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Short communication

Modulation of hydrophobic interactions in denatured whey proteins by transglutaminase enzyme

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Abstract

The role of enzyme crosslinking in mediating formation of hydrophobic association in chemically denatured whey protein isolates (WPI) is examined. WPI samples denatured with dithiothreitol (DTT) and incubated at 50 °C with and without transglutaminase enzyme show dramatic differences in viscosity, with the viscosity of the sample exposed to enzyme being lower by several orders of magnitude than the sample without enzyme. Upon further exposure of both samples to sodium dodecyl sulfate (SDS) to eliminate hydrophobic interactions, we observe no change in the viscosity of the sample previously treated with enzyme, suggesting this sample to have minimal hydrophobic associations. In contrast, the sample without enzyme shows a dramatic drop in viscosity indicating it to have had substantial hydrophobic associations. A similar trend but to a lesser extent is observed at a higher WPI concentration. These results taken together suggest that the formation of enzyme catalyzed ε-(γ-glutamyl)lysine bonds attenuates hydrophobic interactions through steric hindrance and formation of compact molecules that limits exposure of the hydrophobic moieties.

Keywords: Whey protein; Rheology; Transglutaminase; Hydrophobic interactions

1. Introduction

Whey proteins are widely used food ingredients as they can bestow multiple functionalities (Burrington, 1998; de Wit, 1998; Huffman, 1996) such as emulsification, gelation, water binding, solubility, whipping/foaming and thickening, to food products. These functional attributes of whey proteins are dictated by the various chemical and physical bonds that take place among the protein molecules. Chemical bonds typically involve formation of disulphide (Foegeding, Li, & Bottcher, 1998; Gezimati, Singh, & Creamer, 1996a,b; 1997; Roefs & de Kruif, 1994) and enzyme-catalyzed ε(γ-glutamyl)lysine linkages (Burke, Ha, Pysz, & Khan, 2002; Dickinson & Yamamoto, 1996; Eissa, Bisram, & Khan 2004; Faegemand & Qvist, 1999; Wilcox, Clare, & Valentine, 2002; Wilcox & Swaisgood, 2002) while physical bonds occur through hydrophobic, electrostatic and hydrogen bonding interactions. Chemical bonds are strong (200–400 kJ/mol) and permanent, whereas the physical interactions are transient and weaker (5–10 kJ/mol for hydrophobic interactions, 10–40 kJ/mol for hydrogen bonding, and 25–80 kJ/mol for electrostatic interactions) (Israelchvili, 1992; Dickinson, 1997). Nevertheless, hydrophobic interactions in general are known to play an important part in the organization of the constituent molecules of living matter into complex structural entities (Tanford, 1980). Although the exact role of hydrophobic interactions in protein folding is not fully deciphered (Ben-Naim, 1990; Privalov, 1988; Privalov & Gill, 1988), there is no doubt that the conformations of the protein molecules affect and are affected by the extent of the hydrophobic interactions.

In this short note, we address the effect of enzymatic crosslinking of whey proteins by transglutaminase on the extent of hydrophobic interactions under denatured conditions. Protein molecules are denatured by dithiothreitol (DTT) and the rheological properties are monitored in the absence and presence of transglutaminase. The results are intriguing as they seemingly contradict earlier results but can be easily embraced in terms of changes in hydrophobic interactions caused by enzyme crosslinking.

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2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) was obtained from Davisco Food International, (LeSueur, MN). A commercial version of transglutaminase enzyme (1% enzyme and 99% maltodextrin, by weight) was supplied by Ajinomoto Co. (Japan). Sodium azide, dithiothreitol (DTT) and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Co. (St. Louis, MO). De-ionized water (>15 MΩ) was used in all the experiments.

2.2. Preparation of protein samples

WPI powder was dissolved in de-ionized water to the desired final concentration with no pH adjustment (pH$\sim$6.9) and stirred for complete solubility. Sodium azide (0.02% by wt.) was added to prevent bacterial growth. DTT was then added (20 mM) and stirred. For the samples treated with enzyme, an enzyme concentration of 10 U/gm protein was used. The unit of enzyme activity was measured using the hydroxamate method (Eissa, Bisram, & Khan, 2004). All samples were incubated at 50 °C.

2.3. Rheological measurements

Rheological measurements were carried out using a Dynamic Stress Rheometer (DSR) (TA Instruments, New Castle, DE). Parallel plate geometry with 40 mm diameter plates and $\sim$1 mm gap was used for the steady shear experiments whereas standard couette geometry was used for the dynamic experiments.

