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THERMAL STABILITY OF WHEY PROTEINS STUDIED BY DIFFERENTIAL SCANNING CALORIMETRY

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ABSTRACT

The thermal stability of the bovine whey proteins; β-lactoglobulin (β-lg), α-lactalbumin (α-la) and serum albumin (BSA), was studied individually and in mixtures in the temperature range 25-140°C by differential scanning calorimetry. The thermal denaturation temperature (T_D) and the transition enthalpies (ΔH_{app}) were determined at different pH-values (3.0-10.0) in simulated milk ultrafiltrate (SMUF).

β-Lg was, except at pH 9.0 and 10.0, the most thermostable protein at all pH-values. At acidic pH-values BSA was the least thermostable. At alkaline pH-values, however, α-la had lower thermal stability than BSA. α-La exhibited double peak behaviour at acidic pH-values and ΔH_{app} was dependent on Ca-content. Mixtures of the proteins were studied at pH 4.0, 5.0 and 6.6. In general, when mixed, the proteins seemed to denaturate independently of each other.

INTRODUCTION

Calorimetric techniques have been found useful for studying the effect of heat on proteins in aqueous solutions (Ref.1). Several investigations have been published on the whey proteins - β-lactoglobulin (β-lg), α-lactalbumin (α-la), and bovine serum albumin (BSA) by use of differential scanning calorimetry (DSC) (cf. Refs. 2-9). These studies often report results at single pH-values primarily in the neutral pH-range. Furthermore, the thermal stability of these proteins was examined in widely different buffer solutions, making a comparison of their thermal stabilities impracticable.

This investigation by DSC reports the thermal stability of β-lg, α-la and BSA in simulated milk ultrafiltrate (SMUF) in the pH-range 3.0-10.0. SMUF was used to model the natural milk medium. For comparison, a salt-free solution (glass distilled water) was also examined.

Mixtures of whey proteins have not previously been studied by DSC. As this will give some insight into the structure and stability of proteins in milk as well as changes in the properties of milk during heat treatment, the thermal stability of various mixtures of β-lg, α-la and BSA was examined at some pH-values of technological importance (pH 4.0, 5.0 and 6.6).

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MATERIALS AND METHODS

Reagents

β-Lg (L-6879, lot no 111F-8025), α-la (L-6010, lot no 52F-8075-1) and BSA (A-0281, lot no 63F-9350) were all obtained from Sigma Chemical Co. β-Lg was desalted by dialysis against glass distilled water and freeze-dried to constant weight. BSA was saltfree and essentially free of fatty acids. No contamination of β-lg or BSA with other proteins could be detected by SDS-polyacrylamide gel electrophoresis. α-La contained less than 0.3 mol of calcium per mol of protein and was ~ 90% pure, contaminated primarily by β-lg. Simulated milk ultrafiltrate (SMUF) was prepared according to Jenness and Koops (Ref.10). The salts were purchased from Merck AG and were of analytical grade.

Differential scanning calorimetry

Thermal denaturation was measured by differential scanning calorimetry in the temperature range 25-140°C at a scanning rate of 10°C/min. A Perkin Elmer DSC-2C with Du Pont coated aluminium sample pans (no. 900796.901) were used. A sealed sample pan which contained a volume of SMUF (or water) equal to that of the sample (20 µl) was used as reference. The protein concentration was 5% (w/v) in all experiments.

The thermal denaturation temperature (T_D) is defined as the intersection of the extrapolated lower temperature side of the DSC peak and the base line. Calibration of the DSC and calculation of the apparent heat of denaturation (ΔH_{app}) have been described earlier (Ref.11). Reported values are the means of 5-10 independent replicates. The average standard deviation of T_D and ΔH_{app} was $\pm$ 1.5°C and $\pm$ 0.5 cal/g, respectively.

RESULTS AND DISCUSSION

Individual whey proteins at different pH-values

β-Lactoglobulin. Figure 1a shows two transitions at pH 7.0, 8.0 and 9.0. High temperature peaks appeared also at other pH-values but were found to be reproducible only between pH 7.0-9.0. The denaturation temperature of the low temperature peak reached a maximum at 82°C (pH 4.0). T_D then decreases with increasing pH. DeWit and Klarenbeek (Ref.4) explained the high temperature peak as a partial stabilization of the β-lg structure during denaturation near 80°C, followed by destabilization (induced by a breakdown of disulfide bonds) of the residual protein structure in the range 130-140°C.

β-Lg exists as monomer, dimer or octamer depending upon pH (Ref.12). These aggregation phenomena seem not to influence the thermal denaturation behaviour of β-lg (according to DSC), since no anomalous peaks appear. A broadening of the DSC-peaks at extreme pH-values is common for most proteins (Ref.11).

T_D of β-lg in water (Ref.5) and SMUF is similar at most pH-values. However, a few degrees lower values were observed in water at extreme alkaline pH-conditions. ΔH_{app} of the low temperature transition in SMUF reached a maximum at pH 3.0 (4.3 cal/g protein) (Table 1). At neutral and alkaline pH-values, ΔH_{app} in water is much lower (0.3-2.5 cal/g) than in SMUF, suggesting that the added ions stabilize β-lg against the pH induced transition reported close to pH 7 (Ref.13).

