Food Hydrocolloids 30 (2013) 12–18

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

# Effect of microbial transglutaminase treatment on thermal stability and pH-solubility of heat-shocked whey protein isolate

Srinivasan Damodaran*, Kingsley K. Agyare [1]

*Department of Food Science, University of Wisconsin-Madison, 1605 Linden Drive, Madison, WI 53706, USA*

ARTICLE INFO

*Article history:*
Received 9 September 2011
Accepted 13 April 2012

*Keywords:*
Microbial transglutaminase
Whey protein
Thermal stability
pH-solubility

ABSTRACT

Whey protein isolate (WPI) dispersions (5% protein, pH 7.0) were subjected to heat-shock at 70 °C for 1, 5 and 10 min. The heat-shocked WPI dispersions were treated with microbial transglutaminase (MTGase) enzyme, and thermal properties and pH-solubility of the treated proteins were investigated. Heat-shocking of WPI for 10 min at 70 °C increased the thermal denaturation temperature ($T_d$) of β-lactoglobulin in WPI by about 1.5 °C. MTGase treatment (30 h, 37 °C) of the heat-shocked WPI significantly increased the $T_d$ of β-lactoglobulin by about 6.3–7.3 °C when compared with heat-shocked only WPI at pH 7.0. The $T_d$ increased by about 13–15 °C following pH adjustment to 2.5; however, the $T_d$ of heat-shocked WPI was not substantially different from heat-shocked and MTGase-treated WPI at pH 2.5. Both the heat-shocked and the heat-shocked-MTGase-treated WPI exhibited U-shaped pH-solubility profiles with minimum solubility at pH 4.0–5.0. However, the extent of precipitation of MTGase-treated WPI samples at pH 4.0–5.0 was much greater than all heat-shocked and native WPI samples. The study revealed that while MTGase cross-linking significantly enhanced the thermal stability of β-lactoglobulin in heat-shocked WPI, it caused pronounced precipitation at pH 4.0–5.0 via decreasing the hydrophilic/hydrophobic ratio of the water-accessible protein surface.

© 2012 Elsevier Ltd. All rights reserved.

## 1. Introduction

Whey protein is an important food ingredient of high nutritional value and unique functional properties. It has applications in several food products (De Wit, 1998; Fitzsimons, Mulvihill, & Morris, 2007), including meal replacement bars and protein beverages (Childs, Yates, & Drake, 2007; LaClair & Etzel, 2009, 2010). Heat treatments during processing inevitably cause partial denaturation of whey proteins, which often impairs their functionality in various applications (De Wit, 1990). However, it is possible that the loss of functionality can be minimized by appropriate chemical, physical, and enzymatic modification of whey proteins. Recently, transglutaminase-mediated cross-linking has been employed to improve the hydration, thermal and surface-related properties of milk proteins (Lorenzen, 2000; Soeda, Hokazono, Kasagi, & Sakamoto, 2006; Tang, Yang, Chen, Wu, & Peng, 2005).

Transglutaminase (EC 2.3.2.13) is an enzyme that catalyzes an acyl transfer reaction between γ-carboxyamide moiety of protein-bound glutamine residue (acyl donor) and a primary amine (acyl acceptor). When lysine residues act as acyl acceptors, ε-(γ-glutamyl) lysine "isopeptide" covalent bonds are formed in proteins, leading to intra- and inter-molecular cross-linking. The enzyme also catalyzes hydrolysis of the γ-carboxyamide group of glutaminyl residues, resulting in deamidation (Ando et al., 1989; Yokoyama, Nio, & Kikuchi, 2004), which may greatly alter the electrostatic properties of the protein as glutamine residues are abundantly present in most proteins. Alteration in the net charge of β-lactoglobulin has been shown to affect its surface active properties (Song & Damodaran, 1991). The inter-molecular cross-linking, which increases the weight average molecular weight of the protein, may improve the gelation and emulsifying properties (Liu & Damodaran, 1999; Wang & Damodaran, 1990) and the thermal stability of proteins. A few studies have reported that transglutaminase-mediated cross-linking of native β-lactoglobulin enhanced its thermal stability (Agyare & Damodaran, 2010; Tang & Ma, 2007; Tanimoto & Kinsella, 1988); however, it was not clear whether the improved thermal stability was due to intra- or inter-molecular cross-linking.