3. Results and discussion

WPI samples (8%) containing 20 mM DTT doped either with or without transglutaminase enzyme were heated at 50 °C for 5 h. Comparison of these two types of samples in Fig. 1 shows that the steady shear viscosity of the enzyme-treated sample is three orders of magnitude lower than that of the sample containing no enzyme. This is rather interesting as earlier work by us (Eissa, Bisram, & Khan, 2004; Eissa, 2005; Eissa & Khan, 2005) showed that thermally denatured whey proteins, more specifically β-lactoglobulin, could be crosslinked using transglutaminase into high molecular weight aggregates or polymers having higher viscosity. However, in this case, enzymatic treatment in the presence of DTT, which cleaves disulfide bonds, causes the viscosity to be drastically lower compared to the sample with no enzyme. This suggests that the presence of enzyme has a considerable effect on the physical interactions between protein molecules. To examine the effect of hydrophobic interactions, we added 2% SDS to the previous samples. The viscosity of the sample containing no enzyme decreases drastically by several orders of magnitude upon addition of SDS (Fig. 1). On the other hand, the sample with enzyme shows little change in viscosity, indicating that the enzyme-treated sample had minimal hydrophobic associations to begin with. The significance of these results can be explained as follows. Upon addition of DTT, the protein molecules are denatured and considerable hydrophobic associations take place. The fact that we have this scenario is validated by the fact that disruption of hydrophobic association by addition of SDS reduces the sample viscosity by over four orders of magnitude. On the other hand, the presence of enzyme (in the DTT denatured sample), causes reaction between glutamine and lysine

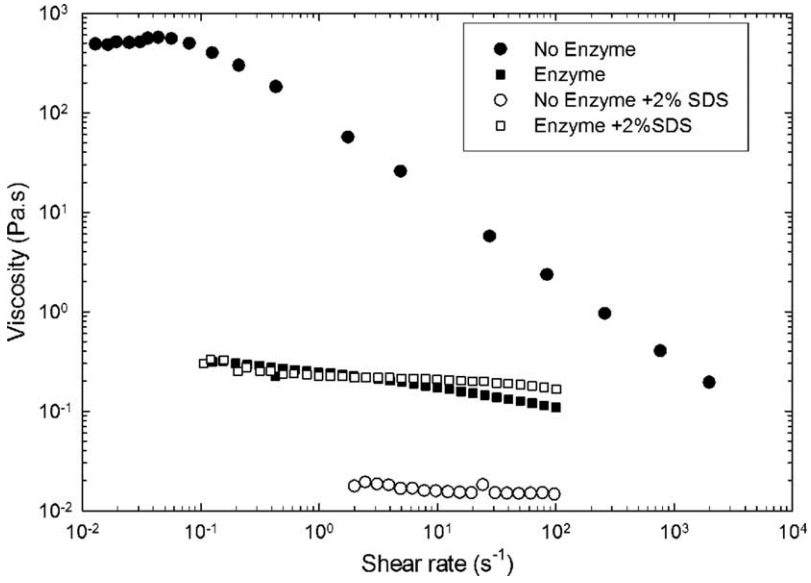

Fig. 1. Steady shear viscosities of 8% whey protein isolate samples (20 mM DTT) in the absence and presence of transglutaminase enzyme after incubation at 50 °C for 5 h. Measurements were taken at 25 °C.

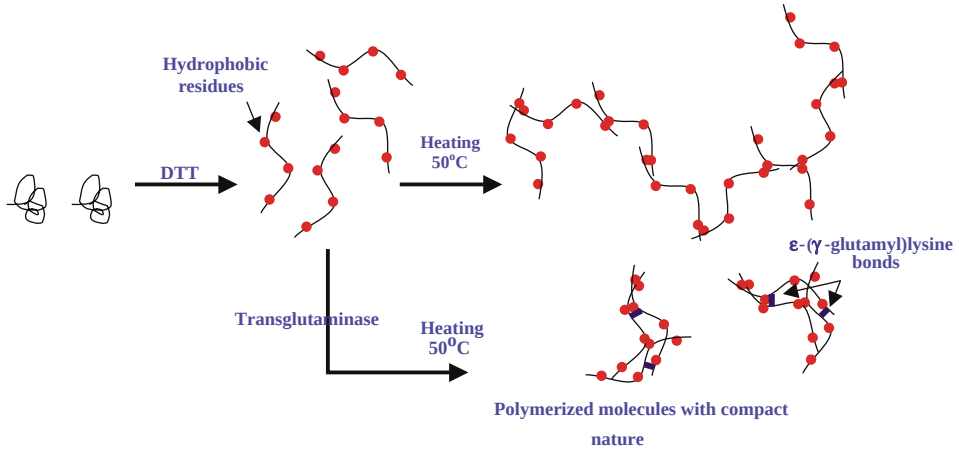

Fig. 2. Schematic diagram illustrating how enzyme catalyzed crosslinking with transglutaminase could lead to more compact protein structures.

residues creating ε-(γ-glutamyl)lysine bonds. The significantly lower viscosity of this sample (Fig. 1) compared to its counterpart without enzyme suggests that the hydrodynamic volume swept by hydrophobically associated protein molecules is much higher than that of the molecules crosslinked by enzyme. The fact that further addition of SDS to the enzyme-containing sample has no effect on viscosity indicates that the enzyme crosslinks effectively limits formation of hydrophobic associations. In this regard, Tanimoto and Kinsella (1988) has shown that β-lactoglobulin crosslinked by transglutaminase in the presence of 10 mM DTT contains intramolecular bonds that impedes unfolding of the molecules upon heating, hence suggesting a compact nature of the polymerized β-lactoglobulin molecules (Fig. 2) leading to a smaller hydrodynamic volume. We may also expect the compact protein structures to hide the hydrophobic residues in the interior part of the aggregates and prevent hydrophobic associations as schematically illustrated in Fig. 2.

