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COMMUNICATION

Thermal Properties of Yak α-Lactalbumin and β-Lactoglobulin: a DSC Study

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Abstract A differential scanning calorimetry (DSC) method was used to investigate the denaturation temperature of yak α-lactalbumin (α-La), β-lactoglobulin (β-Lg), and a mixture of two proteins and the thermal properties of α-La and β-Lg in the presence of glucose, lactose, sucrose, NaCl, $CaCl_2$, and at various pH (4.0–10.0). The denaturation temperature (T_d) of α-La increased from 52.1 °C in the absence of β-Lg to 53.9 °C in the presence of β-Lg, while the T_d of β-Lg decreased from 81.4 °C in the absence of α-La to 79.9 °C in the presence of α-La. α-La was thermal stable in the range of pH 4.0–10.0, while β-Lg was more thermal stable in acidic pH than in alkaline pH. Sugars, Na^+, and Ca^{2+} influenced the stabilization of the two proteins against thermal denaturation with greatly influenced for β-Lg. α-La kept reversibility in the presence of sugars, NaCl, $CaCl_2$, and over a wide pH range (4.0–10.0), with most of the reversibility values being greater than 90%. In contrast, β-Lg was completely irreversible whether in its native state or in the presence of the additives.

Keywords Yak milk · α-Lactalbumin · β-Lactoglobulin · DSC · Thermal denaturation

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Introduction

The yak is mainly developed on the Qinghai-Tibetan Plateau at elevation of between 2000 and 5000 m, extending over 2.5 million square kilometers and often called the "roof of the word" (Wiener et al. 2003). The yak belongs to *Bos grunniens* and the same genus as other domestic cattle (Wiener et al. 2003). However, yak milk is significantly different from bovine milk in terms of composition (Li et al. 2010). Yak milk resembles buffalo milk, as it has higher solids, protein, and fat contents than both bovine and goat milk (Li et al. 2010; Walstra et al. 2005; Kanekanian 2005) and serves as a superior source of vitamins and specific enzymes (Ma et al. 2013).

When milk protein is heated at over 65 °C, there is a formation of protein complexes between denatured whey proteins and κ-casein (κ-CN) that could be bound on the surface of casein micelles or soluble in the serum phase of milk soluble and micelle-bound whey protein/κ-casein complexes are produced in milk (Pesic et al. 2014). β-Lactoglobulin (β-Lg) and α-lactalbumin (α-La) are the major proteins in whey protein concentrates and isolates, so they contribute greatly to the thermal behavior of these ingredients (Vardhanabhuti and Foegeding 2008). β-Lg is the dominant protein in whey protein as a globular milk protein with a free sulfhydryl group (Sawyer 2013). α-La is the second major protein of whey. α-La is a globular, calcium-binding protein containing four intramolecular disulfides with and no free sulfhydryls (Brew 2013).There has been extensive research on heat denaturation and aggregation of β-Lg and α-La. Factors influencing the denaturation of β-Lg and α-La include pH, ionic strength and the nature of ions, the presence of calcium, concentration and purity of the protein, dielectric constant, temperature, and genetic variants (Sawyer 2013; Relkin 1996; Mounsey and O'Kennedy 2012; Vardhanabhuti and Foegeding 2008; Brew 2013).

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α-La denatures at relatively low temperature but does not rapidly aggregate because of its lack of free thiol groups (Vardhanabhuti and Foegeding 2008). From DSC, the denaturation temperatures of apo-α-La (Ca^{2+}-free) and holo-α-La (Ca^{2+}-bonding) were at 39.6 and 64.8 °C, respectively. When reheated at pH 3, apo-α-La was partially reversible (14%) while holo-α-La was completely reversible. At pH 9, both forms of α-La were completely irreversible (Boye et al. 1997). Based on the influencing factors, in dairy industry, one common approach to stabilize proteins during heating is the addition of ingredients such as follows: sugars, surfactants, salts, and polymers (Vardhanabhuti and Foegeding 2008), and these factors effected the aggregation and gelation of whey protein (Boye et al. 1995). It has been reported that the addition of sugar can be used to control the heat-induced denaturation and aggregation behavior of globular proteins in solution (Liang et al. 2014). The addition of NaCl to β-Lg solutions prior to heating inhibited denaturation/aggregation on heating at pH 5.0–6.0 but promoted these reactions at pH 6.0–7.0 (Mounsey and O'Kennedy 2007).

