Short Communication

Conversion of β-Casein to a Stabilizer for αs1-Casein by Enzymatic and Chemical Modifications

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In milk, casein exists as a constituent of casein micelle, a stable colloidal particle which contains calcium and inorganic phosphate. A similar structure can be reconstituted by adding CaCl2 to a neutral solution of casein. Functions of individual casein components in the casein micelle formation have been studied by using such a reconstituted system. It has been proved that the casein micelle was formed in such a manner that calcium-sensitive αs1- and/or β-casein was protected from precipitation by κ-casein.1,2 We have reported that β-casein after enzymatic dephosphorylation lost the ability to form a precipitate in the presence of calcium and that dephosphorylated β-casein gained a new function by which αs1-casein and/or β-casein was protected from precipitation by κ-casein.3,4 In this paper, the stabilizing ability of dephosphorylated β-casein was compared with that of κ-casein and an attempt to increase the stabilizing ability of dephosphorylated β-casein was performed.

Preparation of casein components,5 dephosphorylation of β-casein,3 and stabilization test of αs1-casein4 were performed as described in the previous reports. Lactosylation of β- and dephosphorylated β-caseins was performed by the method of Gray.6 Galactose content of lactosylated β-casein was determined by phenol-sulfuric acid method using galactose as standard.7 Aggregation of casein micelle by the addition of soybean lectin was tested essentially in the same way as in the aggregation test using wheat germ lectin.8 The ability of dephosphorylated β-casein to protect αs1-casein from the calcium-induced precipitation by forming a micelle-like particle is shown in Fig. 1. Dephosphorylated β-casein shows a marked protective effect, while native β-casein does not. It is evident, however, that κ-casein is much more effective than dephosphorylated β-casein; an equal amount (in weight) of dephosphorylated β-casein is required to stabilize αs1-casein completely but κ-casein stabilizes 10-fold αs1-casein. The micelle-like particle formed with dephosphorylated β-casein and αs1-casein in the presence of calcium was viewed with an electron microscope to be compared with the micelle formed with κ-casein and αs1-casein. Both particles were found to be similar in structure and in size (Fig. 2). Hill et al.9 have suggested that an amphiphilic primary structure of κ-casein has an important bearing on its stabilizing ability. Dephosphorylated β-casein also has an amphiphilic primary structure.10 In considering the stabilizing ability of dephosphorylated β-casein and the loss of stabilizing ability of κ-
Fig. 2. Electronmicrograph of Casein Micelles.
(A) Casein micelle formed by the combination of κ- and αs1-casein in a weight ratio of 1:10 (B) Casein micelle formed by the combination of dephosphorylated β- and αs1-casein in a weight ratio of 1:1. Casein micelles were formed in 10mM imidazole buffer, pH 7.1, containing 70mM KCl and 20mM CaCl2 at 37°C. The micelles were fixed with 1% glutaraldehyde for 10 min at room temperature, diluted 10 times with water, air dried and shadowed with platinum-vanadium. An JOEL JEM 100-C electronmicroscope was employed. Bars in the picture represent 1 μm.

casein after its chemical phosphorylation, we have postulated as another structural requirement for this stabilizing ability that phosphate content must be low.4,11) It seems that the stabilizing ability of dephosphorylated β-casein is of little importance in the stability of casein micelle in milk since dephosphorylated β-casein is not present in milk. It was found that a component of rat casein named C3-casein could stabilize bovine αs1-casein in a 1:1 weight ratio.12) Pelissier et al.13) have reported that the N-terminal sequence of the rat caseins named A1 and A2, which probably correspond to C3-casein, was similar to that of bovine β-casein. It is possible that the stabilizing ability of rat C3-casein is related to that of dephosphorylated β-casein. The stabilizing ability of dephosphorylated β-casein for αs1-casein was about one tenth that of κ-casein. One of the causes for this may be that the length of the hydrophilic portion of dephosphorylated β-casein is shorter than that of κ-casein.9,14) In order to increase its hydrophilic nature, we tried to bind lactose covalently to the amino groups of dephosphorylated β-casein. After an incubation of 10 days at 37°C, 10.4 mol of galactose were bound to one mole of the protein. The stabilizing ability of lactosylated-dephosphorylated β-casein was about 2.5 times that of dephosphorylated β-casein. Lactosylated β-casein exhibited no stabilizing ability for αs1-casein. The stabilizing ability of κ-casein for αs1-casein was impaired by its lactosylation. This will be described in detail elsewhere.

We have shown that casein micelle is aggregated by the addition of wheat germ lectin which binds to the sialic moiety of κ-casein, indicating the existence of κ-casein near the surface of the micelle.8) Soybean lectin, which has multiple binding sites for galactose moiety, was added to the micelle composed of lactosylated-dephosphorylated β-casein and

Fig. 3. Aggregation of Casein Micelle by the Addition of Soybean Lectin.
Soybean lectin (Pharmacia Co.) was added to the micelle formed by the combination of lactosylated-dephosphorylated β- and αs1-casein in a weight ratio of 0.6:1 in 10mM imidazole buffer, pH 7.1, containing 70mM KCl and 20mM CaCl2 at 37°C. After the incubation for 30 min, the mixture was centrifuged at 2000 rpm for 1 min. Protein in the supernatant was determined from the absorbance at 280 nm. Initial concentration of casein was 3 mg/ml.
as1-casein. As shown in Fig. 3, the micelle was sensitively aggregated by the addition of soybean lectin. The micelle composed of dephosphorylated β-casein and as1-casein was not aggregated by this lectin. Therefore, it is likely that lactose moiety of lactosylated-dephosphorylated β-casein is located near the surface of the micelle as well as sialic acid moiety in the casein micelle containing κ-casein. Thus, through the above mentioned enzymatic and chemical modifications, β-casein was converted into a stabilizer for as1-casein. This may be useful in the study of the function and structure of casein components.

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REFERENCES


