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The mechanisms of the heat-induced interaction of whey proteins with casein micelles in milk

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Abstract

The heat-induced interactions between whey proteins and casein micelles were investigated by defining the final product of the reaction when milk was heated at temperatures up to 90°C. By looking at the changes of the interactions in skim milk and in resuspended casein micelles, to which different amounts of whey protein had been added, information on the mechanisms that determine the heat-induced protein–protein interactions in milk was derived. The ratio of α-lactalbumin and β-lactoglobulin to κ-casein and the ratio of α-lactalbumin to β-lactoglobulin found in the micellar pellet were used as indices of these heat-induced reactions occurring in milk. The results suggested that at these low temperature (70–90°C) with batch heating conditions, whey proteins form soluble complexes which act as intermediates in the heat-induced association of α-lactalbumin and β-lactoglobulin with the micelles. The presence of β-lactoglobulin was necessary for any association of whey protein with casein micelles to occur; furthermore, the amount of β-lactoglobulin found in the micellar pellet after heating seemed to be limited by a discrete number of binding sites available on the micelles.

1. Introduction

During heat treatment of milk, various reactions take place, including denaturation and aggregation of whey protein, and formation of complexes between whey proteins, caseins and fat globules. The denaturation of α-lactalbumin (α-la) and β-lactoglobulin (β-lg) in milk and in various model systems has been widely studied (Hillier & Lyster, 1979; Dannenberg & Kessler, 1988; Parris, Purcell & Ptashkin, 1991; Qi, Brownlow, Holt & Sellers, 1995). However, understanding the denaturation of single proteins provides insight only into the initial stage of a complex series of reactions occurring during heat treatment of milk. For this reason, a different approach is needed, aimed at determining the intermediates formed and the effect that changes in environmental conditions have on protein–protein interactions during heating.

When whole milk is heated, whey proteins interact not only with casein micelles but also with fat globules (Dalgleish & Banks, 1991). Recently, it has been demonstrated that, although whey protein binding to milk fat globules is rapid, the amounts found associated with the globules are low, compared to the amounts bound to casein micelles (Corredig & Dalgleish, 1996a). The complexes formed between whey proteins and casein micelles are by far the most important product of the heat-induced reactions, and the free sulphydryl group present in the native structure of β-lg seems to play an important role in the interaction (Fox, 1992). The main complex formed during heat treatment of milk is the β-lg/κ-casein complex (Hill, 1989; Jang & Swaisgood, 1990). However, not only β-lg but also α-la participate in the reaction with casein micelles (Law, Horne, Banks & Leaver, 1994). It has been reported that the degree of denaturation of α-la, which on its own denatures semi-reversibly (deWit & Klarenbeek, 1984), increases when β-lg is present (Elfagm & Wheelock, 1977).

In spite of extensive research on heat treatment of milk, details on the mechanisms of the heat-induced interactions of α-la and β-lg with casein micelles are not yet clear. The present paper describes the effects of the addition of whey proteins to skim milk and resuspended casein micelles, on the interaction between α-la, β-lg and

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casein micelles during heating of milk at temperatures up to 90°C.

2. Experimental

Fresh whole milk was collected from the Elora Research Station of the University of Guelph. After addition of 0.2 g l^{-1} of sodium azide as a preservative, the milk was skimmed by low-speed centrifugation (3000 *g* for 20 min). Individual α-la and β-lg were prepared by ion exchange chromatography (Sepharose fast-flow Q, Pharmacia Biotech, Baie d'Uré, Quebec, Canada), with elution with 4 l of 20 mmol l^{-1} Tris pH buffer (0–1 M NaCl gradient). After dialysis the isolated proteins were freeze-dried and added in the dried form to the skim milk before heating. The effect of changing the protein concentrations of the original milk on the composition of the heat-induced complex between α-la, β-lg and casein micelles was studied by adding purified α-la and β-lg to skim milk before heating. In addition, a model system of casein micelles resuspended with milk ultrafiltrate was used. Casein micelles were isolated by centrifuging untreated skim milk at 60 000*g* for 40 min (L870M, Beckman, Palo Alto, CA). Milk ultrafiltrate was prepared by using a laboratory filtration unit (TCF 10, Amicon, Mississagua, ON) with a 10 000 Da cut-off membrane. Purified α-la and β-lg were added in different amounts to this dispersion prior to heating.

