Thermal Properties of Whey Protein Aggregates

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ABSTRACT

Aggregation of 10% whey protein solution was induced by addition of calcium salt, acidification, or proteolysis at 45°C. Effects of the preaggregation on thermal properties of whey proteins were examined by differential scanning calorimetry. The different types of aggregates had three common effects: 1) one endothermic peak, representing denaturation of whey protein aggregates, instead of two endothermic peaks representing α- lactalbumin and β-lactoglobulin in the control; 2) a narrower range (~10°C) of denaturation temperature than the control (~20°C); and 3) significantly greater enthalpy values (~4 J/g) than the control (<2 J/g). Denaturation temperatures (T_D, T_0) of the aggregates were also different from those of α-lactalbumin (67°C) and β-lactoglobulin (76°C) of the control. Aggregates induced by calcium salt (~74°C) and protease (~73°C) had intermediate denaturation temperatures. The pH-induced aggregates had high denaturation temperatures (80 to 91°C) at low pH (3.5 to 5.7). An exothermic peak was detected during calcium salt- or protease-induced aggregation of whey proteins at 45°C. Thus, the preaggregation changed thermal properties of whey proteins. This information on thermal properties of the aggregates may help in the design of appropriate heat processing for the application and manufacture of whey protein products.

(**Key words:** whey proteins, aggregation, differential scanning calorimetry, denaturation)

Abbreviation key: BLP = *Bacillus licheniformis* protease, **DLS** = dynamic light scattering, **DSC** = differential scanning calorimetry, **GDL** = glucono-Δ-lactone, **ΔH** = enthalpy value of denaturation, **T_0** = onset denaturation temperature, **T_D** = denaturation temperature, **WP** = whey proteins, **WPS** = WP solution, **WPI** = WP isolate, **WPC** = WP concentrates.

INTRODUCTION

Proteins maintain their native structure by chemical forces such as hydrophobic, ionic, hydrogen, and disulfide bonds. The chemical bonds are highly dependent upon the environment. As environmental conditions change, some of the original bonds may be altered, and new bonds may form. The proteins then assume new conformations. During this process, rupture of hydrogen bonds may lead to endothermic reactions, and disruption of hydrophobic bonds may lead to exothermic reactions (10, 22, 36, 34). Such enthalpy changes can be detected by differential scanning calorimetry (**DSC**). Information on protein thermal properties is very important for food processing strategies and heat processing design.

Thermal properties of individual whey proteins (**WP**) (e.g., β-LG and α-LA) have been well investigated with DSC (2, 4, 6, 29, 31). At neutral pH, denaturation temperatures of β-LG and α-LA are reported to be around 78°C and 64°C, respectively (31). Lowering pH significantly increases the denaturation temperature of β-LG (85°C at pH 3) (4), but decreases denaturation the temperature of α-LA (58.6°C at pH 3.5) (2). α-Lactalbumin is a calcium-binding protein and has two binding sites. The transition temperature and denaturation enthalpy are strongly dependent on the occupation of the calcium sites. Chelating calcium with EDTA led to significant decreases in temperature and enthalpy of α-LA denaturation. The β-LG is also extremely sensitive to the presence of calcium in solution (6).

Native whey proteins can easily form aggregates in acidic or salt environments or by proteolysis at temperatures (20 to 45°C) far below the thermal denaturation temperatures of proteins (14, 28, 37). How such preaggregation affects the thermal behavior of the proteins either in pure solutions or in mixtures is not known. This information is very important because aggregation followed by heat occurs in the use of WP products in foods. When the WP products are added to vary the pH or salt content of foods, the pH and salt could cause aggregation of WP before heat processing (13, 14, 37). Today the food industry mainly utilizes mixtures of WP such as WP concentrate (**WPC**) and WP isolate (**WPI**) rather than pure α-LA or β-LG. Limited information is

available on the thermal properties of these mixed products.

The results of preaggregation on thermal behavior of WP may help to clarify the gelation mechanism of WP. Upon application of heat, the aggregated WPS can form gels (13, 14, 15). Such gelation may involve three events: 1) aggregation of WP molecules promoted by salt, acid, or enzyme; 2) thermal denaturation of the aggregates; and 3) formation of a gel network. Three different events could occur for gelation of WP without preaggregation: 1) denaturation of WP molecules, 2) aggregation of the denatured WP, and 3) gelation of the aggregates (12, 33). The latter gelation followed a two-step theory of gelation of Ferry (8), but the former gelation might not have. In addition, the former three events led to formation of particulate gels (13, 14, 15, 16), and the latter three events led to formation of fine-stranded gels (12, 16, 20).

The purpose of this research was to study thermal denaturation characteristics of types of WP aggregates induced by acid, calcium salt, or protease and to investigate how type and size of these aggregates affect denaturation temperature and enthalpy value of WP.

