Electrophoretic Behaviors of α-Lactalbumin and β-Lactoglobulin Mixtures Caused by Heat Treatment**

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ABSTRACT : In order to study the reaction behaviors of bovine α-lactalbumin (α-La), β-lactoglobulin (β-Lg), and their mixtures during heat treatment, samples were analyzed using native-polyacrylamide gel electrophoresis (Native-PAGE), sodium dodecylsulfate (SDS)-PAGE, and two-dimensional (2-D)-PAGE. The electrophoresis demonstrated that the loss of native-α-La increased as temperature increased, and that the loss of apo-α-La was slightly higher than that of holo-α-La. The tests also showed that during heat treatment, a mixture of α-La and β-Lg was less stable than α-La *alone*. As such, it was assumed that β-Lg induced holo-α-La to be less stable than apo-α-La during heat treatment. The reaction behavior of α-La (holo-, apo-form) during heat treatment showed similar patterns in the 2-D-PAGE electropherogram, but the mixture of α-La and β-Lg created new bands. In particular, the results showed a greater loss of native α-La in the holo-α-La and β-Lg mixture than in the apo-α-La and β-Lg mixture. Thus, it can be concluded that the holo-α-La and β-Lg mixture was more intensively affected by heat treatment than other samples, and that free sulphydryl groups took part in the heat-induced denaturation. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 7 : 1041-1045)***

Key Words : α-lactalbumin, β-lactoglobulin, Heat Treatment, Electrophoresis, 2-D PAGE, SH Group

INTRODUCTION

Bovine α-lactalbumin (α-La), with about 14.2 kDa molecular weight, is present at concentrations ranging from 1 to 1.5 g L^{-1}, and it consists of 123 amino acid residues. α-La is nearly spherical and has a highly compact, globular structure with four disulfide bonds (Wong et al., 1996). α-La is also susceptible to heat denaturation (Morr, 1976; Permyakov and Berliner, 2000; Ha, 2001). Ruegg et al. (1977) showed that this occured at 65.2°C and a pH 6.7, but that 80 to 90% of the denaturation can be reversed with cooling. The calcium-bound native α-La is holo-α-La, while the calcium-free α-La is apo-α-La (Hiraoka et al., 1980; Bryant and Andrews, 1984). Few results about reaction behavior of holo-α-La and apo-α-La during heat treatment have been reported (Bernal and Jelen, 1984; Relkin, 1996).

Bovine β-lactoglobulin (β-Lg) is the most abundant in the whey proteins, comprising about 50% of the total whey proteins, and is present at concentrations of 2 to 4 g L^{-1} in bovine milk. β-Lg B, a polypeptide of 162 amino acid residues, has a molecular mass of 18.3 kDa, and has been sequenced (Eigel et al., 1984). This protein exists in solution as a 36.6 kDa dimer between pH 4 and 6.5 and has an isoelectric point of 5.2. Below pH 3.5 and above 7.5, the dimer dissociates into a slightly expanded monomer. Between pH 3.5 and 5.2, the dimer of β-Lg A polymerizes into a 146 kDa octamer at low temperatures (Swaisgood, 1996).

High temperature processing and storage causes disulfide bond interchanges to occur, and this facilitates protein polymerization by forming covalent intermolecular disulfide bonds (Shimada and Cheftel, 1989; McSwiney et al., 1994; Holt, 2000; Singh et al., 2000).

The heat denaturation and aggregation of α-La and β-Lg have been studied extensively in milk and whey systems (Hillier and Lyster, 1979; Parris et al., 1991; Relkin, 1996; Havea et al., 1998; Oldfield et al., 2000), where almost all of the β-Lg was incorporated into the aggregates via disulfide bonds and, to a considerable extent, via hydrophobic interactions (Havea et al., 1998; Oldfield et al., 2000).

The heat-induced interactions between α-La and β-Lg have also been studied in model systems (Matsudomi et al., 1992; Dalgleish et al., 1997; Schokker et al., 2000; Havea et al., 2001). At a constant protein concentration, mixtures of α-La and β-Lg produced firmer gels than β-Lg by *alone* (Matsudomi et al., 1992; Getzimati et al., 1997). The large aggregates seemed to be caused by disulfide bonds as reported recently (Matsudomi et al., 1992; Dalgleish et al., 1997; Havea et al., 1998). But the detailed reaction behaviors of holo-α-La, apo-α-La, and β-Lg during heating have not yet been reported.

