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HEAT-INDUCED INTERACTIONS OF β-LACTOGLOBULIN, α-LACTALBUMIN AND CASEIN MICELLES.

A THESIS
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ABSTRACT

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

1. β-lactoglobulin (0.4% w/v),
2. α-lactalbumin (0.15% w/v),
3. β-lactoglobulin (0.4% w/v) and casein micelles (~2.6% w/v),
4. α-lactalbumin (0.15% w/v) and casein micelles (~2.6% w/v),
5. β-lactoglobulin (0.4% w/v) and α-lactalbumin (0.15% w/v) and
6. β-lactoglobulin (0.4% w/v), α-lactalbumin (0.15% w/v) and casein micelles (~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 β-lactoglobulin was heated alone and examined by native-PAGE, the quantity of native protein decreased with increasing heating time at 80°C. Addition of α-lactalbumin to the β-lactoglobulin solution increased the loss of β-lactoglobulin during the initial stages of heating. Addition of casein micelles to the β-lactoglobulin solution markedly increased the loss of native β-lactoglobulin throughout the heating period. The loss of β-lactoglobulin from the mixture containing β-lactoglobulin, α-lactalbumin and casein micelles was similar to that from the mixture of β-lactoglobulin and casein micelles. The loss of β-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 β-lactoglobulin, but the effects of the addition of casein micelles and α-lactalbumin were generally similar to those observed at 80°C.

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