Globular proteins in the native state are less susceptible substrates to transglutaminase action and require partial or complete unfolding to undergo enzymatic cross-linking (Han & Damodaran, 1996). Unfolding of globular proteins may be induced by thermal treatment at or above the thermal denaturation temperature or by addition of reducing agents that cleave disulfide

* Corresponding author. Tel.: +1 608 263 2012; fax: +1 608 262 6872.
*E-mail address:* sdamodar@wisc.edu (S. Damodaran).
[1] Present Address: McCain Foods Limited, 395 Centreville Road, Florenceville-Bristol, New Brunswick, Canada E7L 3K5.

0268-005X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodhyd.2012.04.012

bonds (Abourmahmoud & Savello, 1990; Tang & Ma, 2007). In the present study we investigated the efficacy of microbial transglutaminase (MTGase) enzyme in modifying the thermal and pH-solubility properties of heat-shocked (70 °C) whey protein isolate. The objective of this study was to understand how partial denaturation of WPI improves its susceptibility as a substrate for enzymatic cross-linking by MTGase. An additional objective of the study was to determine the improvement in the thermal stability of WPI as well as its pH-solubility characteristics as a function of the extent of cross-linking. A basic understanding of this structure–reactivity relationship of proteins may facilitate the use of MTGase in several food applications, including improvement of the stability of protein-stabilized emulsions in which the adsorbed proteins are in a partially unfolded state. It is hypothesized that partial unfolding of whey proteins resulting from the heat-shock treatment may enhance MTGase cross-linking and lead to substantial increase in the thermal stability of whey protein isolate.

## 2. Materials and methods

### 2.1. Materials

Whey protein isolate (WPI, BiPRO) was obtained from Davisco Foods International, Inc. (Le Sueur, MN). According to the manufacturer, the WPI contained >95% protein, 3% moisture, and <1% ash and lactose. Microbial transglutaminase (MTGase) used in this study was Activa®-TI (99% maltodextrin and 1% MTGase) (100 U/g of solid), donated by Ajinomoto Food Ingredients (Eddyville, IA). The crude enzyme was used without further purification. All chemicals used were analytical grade.

### 2.2. Methodology

#### 2.2.1. Experimental design

WPI solution (5% w/w) was heat-shocked at 70 °C for 1, 5, and 10 min. Each of these heat-shocked solutions was treated with MTGase at 37 °C for 0, 1, 4, 8, 20, 30, and 44 h. The native WPI and WPI incubated at 37 °C for 30 h were used as controls. The thermal denaturation temperature, the SDS-PAGE profile, and the pH-solubility profile of these samples were analyzed. In addition, the effect of acidification at pH 2.5 on thermal denaturation of WPI with and without MTGase treatment (30 h) was examined. The experimental details are provided below.

#### 2.2.2. WPI sample preparation and preheat treatment

All reagent solutions were prepared with ultrapure water (resistivity = 18.2 MΩ cm) from the Milli-Q Plus purification system (Millipore Corp., Billerica, MA, USA).WPI dispersions (5% protein) were prepared by dispersing WPI powder in 10 mM phosphate buffer (pH 7.0), containing 0.002% sodium azide, and stirring for 2 h at 23 ± 1 °C (designated as untreated native WPI). Heat-shock treatment was conducted using glass tubes (25 mm internal diameter) that contained 50 mL aliquots of the WPI solution (5% protein). The glass tubes were inserted into a 70 °C water bath, and the temperature of the WPI dispersion was monitored with a thermocouple until it reached 70 °C in about 100 s. The WPI dispersion was then maintained in the water bath at 70 °C for the specified times, i.e., 1, 5 and 10 min, and designated thereafter as "70/1", "70/5" and "70/10" for dispersions, respectively. The heat-shocked solutions were immediately cooled to 20 °C in ice slurry and were subsequently treated with MTGase. To determine whether mere long-time incubation of WPI samples at 37 °C without MTGase caused any change in its thermal denaturation behavior, we incubated the native and heat-shocked WPI samples at 37 °C for 30 h. These samples are marked as "30h N".

