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Properties of Casein Micelles in Transglutaminase Treated Raw Skim Med Bovine Milk

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Introduction

Transglutaminase (TGase; EC 2.3.2.13) treatment influences several properties of milk; e.g., it impairs rennet-induced coagulation, enhances the heat stability and increases the resistance of casein micelles to disruption by urea, citrate or high pressure treatment. TGase treatment of milk also enhances the properties of yoghurt made therefrom. Although it has been established that treatment with TGase influences various properties of milk, information on the mechanism of TGase-induced crosslinking of caseins in the micelles is lacking. The aim of the study presented in this communication was to elucidate this mechanism.

Materials and Methods

Raw whole bovine milk was obtained from a dairy industry co. and defatted by centrifugation at 2,000 × g for 20 min at 4°C, followed by filtration of the supernatant through glass wool. Sodium azide (0.05%, w/v) was added to the skimmed milk to prevent microbial growth. All experiments were repeated on three individual milk samples. Ca^{2+}-independent TGase, with a declared activity of ~1,000 units g^{-1}, a gift from Ajinomoto Europe Sales (Stubbenhuk 3, D-20459, Hamburg, Germany). Following incubation with TGase, the milk was centrifuged at 5,000, 10,000, 20,000, 40,000 or 100,000 × g for 60 min at 20°C. The protein content of the milk and its centrifugal supernatants was determined using the Kjeldahl method (IDF) and the protein composition was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Results and Discussion

Electrophoretograms of milk incubated with TGase for up to 24 h at 30°C (Fig. 1) showed that the intensity of the bands of the caseins decreased with incubation time, indicating progressive TGase-induced crosslinking of caseins with increasing incubation time, due to the failure of crosslinked protein to enter the gel. After incubation for 24 h, only trace amounts of caseins were observed in the electrophoretogram (Fig. 1). Cross-linking of β- and κ-casein was more rapid than that of α_{s1}-casein, as previously observed. increasing centrifugal force (Fig. 2), as did the level of α_{s1}-, β- and κ-casein in the centrifugal supernatant of milk (Fig. 3); only trace amounts of caseins were observed in the supernatant of milk centrifuged at 100,000 × g (Fig. 3). Decreases in the level of total protein (Fig. 2) or individual caseins (Fig. 3) with increasing centrifugal force were due to the fact that smaller casein micelles can be sedimented at a higher centrifugal force. The amount of protein sedimentable at a 5,000, 10,000 or 20,000 × g increased with increasing incubation time with TGase; the amount of protein sedimentable at 5,000 × g in milk The protein content of the centrifugal supernatant of control milk decreased with treated with TGase for 8 h was comparable to that in control sedimentable at ≥40,000 × g. The higher level of sedimentable protein in TGase-treated milk than in untreated milk (Fig. 2) suggests that treatment with TGase results in the formation of sedimentable aggregates of milk protein. At a given centrifugation speed, considerably less casein was non-sedimentable

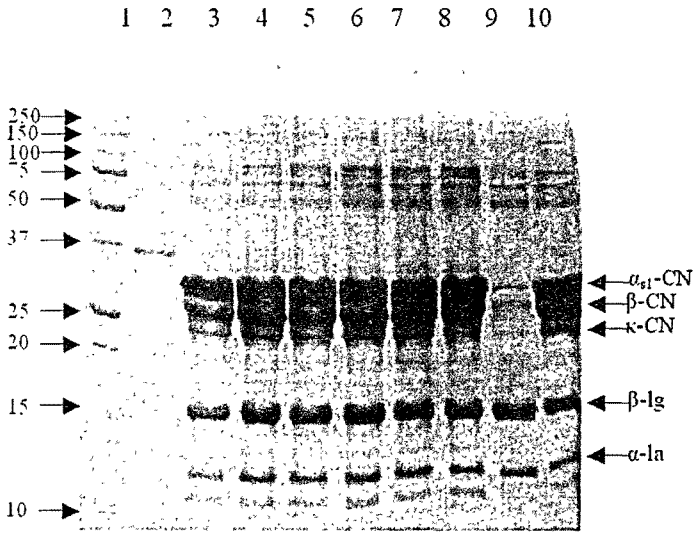

Fig. 1. SDS-PAGE electrophoretogram of molecular weight marker (lane 1), transglutaminase (lane 2) skim milk control (lanes 3 and 10) or following incubation with transglutaminase for 0 (lane 4), 1 (lane 5), 2 (lane 6), 4 (lane 7), 8 (lane 8) or 24 (lane 9) h at 30°C.