The growing increase of yak milk production in recent years has enhanced the study of its properties by the scientific community. There are reported works on yak milk compositions and processing properties (Zhang et al. 2008; Li et al. 2010; Wu et al. 2009; Cui et al. 2016) and milk thermal stability (Li et al. 2014; Yang et al. 2014; Xu et al. 2015); however, there is no information on the thermal denaturation of α-La and β-Lg about yak milk. In this paper, a differential scanning calorimetry (DSC) was used to investigate the thermal properties of α-La and β-Lg at different pH, the presence of sugars, NaCl, and $CaCl_2$, in order to facilitate yak milk utilization and its application in industry.

Materials and Methods

Sample Preparation and DSC Analysis

α-La and β-Lg were separated from yak milk as described by Alomirah et al. (Alomirah et al. 2004). The contents of α-La and β-Lg were analyzed using reversed-phase high-performance liquid chromatography (Li et al. 2010). The purity of α-La and β-Lg was approximately 90% separately. All other chemical reagents were of analytical grade. The water used was deionized water.

Based on the preliminary test and described by Boy et al. (Boye et al. 1997), 20% α-La and 10% β-Lg were used in the following test. α-La and β-Lg were separately dissolved in 20 mM phosphate buffer (pH 7.0) to obtain 10% (*w*/*v*) protein solution. The mixtures of α-La and β-Lg (2:1) were dissolved in a 20-mM phosphate buffer (pH 7.0) to obtain 30% (*w*/*v*) protein solution. The protein solutions of the α-La, β-Lg, and α-La/β-Lg mixture were used in order to determine the denaturation temperature of two proteins.

To study influence of pH on the thermal properties of α-La and β-Lg, the two proteins were separately dissolved in 20 mM phosphate buffers with different pHs (4.0–10.0, with one pH unit interval) to a final concentration of 10% (*w*/*v*) and then samples were subjected DSC analysis.

To study the effect of sugars on the thermal properties of α-La and β-Lg, the two proteins were separately dissolved in sugar solutions (sucrose, lactose, and glucose) of different concentrations (0.25, 0.5, 0.75, and 1.0 M). The final concentration of α-La and β-Lg was 10%, and pH was 7.0, separately.

To study the effect of Na^+ and Ca^{2+} on the thermal properties of α-La and β-Lg, the two proteins were separately dissolved in NaCl and $CaCl_2$ water solutions with different concentrations (0.25, 0.5, 0.75 and 1.0 M). The final concentration for both α-La and β-Lg was 10%, and the pH was ~ 7.0 and ~ 7.1, respectively.

All samples were subjected to DSC (1 STARe SYSTEM, METTLER TOLEDO, Switzerland). The DSC analysis procedure used was as described by Boye and Alli (Boye and Alli 2000). Each solution was placed in preweighed DSC pans and scanned from 10 to 100 °C at a programmed heating rate of 5 °C/min. For each run, a sample pan containing the buffer or solution used for dissolving the proteins was used as reference. Peak temperature of denaturation (T_d) and heat of transition or enthalpy (ΔH) (area underneath peak) was computed from each thermal curve (ΔH values are based on the total weight of the protein solutions). After heating, the samples were allowed to cool to room temperature in the DSC, and the heating cycle was repeated under the same experimental conditions. The degree of reversibility was determined from the ratio of the areas under the second and first endothermal peaks (Relkin et al. 1993). All DSC experiments were done in triplicate.

Statistical Analysis

All experiments were conducted in triplicate. Data were analyzed using One-Way Analysis of variance (ANOVA) test, and the means obtained were compared using Fisher's least significant difference test ($P < 0.05$).