#### 2.2.3. Transglutaminase treatment

MTGase treatment was performed as outlined in our previous report (Agyare & Damodaran, 2010). Specifically, heat-shocked WPI dispersion (5% protein) was incubated with MTGase (50 U/g of protein substrate) at 37 °C for various times (designated as MTGase-treated WPI). The MTGase stock solution (50% w/w) contained 5 mM β-mercaptoethanol in order to maintain the active site SH group of the enzyme in the reduced state (Ando et al., 1989). The final concentration of maltodextrin and β-mercpatoethanol in the reaction mixture was 2% and 0.2 mM, respectively. At these low levels, these additives did not affect the thermal properties of WPI. When a specific incubation time was reached, the MTGase activity was inhibited by mixing an aliquot of 1 M stock solution of $NH_4Cl$ so that the final concentration of $NH_4Cl$ was 10 mM in the reaction dispersion (Færgemand, Murray, & Dickinson, 1997). In the case of '0 h' sample, $NH_4Cl$ was added to the reaction mixture soon after the addition of MTGase. When required, the pH of heat-shocked and heat-shocked-MTGase-treated WPI dispersions was adjusted to 2.5 by adding 2.0 M phosphoric acid.

#### 2.2.4. Differential scanning calorimetry (DSC)

Thermal stability of all WPI was measured using a Micro DSC VII (Setaram, Caluire, France). The WPI solutions (5% protein) were accurately weighed (ca. 700 mg) into pre-weighed DSC vessels (Hastelloy C276) and securely closed with the stopper. For each run, a capped DSC vessel containing the buffer (ca. 700 mg) used for dissolving the protein was used as reference. Thermal scanning from 20 to 120 °C was performed at a constant heating rate of 1 °C/min. The temperature at maximum heat flow ($T_d$) and maximum heat flow (heat of transition or enthalpy, $\Delta H$) were determined using the Setsoft software (version 1.40) supplied by the DSC manufacturer. The DSC was calibrated using cyclohexane, phenyl ether and o-terphenyl standards recommended by the DSC manufacturer. DSC experiments were carried out at least in duplicate for each sample.

#### 2.2.5. Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of protein samples under reducing conditions was performed as described previously (Agyare & Damodaran, 2010) using a 12% acrylamide separating and a 4% acrylamide stacking slab gel. Protein solutions (0.2% protein) were dissolved in an equal-volume of sample buffer (4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.125 M Tris, pH 6.8). After heating in boiling water for 3 min, aliquots containing 20 μg of protein per lane were loaded onto the gel and electrophoresed in a Mini-PROTEAN 3 apparatus (Bio-Rad Laboratories, Hercules, CA). A mixture of molecular weight standards (6.5–200 kDa) (Sigma–Aldrich Co., St. Louis, MO, USA) was also run along with the samples. Molecular weights (MW) of protein bands in samples were estimated from the regression line obtained by plotting the log (MW) *versus* relative mobility ($R_f$) of the protein standards.

#### 2.2.6. pH-Solubility measurement

The pH-solubility profile of the native and some of the treated WPI samples was determined according to the turbidity method described elsewhere (Zhu & Damodaran, 1994). Specifically the turbidity of 0.1% protein dispersion was measured at 500 nm at various pHs, namely, pH 2.0–7.0. Experiments were run in triplicate for each sample. The results are presented as % transmittance. Since the MTGase-treated samples contained 2% maltodextrin and 0.2 mM β-mercaptoethanol in the reaction mixture, we checked the effects of maltodextrin and 2-mercaptoethanol at these concentration levels on thermal stability and pH-solubility of native WPI and found to have no measurable effect.

Download English Version:

https://daneshyari.com/en/article/604720

Download Persian Version:

https://daneshyari.com/article/604720

Daneshyari.com