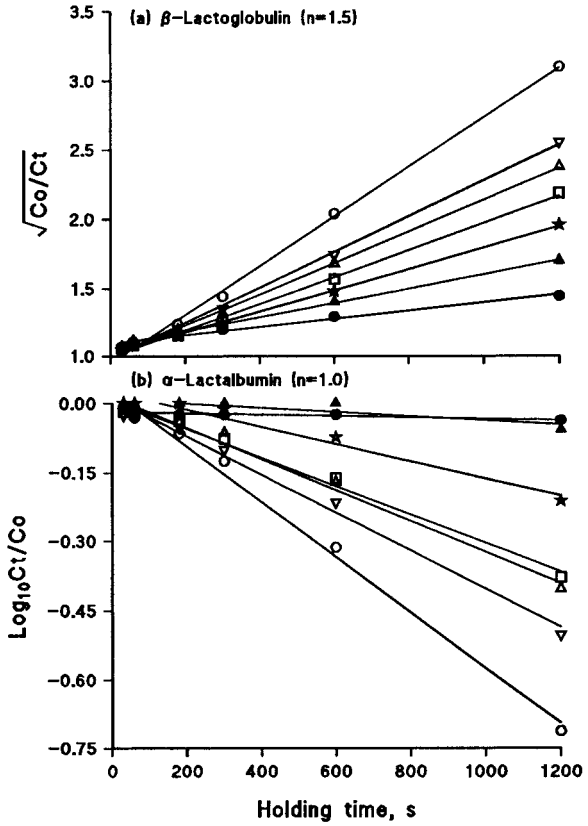

Figure 2. Kinetics plots for denaturation of whey proteins with different concentrations of total protein in skim milk (data Figure 1). (a) β-Lactoglobulin represented with a reaction order of 1.5. (b) α-Lactalbumin represented with a reaction order of 1.0. Symbols as in Figure 1.

rate constants relating to denaturation of β-lactoglobulin and α-lactalbumin (Anema and McKenna, 1996). The occurrence of fractional values for orders of reaction of β-lactoglobulin and the change in slope found in Arrhenius plots for β-lactoglobulin and α-lactalbumin (Lyster, 1970; Dannenberg and Kessler, 1988) indicate that denaturation of these whey proteins is complex and may involve several simultaneous reactions with different temperature dependence. In particular, different heating conditions may affect the relative predominance of the unfolding and aggregation steps.

The rate constants for denaturation of β-lactoglobulin and α-lactalbumin were calculated from the slopes of the respective lines (Figure 2), as described by Dannenberg and Kessler (1988). In a preliminary experiment in which 6 samples of a single skim milk were heated, the coefficients of variation in the determination of the denaturation rate constants of β-lactoglobulin and α-lactalbumin were 2.8 and 3.3%, respectively. Values for the rate constants (k) of the two whey proteins were plotted as a function of total protein concentration (Figure 3). Results show that there was an almost linear increase in the rate constants for denaturation of the two whey proteins with increasing total protein concentration. For a reaction where $n \neq 1$, such as the denaturation of β-lactoglobulin, the value of the rate constant is known to vary with the initial concentration. Denaturation of α-lactalbumin appears to follow first order kinetics, which normally would be independent of the initial protein concentration. However, Hillier and Lyster (1979) have shown that denaturation of α-lactalbumin is probably a pseudo-first-order reaction, involving an unfolding step followed by an irreversible,

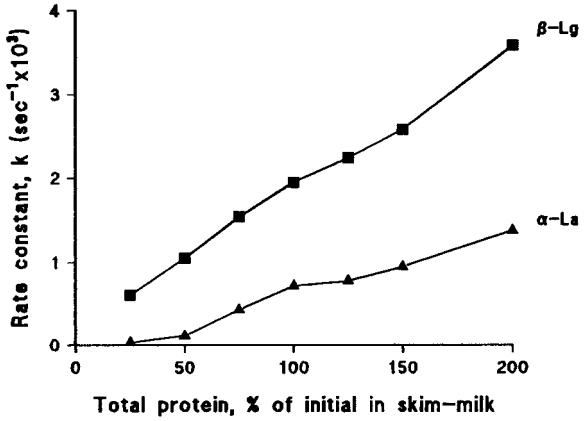

Figure 3. Rate constants for denaturation of β-lactoglobulin ($n = 1.5$) and α-lactalbumin ($n = 1.0$), determined from Figure 2, plotted as a function of total protein in skim milk.

and concentration dependent, step in which new disulfide links are formed.