MATERIALS and METHODS

Protein

Whey protein isolate (BiPro; Davisco Int. Inc., Le Sueur, MN) was used in this study. The WPI contained 95% protein (dry basis) and ≤5% moisture. A 10% WP solution (**WPS**) was prepared by adding 11.1 g of WPI powder to distilled water to a final volume of 100 ml and stirring for 30 min.

Reagents

Chemical reagents, $CaCl_2$ and the acidulant glucono-Δ-lactone (**GDL**), were purchased from Sigma Chemical Co. (St. Louis, MO). In addition, the protease from *Bacillus licheniformis* (8.25 Anson U/g) was from Novo Nordisk A/S (Bagsvaerd, Denmark).

Size Exclusion HPLC

The 10% WP solution (pH 7.0) was eluted by size exclusion HPLC with an HPLC system (Waters, Division of Millipore, Milford, MA) mounted with a column (Progel™-TSK G3000 SWXL; Supelco, Bellefonte, PA) (12). The 10% WPS was diluted 1:19 with distilled water, and 25 μl of sample was injected onto the column. Individual WP were identified by analysis of standards. Constitution of WP in the WPI was evaluated by calculating the percentage of peak area of each WP as a function of the total peak area.

Aggregation of WPS

CaCl₂. The 10% WPS were adjusted to various concentrations (5 to 100 mM) of $CaCl_2$ with 2 M $CaCl_2$ solution and incubated in a 45°C water bath for 1 h, which could have led to differing extents of aggregation (13). Immediately after cooling the samples to 20°C, mean aggregate sizes in the samples were determined by dynamic light scanning (**DLS**), and thermal analysis of samples was performed with DSC. The 10% WPS, without addition of $CaCl_2$ but incubated at 45°C for 1 h, served as a control.

The $CaCl_2$-induced aggregation of WP is also time dependent (37). Therefore, 10% WPS was incubated with 20-mM $CaCl_2$ at 45°C for 4 h. During incubation, samples were withdrawn at fixed intervals for DSC and DLS analyses.

pH. Various concentrations (0.2 to 10.0%, wt/vol) of GDL powder were added to 10% WPS. The WPS were then incubated in a 45°C water bath for 1 h. All GDL were hydrolyzed to the gluconic acid within 45 min at 45°C, which resulted in the quiescent acidification and homogenous aggregation of the WPS (14). After cooling to 20°C, pH of the samples was 6.8 to 3.5. The samples were immediately analyzed by DSC and DLS.

Bacillus licheniformis protease. The 10% WPS was incubated in a 45°C water bath for 4 h with *Bacillus licheniformis* protease (BLP) at a 1% (wt/wt) ratio of enzyme powder to the substrate. During the incubation, protease-induced aggregation occurred (28), and samples were taken at fixed-time intervals for DSC and DLS analyses. A 10% WPS, with addition of inactivated BLP, served as a control. Selected samples were analyzed by size exclusion HPLC. The level of degradation (D%) of individual WP was calculated as follows (28): D% = $(1 - A/A_0) \times 100$ where A = area of peak of individual WP (β-LG and α-LA) in hydrolyzed samples, and A_0 = peak area of the same protein in the control sample (unhydrolyzed).

DLS

The mean aggregate sizes were measured by an ultrafine particle analyzer (Macrotrac; Leeds & Northrup Instruments, St. Petersburg, FL) as described by Ju and Kilara (13). The samples were diluted to 0.2% WP with distilled water. Particle size determinations were replicated two times.

DSC

The thermal characteristics of the control and the preaggregated 10% WPS were examined by a DSC-4 (Perkin-Elmer Corp., Norwalk, CT) equipped with thermal analysis software (Version 4.00, Pyris-I-DSC, Per-

kin-Elmer Corp.). About 50 μl of sample was placed into a stainless steel pan (large volume capsule) and accurately weighed (48.5 to 52.5 mg), and was scanned during temperature increases from 20 to 120°C at 10°C/min. A WPS that was previously denatured was used as a reference. Onset denaturation temperature ($\mathbf{T_0}$), denaturation temperature ($\mathbf{T_D}$), and enthalpy value of denaturation ($\mathbf{\Delta H}$) were computed from each thermogram by the software. The T_0 was estimated by extrapolation of maximum deflection of the curve onto the baseline. The T_D was the temperature of the peak maximum in the curve. The ΔH was calculated by stepwise integration of the area of the peak.

Aggregation has been reported to be an exothermic process (21). The aggregation at 45°C induced by $CaCl_2$, pH, or BLP was also monitored by DSC. Immediately after 10% WPS was mixed with calcium salt, acid, or protease at 20°C, 50 μl of the sample was weighed into a pan. The samples were scanned between 20 to 45°C, with increases of 10°C/min, and were maintained at 45°C for 1 or 4 h to observe the exothermic reaction during the aggregation process.