Results and Discussion

The Denaturation Temperature of Yak α-La and β-Lg

The DSC thermograms of yak α-La, β-Lg, and the α-La/β-Lg mixture are shown in Fig. 1. The thermograms of both α-La and β-Lg had single peak temperature of denaturation (T_d) at 52.1 and 81.4 °C, respectively. Two endothermic transitions of

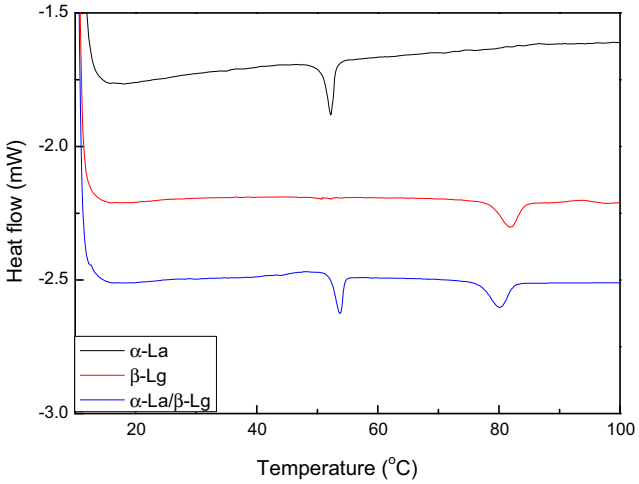

Fig. 1 DSC thermograms for α-La, β-Lg, and the α-La/β-Lg

the α-La/β-Lg mixture were observed at 79.9 and 53.9 °C which corresponded to the denaturation temperature of α-La and β-Lg, respectively. The T_d of α-La was increased by 1.8 °C, and the T_d of β-Lg was decreased by 1.5 °C when α-La and β-Lg were heated together. These results suggested that thermal stability of α-La was increased slightly, and the thermal stability of β-Lg was decreased slightly in the α-La/β-Lg mixture. α-La has a strong Ca^{2+}-binding site, which may stabilize the molecule against irreversible thermal denaturation. Removal of Ca^{2+} from α-La (apo-α-La) causes a local unfolding of the Ca^{2+}-binding loop and a change of a part of the backbone chain from a rigid coordination complex to an unordered loop (Brew 2013). Because the α-La separated from yak milk did not remove binding Ca^{2+} in this experiment, the thermogram of α-La showed one thermal transition at 52.1 °C corresponding to the denaturation of holo-α-La (Ca^{2+}-bonding). The similar results were found in bovine α-La/β-Lg mixture, which showed a significant increase of 2.5 °C in the T_d of α-La and a decrease of 2.8 °C in the T_d of β-Lg when the mixture of α-La/β-Lg was heated (Boye and Alli 2000). The unfolding of α-La on denaturation initiated cross-linking interactions with exposed sites on the β-Lg molecule which enhanced the unfolding of β-Lg (Boye and Alli 2000). Bovine β-Lg denatures through an initial dissociation from dimer to monomer followed by a change in the polypeptide chain conformation and subsequent aggregation (Brew 2013). Although there are no reported works concerning thermal denaturation of yak α-La and β-Lg, their thermal denaturation behaviors are similar to reported bovine β-Lg and α-La (Boye and Alli 2000; Haug et al. 2009; Boye et al. 1997).

Effect of pH on the Thermal Properties of Yak α-La and β-Lg

The peak temperature of denaturation (T_d), enthalpy (ΔH), and reversibility degree of yak α-La and β-Lg in phosphate buffers with different pH was shown in Table 1. Increasing the pH from 4.0 to 10.0, the T_d and ΔH of α-La were not significantly ($P < 0.05$) affected and were in the range of 52.1–52.3 °C and 1.1–1.5 J/g, respectively. When the α-La buffers were reheated, the reversibility of α-La was 100% in the pH 4.0–6.0, while the reversibility of α-La was 94–89% within pH 7.0–10.0. The results revealed that α-La is the reversible heat denatured whey protein (reversibility > 90%). As a Ca-binding protein, certain carboxyl groups of α-La molecule are involved in binding of calcium ions and loss of tightly bound calcium which is responsible for the conformational change of α-La around different pH (Dewit and Klarenbeek 1984). The thermal properties of yak milk α-La are different from bovine milk α-La. In a bovine milk α-La study, it was observed that the thermal stability of holo-α-La decreased with increasing of pH (3.0–9.0) and with 2.3 °C shift (Boye et al. 1997). The bovine holo-α-La was completely reversible at pH 3.0, while it was partially reversible (38%) at pH 9.0 (Boye et al. 1997). Increasing the pH 4.0 to 10.0 resulted in a significant decrease ($P < 0.05$) in the T_d of β-Lg from 82.2 °C at pH 4.0 to 79.8 °C at pH 10.0 which indicated that β-Lg had higher thermal stability in lower pH. At acidic pH, the free thiol group is not reactive but the hydrophobic residues of the folded state, which were initially buried, come