Previous studies, in which the milk protein has been concentrated up to six-fold by ultrafiltration, have shown that the level of denaturation of total whey protein increases with total protein concentration (Pierre *et al.*, 1977; McMahon and Yousif, 1993). In studies in which both proteins and nonprotein material were concentrated by evaporation, the level of denaturation of total whey protein actually decreased with total solids (McKenna and O'Sullivan, 1971). However, Pierre *et al.* (1977) showed that the increase in concentration of lactose, which occurs on evaporation but not during ultrafiltration, reduces the rate of denaturation of total whey protein.

Whey Protein Concentration. The concentration of whey protein in skim milk was increased up to 2.5 times the original level by the addition of freeze-dried whey proteins. The concentrations of casein and nonprotein material were kept constant. Levels of denaturation of total whey proteins increased as the concentration of whey in skim milk was increased (Figure 4a). On heating at 80 °C, denaturation of the immunoglobulins occurred rapidly at all concentrations of whey protein, and no increase in the rate of denaturation could be detected. However, the rates of denaturation of serum albumin/lactoferrin (results not shown), β-lactoglobulin (Figure 4b), and α-lactalbumin (Figure 4c) all increased with increasing concentration of whey protein. Results from analysis of variance showed that the effect of concentration on rates of denaturation of total whey protein, β-lactoglobulin, and α-lactalbumin was significant at the $P < 0.001$ level. At all concentrations of whey proteins, the orders of reaction, determined as above, for the denaturation of β-lactoglobulin and α-lactalbumin were 1.5 and 1.0, respectively. The rate constants for denaturation of β-lactoglobulin and α-lactalbumin increased with increasing total whey protein concentration (Figure 5). When the whey protein concentration of the original skim milk was doubled, values of k for denaturation of β-lactoglobulin and α-lactalbumin increased by 38% and 67%, respectively. These increases were smaller than those found on doubling the concentration of total protein (Figure 3), when values of k for β-lactoglobulin and α-lactalbumin increased by 84% and 92%, respectively.

Few studies have been carried out on the effect of changing only the whey protein concentration in milk. Lyster (1970) found that doubling the concentration of β-lactoglobulin A in milk had no effect whereas doubling