Statistical Analysis

Data of T_0, T_D, and ΔH were analyzed using ANOVA (26) to determine differences between treatment means. Means were separated by Tukey's significant difference test ($P < 0.05$). All experiments were replicated two times.

RESULTS AND DISCUSSION

Thermal Behavior of Control WPI

The major WP, β-LG, α-LA, and BSA exist as monomers or dimers at neutral pH (6). Size exclusion HPLC revealed the presence of two major native proteins in the WPI: β-LG (56.6%) and α-LA (37.8%) (Figure 1A). The BSA (2.7%), Ig, and soluble protein aggregates (denatured) altogether were <6% of the protein. Two separated endothermic areas were detected by DSC upon heating from 20 to 120°C and represented denaturation of α-LA and β-LG (Figure 1B). Denaturation of α-LA occurred between 59 to 71°C, and denaturation of β-LG occurred between 71 to 79°C. The denaturation temperatures were 67.1°C and 76.3°C for α-LA and β-LG, respectively. These were consistent with previous results from research of the single protein (31) and suggested that denaturation of individual WP molecules in the mixture was independent (21). Similar phenomena were observed for mixtures of standard proteins (α-LA and β-LG; result not shown), mixtures of soy proteins (7S and 11S) (1, 9), and for a mixture of β-LG and lactoferrin (32). The denaturation temperature of BSA
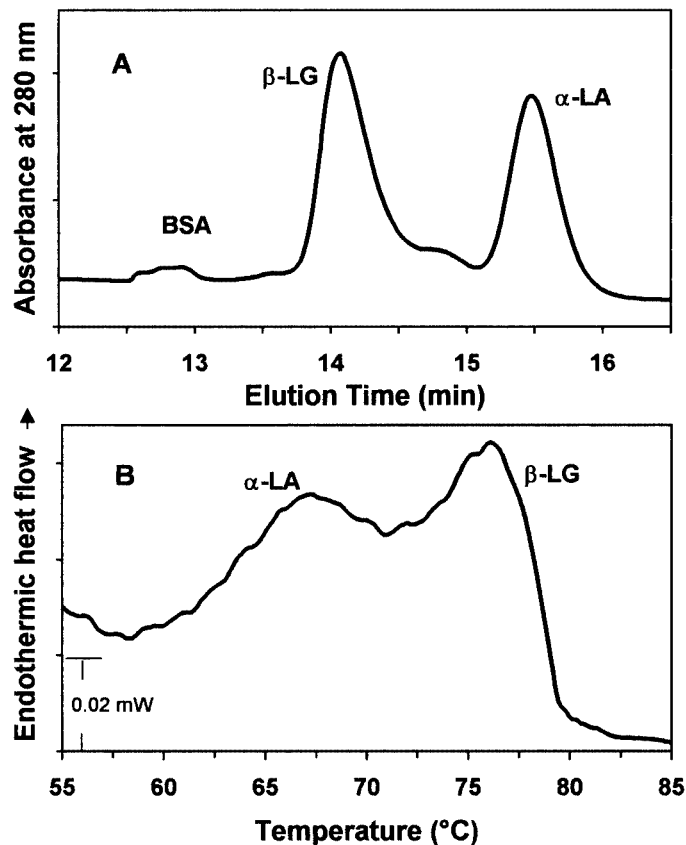

Figure 1. Size exclusion HPLC (A) and typical differential scanning calorimetry thermogram (B) of original whey protein isolate solution.

has been reported to be between that of α-LA and β-LG (6).

A rescan of the cooled WPI sample revealed no denaturation peak and indicated an irreversible denaturation of individual WP caused by the first heating. Denatured WP molecules form coaggregates after heating and cooling, as evidenced by HPLC (11, 12, 24, 25). Formation of the aggregates might prevent renaturation of the denatured proteins. For example, heat treatment alone of α-LA did not lead to the formation of aggregates (5, 25). The isolated α-LA was reported to be 80 to 90% renatured upon heating at 60°C or 65°C (7, 34). Heat treatment of β-LG or BSA can result in formation of aggregates (24, 25). The two proteins were not observed to renature (34).

Effect of CaCl₂-Induced Aggregation

Adding 10 to 100 mM $CaCl_2$ led to an aggregation of the 10% WPS, which became white; DLS measured large aggregates (149 to 3126 nm; Table 1). The DSC detection showed only one endothermic peak for all of the preaggregated WPS (Figure 2). This endothermic

TABLE 1. Effects of aggregation, induced by $CaCl_2$ at varying concentrations, on thermal properties of 10% whey protein isolate solution.