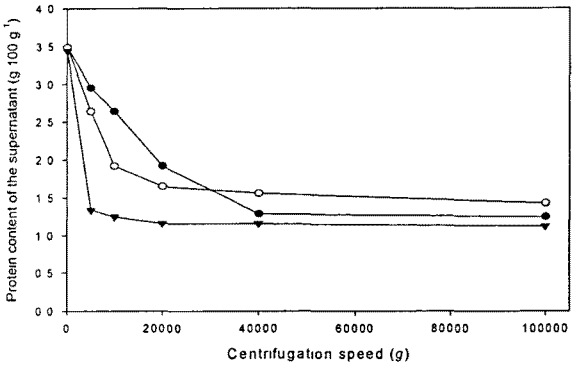

Fig. 2. Protein content of centrifugal supernatants prepared from skim milk control (- • -) or incubated with transglutaminase at 30°C for 1 (-○-) or 8 (-▼-).
Values are means of data from triplicate experiments on individual milk samples.

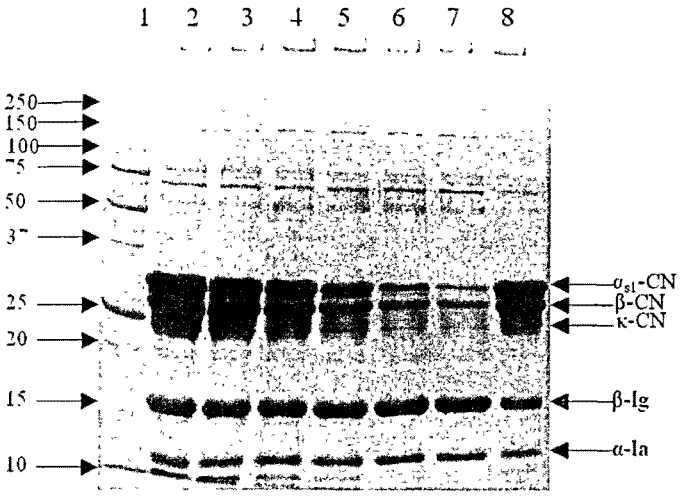

Fig. 3. SDS-PAGE electrophoretogram of molecular weight markers (lane 1), or the centrifugal supernatant from skim milk after centrifugation at 0 (lanes 2, 8), 5,000 (lane 3), 10,000 (lane 4), 20,000 (lane 5), 40,000 (lane 6) or 100,000 (lane 7) × g for 60 min at 20°C.

in milk incubated with TGase for 8 h (Fig. 4) than in control milk (Fig. 3). In TGase-treated milk, some caseins remained in the supernatant after centrifugation at 5,000 × g,

but after centrifugation at a higher speed only trace amounts of caseins remained in the supernatant (Fig. 4). The level of whey proteins in the centrifugal supernatant was not influenced by centrifugation speed (Fig. 3 and 4) or treatment with TGase prior to centrifugation (Fig. 4). The higher level of protein sedimentable at 100,000 × g in milk treated with TGase for 8 h than in untreated milk (Fig. 2) suggests that some protein becomes sedimentable on treatment with TGase; this fraction consists of caseins, because their level in the supernatant of TGase-treated milk was lower than in the supernatant of untreated milk (Fig. 3).

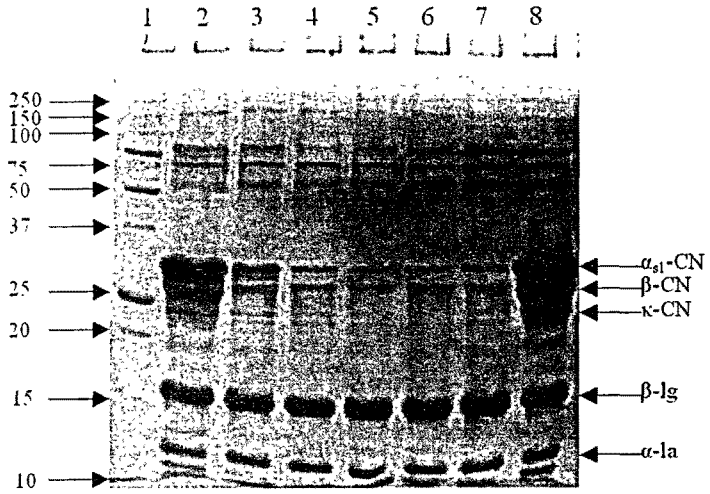

Fig. 4. SDS-PAGE electrophoretogram of molecular weight markers (lane 1), the supernatant of raw skim bovine milk incubated with TGase for 8 h at 30°C, followed by centrifugation at 0 (lane 2), 5,000 (lane 3), 10,000 (lane 4), 20,000 (lane 5), 40,000 (lane 6) or 100,000 (lane 7) × g for 60 min at 20°C, or control raw skim bovine milk (lane 8).

Conclusion

Treatment of milk with TGase can enhance the stability of casein micelles considerably against disruption induced by removal of MCP. TGase-treated micelles are also more stable against disruption by high hydrostatic pressure and addition of urea, which disrupts intramicellar hydrogen bonds. Furthermore, results suggest that TGase-induced cross-linking may be used to incorporate additional casein into the micelles, an area that may be of interest for further study. These results provide further fundamental understanding of the mechanism how TGase influences the proteins in milk, which is required for optimizing

potential applications of TGase in dairy processing.

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