Table 1 Effect of pH on the thermal properties of α-La and β-Lg

pH	α-La			β-Lg		
	T_d	ΔH (J/g)	Reversibility (%)	T_d	ΔH (J/g)	Reversibility (%)
4.0	52.3 ± 0.1^{b}	1.1 ± 0.2^{a}	100.0	82.2 ± 0.1^{e}	3.1 ± 0.5^{a}	0
5.0	52.3 ± 0.0^{b}	1.2 ± 0.1^{a}	100.0	82.0 ± 0.1^{de}	3.2 ± 0.3^{a}	0
6.0	52.2 ± 0.0^{b}	1.1 ± 0.1^{a}	100.0	81.8 ± 0.2^{d}	3.3 ± 0.1^{a}	0
7.0.	52.2 ± 0.1^{b}	1.2 ± 0.2^{a}	100.0	81.0 ± 0.1^{c}	3.7 ± 0.1^{ab}	0
8.0	52.2 ± 0.0^{b}	1.3 ± 0.1^{ab}	93.8	80.1 ± 0.1^{b}	4.0 ± 0.1^{b}	0
9.0	52.1 ± 0.1^{a}	1.2 ± 0.1^{a}	91.7	79.9 ± 0.2^{ab}	3.2 ± 0.1^{a}	0
10.0	52.1 ± 0.1^{a}	1.5 ± 0.1^{b}	89.1	79.8 ± 0.1^{a}	3.1 ± 0.1^{a}	0

Different letters in the same column indicate that values are significantly different ($P < 0.05$)

into contact with aqueous medium and form new hydrogen bonds. This phenomenon is thought to be responsible for the increase in heat stability at acidic pH relative to heat stability at neutral pH (Boye and Alli 2000; Kella and Kinsella 1988; Relkin 1996). At alkaline pH, the oxidation of free thiol groups via intra and/or intermolecular SH/S-S exchange reactions was responsible for the reduction in heat stability of β-Lg, occurring in parallel with the unfolding and aggregation processes (Relkin 1996). This indicated that it is easier for β-Lg with lower thermal stability to form aggregation and gel at alkaline pH (9.0–10.0) (Boye et al. 1995). At acidic (pH 4.0–6.0) and alkaline (pH 9.0–10.0) ranges, the transition enthalpy values (ΔH) were lower than that within pH 7.0 and pH 8.0. ΔH correlated with the content of ordered secondary structure of a protein and can be used to monitor the proportion of protein denaturation (Arntfield and Murray 1981). A lowering of ΔH in the acid range could be explained by a weakening of the structure due to an increase in the number of positive charges, and in the alkaline, an excess of repulsive negative charges was involved and decreased in the thermal stability of β-Lg (Arntfield and Murray 1981). When the samples were reheated, β-Lg was completely irreversible within pH 4.0–10.0. The β-Lg contains two disulfide bridges and one free thiol group per monomer. The thiol group is capable of interacting to form new disulfide bonds, and the rate of reaction depended on pH, temperature, and other environmental conditions. Heating results in intramolecular or intermolecular disulfide exchange reactions with β-Lg itself or with other thiol-containing proteins, which may result in irreversible changes of protein structure and effect solubility and functionality of this protein (Dewit and Klarenbeek 1984; Boye and Alli 2000). This experiment revealed that the thermal stability of yak milk α-La was stronger than that of β-Lg in the pH 4.0–10.0, and the solubility and functionality of these two proteins were different in whey protein.