$CaCl_2$ content (mM)	Mean aggregate size (nm)	T_0[1] (°C)	T_D[1] (°C)	ΔH[1] (J/g)
0	8	(58.1)[2], 70.2[a]	(67.1), 76.3[a]	0.79 + .86 = 1.65[a]
5	10	(59.2), 70.5[a]	(66.8), 76.2[a]	0.73 + 1.02 = 1.75[a]
10	149	69.6[b]	74.8[b]	5.13[b]
20	470	68.5[c]	74.3[b]	4.50[c]
25	1252	69.3[b]	74.6[b]	4.39[c]
30	1851	69.4[b]	75.1[b]	4.28[c]
40	2639	70.3[a]	75.9[ab]	4.20[c]
50	2821	70.8[a]	75.8[ab]	4.13[c]
80	2987	71.9[d]	77.6[c]	4.36[c]
100	3126	71.4[d]	77.8[c]	4.33[c]

[a,b,c]Means within a column followed by the same superscript letter are not significantly different ($P \leq$ 0.05).

[1]T_0 = onset denaturation temperature, T_D = denaturation temperature, and ΔH = enthalpy value of denaturation.

[2]Data in parentheses represent temperature of α-LA denaturation.

peak represented the denaturation of protein aggregates. Denaturation of the aggregates occurred at a narrow temperature range of ~12°C, as compared with ~21°C in the control, and suggested that denaturation
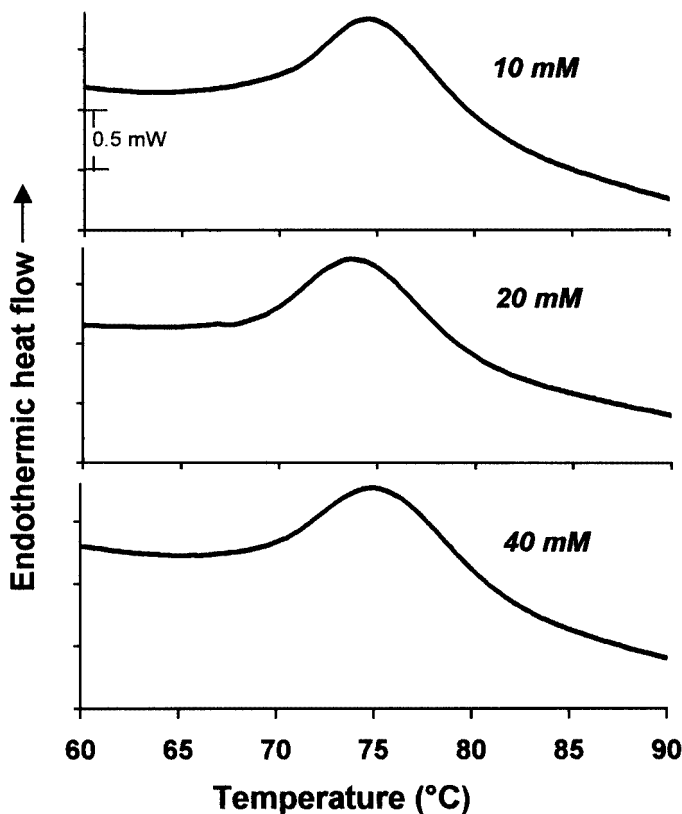

Figure 2. Effect of $CaCl_2$ (10 to 40 mM)-induced aggregation of whey protein isolate solution shown on a differential scanning calorimetry thermogram. Protein concentration, 10%; solvent, distilled water; heat range, 20 to 120°C; heating rates, 10°C/min.

of the aggregates was highly cooperative (10, 21). The $CaCl_2$-induced aggregation changed the thermal behavior of the original WPI.

Addition of 5 mM $CaCl_2$ to 10% WPS did not result in protein aggregation (12, 35). The WPS was still brown and transparent, and the mean particle size did not increase from that of the original WPS (Table 1) after the incubation. The DSC detected two separate endothermic peaks as the control (Table 1).

Upon preaggregation, ΔH greatly increased when compared with the control (Table 1) and indicated that a higher energy was required to denature the WP aggregates. Denaturation temperatures of the aggregates were over 10°C greater than were those of α-LA, and also were different from that of β-LG in the unaggregated control. Adding 10 to 30 mM $CaCl_2$ led to the formation of stable aggregates, and no sediment was observed after 2 d of storage at room temperature (18 to 20°C). The stable aggregates had significantly ($P <$ 0.05) lower T_0 and T_D than did those of β-LG in the control (Table 1). Larger aggregates that were formed upon the addition of 80 to 100 mM $CaCl_2$ caused precipitation even during incubation. This aggregated solution had significantly ($P < 0.05$) higher T_0 and T_D than did those of the β-LG in the control.

