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Electrophoretic Properties of Heat-Induced Bovine α-Lactalbumin

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Abstract

In order to study the reaction behavior of bovine holo- and apo- α -lactalbumin (α -La) during heat treatment at 65～100°C, the samples were analysed by first (1D)- and second-dimensional (2D) native-polyacrylamide gel electrophoresis (Native-PAGE) and sodium dodecylsulfate (SDS)-PAGE. When holo- α -La or apo- α -La were heated, they formed non-native, monomers, dimers and trimers. The apo- α -La was more heat-sensitive than holo- α -La. The monomers seemed to have the same composition as the native α -La, but many of the disulfide bonds could be non-native.

Key words: bovine whey, α -lactalbumin, heat treatment, electrophoresis

INTRODUCTION

Bovine milk contains about 3.5% of protein and the protein contains about 20% of whey protein. The whey protein of bovine milk consists of four main proteins: β-lactoglobulin (β-Lg, 50%), α -lactalbumin (α -La, 20%), blood serum albumin (BSA, 10%) and immunoglobulins (Ig, 10%) (1).

α -La, with about 14 kd 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 of four disulfide bonds (2). α -La is relatively rich in tryptophan (four residues per mole). It is also rich in sulfur (1.9%) which is present in cystine and methionine; it contains no cysteine (sulphydryl group) (3).

α -La is susceptible to heat denaturation (4). Ruegg et al. (5) showed that α -La is denatured at 65.2°C and pH 6.7, and 80 to 90% of the denaturation reversed upon cooling. α -La was denatured at 62 to 63°C and was 90% renatured at pH 6.5 (6). This high degree of renaturation is probably due to the apparent high heat resistance attributed to α -La (7). Chaplin and Lyster (8) reported that heating α -La solutions to 77°C and immediate cooling resulted in only 10% of irreversible protein denaturation. Heating an α -La solution to 95°C and holding at this temperature for 15 min allowed only 40% of the protein to be renatured.

α -La is a good Ca-binding protein. Its ability to bind Ca^{+2} is attributed to the presence of three aspartyl groups that are bound together in its tertiary structure (9). Ca^{+2} ion is strongly bound by α -La in a cleft surrounded by three Asp residues with abnormally low pK values (10), shown as Asn residue 82, 87, and 88 in the amino acid sequence of α -La (11).

α -La undergoes a conformational transitions at ≤pH 4, which is probably due to the replacement of this tightly bound Ca^{+2} ion with a H^{+1} ion at this pH (12).

The calcium-bound native α -La is holo- α -La, while the calcium-free α -La is apo- α -La (13).

Previous work has shown that when α -La is heated in solution, it can repeatedly undergo the same thermal transition without forming a gel (5,14). When α -La binds less than 1 mole of calcium per mole of protein, the thermal transition temperature of this apo- α -La decreases to about 35°C (15).

Addition of calcium ions to the apo- α -La increases the transition temperature to about 66°C (14). More extensive heating (100°C for at least 10 min) in the absence of extra calcium gives rise to disulfide-bonded α -La dimers, trimers etc. as well as some altered monomeric protein (8). Chaplin and Lyster (8) also noted that in alkaline (native)-polyacrylamide gel electrophoresis (PAGE) the dimer had a mobility comparable with that of the native monomer, but in sodium dodecyl sulfate (SDS)-PAGE the dimer had the expected mobility, i.e. lower than that of monomer.

Few results about reaction behavior of holo- α -La and apo- α -La during the heat treatment have been reported.

α -La can be used in infant formulas, food fortifiers and functional foods. Therefore, studying its physicochemical behavior and its properties of α -La is very important for better applications.

The objective of this study is to investigate any differences in heat-induced changes between holo- α -La and

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apo- α -La by using electrophoretic techniques.

MATERIALS AND METHODS

Holo- α -La (L5385, Ca-bound), apo- α -La (L6010, Ca-free), 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).