Effect of Sugars on the Thermal Properties of Yak α-La and β-Lg

The denaturation temperature (T_d), enthalpy (ΔH), and reversible degree of yak α-La and β-Lg in the presence of sucrose, lactose, and glucose were shown in Table 2. In comparison with control (no sugar in α-La and β-Lg water solution), the T_d of α-La was not affected by different concentrations of glucose, but the transition ΔH and the reversibility of α-La had a slight increase with the increase of glucose concentrations (0.25–1.0 M). In the presence of sucrose and lactose, the T_d of α-La elevated with the increase of two sugar concentrations which had a 0.8 °C increase, while the reversible degrees of α-La were 96–100% in the sucrose solution and 83–100% in lactose solution. In the higher sugar concentrations (0.75–1.0 M), the reversible degrees of α-La were in the range of 94–100%. The results indicated that sucrose and lactose had a greater stabilizing effect on the denaturation of α-La than glucose in the higher sugar concentration (0.75–1.0 M). The T_d of β-Lg was 85.3, 87.1, and 86.7 °C for glucose, sucrose, and lactose solutions (1.0 M), respectively. These values are all significantly ($P < 0.05$) higher than 80.8 °C observed in the absence of any sugar. The results indicated that sugars had a stabilizing influence on the thermal stability of β-Lg, and this effect was concentration-dependent. When the samples were reheated, β-Lg was completely irreversible within and without sugar solution which showed that β-Lg loss native structure during heating of β-Lg sugar solutions. The stabilizing influence of the sugars was greater for β-Lg than for α-La, with sucrose and lactose having the greater stabilizing effect than that of glucose. Similar results were reported when individual β-Lg and α-La were heated separately and α-La/β-Lg mixture was heated together; however, the difference in the stabilizing influence was that glucose was greater than that of sucrose and lactose (Boye et al. 1996; Boye and Alli 2000; Boye et al. 1997). Sugar modified thermal stability of whey protein mainly by perturbing the thermodynamic behavior of the solvent (i.e., general solvent effects) without involving direct, specific sugar-protein interactions (Panzica et al. 2012). Increasing concentration of sugars, there is an increase in solvent cohesive force which increases the energy required for cavity formation for the associated structures in the solvent (Kulmyrzaev et al. 2000; Beg et al. 2016).

Effect of NaCl on the Thermal Properties of Yak α-La and β-Lg

In solution, ions exert their influence by affecting the net charge of proteins, hydration, and electrostatic interactions and alter the native conformation of food proteins and affect their temperature of denaturation (Arntfield et al. 1990). Table 2 shows the DSC analysis results of yak α-La and β-Lg in NaCl water solution (pH ~ 7.0) with different Na^+ concentration (0.25–1.0 M). In comparison with the absence of NaCl, the T_d of α-La shifted to 52.4–52.5 °C with increasing of 0.3–0.4 °C in NaCl solution (0.25–1.0 M), which suggested that Na^+ did not have the stabilizing effect on the thermal denaturation of α-La. Although, T_d of α-La did not affect by Na^+ concentration, in 0.25–0.75 M, the transition ΔH increased and the percent reversibility decreased with the increase of Na^+ concentration. The reversibility of α-La was decreased from 80.7% (0.25 M) to 68.1% (0.75 M), which indicated that the α-La was more irreversibly denatured at higher concentrations of Na^+. Na^+ affected the electrostatic interactions between protein and protein due to the charge changes in the protein. Complex coacervation of oppositely charged proteins can be limited at very low-ionic strength due to the high-charge density, causing the molecules to be in the extended state (Burgess 1990). However, in 1.0 M Na^+ solution, the ΔH and the reversibility of α-La were 1.2 J/g and