As previously reported (13), the mean aggregate sizes continuously increased during the 3-h incubation of 10% WPS with 20 mM $CaCl_2$ at 45°C (Table 2). The increases in mean aggregate size from 149 to 554 nm resulted in increased enthalpy values of denaturation ($P < 0.05$). Further increases in mean aggregate size (up to 823 nm) led to decreased enthalpy values of denaturation ($P < 0.05$) but did not significantly change the temperatures (T_0 and T_D) of denaturation (Table 2).

TABLE 2. Effects of incubation of 10% whey protein isolate solution with 20 mM CaCl$_2$ at varying times on thermal properties.

Incubation time (min)	Mean aggregate size (nm)	T_0[1] (°C)	T_D[1] (°C)	ΔH[1] (J/g)
5	149	67.6[a]	74.2[a]	4.31[a]
10	228	68.0[a]	74.7[a]	4.93[b]
20	258	68.8[a]	74.3[a]	4.56[ab]
30	294	68.1[a]	74.2[a]	4.77[ab]
40	331	67.9[a]	74.8[a]	4.53[a]
50	405	67.7[a]	74.2[a]	4.45[a]
60	470	68.2[a]	74.3[a]	4.49[a]
90	554	67.5[a]	73.8[a]	4.37[a]
120	623	68.1[a]	74.2[a]	4.08[ac]
150	709	67.9[a]	73.7[a]	3.94[c]
180	823	68.6[a]	74.6[a]	3.85[c]

[a,b,c]Means within a column followed by the same superscript letter are not significantly different ($P \leq 0.05$).

[1]T_0 = onset denaturation temperature, T_D = denaturation temperature, and ΔH = enthalpy value of denaturation.

Protein aggregation is considered to be an exothermic process (23). The DSC detected an exothermic peak during aggregation induced by CaCl$_2$ at 45°C (Figure 3). The time of occurrence of this exothermic event depended on the concentration of CaCl$_2$. It occurred at ~7 min after addition of 40 mM CaCl$_2$, but ~13 and 19 min upon addition of 30 and 20 mM CaCl$_2$, respectively. The exothermic peak areas (Figure 3) showed large enthalpy values (–2.48 to –4.37 J/g). The exothermic peak was not detected upon addition of 50 mM CaCl$_2$, which might have been due to a rapid initial aggregation before the detection of DSC (13). In addition, the 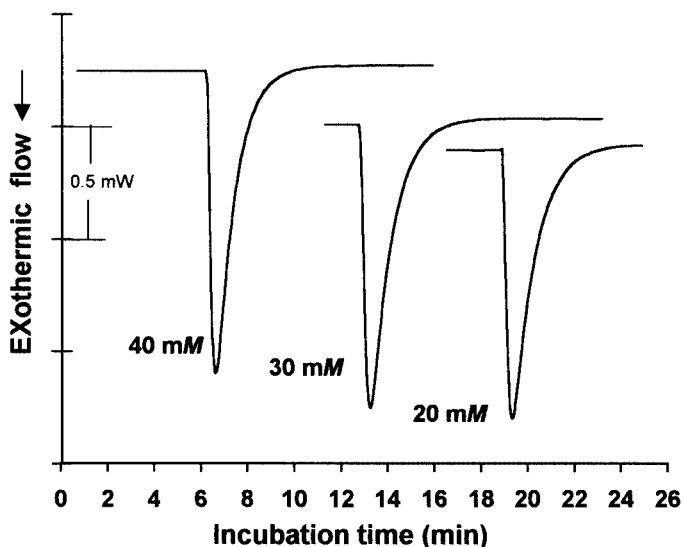exothermic reaction might also have depended on the incubation temperature. For example, the exothermic peak appeared at 25 min and 37°C. Upon addition of 20 mM CaCl$_2$, the peak appeared at 6.2 h and 5°C (result not shown). This phenomenon may suggest that the aggregation of WP involves disruption of hydrophobic bonds (22). Higher temperatures favored hydrophobic interactions and speed aggregation rate.

Effect of pH-Induced Aggregation

Aggregation was not detected in the pH range of 6.2 to 7.0, and the mean aggregate sizes did not change much in this pH range (Table 3), which suggested that proteins in the WPS were still in their molecular state (no aggregation). Previously, the transition of WP molecules to aggregates was reported at pH 6.0 (14, 20). The DSC detected two separated endothermic peaks at pH > 6.0. Compared with the control, the endothermic peak area (ΔH) of α-LA decreased, and the area of β-LG increased with the lowering of pH from 7.0 to 6.2 (Table 3), which suggested that interaction of proteins occurred during heating. Also, total ΔH or sum of α-LA and β-LG ΔH increased, and denaturation temperatures (T_0 and T_D) of both proteins slightly increased with lowered pH.