Samples were heated in test tubes in a temperature controlled water bath (in a range between 70 and 90°C), for different lengths of time. After heat treatment the samples were rapidly cooled in an ice bath.

The final complex resulting from the heat-induced interactions between whey protein and casein micelles was separated from the heated samples by ultracentrifugation and analyzed by SDS-PAGE electrophoresis as described by Corredig and Dalgleish (1996b). The amounts of α-la and β-lg bound to the micelles were quantified by relating these proteins to the amount of κ-casein present in the micellar pellet. These ratios were taken as indices of the reaction between whey proteins and caseins. The variation of the ratio of associated α-la/β-lg with protein concentration in milk was also investigated. Previous authors have suggested the use of the α-la/β-lg as a means of estimating the extent of heat treatment in skim milk (Law et al., 1994; Parris, Hollar, Hsieh & Cockley, 1997).

3. Results and discussion

The extent of the interaction of whey proteins with casein micelles increased with time and temperature when milk was treated by batch heating at temperatures between 75 and 90°C. In general, the amounts of whey protein associated with the micellar pellet increased as the time of heating increased up to a maximum value, and higher temperatures caused faster protein–protein interactions. Previous work has indicated that changes in the environmental conditions strongly affected the heat-induced interactions of both α-la and β-lg with casein micelles (Corredig & Dalgleish, 1996a,b). Analysis of variance demonstrated that not only time and temperature of heating, but also the amount of whey protein present in skim milk significantly affected the amounts of α-la/κ-casein and β-lg/κ-casein associated with the micelles.

The observed association of α-la and β-lg with casein micelles after heat treatment is in agreement with previous literature (Law et al., 1994). During heating at temperatures lower than 90°C, the two whey proteins not only interacted with the micelles, but also had a very similar kinetic behaviour (Fig. 1). This temperature (90°C) seemed to be critical in the mechanisms of the reaction, in agreement with the known change in the slope of the Arrhenius plot of the kinetics of whey protein denaturation (Hillier & Lyster, 1979; Dannenberg & Kessler, 1988). When milk was heated at low temperatures, the two whey proteins behaved similarly, and their amounts in the micellar pellet were directly related to their concentrations in the original milk (Fig. 1). The results displayed in Fig. 2 confirmed this hypothesis. When 2 g l^{-1} of α-la were added to skim milk, the concentrations of α-la and β-lg were comparable, they had the same kinetics of interaction, and similar amounts were found associated with the micellar pellet after treatment. When skim milk with added β-lg (2 g l^{-1}) was heated at 80°C (Fig. 2), the maximum value of β-lg/κ-casein was reached after only a few minutes of heating. However, the amount of β-lg/κ-casein reached a plateau value not significantly different from that of control milk.

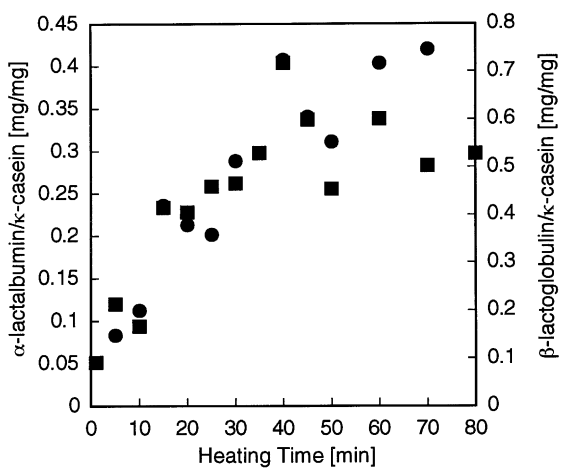

Fig. 1. Weight ratio of α-lactalbumin/κ-casein (filled squares, left-hand axis), and β-lactoglobulin/κ-casein (filled circles, right-hand axis) in the micellar pellet of skim milk heated at 80°C as a function of time. Results are the average of four independent experiments.