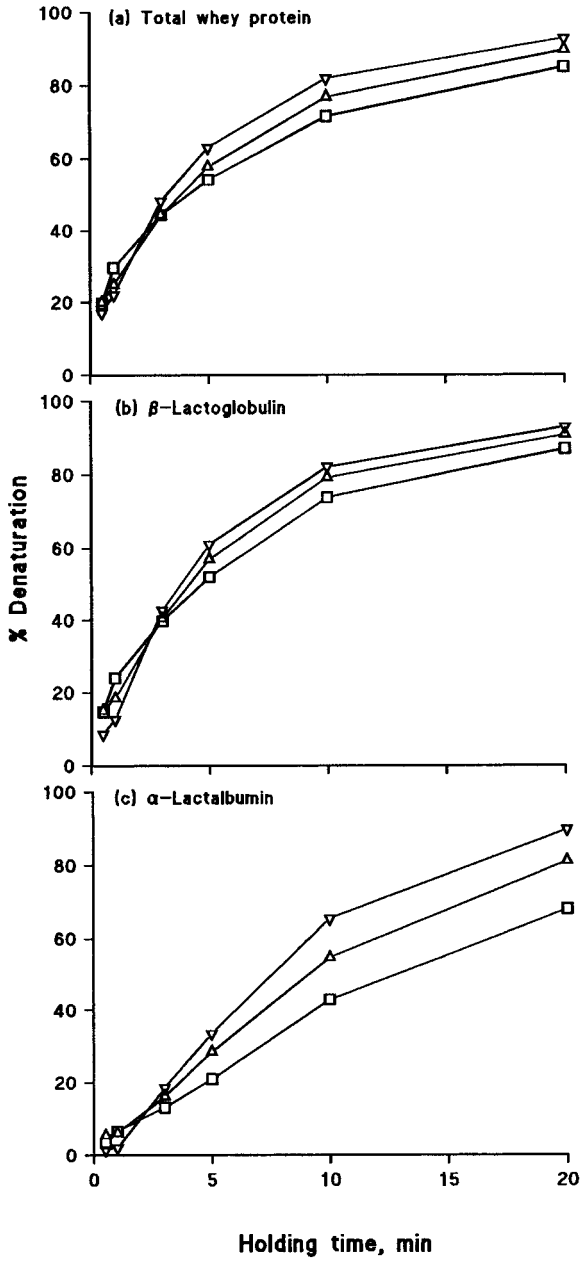

Figure 4. Effect of increasing whey protein concentration in skim milk on rates of denaturation of (a) total whey protein, (b) β-lactoglobulin, and (c) α-lactalbumin. (□) Skim milk. Concentrations of total whey protein as percentages of that in the original skim milk: (△) 146%, (▽) 259%. Rates of denaturation for concentrations of whey protein 123% and 178% of that in skim milk showed exactly the same trends as the above data (see Figure 5) but have been omitted for clarity of presentation.

the concentration of β-lactoglobulin B gave an increased rate of denaturation of that protein. Increasing the β-lactoglobulin concentration also gave a slight increase in the rate of denaturation of α-lactalbumin.

Casein Concentration. The concentration of casein in skim milk was reduced, while maintaining a constant level of whey protein and nonprotein material, by adding casein-free acid filtrate that had been adjusted to pH 6.7 and dialyzed against skim milk. In a further experiment to increase the casein concentration without changing the whey protein concentration, casein micelles centrifuged from skim milk were added back to skim milk. On warming to 80 °C in the absence of casein, or when the casein concentration was reduced to one-tenth of that in skim milk, the whey proteins

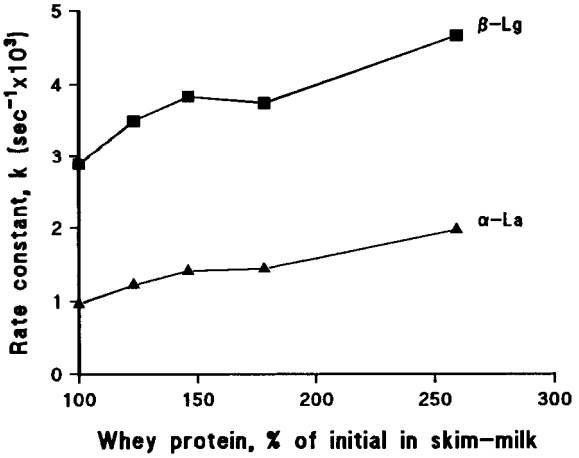

Figure 5. Rate constants for denaturation of β-lactoglobulin ($n = 1.5$) and α-lactalbumin ($n = 1.0$) plotted as a function of total whey protein concentration in skim milk.

were denatured more slowly than in skim milk, but readily coagulated. Patocka *et al.* (1993) similarly found that, on heating blends of ultrafiltered whey with skim milk, coagulation occurred when the casein/total whey protein ratio was less than 1. As the casein concentration was increased up to the levels in skim milk, the rates of denaturation of total whey protein, β-lactoglobulin, and α-lactalbumin also increased (Figure 6), but the higher levels of casein prevented visible coagulation. Results of analysis of variance showed that, at concentrations below that in skim milk, increasing casein concentration had significant effects on the rates of denaturation of total whey protein ($P < 0.001$), β-lactoglobulin ($P < 0.001$), and α-lactalbumin ($P < 0.05$). In the absence of casein, denaturation of the immunoglobulin and serum albumin/lactoferrin fractions occurred more slowly than in skim milk. At a concentration of casein 9.1% of that in skim milk, denaturation of these fractions occurred rapidly, and increasing the concentration of casein above this initial level gave no appreciable increase in their rates of denaturation.