Table 2 Effect of sugars on the DSC properties of α-La and β-Lg

Treatment	α-La			β-Lg		
	T_d (°C)	ΔH (J/g)	Reversibility (%)	T_d (°C)	ΔH (J/g)	Reversibility (%)
Control (0 M)	52.1 ± 0.1^{a}	1.1 ± 0.1^{a}	100	80.8 ± 0.1^{a}	2.3 ± 0.1^{a}	0
			Glucose (M)			
0.25	52.1 ± 0.1^{a}	1.3 ± 0.1^{b}	91.8	82.7 ± 0.0^{b}	2.5 ± 0.1^{d}	0
0.5	52.1 ± 0.1^{a}	1.3 ± 0.1^{b}	93.2	83.2 ± 0.1^{c}	2.7 ± 0.0^{c}	0
0.75	52.2 ± 0.1^{ab}	1.5 ± 0.1^{c}	93.8	84.6 ± 0.1^{d}	2.4 ± 0.1^{b}	0
1.0	52.3 ± 0.1^{b}	1.6 ± 0.1^{c}	100	85.3 ± 0.1^{d}	2.0 ± 0.1^{a}	0
			Sucrose (M)			
0.25	52.2 ± 0.1^{a}	1.2 ± 0.1^{b}	100	83.2 ± 0.0^{b}	2.9 ± 0.3^{b}	0
0.5	52.5 ± 0.1^{b}	1.0 ± 0.1^{a}	96.7	84.5 ± 0.2^{c}	3.2 ± 0.4^{b}	0
0.75	52.7 ± 0.2^{bc}	1.1 ± 0.0^{a}	100	85.7 ± 0.1^{d}	2.3 ± 0.1^{a}	0
1.0	52.9 ± 0.1^{c}	1.0 ± 0.0^{a}	95.8	87.1 ± 0.1^{e}	2.2 ± 0.1^{a}	0
			Lactose (M)			
0.25	52.3 ± 0.1^{a}	1.2 ± 0.2^{ab}	83.7	83.3 ± 0.1^{b}	2.2 ± 0.1^{a}	0
0.5	52.3 ± 0.2^{a}	1.2 ± 0.2^{ab}	86.3	84.4 ± 0.2^{c}	2.1 ± 0.1^{a}	0
0.75	52.8 ± 0.2^{b}	1.4 ± 0.3^{ab}	100	85.4 ± 0.1^{d}	2.3 ± 0.1^{a}	0
1.0	52.9 ± 0.1^{b}	1.5 ± 0.1^{b}	96.7	86.7 ± 0.1^{e}	2.7 ± 0.1^{b}	0

Different letters in the same column indicate that values are significantly different ($P < 0.05$)

97.6%, respectively, which were similar to those in the water solution without NaCl. At high-salt concentration, the net charge carried by the protein is reduced, resulting in a decrease of the electrostatic interaction between macromolecules and the coacervation is suppressed (Burgess 1990). Increasing the concentration of NaCl appeared to increase the thermal stability of β-Lg (Table 3), and the thermal stability of β-Lg was concentration-dependence. The peak temperature of denaturation increased from 82.7 °C in 0.25 M NaCl to 83.7 °C in 1.0 M NaCl. For β-Lg/Na^+ solution, regions of high-surface charge density can act as nucleation centers for further unfolding since they are the least stable parts of the fold (Qi et al. 1997; Relkin and Mulvihill 1996). When the samples reheated in NaCl solution, β-Lg was completely irreversible which involved thiol/disulfide interchange and hydrophobically driven association reactions (Vardhanabhuti and Foegeding 2008).

Effect of $CaCl_2$ on the Thermal Properties of Yak α-La and β-Lg

Table 4 shows the DSC parameters of α-La and β-Lg heated in the presence of different concentrations of Ca^{2+} water solution (0.25–1.0 M, pH ~ 7.1). In this experiment, purified yak α-La is holo-α-La (Ca^{2+}-bonding). The T_d of α-La was slightly affected by Ca^{2+}. Compared with the control solution (Ca^{2+}, 0 M), the T_d of α-La only had 0.6 °C increase in 1.0 M Ca^{2+} solution, and the transition ΔH of α-La increased from 1.1 J/g (0 M) to 2.6 J/g (1.0 M). When the samples were reheated, the reversibility of α-La was more than 90% in 1.0 M Ca^{2+} solution which indicated that the ability of α-La to renature after heat treatment is directly related to the presence of Ca^{2+} (Boye et al. 1997). α-La contains a tightly-bound Ca^{2+}, and calcium binding was thought to be a unique feature of α-La. Calcium

Table 3 Effect of Na^+ concentration on the thermal properties of yak α-La and β-Lg

