Note

Dissociation of Large and Small Bovine Casein Micelles by Dialysis

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Casein micelles range from 30 to 300 nm in diameter, and the composition of the micelles varies according to the diameter.^{1,2)} Investigation of the micelle size is important for clarification of the micelle structure. Slattery and Evard³⁾ reported that the protein composition of a subunit of a large micelle must be different from that of a small micelle. However, they did not take the salt content of micelles into consideration. It has been reported that the salt content varies with the micelle size,^{2,4)} and that a large micelle becomes small particles through medium ones liberating salts on dialysis.⁵⁾ Therefore, in order to clarify the correlation between large and small micelles, we investigated the change of micelle composition with dissociation by dialysis.

Skimmilk containing less than 0.01% of fat was prepared from bulk milk of Holstein cows. A large casein micelle fraction (average diameter 120 nm) was prepared from skimmilk by centrifugation at $15,000 \times g$ using the same method as in the previous paper.⁵⁾ A small casein micelle fraction (average diameter 60 nm) was prepared by centrifugation at $100,000 \times g$ for 30 min from the supernatant of skimmilk centrifuged at $60,000 \times g$ for 30 min. Each fraction was dispersed in 0.01 M imidazole buffer (pH 6.8, ionic strength 0.07) containing 0.02% NaN_3 with a protein concentration of 3% by a Potter-Elvehjem homogenizer. Proteolysis was inhibited by the addition of soybean trypsin inhibitor (Miles Labo. Ltd.) (5 mg/100 ml) to each suspension.⁷⁾ 20 ml portions of these casein micelle suspensions were each tightly packed in a visking tube, and dialyzed against 0.01 M imidazole buffer at 5°C or 37°C. The buffer was renewed at 2, 5, 10, 24, 36, and 48 hr after starting dialysis. After dialysis for 5, 10, 24, 48, and 72 hr, the dialyzates were centrifuged at $100,000 \times g$ for 30 min. The micelles of above 30 nm were precipitated by this centrifugation.⁴⁾ The quantities of protein, calcium, and inorganic phosphorus in the sedimented pellets were determined according to the methods described in the previous paper.⁴⁾ The protein composition of the sedimented pellets was analyzed by disc gel electrophoresis in which the separation gel consisted of upper (4.5% gel) and lower (7% gel) layers. A riboflavine was used as the polymerization initiator of the separation gel. The ratio of

the proteins was calculated from the densitometric traces of stained gels with a Fujiriken FD-A IV densitometer.

Large and small micelles were dissociated by dialysis both at 5°C and 37°C. The protein content in the residual micelles which were precipitated at $100,000 \times g$ for 30 min was measured at regular time intervals of dialysis. The small micelles were dissolved rapidly up to 20 hr, slowly thereafter, and incompletely over 72 hr at both temperatures, as shown in Fig. 1. The large micelles at 5°C were dissolved slowly and then completely over 72 hr. The dissolution of the large micelles at 37°C was similar to that

FIG. 1. The Quantities of Casein in Residual Micelles after the Dissolution of Large (○) and Small (△) Micelles by Dialysis.

Each solution after dialysis was centrifuged at $100,000 \times g$ for 30 min, and the protein content in the pellets was measured. ● ▲, dialysis at 5°C; ○ △, at 37°C.

FIG. 2. The Salt Contents of Residual Micelles after the Dissolution of Large (○) and Small (△) Micelles by Dialysis.

Each solution after dialysis was centrifuged at $100,000 \times g$ for 30 min, and calcium and inorganic phosphorus contents in the pellets were measured. ● ▲, dialysis at 5°C; ○ △, at 37°C.

of the small micelles. That is, the small micelles were dissolved more rapidly than the large micelles, and the dissolution at 37°C was more rapid than that at 5°C. The rapid dissolution of the small micelles was considered to be due to the large specific surface area.

It was reported that the large micelle fraction (120 nm) changed to 80, 50, and 40 nm particles after dialysis for 5, 10, and 24 hr, respectively, at 20°C,⁵⁾ and that the small micelle fraction (60 nm) was reduced and changed to 40 nm particles on dialysis for 12 hr both at 37°C or 5°C.⁶⁾ The micelle size decreased in proportion to the dissociation of micelles.

The changes of calcium (Ca) and inorganic phosphorus (Pi) contents in micelles on dialysis were followed at regular time intervals, and are shown in Fig. 2. The salt content in the large micelles was more than that in the small micelles. The salt content in the residual micelles decreased in parallel to protein content in the residual micelles (Fig. 1) of which the size decreased in parallel to the dissociation, and became constant at 3~4 mg Ca/g protein and 1~2 mg Pi/g protein except for the large micelles at 5°C. These parallel relations among the salt content, the protein content and micelle size suggest that the micelle formation was closely related to the salt content. We^{8,9)} and Dosako *et al.*¹⁰⁾ have pointed out previously that a conformational change of caseins, of which the hydrophobic sites^{8,9)} and protein binding sites¹⁰⁾ are exposed to solvent, is induced by binding of salts, especially calcium. The quantity of salts bound seems to be a main factor responsible for the formation of the micelles of larger size.

Casein compositions of the residual micelles after dialysis at 5°C are shown in Fig. 3. The β -casein content in the residual micelles was less than that in the native micelles. The small micelles contained more κ -casein than the large micelles. The κ -casein content was not changed in the residual micelles by dialysis. A slight change of micelle size from a small micelle (60 nm) to a residual micelle (40 nm)⁶⁾ did not affect the casein composition. The κ -casein content of the residual micelles from the large micelles increased with the dissociation of micelles, and became constant after dialysis for 10 hr. This constant value was identical with that for the small micelles. This means that the small particles which were formed by dissociation of a large micelle have a similar casein composition to a small micelle. The β -casein content of the residual micelles after dialysis at 37°C also decreased with the dissociation as well as that at 5°C.

To summarize our interpretation of the results, the quantity of salts bound to micelles was considered to be proportional to the micelle size, and the κ -casein content of small particles which were formed from large micelles by

FIG. 3. The Casein Composition of Residual Micelles after the Dissolution of Large (A) and Small (B) Micelles by Dialysis at 5°C.

□, α_{s1} -casein; ■, β -casein; ▨, κ -casein.

dialysis was nearly identical with that of a small micelle. Therefore, it is considered that the main factors for formation of large and small micelles are the salt and κ -casein contents, respectively.

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