Over the concentration range for casein, the reaction orders for β-lactoglobulin and α-lactalbumin were close to 1.5 and 1.0, respectively. On plotting values of the rate constants for denaturation of β-lactoglobulin and α-lactalbumin against casein concentration (Figure 7), it can be seen that there was a substantial increase in the rate of denaturation of β-lactoglobulin and a small increase in that of α-lactalbumin with casein concentration up to about the level in skim milk. On increasing the micellar casein concentration up to twice that in skim milk, but maintaining the whey protein and nonprotein concentrations, there was no significant increase in the rate constants for denaturation of β-lactoglobulin and α-lactalbumin (Figure 7).

Kessler *et al.* (1992) similarly found that, as the concentration of casein was increased to that in skim milk, with a constant whey protein content, the rate of denaturation of β-lactoglobulin increased. These workers found that as the casein/whey protein ratio was increased, the value for the reaction order of β-lactoglobulin decreased from 2.0 to 1.5.

Effect of β-Lactoglobulin Phenotype. Twenty-three samples of milk were obtained from 19 cows at different stages of lactation. Five samples contained only the A variant of β-lactoglobulin, 10 contained the A and B variants, and 8 contained only the B variant. Analysis of variance showed that the composition of

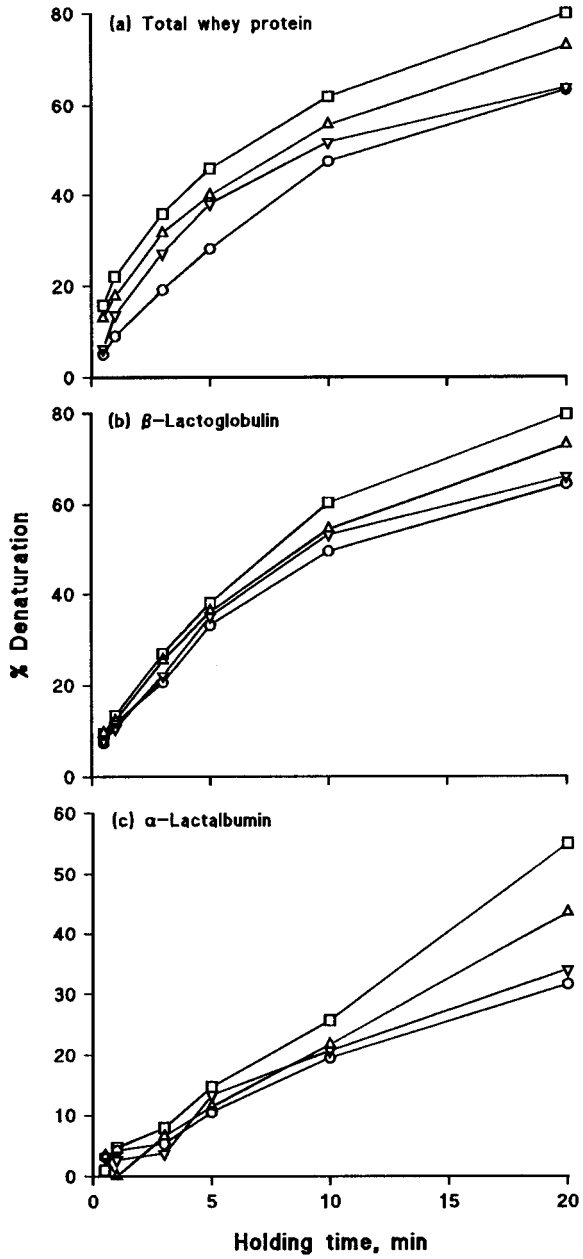

Figure 6. Effect of casein concentration in skim milk on rates of denaturation of total whey protein, β-lactoglobulin, and α-lactalbumin. (□) Skim milk. Concentrations of total casein as percentages of that in the original skim milk: (○) 0%, (▽) 9.1%, and (△) 33.3%. Rates of denaturation for concentrations of casein 20.0% and 50.0% of that in skim milk showed exactly the same trends as the above data (see Figure 7) but have been omitted for clarity of presentation. Rates of denaturation for concentrations of casein above that in skim milk were similar to those for skim milk (see Figure 7).