In the pH range of 3.5 to 5.7, aggregation occurred, and one endothermic peak was detected (Figure 4). The WPS were visually observed to be white and opaque except at pH 3.5. Measured aggregate sizes gradually increased from pH 5.7 (198 nm) to 5.2 (2253 nm) and then decreased to 86 nm at pH 3.5. The aggregation from 5.7 to 3.5 led to significant increases of T_0, T_D, and ΔH (Table 3, $P < 0.05$). Higher denaturation tem-

Figure 3. Differential scanning calorimetry exothermic curves obtained by incubation of 10% whey protein isolate solution with 20, 30, or 40 mM at 45°C for 1 h.

TABLE 3. Effects of pH-induced aggregation on thermal properties of 10% whey protein isolate solution.

pH	Mean aggregate size (nm)	T_0[1] (°C)	T_D[1] (°C)	ΔH[1] (J/g)
7.0	8	(58.1)[2], 70.2[a]	(67.1), 76.3[a]	0.79 + .86 = 1.65[a]
6.8	13	(60.2), 72.0[b]	(66.7), 78.3[b]	0.51 + 1.59 = 2.10[b]
6.5	15	(62.9), 73.5[bc]	(66.3), 79.2[bc]	0.46 + 2.01 = 2.47[c]
6.2	22	(63.2), 75.7[c]	(67.1), 80.1[c]	0.34 + 3.25 = 3.59[d]
5.7	198	76.2[c]	79.8[c]	4.38[f]
5.5	1312	76.3[c]	80.1[c]	4.50[f]
5.2	2253	76.5[c]	81.2[d]	4.58[f]
5.0	2156	77.9[d]	82.9[e]	4.93[g]
4.8	1768	79.6[e]	84.9[f]	5.06[g]
4.5	976	82.0[f]	85.9[g]	5.42[h]
4.2	325	83.6[g]	88.3[h]	5.96[i]
4.0	102	86.1[h]	91.0[i]	5.68[hi]
3.5	86	86.0[h]	91.2[i]	5.94[i]

[a,b,c,d,e,f,g,h,i]Means within a column followed by the same superscript letter are not significantly different ($P \leq 0.05$).

[1]T_0 = onset denaturation temperature, T_D = denaturation temperature, and ΔH = enthalpy value of denaturation.

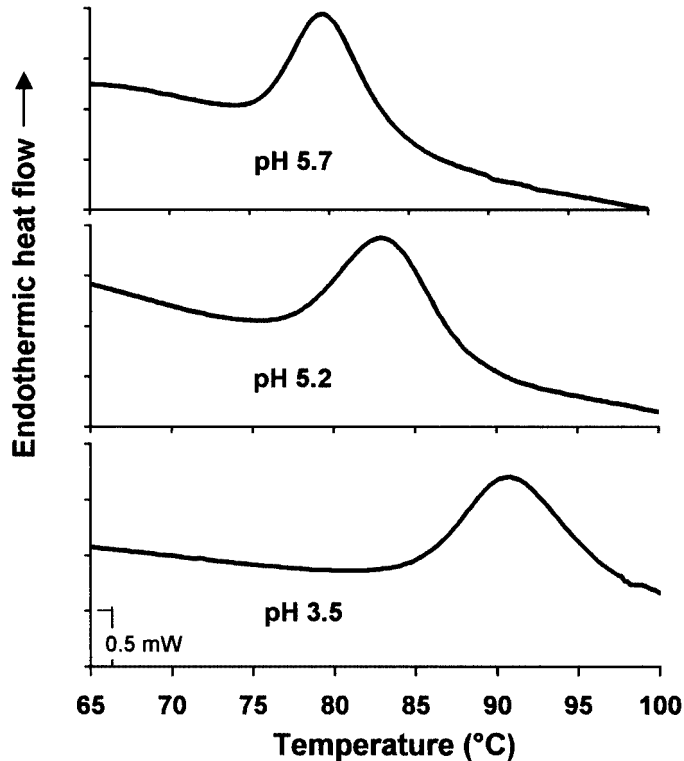[2]Data in parentheses represent temperature of α-LA denaturation.

Figure 4. Effect of pH (3.5 to 5.7)-induced aggregation of whey protein isolate solution shown on a differential scanning calorimetry thermogram. Protein concentration, 10%; solvent, distilled water; heat range, 20 to 120°C; heating rates, 10°C/min.

perature at lower pH was also reported for the pure proteins of β-LG (3, 4), but their values did not appear to vary as greatly as we observed for the WPI (Table 3). Differences in protein enthalpy values at various pH are attributed to differences in the extent of denaturation (10). Exothermic reaction was not observed during quiescent acidification of the 10% WPS from pH 7.0 to 4.0, probably because only electrostatic interactions were involved in the pH-induced aggregation.