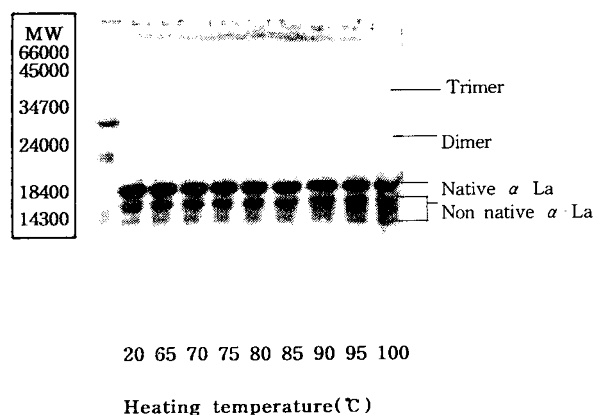
The protein concentration was adjusted to 2.0 mg mL^{-1} using 15 mM phosphate buffer, pH 6.8. The samples were heated at 65 ~ 100°C in small plastic (Eppendorf) vials for 10 min and cooled immediately in iced water.

The heated samples were analysed by one-dimensional (1D) and two-dimensional (2D) PAGE as described by Havea et al. (16). The 1D PAGE was either run on alkaline- (17) or SDS-PAGE (18). After preparing the appropriate gel, 10~20 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 top of a SDS gel and run in a second dimension. For the SDS-reduced 2D-PAGE, a strip from a SDS gel containing the protein bands that had been separated in a first dimensional run, was cut, 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 rinsed with water to remove excessive 2-mercaptoethanol, then used for a second dimensional run on another SDS gel (16,19).

RESULTS AND DISCUSSION

Heat-induced changes of holo- α -La in pH 6.8 phosphate buffer for 10 min at temperatures from 65 to 100°C were analysed using native (alkaline)- and SDS-PAGE, to obtain the patterns shown in Figs. 1A and 1B, respectively. As the heating temperature was increased, little change was noted until a temperature of 80°C was reached. Between 85 and 100°C, there was a gradual decrease in the intensity of the original band and an increase in the number and intensities of some new bands. At the highest heating temperature (100°C), there were at least three higher mobility bands and some lower mobility bands (non-native α -La, Fig. 1A) in addition to the original band. Only a few new low intensity bands of lower mobility were observed in the SDS-PAGE patterns (bands above the SDS-dimeric α - La, Fig. 1B). Binding to Ca^{+2} ions stabilizes the conformation of α -La (9) and reduces its electrophoretic mobility (20). Apenten (21) reported that Ca^{+2} binding preferentially stabilizes one domain of holo- α -La against heat-denaturation. Cation binding to the strong calcium site increases protein stability of α -La against the action of heat and various denaturing agents (22).

(A)



(B)

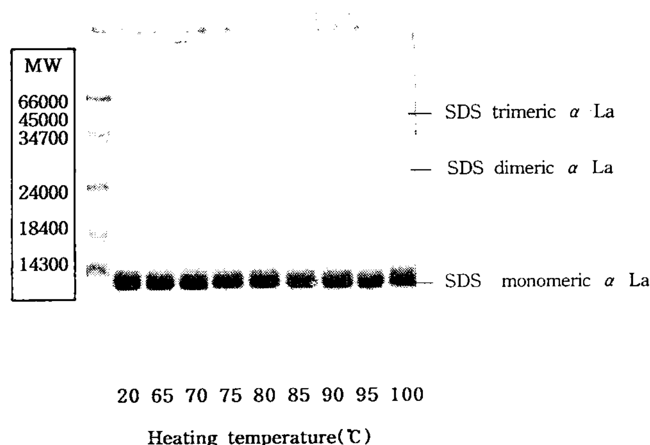


Fig. 1. Electrophoretic patterns of holo- α -La in pH 6.8 phosphate buffer after 10-min heat treatment between 65 and 100°C. (A): holo- α -La, Native-PAGE, (B): holo- α -La, SDS-PAGE.

When apo- α -La was heated similarly, the results shown in Figs. 2A and 2B, respectively, were obtained. It is clear that the loss of native-like protein was greater for the apo protein than for the holo protein (cf. Figs. 1A and 1B with Figs. 2A and 2B, respectively) after heat treatment. This result is in good agreement with other reports (8,15,22). Also, more SDS dimers and timers were formed (Fig. 2B) by heat treatment of the apo protein. The monomers were likely to have the same composition as the native α -La, but many of the disulfide bonds could be non-native.