The present finding may look seemingly contradictory to previous work that showed an increase in viscosity with enzymatic treatment in the presence of DTT (Faegemand & Qvist, 1999; Wilcox, Clare, & Valentine, 2002; Wilcox & Swaisgood, 2002). However, these authors conducted the enzymatic treatment at 40 °C, which is less favorable to hydrophobic interactions than 50 °C, as the hydrophobic interactions are strengthened at elevated temperatures (Ben-Naim, 1980). Dickinson and Yamamoto (1996) showed that enzymatic crosslinking resulted in gel formation at 13 and 14% protein, with elastic modulus

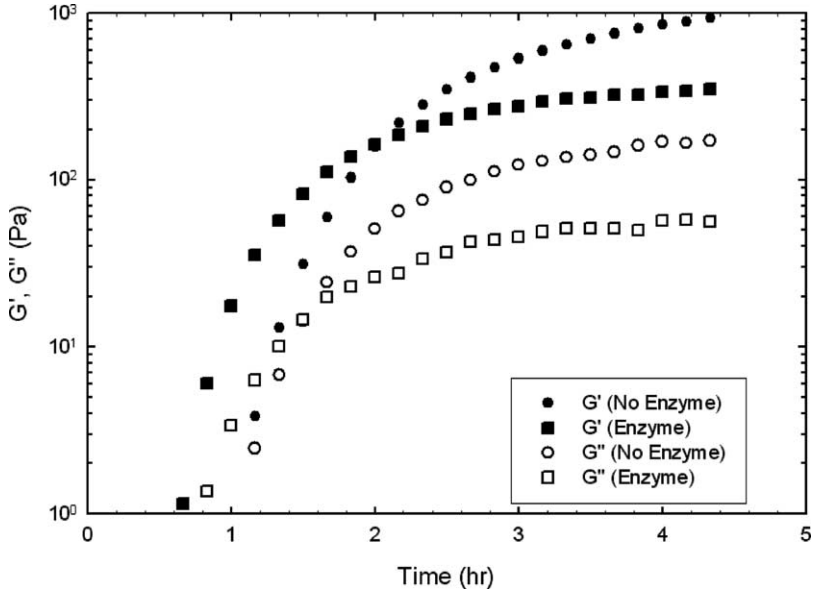

Fig. 3. Evolution of elastic (G') and viscous (G'') moduli of 10% whey protein isolate samples (20 mM DTT) in the absence and presence of transglutaminase enzyme while in situ incubation at 50 °C. Measurements were taken in the linear viscoelastic regime at a frequency of 1 rad/s.

(G') in the order of 10^3 Pa, while samples without enzyme showed no gelation; however, the authors did not use DTT for the sample without enzyme. On the other hand, Truong, Clare, Catignani, and Swaisgood (2004) found whey protein gels with enzyme treatment to be weaker than those formed with no enzyme treatment. They attributed their findings to the formation of extensive intra- and interchain crosslinking leading to polymers that were 'too large for effective network development'. However, this issue was not examined further, and we believe that a major reason behind their observation is the lower extent of hydrophobic interactions in the case of enzyme treatment.

We find that trends similar to Fig. 1 are also observed when 10% whey protein solution is heated in the presence of DTT. This solution undergoes gelation upon incubating at 50 °C. Fig. 3, which shows results of in situ experiments, reveals that the gel elastic modulus (G') for the enzyme treated sample is lower than that without enzyme treatment. However, the difference in the values of G' is not as substantial as the difference in viscosity observed for the samples at 8% concentration. This is possibly due to the involvement of other types of interactions such as hydrogen bonding and/or presence of intermolecular crosslinks at the higher protein concentration. These results indicate that the concentration of protein affects the extent of modulation of hydrophobic interactions by transglutaminase enzyme. Finally, we need to keep in mind that the results presented in this manuscript may depend on the type and processing history of the whey proteins used.

4. Conclusions

This study revealed that the presence of transglutaminase catalyzed ε-(γ-glutamyl)lysine bonds in WPI denatured using DTT impedes hydrophobic association formation. This was validated from comparison of viscosity data of 8% WPI containing DTT with and without enzyme. In the absence of enzyme, highly shear thinning viscosity was obtained versus Newtonian viscosity three decades lower in magnitude in the presence of enzyme. Upon SDS addition, no change in viscosity occurred in the presence of enzyme, indicating presence of minimal hydrophobic associations with enzyme treatment. In contrast, SDS addition to samples containing no enzyme dropped viscosity drastically, revealing the presence of dominant hydrophobic interactions in the absence of enzyme. These results can be explained on the basis that use of DTT cleaves disulfide linkages enabling formation of significant hydrophobic associations. Presence of enzyme produces crosslinks that prevents exposure of the hydrophobic moieties through steric hindrance and creation of compact molecules. A similar trend was noticed in the dynamic properties at a higher concentration (10%) where gels were formed, but to a lesser extent, possibly due to the involvement of other modes of interactions.

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