whey protein from AB cows was similar to that from AA cows, but whey protein from BB cows contained significantly less β-lactoglobulin than that from AB cows (Table 1). The concentrations of whey proteins in milks from AA and AB cows were not significantly different, but milk from BB cows contained significantly less β-lactoglobulin. These results confirm previous findings that the level of expression of the A allele is higher than that of the B allele (Jakob, 1994).

On heating milks at 80 °C, there was considerable variation in the rates of denaturation of total and individual whey proteins within each of the phenotypes. Mean values and standard deviations of the rate constants for denaturation of β-lactoglobulin and α-lactalbumin are shown in Table 1. There were no obvious differences in the rates of denaturation of individual whey proteins due to the effect of phenotype, and results from analysis of variance showed that the respective rate constants for denaturation of β-lactoglobulin and α-lactalbumin in the three phenotypes were not significantly different (Table 1).

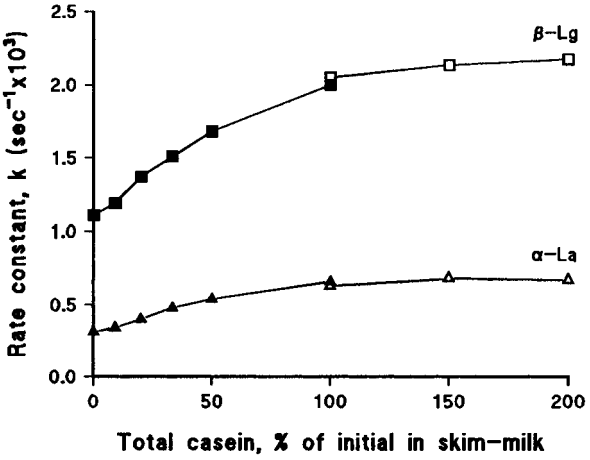

Figure 7. Changes in the rate constants for denaturation of β-lactoglobulin ($n = 1.5$) and α-lactalbumin ($n = 1.0$) in skim milk with total casein concentration. Filled symbols, effect of reducing the casein concentration below that in skim milk. Open symbols, effect of increasing the casein concentration above that in skim milk. Results for reduced and increased casein concentrations were obtained from two different milk samples.

Table 1. Effect of β-Lactoglobulin Phenotype on the Rates of Denaturation of β-Lactoglobulin and α-Lactalbumin and on the Composition and Content of Whey Proteins in Milk

phenotype	AA		AB		BB	
no. of samples	5		10		8	
	rate constant (k $s^{-1} \times 10^3$)					
	mean	SD	mean	SD	mean	SD
β-lactoglobulin	2.46	0.98	2.68	0.59	2.49	0.43
α-lactalbumin	0.72	0.30	0.89	0.17	0.78	0.23
	% of total protein					
immunoglobulins	11.1	2.5	9.3	1.8	10.7	1.9
serum albumin/ lactoferrin	12.0	3.8	11.4	2.4	14.2	5.1
β-lactoglobulin	61.2	3.4	63.6	4.0	56.3[a]	4.9
α-lactalbumin	15.7	3.0	15.7	2.9	18.8	3.6
	concentration (g L^{-1})					
immunoglobulins	0.80	0.28	0.64	0.19	0.64	0.22
serum albumin/ lactoferrin	0.87	0.40	0.78	0.23	0.87	0.51
β-lactoglobulin	4.27	0.38	4.28	0.64	3.26[a]	0.60
α-lactalbumin	1.09	0.13	1.05	0.18	1.07	0.09
total whey protein	7.02	0.97	6.74	0.98	5.83	1.18

[a] Values significantly different ($P < 0.003$) from those for β-lactoglobulin AB.