The electrophoretic patterns show a similar behavior to the results of Hong and Creamer (23). The metal-free protein (apo- α -La) is denatured at quite low temperatures

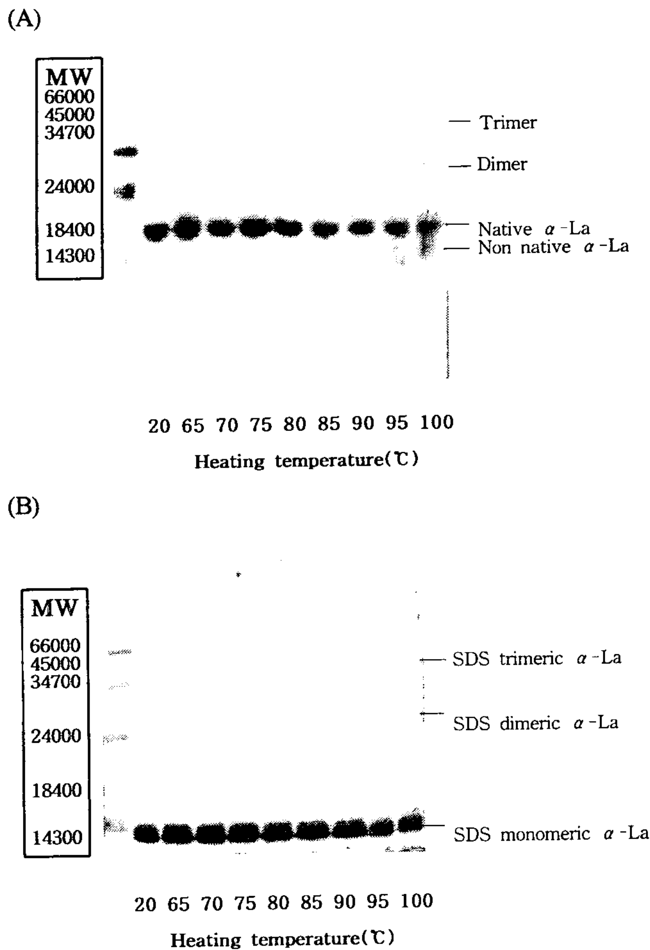


Fig. 2. Electrophoretic patterns of apo- α -La in pH 6.8 phosphate buffer after 10-min heat treatment between 65 and 100°C. (A): apo α -La, Native-PAGE, (B): apo α -La, SDS-PAGE.

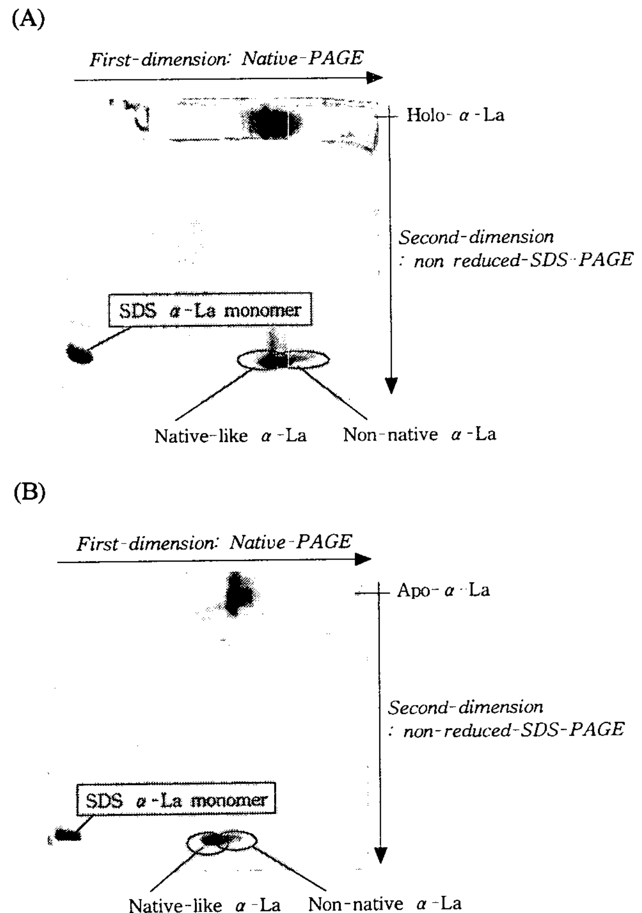


Fig. 3. 2D-PAGE patterns (*Native→ non-reduced SDS-PAGE*) of holo- and apo- α -La heated at 85°C for 10 min. (A): holo- α -La, (B): apo- α -La.

and does not renature on cooling (3). However, the detailed mechanisms of those behaviors are not reported.