In this study, we investigated the electrophoretic reaction behavior of bovine α-La, β-Lg, and their mixtures during heat treatment to elucidate how the whey proteins affect each other and to provide for dairy industry with more information.

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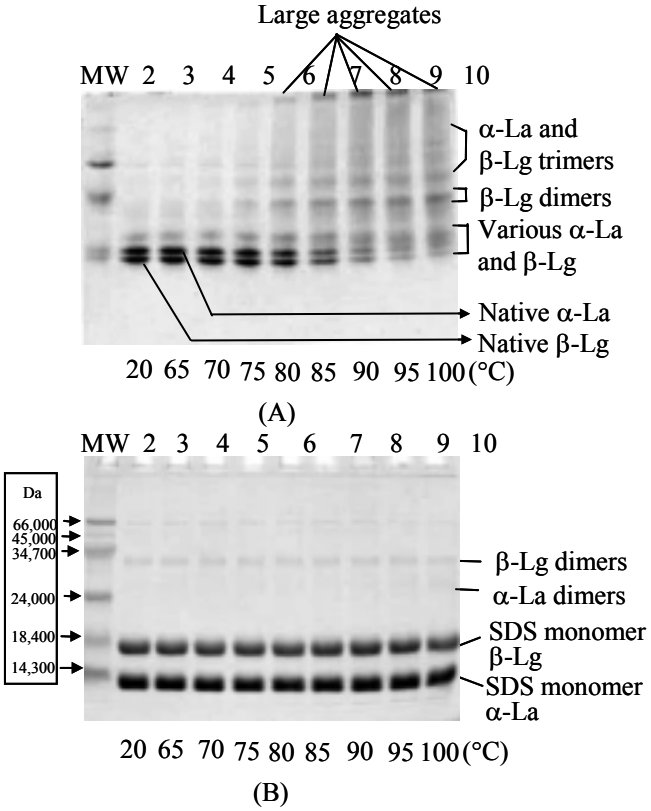

Figure 1. The effect of heating mixtures of β-Lg and holo-α-La at temperatures between 65 and 100°C for 10 min. (A); holo-α-La and β-Lg, Native-PAGE, (B); holo-α-La and β-Lg, SDS-PAGE.

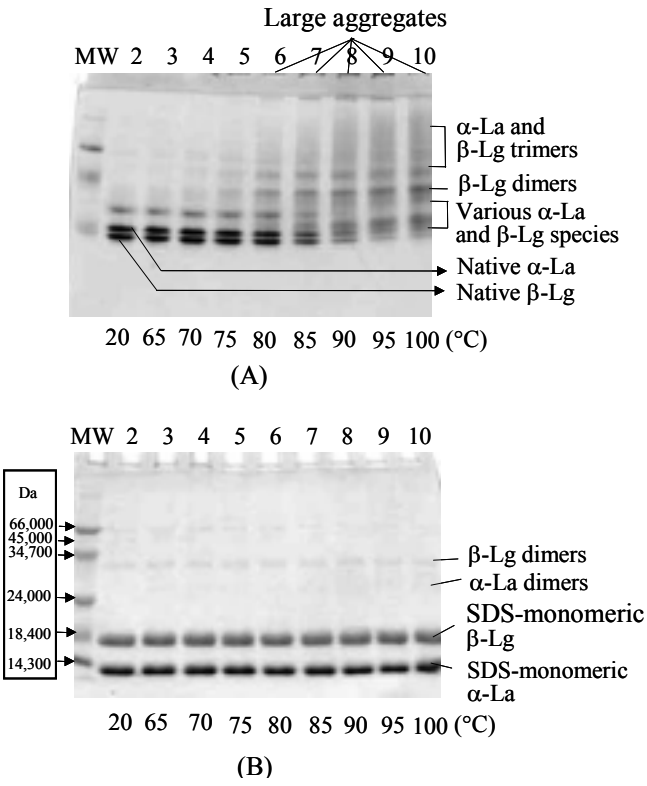

Figure 2. The effect of heating mixtures of β-Lg and apo-α-La at temperatures between 65 and 100°C for 10 min. (A); apo-α-La and β-Lg, Native-PAGE, (B); apo-α-La and β-Lg, SDS-PAGE.