Various studies have been carried out on the effect of phenotype on the rates of denaturation of β-lactoglobulin heated in buffered systems (Jakob and Puhan, 1992). Although there are some conflicting reports, it has been fairly well established that, on heating below 90 °C at normal concentrations, the A variant is more heat resistant than the B variant (Jakob and Puhan, 1992). There have been few comparable studies, however, on the effect of β-lactoglobulin phenotype on denaturation of the whey proteins in milks of different cows. Lyster

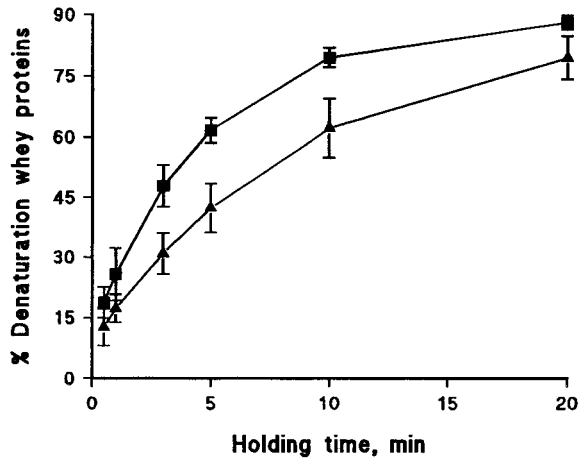

Figure 8. Variation in the rates of denaturation of total whey protein in skim milk from cows at different stages of lactation. Mean values and standard deviations are given for (■) 3 cows in late lactation and (▲) 17 cows in midlactation.

(1970), in a study of three different cows, found that the rates of denaturation of β-lactoglobulin followed the order BB > AB > AA. Dannenberg and Kessler (1988) similarly found that between 70 and 90 °C the rate of denaturation of the B variant was greater than that of the A variant. In the present study, there was considerable variation within each phenotype partly due to variation in the concentration of total whey protein.

Stage of Lactation. Of 23 milk samples from 19 different cows, 3 were from cows about 1 month from the beginning of lactation, 3 were from cows about 330 days in lactation, and the remainder were from cows in midlactation (103−266 days). There was considerable variation in the rates of denaturation of total whey protein in the milks of different cows at each stage of lactation. The rates of denaturation of total whey protein in milks from cows in early and midlactation were similar (results not shown) but were considerably higher in milks of cows in late lactation (Figure 8). The rates of denaturation of the immunoglobulins and the serum albumin/lactoferrin fraction were also slightly higher in milk from cows in late lactation.

Mean values and standard deviations of the rate constants for denaturation of β-lactoglobulin and α-lactalbumin at each stage of lactation are shown in Table 2. Results from analysis of variance showed that the respective values of the rate constants for denaturation of β-lactoglobulin and α-lactalbumin in early and midlactation milks were not significantly different but that they were significantly higher in late lactation milks.

Analysis of variance showed that whey protein from cows in late lactation contained significantly more serum albumin/lactoferrin and less α-lactalbumin than that from cows in midlactation (Table 2). Compared with milk from cows in midlactation, that from cows in late lactation contained significantly higher concentrations of immunoglobulins, serum albumin/lactoferrin, and total whey protein. Results of this, and previous studies have shown that the order of susceptibility of the whey proteins to denaturation is immunoglobulins > serum albumin > β-lactoglobulin > α-lactalbumin. The higher content of the more easily denatured whey proteins in whey protein from cows in late lactation, therefore, would tend to increase overall rates of denaturation. The higher concentration of total whey proteins in milk from cows in late lactation may also account for some of the increase in rates of denaturation.

