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**HEAT-INDUCED INTERACTIONS OF  $\beta$ -LACTOGLOBULIN,  
 $\alpha$ -LACTALBUMIN AND CASEIN MICELLES.**

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## ABSTRACT

The denaturation and aggregation of  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin were studied in the following mixtures, designed to simulate the protein concentrations and ionic environment in normal skim milk.

1.  $\beta$ -lactoglobulin (0.4% w/v),
2.  $\alpha$ -lactalbumin (0.15% w/v),
3.  $\beta$ -lactoglobulin (0.4% w/v) and casein micelles ( $\sim$  2.6% w/v),
4.  $\alpha$ -lactalbumin (0.15% w/v) and casein micelles ( $\sim$  2.6% w/v),
5.  $\beta$ -lactoglobulin (0.4% w/v) and  $\alpha$ -lactalbumin (0.15% w/v) and
6.  $\beta$ -lactoglobulin (0.4% w/v),  $\alpha$ -lactalbumin (0.15% w/v) and casein micelles ( $\sim$  2.6% w/v)

Proteins were dissolved in SMUF, pH 6.7, and heated at 80 and 95°C for various times and centrifuged at 100,000 g for 60 min. The supernatants and pellets obtained were analysed using gel electrophoresis under non-dissociating (Native-PAGE in the absence of dissociating and reducing agents), dissociating but non-reducing (SDSNR-PAGE) and dissociating and reducing conditions (SDSR-PAGE).

When  $\beta$ -lactoglobulin was heated alone and examined by native-PAGE, the quantity of native protein decreased with increasing heating time at 80°C. Addition of  $\alpha$ -lactalbumin to the  $\beta$ -lactoglobulin solution increased the loss of  $\beta$ -lactoglobulin during the initial stages of heating. Addition of casein micelles to the  $\beta$ -lactoglobulin solution markedly increased the loss of native  $\beta$ -lactoglobulin throughout the heating period. The loss of  $\beta$ -lactoglobulin from the mixture containing  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin and casein micelles was similar to that from the mixture of  $\beta$ -lactoglobulin and casein micelles. The loss of  $\beta$ -lactoglobulin from these protein mixtures could be described by second-order reaction kinetics. Heating these mixtures at 95°C caused very rapid loss of native  $\beta$ -lactoglobulin, but the effects of the addition of casein micelles and  $\alpha$ -lactalbumin were generally similar to those observed at 80°C.

When  $\alpha$ -lactalbumin was heated at 80°C either alone or in the presence of casein micelles, there was only a slight loss of the native  $\alpha$ -lactalbumin. However the

corresponding losses of native  $\alpha$ -lactalbumin were considerably greater on heating at 95°C. At both temperatures, the addition of  $\beta$ -lactoglobulin increased the rate of loss of  $\alpha$ -lactalbumin substantially. The addition of casein micelles to the mixture of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin had little further effect on the loss of native  $\alpha$ -lactalbumin. The rates of loss of  $\alpha$ -lactalbumin at 95°C in all mixtures could be adequately described by first-order kinetics.

When  $\beta$ -lactoglobulin was heated either alone or in the presence of casein micelles and examined by SDSNR-PAGE, the loss of SDS-monomeric  $\beta$ -lactoglobulin was less than the loss of native  $\beta$ -lactoglobulin. In contrast, when  $\alpha$ -lactalbumin was added to  $\beta$ -lactoglobulin or  $\beta$ -lactoglobulin and casein micelles mixture, the loss of SDS-monomeric  $\beta$ -lactoglobulin was comparable to that of native  $\beta$ -lactoglobulin. The difference between native and SDS-monomeric  $\beta$ -lactoglobulin represents aggregates that are linked by non-covalent (hydrophobic) interactions. Thus the protein mixtures containing  $\alpha$ -lactalbumin, contain no or little non-covalently linked  $\beta$ -lactoglobulin aggregates, and consequently, all the  $\beta$ -lactoglobulin aggregates would be disulphide linked.

The results for the loss of SDS-monomeric and native  $\alpha$ -lactalbumin at 95°C showed that both non-covalent and disulphide-linked aggregates of  $\alpha$ -lactalbumin were present in all the protein mixtures studied.

When  $\beta$ -lactoglobulin solution was heated at 95°C, large aggregates were formed which could be sedimented at 100,000 g for 60 min. Addition of casein micelles to  $\beta$ -lactoglobulin solution caused greater sedimentation of  $\beta$ -lactoglobulin. Similar results were obtained when the mixture containing  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin and casein micelles was heated at 95°C. In contrast, the mixture containing  $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin behaved in a similar manner to  $\beta$ -lactoglobulin alone.

When  $\alpha$ -lactalbumin was heated at 95°C alone or in the presence of casein micelles, it did not interact to form large sedimentable aggregates. However when  $\beta$ -lactoglobulin was added to the above protein solutions, there was a considerable increase in sedimentation of  $\alpha$ -lactalbumin.

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