In order to identify more clearly, the various species observable in Figs. 1A and 2A, heated samples of holo- α -La were examined using 2D PAGE and the results shown in Figs. 3A and 4A obtained. The non-native α -La had separate bands in native-PAGE system, but it appeared to become a single band in the SDS-PAGE system.

The 2-D electrophoretic patterns of heated apo- α -La are shown in Figs. 3B and 4B. The size of spots of holo- α -La from the 2D-gel was bigger than that of apo- α -La. It suggested that the holo- α -La was more heat stable than apo- α -La. This result is consistent with the previous result that the mobility of non-native monomers was faster than that of native monomers (ref. Figs. 1 and 2).

The series of spots that made the high mobility stripes in Figs. 4A and 4B, called "reduced SDS α -La" were all reduced α -La monomer molecules and they would all have the same composition and structure in the SDS buffer. The large spot at the right-hand end was monomeric prior to reduction and corresponded to the most mobile bands in the sample strip at the top of Figs. 4A and 4B, labelled "SDS α -La monomers". Moving left on the "reduced SDS α -La" band, the next two spots, which corresponded to a pair of bands in the sample strip at the top of Figs. 4A and 4B, were consequently labelled as "SDS dimeric α -La". It was not easy to see these bands in the vertical 1D sample pattern in Figs. 3A and B. They had similar mobilities to that of native α -La in Native (alkaline)-PAGE (Figs. 1A and 2A), confirming the earlier results of Chaplin and Lyster (8) and Havea et al. (24). A spot of trimers was also appeared on the middle of 2D gels.

Studies of the reversible changes in the spectral properties of the protein implicate protein conformational changes that are pH, calcium and ionic strength dependent (7,9,15).

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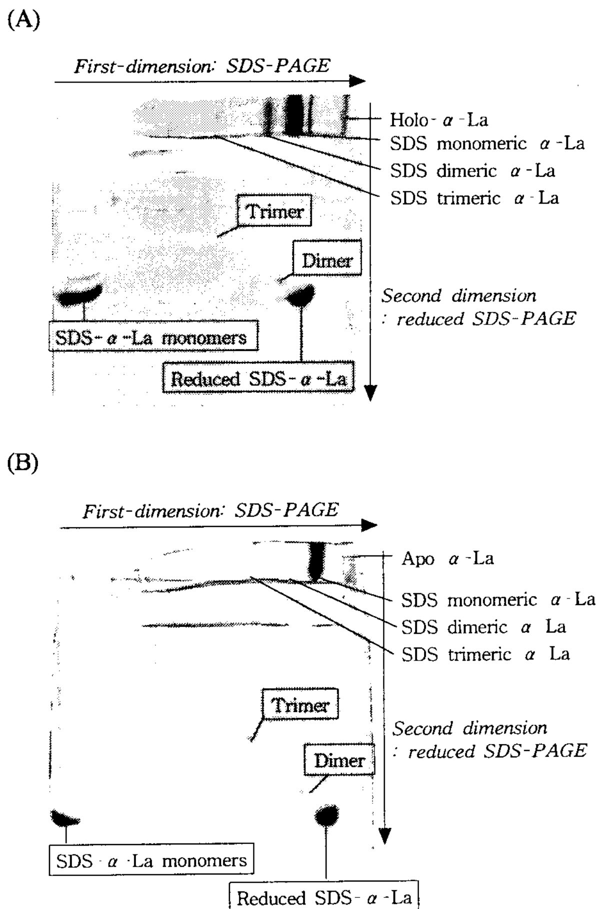

Fig. 4. 2D-PAGE patterns (*SDS → reduced SDS-PAGE*) of holo- and apo- α -La heated at 85°C for 10 min. (A): holo- α -La, (B): apo- α -La.