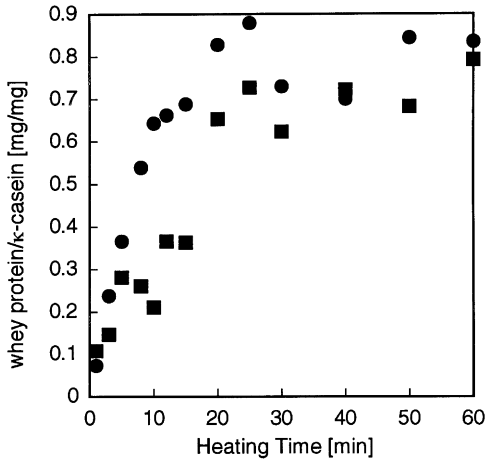

Fig. 2. Weight ratio of whey protein/κ-casein in the micellar pellet of skim milk heated at 80°C, as a function of time. Filled circles, β-lactaglobulin/κ-casein in skim milk with 2 g l^{-1} of β-lg added; filled squares, α-lactalbumin/κ-casein in skim milk with 2 g l^{-1} of α-la added. Results are the average of three independent experiments.

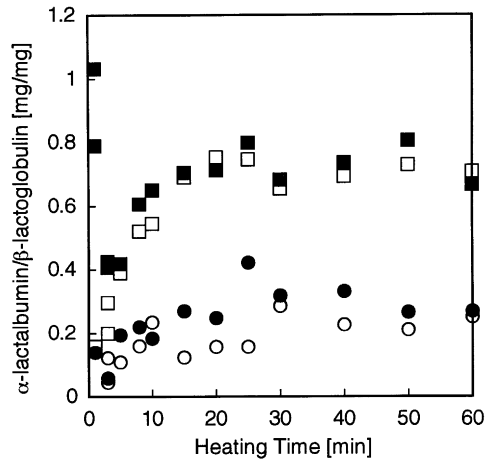

Fig. 3. Weight ratio of α-lactalbumin/β-lactoglobulin associated with the micellar pellet after heating of skim milk at 80°C, as a function of time and different amounts of whey protein added: filled circles, control skim milk; filled squares, addition of α-lactalbumin (2 g l^{-1}); Open circles, addition of β-lactoglobulin (2 g l^{-1}); open squares, addition of α-lactalbumin (1 g l^{-1}) and β-lactoglobulin (1 g l^{-1}). Results are the average of three independent experiments.

This result suggested that only a certain number of sites were available for the interaction of β-lactoglobulin with the micelles. This behaviour differed from that of α-la, which increased its amount associated with the micellar pellet after heating under the same conditions.

The presence of both whey proteins in the micellar pellet after heating, and their similar kinetic behaviour when milk was heated at low temperature (up to 90°C) suggested that, under these conditions, the formation of soluble aggregates of whey protein may occur, whereby the two whey proteins, α-la and β-lg, react with each other, form intermediate complexes, and then interact with casein micelles. It is known that, at low temperature, some time is necessary for β-lg to modify its structure (Jang & Swaisgood, 1991) and this allowed α-la to participate in the reaction with the casein micelles. The results of the experiments involving heating of resuspended micelles with different amounts of α-la and β-lg suggested that, while the presence of α-la did not influence the interaction of β-lg with the micelles, β-lg was needed for the association of α-la with the micelles.

The ratios of α-la/β-lg found in the micellar pellet after heating at 80°C are shown in Fig. 3 as a function of α-la and β-lg concentration in skim milk. In all the heating experiments performed on skim milk with no protein added the ratios of α-la/β-lg reflected their original composition. When α-la was added at 2 g l^{-1}, so that the two whey proteins had a similar concentration, the ratio of α-la/β-lg associated with the micelles was higher than in the control milk, and reached a value of approximately 0.8 mg of α-la/mg β-lg. When both α-la (1 g l^{-1}) and β-lg (1 g l^{-1}) were added to skim milk the ratio of α-la/β-lg was different from the control and not significantly different from that of skim milk with more α-la added (Fig. 3). These results confirmed the hypothesis that only a discrete number of binding sites is available for the interaction of β-lg with casein micelles. Furthermore, when additional β-lg was added to skim milk, the ratio between α-la and β-lg associated with the micelles did not change significantly from that of control milk. These results were confirmed by the studies performed on resuspended micelles to which different amounts of whey protein had been added.

In summary, under the heating conditions carried out in this study (low-temperature batch heating) two main interactions are recognized: (i) a direct interaction of β-lg with casein micelles, via κ-casein binding; (ii) a reaction between α-la and β-lg with the micelles, through an intermediate formed between the two whey proteins in solution. The results from both skim milk heating and resuspended micelles experiments showed that the amount of β-lg which interacted with the micelles was limited by the number of binding sites available. On the other hand, the amount of α-la associated with the micellar pellet seemed to depend on its concentration in milk.

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