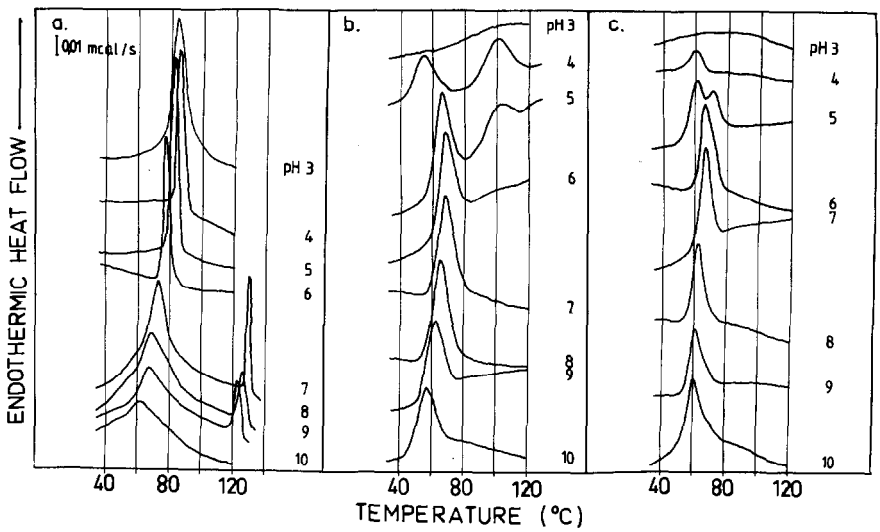

Fig. 1. Typical DSC-thermograms of individual whey proteins in SMUF at various pH-values. (a) β-lactoglobulin, (b) α-lactalbumin and (c) bovine serum albumin.

α-Lactalbumin. Only a marginal thermal transition was observed at pH 3.0 (Fig. 1b), indicating that the protein has been unfolded prior to heating. This observation confirms that α-la is subjected to acid denaturation (Ref.9).

The thermograms at pH 4.0 and 5.0 show two sharp transitions, which are absent at other pH-values. The thermogram at pH 4.0 gives denaturation temperatures at 43°C and 85°C. These transitions have similar ΔH_{app}-values (Table 1). Kronman et al. (Ref.14) reported two Ca^{2+} binding sites for bovine α-la, a stronger and a weaker site. Binding of calcium seems to be essential for stabilization of the tertiary structure. This is confirmed by the lower ΔH_{app} values registered in glass-distilled water (0.4 and 0.7 cal/g) compared to those in SMUF (Table 1). An acidic transition is associated with a weakening of Ca^{2+} binding with a subsequent change of conformation. The presence of two transition peaks can thus be attributed to this calcium binding/conformation change phenomena or, less probably, to the aggregation behaviour reported for α-la (Ref.13).

The low temperature peak of α-la has a maximum T_D of 59°C (pH 6.0) which decreases to ∿ 45°C at extreme alkaline and acidic pH-values. ΔH_{app} reached a maximum around pH 7-8 (Table 1). Compared to SMUF, the T_D:s in water were 3°C higher at pH 8.0-10.0. Two transitions were also registered in water at pH 4.0 and 5.0.

Bovine serum albumin. No thermal transition was observed at pH 3.0 (Fig.1c), which is explained by the pH-induced transition of BSA at acidic pH-values. The transitions at other pH-values gave single peaks, except at pH 5.0, where a double-peak appeared. T_D has its maximum at neutral pH-values (∿ 60°C) while maximum ΔH_{app} is seen at pH 5.0 (Table 1). The reduced thermal stability above pH 6.0 parallels the increased reactivity of the free thiol group of BSA.

The maximum temperatures of the peaks at pH 5.0 differ by 10°C. The reason for the double peak appearance at pH 5.0 is difficult to elucidate. As a double-peak was also observed in water (pH 6.0), binding of electrolyte ions cannot account for this phenomenon. The transition temperature of BSA is dependent on ionic strength (Ref.15), giving T_D-values in SMUF that are ∿ 10°C higher than in water at pH 6.0-7.0.

Comparison of the proteins at different pH-values

Figure 2 and Table 1 summarize the T_D- and ΔH_{app}-values of the three whey proteins in SMUF at different pH-values. In general, the thermal stability of the proteins decreases towards extreme acidic and alkaline pH-values.

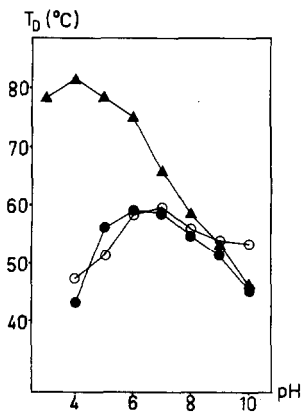

Fig. 2. The denaturation temperature, T_D, of β-lactoglobulin (▲), α-lactalbumin (●) and bovine serum albumin (○) in SMUF as a function of pH.