Na^+ concentration (M)	α-La			β-Lg		
	T_d (°C)	ΔH (J/g)	Reversibility (%)	T_d (°C)	ΔH (J/g)	Reversibility (%)
0.00	52.1 ± 0.1^{a}	1.1 ± 0.0^{a}	100	80.9 ± 0.1^{a}	2.3 ± 0.1^{a}	0
0.25	52.5 ± 0.1^{b}	1.3 ± 0.2^{a}	80.7	82.7 ± 0.1^{b}	2.0 ± 0.2^{a}	0
0.5	52.5 ± 0.1^{b}	1.3 ± 0.2^{a}	68.9	82.8 ± 0.2^{b}	3.4 ± 0.6^{c}	0
0.75	52.4 ± 0.1^{b}	1.6 ± 0.1^{b}	68.1	83.2 ± 0.2^{c}	2.5 ± 0.1^{ab}	0
1.0	52.4 ± 0.1^{b}	1.2 ± 0.1^{a}	97.6	83.7 ± 0.0^{d}	2.9 ± 0.1^{b}	0

Different letters in the same column indicate that values are significantly different ($P < 0.05$)

Table 4 Effect of Ca^{2+} concentration on the thermal properties of yak α-La and β-Lg

Ca^{2+} concentration (M)	α-La			β-Lg		
	T_d (°C)	ΔH (J/g)	Reversibility (%)	T_d (°C)	ΔH (J/g)	Reversibility (%)
0.00	52.1 ± 0.1^{a}	1.1 ± 0.1^{a}	100	80.9 ± 0.0^{a}	2.3 ± 0.1^{a}	0
0.25	52.4 ± 0.1^{b}	1.5 ± 0.1^{ab}	100	82.0 ± 0.0^{b}	2.3 ± 0.1^{a}	0
0.5	52.5 ± 0.1^{ab}	1.6 ± 0.1^{b}	95.9	82.2 ± 0.1^{c}	2.5 ± 0.2^{a}	0
0.75	52.4 ± 0.1^{b}	1.9 ± 0.1^{b}	98.9	82.8 ± 0.0^{d}	2.6 ± 0.2^{a}	0
1.0	52.7 ± 0.1^{c}	2.6 ± 0.3^{c}	90.8	83.1 ± 0.1^{e}	2.6 ± 0.2^{a}	0

Different letters in the same column indicate that values are significantly different ($P < 0.05$)

strongly enhances the stability of the folded protein and is required for refolding and native disulfide bond formation in the reduced denatured protein (Brew 2013).

The T_d and ΔH of β-Lg increased with the increase of Ca^{2+} concentration which suggested that Ca^{2+} had a stabilizing effect of β-Lg structure. Compared with the control samples (without Ca^{2+}), the increase in enthalpy was contributed by intermolecular hydrophobic interaction in Ca^{2+} solution (Relkin 1996). At pH 7.0 (in this study), the overall charge on the β-Lg is negative and calcium bound to β-Lg carboxylate groups with a threshold affinity, and also, Ca^{2+} may destabilize the β-Lg by hydrophobic interactions (Relkin 1996; Mounsey and O'Kennedy 2012). Like other environmental factors (pH, temperature, NaCl, and sugars), the β-Lg was completely irreversible in $CaCl_2$ solution, when the samples were reheated. The irreversibility of β-Lg is induced by hydrophobic association and partial unfolding, as well as protein denaturation which resulted in reduced protein solubility (Dewit and Klarenbeek 1984).

Conclusions

The denaturation temperature of α-La and β-Lg was at 52.1 and 81.4 °C, respectively. Within the pH range of between 4.0 and 10.0, the T_d and ΔH of α-La were not significantly ($P < 0.05$) affected, and the reversibility of α-La was higher than 90%. In contrast, the T_d of β-Lg was pH-dependent, which was increased with pH elevation. Sugars (glucose, lactose, and sucrose), Na^+, and Ca^{2+} had a slight influence on the thermal stability of α-La, but they significantly ($P < 0.05$) enhanced the thermal stability of β-Lg, and this effect was concentration-dependent. When the samples were reheated in the presence of the additives (sugars, Na^+, and Ca^{2+}), α-La had higher reversible degrees which depended on concentration range 70–100%; however, β-Lg was completely irreversible when it was heated over denaturation temperature with and without additives. The present work demonstrated that yak α-La is a thermal stable protein within a wider pH range, and the thermal stability of yak β-Lg was enhanced with the addition of sugars, Na^+, and Ca^{2+}. Furthermore, this work provides some understanding with respect to the thermal stability of yak milk.

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