Effect of Protease-Induced Aggregation

The incubation of the WPS with BLP had two effects: hydrolysis and aggregation of WP (28). By the chromatographic measurements, about 30 and 33% of α-LA and β-LG had been hydrolyzed after 4 h of incubation at 45°C. Simultaneously, mean aggregate size of WP in the control increased from 18 nm in control to 1427 nm at end of incubation (Table 4).

One endothermic peak was detected for all BLP-treated WPS (Figure 5). The peak reflected the denaturation properties of the BLP-induced aggregates. Although aggregates were not detected during initial 20-min hydrolysis (Table 4), they could be formed rapidly

during heating from 20°C to the temperature at which the enzyme was destroyed (16, 17).

All enzyme-treated WPS had significantly lower T_0 and T_D than those of β-LG in the control (Table 4), but these values were generally greater than those of α-LA. This finding suggested that the enzyme-induced aggregates had intermediate heat stability between the two parent proteins. With increased hydrolysis time or mean aggregate sizes, both T_0 and T_D significantly increased ($P < 0.05$) (Table 4), and ΔH gradually decreased. The initial aggregation (17 to 625 nm in size) resulted in significantly higher ΔH than for the control (Table 4). As the extent of hydrolysis increased, ΔH decreased. At the end of the 4-h incubation, about 30% of the proteins were hydrolyzed to peptides, and ΔH had diminished by more than half compared with the initial treatment (Table 4). This result suggested that the enzyme-induced aggregation might have increased the enthalpy value and that continuous hydrolysis decreased it. The measured ΔH was the combined effect of hydrolysis and aggregation. Considering that the enzyme catalyzed not only during 5 or 10 min of incubation at 45°C, but also during heating from 20°C to 70°C (inactivation temperature of the enzyme is ~70°C) with DSC, it can not be excluded that even short incubation times at the high temperature may lead to a rapid increase in the protein aggregation (13).

An exothermic peak was also detected during the enzyme-induced aggregation (Figure 6). The peak area (−3.91 J/g) appeared around 24 min of incubation of the WPS at 45°C with BLP, around the time that aggregate formation was first detected (Table 4). This result suggests that hydrolysis changed the hydrophobic interaction in the WP, which was followed by subsequent protein aggregation.

All of the aggregates had significantly higher enthalpy values (Tables 1 to 4) than the parent protein molecules. Possibly, denaturation of the protein molecules (Figure 1) only involved disruption of intraprotein bonds, whereas thermal denaturation of the aggregates involved disruption of both inter- and intro-protein bonds. More bonds need greater energy to disrupt them. Aggregates induced at 45° by $CaCl_2$, pH, or protease had a loose microstructure (13, 14, 15) and were considered to be held by noncovalent bonds (13, 19, 28). Also, the denaturation of all of the aggregates was highly cooperative, and their denaturation occurred at narrower temperature ranges than did the control. This result may suggest that molecular proteins lost their characteristics if they formed aggregates.

As the endothermic peak showed (Figure 2, 4, 5), the protein aggregates formed at ambient temperature could be subsequently heat denatured (i.e. the protein aggregation could occur before thermal denaturation).

TABLE 4. Effects of protease-induced aggregation on thermal properties of 10% whey protein isolate solution.

Incubation time (min)	Mean aggregate size (nm)	T_0^1 (°C)	T_D^1 (°C)	ΔH^1 (J/g)
0	18	$(62.3)^2$, 71.2[a]	(65.5), 76.8[a]	0.76 + 0.87 = 1.63[a]
5	17	62.1[b]	71.3[b]	2.75[b]
10	15	60.9[b]	70.5[b]	2.61[b]
20	16	63.7[b]	73.5[bc]	2.31[bc]
30	66	62.1[b]	72.0[bc]	2.19[c]
40	104	62.9[b]	72.7[bc]	2.12[c]
50	125	66.6[c]	73.8[c]	2.02[c]
60	150	65.5[c]	73.7[bc]	2.05[c]
90	264	65.2[c]	73.5[bc]	1.89[d]
120	615	66.1[c]	74.2[c]	1.79[d]
150	1108	66.9[c]	74.7[c]	1.44[ae]
180	1238	68.7[d]	74.2[c]	1.38[ae]
210	1325	69.1[d]	74.7[c]	1.39[ae]
240	1427	68.6[d]	74.5[c]	1.26[e]

[a,b,c,d,e]Means within a column followed by the same superscript letter are not significantly different ($P \leq$ 0.05).

[1]T_0 = onset denaturation temperature, T_D = denaturation temperature, and ΔH = enthalpy value of denaturation.