MATERIALS AND METHODS

Holo-α-La (L5385, Ca-bound), apo-α-La (L6010, Ca-free), β-Lg, the molecular weight standards and the gel buffer salts were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Most of the reagents used for the preparation of electrophoresis gels were obtained from BioRad Laboratories (Hercules, CA, USA). The water was purified using a MilliQ system (Millipore Corp., Bedford, MA, USA).

The protein concentration was adjusted to 2.0 mg mL^{-1} using a 15 mM phosphate buffer with a pH of 6.8. The samples were heated at 65 to 100°C in small plastic (Eppendorf) vials for 10 min and cooled immediately in iced water. In all cases, the mixtures of α–La and β–Lg were on a 1:1 volume ratio.

One-dimensional (1D) and two-dimensional (2D) PAGE was used to analyze the heated samples, as described by Havea et al. (1998). The 1D PAGE was either run on alkaline- (Andrews, 1983) or SDS-PAGE (Laemmli, 1970). After preparing the appropriate gel, 10 to 20 μl samples of 0.2 mg protein/ml solutions diluted with appropriate sample buffer were injected into sample wells and then electrophoresed to separate the proteins. For the alkaline 2D (second-dimensional)-PAGE, a strip from the alkaline gel containing the protein bands that had been separated in a first dimensional run, was cut and rinsed in SDS sample buffer. This strip was placed on the top of a SDS gel and run in a second dimension. For the SDS-reduced 2D-PAGE, a strip from the SDS gel containing the protein bands that had been separated in a first dimensional run, was cut and placed in hot (94°C) solution of SDS buffer containing 2-mercaptoethanol (5 ml/L SDS sample buffer) for 30 s. The gel strip was then rinsed with water to remove the excessive 2-mercaptoethanol, then used for a second dimensional run on another SDS gel (Havea, 1998; Manderson et al., 1998).

RESULTS AND DISCUSSION

Effect of heat on mixture of holo-α-La and β-Lg

When mixture (1:1, v/v) of holo-α-La and β-Lg was heated together and the resultant samples were analyzed by alkaline- and SDS-PAGE, the patterns shown in Figure 1 were obtained. Bands of native α-La and β-Lg began to lose their intensity at the temperature around 80°C, forming dimers, trimers, oligomers, and large aggregates that could not get through the stacking gel (Figure 1A). The

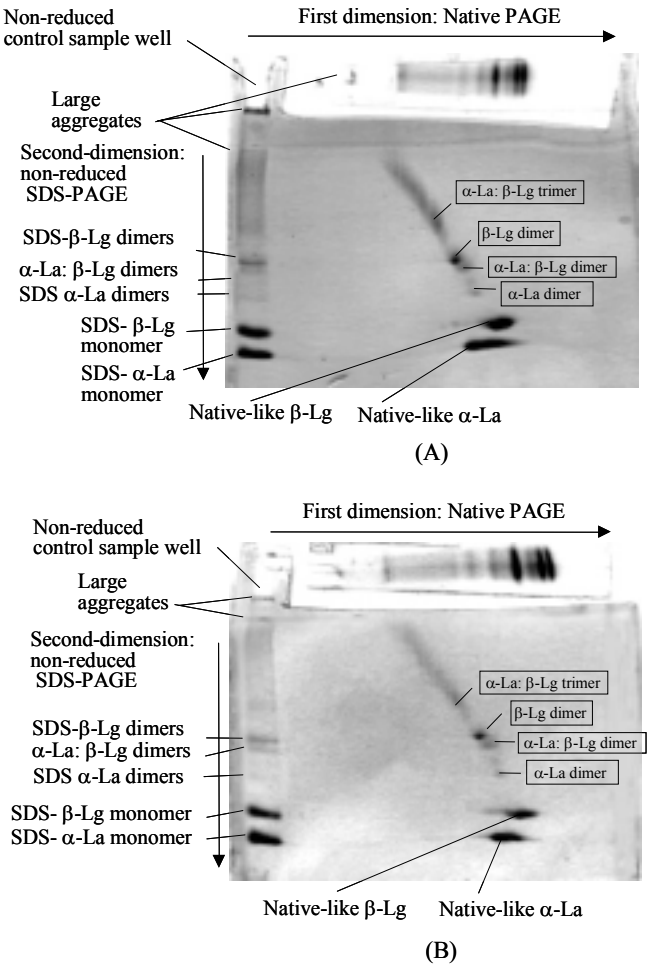

Figure 3. 2D-PAGE analysis (Native→non-reduced SDS-PAGE) of holo-α-La, apo-α-La and β-Lg at temperature of 85°C for 10 min. (A); holo-α-La and β-Lg, (B); apo-α-La and β-Lg.