REFERENCES

1. Fox PF. 2001. Milk proteins as food ingredients. *Int J Dairy Technol* 54: 41-55.
2. Wong DWS, Cairand WM, Paviath AE. 1996. Structures and functionalities of milk proteins. *Crit Rev Food Sci Nutr* 36: 807-844.
3. Fox PF, McSweeney PLH. 1998. *Dairy Chemistry and Biochemistry.* A, Blackie Academic and Professional, London. p 192.
4. Morr CV. 1976. Whey protein concentrates: an update. *Food Technol* 30: 18-42
5. Ruegg M, Moor U, Blanc B. 1977. A calorimetric study of thermal denaturation of whey protein in simulated milk ultrafiltrate. *J Dairy Res* 44: 509-520.
6. de Wit JN, Klarenbeck G, Hontelez-Backx E. 1983. Evaluation of functional properties of whey protein concentrates and whey protein isolates. I. Isolation and characterization. *Neth Milk Dairy J* 37: 37-49.
7. Bernal V, Jelen P. 1984. Effect of calcium binding on thermal denaturation of bovine α -lactalbumin. *J Dairy Sci* 67: 2452-2454.
8. Chaplin LC, Lyster RLJ. 1986. Irreversible heat denaturation of bovine a-lactalbumin. *J Dairy Res* 53: 249-258.
9. Hiraoka Y, Segawa T, Kuwajima K, Sugai S, Murai N. 1980. α -Lactalbumin: a calcium metalloprotein. *Biochim Biophys Res Commun* 95: 1098-1104.
10. Fox PF. 1992. The milk protein system. In *Developments in dairy chemistry-4.* Elsevier Applied Science Publishers, London and New York. p 37.
11. Eigel WN, Butler JE, Ernstrom CA, Farrel Jr HM, Harwalker VR, Jenness R, Whitney RM. 1984. Nomenclature of proteins of cows milk; fifth revision. *J Dairy Sci* 67: 1599-1631.
12. Ha EYW. 2001. Milk, the rich source of bioreactive ingredients for functional nutraceutical and pharmaceutical industry. *Dairy Industry and Technol* 1: 93-124.
13. Bryant DTW, Andrews P. 1984. High-affinity binding of Ca^{2+} to bovine α -lactalbumin in the absence and presence of EGTA. *Biochemical J* 220: 617-620.
14. Relkin P, Launay B, Eynard L. 1993. Effect of sodium and calcium additionon thermal denaturation of apo- α -lactalbumin: a differential scanning calorimetric study. *J Dairy Sci* 76: 36-47.
15. Relkin P. 1996. Thermal unfolding of β-lactoglobulin, α -lactalbumin, and bovine serum albumin. A thermodynamic approach. *Crit Rev Food Sci Nutr* 36: 565-601.
16. Havea P, Singh H, Creamer LK, Campanella O. 1998. Electrophoretic characterization of the protein products formed during heat treatment of whey protein concentrate solutions. *J Dairy Res* 65: 79-91.
17. Andrews AT. 1983. Proteinase in normal bovine milk and their action on casein. *J Dairy Res* 50: 45-57.
18. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
19. Manderson GA, Hardman MJ, Creamer LK. 1998. Effect of heat treatment on the conformation and aggregation of β-lactoglobulin A, B and C. *J.Agric Food Chem* 46: 5052-5061.
20. Thompson MP, Groves ML, Brower DP, Farrell HM, Jenness R, Kotts CE. 1988. The calcium-dependent electrophoretic shift of a-lactalbumin, the modifier protein of galactosyl transferase. *Biochem Biophys Res Comm* 157: 944-988.
21. Apenten RKO. 1995. A three-state heat-denaturation of bovine α -lactalbumin. *Food Chem* 52: 131-133.
22. Permyakov EA, Berliner LJ. 2000. a-Lactalbumin: structure and function. *FEBS Letters* 473: 269-274.
23. Hong YH, Creamer LK. 2002. Changed protein structures of bovine β-lactoglobulin and α -lactalbumin as a consequence of heat treatment. *Int Dairy J* 13: In press.
24. Havea P, Singh H, Creamer LK. 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.

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