[2]Data in parentheses represent temperature of α-LA denaturation.

Molecular α-LA and β-LG in the WPS separately demonstrated the endothermic peaks upon heat denaturation (Figure 1). Once aggregation of WP occurred, the WPS demonstrated only one endothermic peak of the aggregates (Figure 2, 4, 5). All WPC samples have been reported to show a single endothermic peak (6, 30), probably because of aggregation. This aggregation could have resulted during production by the concentration and spray drying of the WPC (27) or during initial heating in the presence of a high percentage of nonprotein components in WPC, such as salt (18, 27). Information on thermal properties of WP aggregates may help in the design of appropriate heat processing for the application and manufacture of WP products.
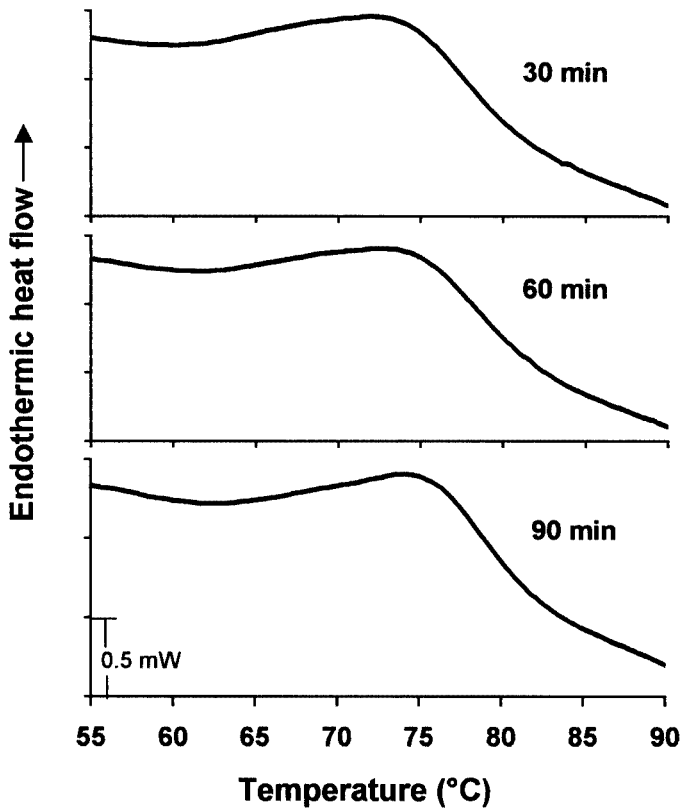

Figure 5. Effect of whey protein isolate aggregation shown on a differential scanning calorimetry thermogram. The aggregation was induced by hydrolyzing 10% whey protein isolate solution with *Bacillus licheniformis* protease at 45°C for varying times (30 to 90 min).
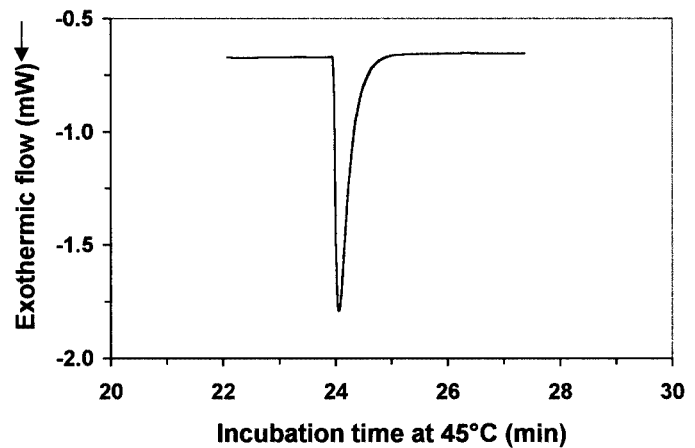

Figure 6. Differential scanning calorimetry exothermic curve obtained by incubation of 10% whey protein isolate solution with a protease from *Bacillus licheniformis* at 45°C.

CONCLUSIONS

The preaggregations induced by calcium salt, pH, or protease changed the thermal properties of WP. We concluded that 1) the aggregates of whey proteins showed one endothermic peak, representing denaturation of the aggregates instead of two peaks representing denaturation of α-LA and β-LG in the control; 2) the protein aggregation could cause significant increases in enthalpy values, requiring greater energy to denature protein aggregates; 3) denaturation of the aggregates might have occurred at a narrow temperature range compared with the denaturation of the parent proteins; and 4) generally, the aggregates had significantly different denaturation temperatures than those of α-LA and of β-LG in the control. The aggregation that was induced by calcium salt or the enzyme at 45°C resulted in an exothermic peak, indicating a change in the hydrophobic properties during formation of these aggregates.

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