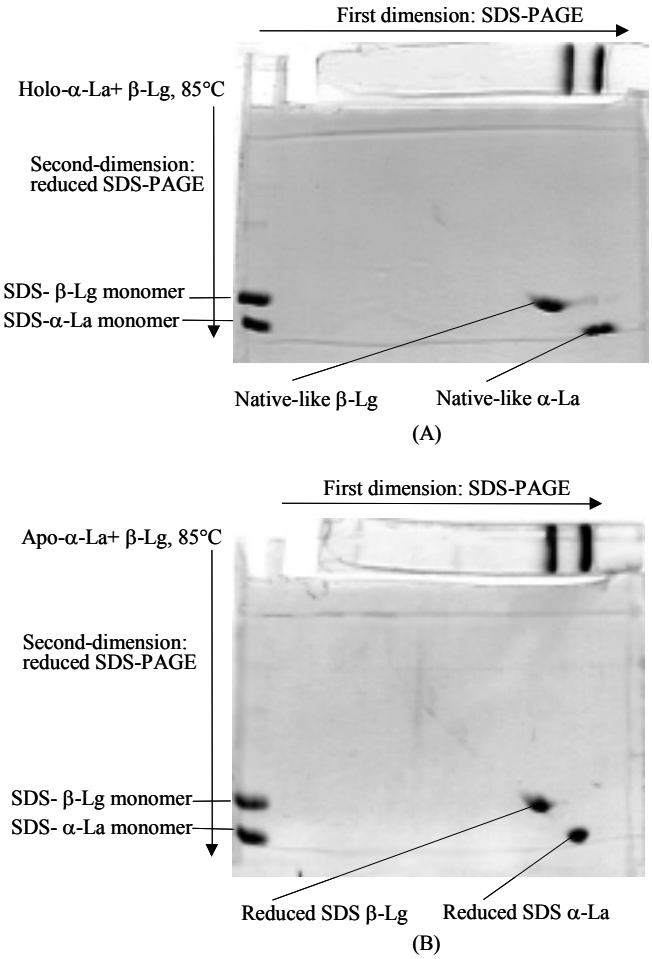

Figure 4. 2D-PAGE analysis (SDS→reduced SDS-PAGE) of holo-α-La, apo-α-La and β-Lg at temperature of 85°C for 10 min. (A); holo-α-La and β-Lg, (B); apo-α-La and β-Lg.

denaturation rate of native α-La was faster than that of native β-Lg, which is consistent with other reports (Havea et al., 2001; Hong and Creamer, 2002). A Comparison of the patterns shown in Figure 1 with those of earlier results (Lee and Hong, 2002) indicates a much greater rate of loss of the native holo-α-La when β-Lg is present. This is due to the substantial overlap of the α-La and β-Lg aggregate bands in the 1D PAGE patterns, as shown by the 2D patterns. The reactions between holo-α-La and β-Lg involve interprotein hydrophobic associations at a molecular level that allow segments of each molecule to interact with the other so that disulfide bond interchange can occur.

Effect of heat on mixture of apo-α-La and β-Lg

The differences between the patterns created by mixtures that contained the apo-α-La and those that contained the holo-α-La were slight but appeared to be moving in the same direction as the patterns created by α-La by itself; that is, apo-α-La reacted faster than holo-α-La (Figures 1 B, 2 B). It is known that α-La reversibly transforms into molten globule conformation at high temperature. The results shown in these Figures, which demonstrate that apo-α-La is more easily converted into non-native forms than holo-α-La, confirm the molten globule intermediate hypothesis (Hirose, 1993; Relkin, 1996; Hong and Creamer, 2002).

It was clear that the loss of native-like protein was greater for the apo-α-La than for the holo-α-La (Figure 1 A and Figure 2 A). In addition, more SDS dimers and higher aggregates were formed when the apo-α-La was given heat treatment (Hong and Creamer, 2002; Lee and Hong, 2002).