TABLE 1
The transition enthalpies (ΔH_{app}) of β-lactoglobulin, α-lactablumin, bovine serum albumin at different pH-values in SMUF.

pH	ΔH_{app} (cal/g protein)		
	β-lg	α-la	BSA
3	4.3		
4	3.2	2.0+2.0	0.8
5	3.5	3.4+1.4	3.3
6	2.4	3.5	2.8
7	3.7+1.8	4.2	2.7
8	2.7+1.2	4.2	2.3
9	2.6+1.3	3.4	2.1
10	0.7	2.6	2.1

pH 3.0-6.0. In this pH-range β-lg is the most thermostable protein with a T_D between 75° and 82°C. α-La is less thermostable than β-lg, while BSA seems to be the least thermostable of these proteins with T_D-values between 47° and 58°C.

pH 7.0-10.0. In this pH-range T_D of β-lg decreases steadily with increasing pH. Notably, BSA has higher thermostability than α-la and is, at pH 9.0 and 10.0, even more thermostable than β-lg.

In conclusion, the generally accepted idea of α-la as the most thermolabile and β-lg as the most thermostable of the whey proteins is thus inappropriate when thermal stability studies are extended to different conditions.

Thermal stability of mixtures of the whey proteins

α-La and β-lg. The DSC-thermogram of mixtures of α-la and β-lg in SMUF at pH 4.0 shows two transitions (Fig. 3a). The small low temperature peak corresponds to the first transition of pure α-la while the large peak corresponds to the transition of pure β-lg. The two peaks in the mixture have almost the same T_D-value as the pure proteins. Notably, the second transition of pure α-la appears only at pH 5.0 in the mixture. The difference between the T_D-values of the two transitions in the mixture decreases when pH increases in analogy to the individual proteins. At pH 6.6 the two transitions overlap each other. Besides the loss of the second peak of α-la, there is no indication of an interaction between the two proteins at these conditions.

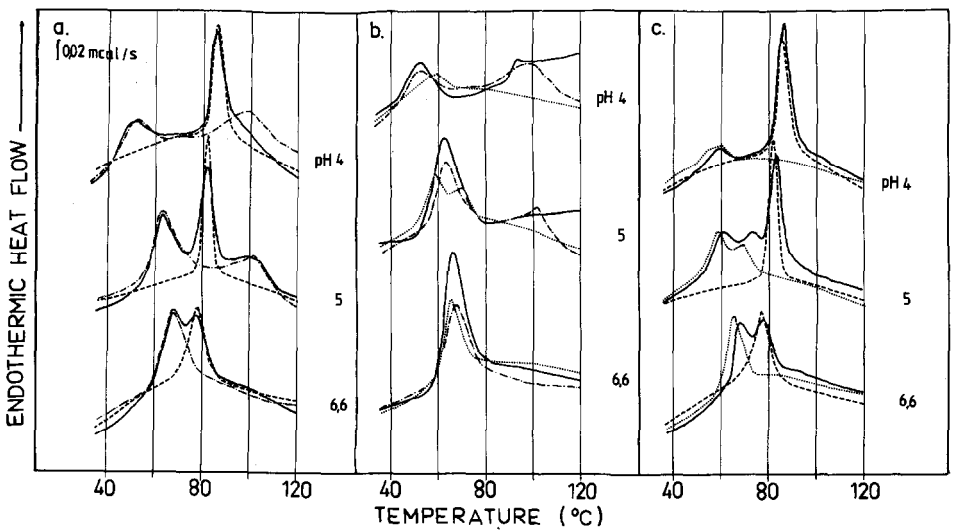

Fig. 3. Typical DSC-thermograms of individual and mixed whey proteins in SMUF at various pH-values. (– – –) β-Lactoglobulin (β-lg), (– · –) α-lactalbumin (α-la), (·····) bovine serum albumin (BSA) and (——) mixed proteins. (a) α-la and β-lg, (b) α-la and BSA and (c) β-lg and BSA.

α-La and BSA. The thermogram of a mixture of α-la and BSA in SMUF at pH 4.0 shows one major peak (Fig. 3b), which is the complex transition of pure α-la and pure BSA. T_D of the mixture is nearly the same as for pure α-la. A small peak remains from the high temperature transition of α-la. Only one peak is observed in the mixture at pH 5.0 while pure BSA shows a double-peak at this pH. T_D of the mixture is equivalent to that of pure α-la and the transition appears between the double-peak of BSA.

The peaks of the pure proteins and the mixture overlapped at pH 6.6 and ΔH_{app} of the mixture is nearly equivalent to the sum of the pure proteins. In conclusion, the thermal transition of mixtures of α-la and BSA indicates some interaction between the two proteins, even if the denaturation temperatures are only marginally influenced.

β-Lg and BSA. The transitions of the mixture correspond to the transitions of the pure proteins (Fig. 3c). When pH was increased from 4.0 to 6.6 the difference between the T_D-value of the peaks diminished. Besides the double peak of BSA at pH 5.0, there is no indication of interaction between the proteins.

When studying the three whey proteins in a mixture no further conclusions can be drawn compared to the results presented here.

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