Table 2. Effect of Stage of Lactation on the Rates of Denaturation of β-Lactoglobulin and α-Lactalbumin and on the Composition and Content of Whey Proteins in Milk[a]

stage of lactation	early		mid		late	
no. of samples	3		17		3	
	rate constant (k $s^{-1} \times 10^3$)					
	mean	SD	mean	SD	mean	SD
β-lactoglobulin	2.84	0.37	2.35	0.51	3.48[1]	0.50
α-lactalbumin	0.67	0.19	0.77	0.18	1.17[1]	0.07
	% of total protein					
immunoglobulins	10.6	1.6	9.7	1.9	12.4	2.7
serum albumin/ lactoferrin	10.3	1.4	11.8	2.3	18.8[1]	6.9
β-lactoglobulin	58.4	6.1	61.7	4.0	56.0	9.4
α-lactalbumin	20.6	3.2	16.8	2.9	12.7[2]	1.4
	concentration (g L^{-1})					
immunoglobulins	0.63	0.05	0.62	0.20	0.99[3]	0.27
serum albumin/ lactoferrin	0.61	0.04	0.76	0.23	1.49[1]	0.56
β-lactoglobulin	3.52	0.96	3.91	0.72	4.39	0.71
α-lactalbumin	1.21	0.06	1.05	0.14	1.00	0.07
total whey protein	5.96	1.08	6.33	1.05	7.86[4]	0.58

[a] Significantly different from midlactation: [1]$P < 0.002$; [2]$P < 0.029$; [3]$P < 0.011$; [4]$P < 0.026$.

There was considerable variation in the rates of denaturation of total (Figure 8) and individual whey proteins (Table 2) in the milks of cows in midlactation. There was some indication of a seasonal change, but part of the variation in rates of denaturation of β-lactoglobulin and α-lactalbumin in milk from cows in midlactation was associated with changes in the concentration of total whey proteins. There were positive correlations between the rate constants for denaturation of β-lactoglobulin ($r = 0.748$) and α-lactalbumin ($r = 0.785$) with total whey protein concentration.

DISCUSSION

On denaturation, the whey proteins lose their globular conformation and undergo aggregation in which they become associated with other whey proteins or casein micelles through hydrophobic interaction, calcium-linked complexes, or disulfide linkage. In this study, rates of denaturation of the whey proteins, based on their loss of solubilty at pH 4.6, increased with increasing concentrations of total protein and total whey protein. The results are consistent, therefore, with the higher concentrations of protein increasing the amount of interaction of unfolded whey proteins with other whey proteins or casein micelles and promoting aggregation.

On heating milk serum in the absence of casein, or when the concentration was reduced to about 10% of that at in skim milk, rates of denaturation of the whey proteins were considerably below that in skim milk, and the whey proteins readily coagulated. On adding small amounts of casein micelles before heating, the rates of denaturation of the whey proteins increased but the presence of casein prevented visible coagulation. The rates of denaturation of the whey proteins increased with casein concentration up to that in skim milk. Results, therefore, confirm previous work which has shown that denatured β-lactoglobulin and α-lactalbumin become closely associated with the surface of the casein micelles, and may become disulfide linked to κ-casein (Sawyer, 1969; Noh and Richardson, 1989). Morr and Josephson (1968) have indicated that the the stabilizing

effect of casein in preventing precipitation does not, initially at least, involve disulfide linkage, but is by formation of calcium-linked complexes between casein and whey protein aggregates. Other workers have shown that there are changes in the surface hydrophobicities of the whey proteins that may lead to their association with the surface of the micelles. Increasing the micellar casein concentration in skim milk by a factor of 2 had little effect on the rates of denaturation of the whey proteins and indicates that, because the surface area of the micelles is considerable, the availability of regions on the surface with which the denatured whey proteins become associated is not rate determining.

The results of this, and previous, work in which milks were concentrated by ultrafiltration show that the rates of denaturation of the whey proteins increase with total protein concentration. However, in other studies in which milks were concentrated by evaporation, or prepared with high concentrations of skim milk powder, rates of denaturation usually decreased with increasing total solids. Studies of whey and skim milk have shown that the decrease in rates of denaturation is due to an increase in the concentration of lactose, which may prevent the formation of complexes (Pierre *et al*., 1977). The present study shows how changing the concentrations of total protein, whey protein and casein, while maintaining a constant concentration of nonprotein material, affects the rates of thermal denaturation of the individual whey proteins in milk, and may provide useful information for selecting optimum heating conditions during processing.

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