Figures 3 A and B (native→non-reduced SDS-PAGE) show 2-D electropherograms of holo-α-La, apo-α-La and β-Lg heated at 85°C, and suggest that the apo-α-La and β-Lg mixture produced less dimers, trimers and large aggregates than the holo-α-La and β-Lg. This implies that the β-Lg induces a greater loss of the holo-α-La than apo-

α-La and is stimulated to form aggregates through denaturation of native α-La. The apparently lower rate of loss of SDS monomeric α-La (Figure 1 B), in comparison with the apparent loss of native-like α-La (Figure 1 A), was caused by the non-native α-Las, which were separate bands in native (alkaline)-PAGE but ran as a single band in the SDS-PAGE system (Figure 3 A).

Figure 4 A and B (native→ reduced SDS-PAGE) show 2-D electropherograms of holo-α-La, apo-α-La and β-Lg heated at 85°C and are not very different from the 2-D electropherograms of the holo- and apo-α-La. The trace spots of oligomers of apo-α-La and β-Lg mixture were slightly clearer than those found in the holo-α-La and β-Lg mixture. Similar results were recently reported by Hong and Creamer (2002).

The holo-α-La showed more stability than the apo-α-La during heat treatment, while the mixtures of holo-α-La, apo-α-La and β-Lg were less stable than α-La itself. These results are consistent with previous report that the holo-α-La itself was more heat stable than apo-α-La (Lee and Hong, 2002). Heated mixtures of α-La and β-Lg contained large aggregates that were held together by disulfide bonds, as well as intermediate-sized aggregates that were held together primarily by disulfide bonds and, to a lesser extent, by covalent bonding (Matsudomi et al., 1992; Dalgleish et al., 1997; Havea et al., 1998).

In the mixtures, β-Lg seems to catalyze more extensively during the heat denaturation of native-like α-La of holo-α-La than that of apo-α-La. The formation of non-native β-Lg monomers, dimers, etc. was not influenced intensively. It is assumed that the free sulphydryl groups of β-Lg take part in the heat-induced aggregation reaction.

In conclusion, the loss of native-like protein was greater for apo-α-La than for holo-α-La. β-Lg induces a greater loss of holo-α-La than apo-α-La, and stimulates native α-La to form aggregates through denaturation. The free sulphydryl groups of β-Lg may take part in the heat-induced denaturation reaction.

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REFERENCES

Andrews, A. T. 1983. Proteinase in normal bovine milk and their action on casein. J. Dairy Res. 50:45-57.

Bernal, V. and P. Jelen. 1984. Effect of calcium binding on thermal denaturation of bovine α-Lactalbumin. J. Dairy Sci. 67:2452-2454.

Bryant, D. T, W. and P. Andrews. 1984. High-affinity binding of Ca^{2+} to bovine α-Lactalbumin in the absence and presence of EGTA. Biochem. J. 220, 617-620.

Dalgleish, D. G., L. van Mourik and M. Corredig. 1997. Heat-induced interaction of whey proteins and casein micelles with different concentration of α-lactalbumin and β-lactoglobulin. J. Agric. Food Chem. 45:4806-4813.

De Wit, J. N., G. Klarenbeck and E. Hontelez-Backx. 1983. Evaluation of functional properties of whey protein concentrates and whey protein isolates. I. Isolation and characterization. Neth. Milk Dairy J. 37:37-49.

Eigel, W. N., J. E. Butler, C. A. Ernstrom, H. M. Farrel Jr, V. R. Harwalker, R. Jenness and R. M. Whitney. 1984. Nomenclature of proteins of cows milk; fifth revision. J. Dairy Sci. 67:1599-1631.

Fox, P. F. 2001. Milk proteins as food ingredients. Int. J. Dairy Technol., 54(2): 41-55.

Gezimati, J., L. K. Creamer and H. Singh. 1997. Heat-induced interaction and gelation of mixtures of β-lactoglobulin and α-lactalbumin. J. Agric. Food Chem. 45(4):1130-1136.

Ha, E. Y. W. 2001. Milk, the rich source of bioreactive ingredients for functional nutraceutical and pharmaceutical industry. Dairy Industry and Technology, 1:93-124.

Havea, P., H. Singh, L. K. Creamer and O. Campanella. 1998. Electrophoretic characterization of the protein products formed during heat treatment of whey protein concentrate solutions. J. Dairy Res. 65:79-91.

Havea, P., H. Singh and L. K. Creamer. 2001. Characterization of heat-induced aggregates of β-lactoglobulin and α-lactalbumin and bovine serum albumin in a whey protein concentrate environment. J. Dairy Res. 68:483-497.

Hillier, R. M. and R. L. J. Lyster. 1979. Whey protein denaturation in heated milk and cheese whey. J. Dairy Res. 46:95-102.

Hiraoka, Y., T. Segawa, K. Kuwajima, S. Sugai and N. Murai. 1980. α-Lactalbumin calcium metalloprotein. Biochim. Biophys. Res. Commun. 95:1098-1104.

Hirose, M. 1993. Molten globule state of food proteins. Trends in Food Sci. & Technol. 4, 48-51

Holt, C. 2000. Molecular basis of whey protein food functionalities. Austral. J. Dairy Technol. 55 (June):53-55.

Hong, Y. H. and L. K. Creamer. 2002. Changed protein structures of bovine β-lactoglobulin and α-lactalbumin as a consequence of heat treatment. Int, Dairy J. 12:345-359.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227:680-685.

Lee, Y. R. and Y. H. Hong. 2002. Electrophoretic properties of heat-induced bovine α-lactalbumin. J. Food Sci. Nutr. 7(1), 48-51.

Manderson, G. A., M. J. Hardman and L. K. Creamer. 1998. Effect of heat treatment on the conformation and aggregation of β-lactoglobulin A, B and C. J. Agric. Food Chem. 46:5052-5061.

Matsudomi, N., T. Oshita, E. Sasaki and K. Kobayashi. 1992. Enhanced heat-induced gelation of β-lactoglobulin by α-lactalbumin. Biosci. Biotech. Biochem. 56(11):1697-1700.

McSwiney, M., H. Singh and O. Campanella. 1994. Thermal aggregation and gelation of bovine β-lactoglobulin. Food Hydrocolloids, 8:441-453.

Morr, C. V. 1976. Whey protein concentrates: an update. Food Technol. 30:18.

Oldfield, D. J., H. Singh, M. W. Taylor and K. N. Pearce. 2000. Heat-induced interactions of β-Lactoglobulin and α-Lactalbumin with the casein micelle in pH-adjusted skim milk. Int. Dairy J. 10:509-518.

Parris, N., C. M. Hollar, A. Hsieh, A. and K. D. Cockley. 1991. Thermal stability of whey proteins in skim milk. J. Agric. Food Chem. 39:2167-2170.

Permyakov, E. A. and L. J. Berliner. 2000. α-Lactalbumin: structure and function. FEBS Letters, 473:269-274.

Relkin, P. 1996. Thermal unfolding of β-Lactoglobulin, α-lactalbumin, and bovine serum albumin. A thermodynamic approach. Crit. Rev. Food Sci. Nutr. 36(6):565-601.

Ruegg, M., U. Moor and B. Blanc. 1977. A calorimetric study of thermal denaturation of whey protein in simulated milk ultrafiltrate. J. Dairy Res. 44:509-520.

Schokker, E. P., H. Singh, D. N. Pinder, G. E. Norris and L. K. Creamer. 1999. Characterization of intermediates formed during heat-induced aggregation of β-lactoglobulin AB at neutral pH. Int. Dairy J. 9:791-800.

Shimada, K. and J. C. Cheftel. 1989. Sulfhydryl group/disulfide bond interchange reactions during heat-induced gelation of whey protein isolate. J. Agric. Food Chem. 37:161-168.

Singh, H., Y. Ye and P. Havea. 2000. Milk protein interactions and functionality of dairy ingredients. Austral. J. Dairy Technol. 55:71-77.

Swaisgood, H. E. 1996. Characteristic of milk. In: Food Chemistry (Ed. O. R. Fennema) 3rd Ed., Marcel Dekker, New York (1996). pp. 841-878.

Wong, D. W. S., W. M. Cairand and A. E. Paviath. 1996. Structures and functionalities of milk proteins. Crit. Rev. Food Sci. Nutr. 36(8):807-844.