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Susceptibility of an industrial α-lactalbumin concentrate to cross-linking by microbial transglutaminase

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Abstract

The susceptibility of an industrial α-lactalbumin concentrate to cross-linking with a microbial transglutaminase from *Streptoverticillium mobaraense* was investigated. At a protein concentration of 0.5% w v^{-1}, the maximum cross-linking was observed at 50°C, pH 5 and at 5 h of incubation time. Results from sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis showed that most of the monomeric form of α-lactalbumin was converted to polymers too large to move into the gel matrix. Addition of ethylenediamine tetraacetic acid or SDS prior to the incubation of protein–enzyme mixture, further enhanced the transglutaminase reaction with the industrial α-lactalbumin. Results from reverse phase chromatography indicated that cross-linking caused a broadening of the α-lactalbumin peak with little change in the average hydrophobicity of the protein. In contrast to the reported results on pure α-lactalbumin, the industrial α-lactalbumin concentrate showed considerable cross-linking with transglutaminase even without the reduction of the disulphide bonds. This difference was attributed to the partially unfolded secondary structures in the industrial α-lactalbumin concentrate.
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Keywords: Transglutaminase; α-lactalbumin; Cross-linking

1. Introduction

Transglutaminases (EC 2.3.2.13) are part of a class of enzymes known as the aminoacyltransferases that catalyse acyl transfer reactions from glutamine residues in proteins or peptides to primary amines (Folk & Chung, 1985). As a result of this cross-linking, large molecular weight polymers with altered functionality are produced. Many food applications have been suggested for transglutaminase. Formation of protein gels with unique functionality is probably the most commonly reported application of transglutaminase. As transglutaminase can form intra- and inter-protein cross-links, the possibility of inter-molecular interactions can be explored in cross-linking proteins from two or more different sources, i.e. milk and soy or wheat protein. This would help in improving the functional or nutritional properties of one or the other protein. Also specific peptides or amino acids can be incorporated into protein and hence into a food formulation more effectively than by the simple addition of such peptides and amino acids.

Transglutaminase preparations can be derived from either animal or microbiological sources (Motoki & Seguro, 1998). Production of transglutaminases from animal sources appears to be industrially uneconomical as yields are low (Zhu, Bol, Rinzema, Tramper, & Wijngaards, 1999). Also, the animal-derived transglutaminase appears to require Ca^{2+} for its reaction, making it unpopular with the food industry, as Ca^{2+} plays a destabilising role in many food systems. The microbial transglutaminases can either be produced by the genetic manipulation of specific microorganisms or by selecting microorganisms that produce sufficient transglutaminase without genetic manipulation. The former approach seems less attractive at present because of food regulations and limited consumer acceptance for foods containing genetically modified additive in some countries. Ando et al. (1989) showed that the latter approach was industrially viable when they extracted transglutaminase from *Streptoverticillium mobaraense* and this

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transglutaminase has also been found to be Ca^{2+}-independent (Motoki & Seguro, 1998).

Milk proteins have shown potential as substrates for cross-linking by transglutaminase (Ikura, Kometani, Yoshikawa, Sasaki, & Chiba, 1980; Nio, Motoki & Takinami, 1985; Sakamoto, Kumazawa, & Motoki, 1994; Faergemand & Qvist, 1997; Faergemand, Otte, & Qvist, 1997; Sharma, Lorenzen, & Qvist, 2001). Caseins especially appear to be readily cross-linked because of their flexible, random-coil structures and the absence of any disulphide bonds in the α_{s1}- and β-caseins. Due to their compact globular structures, whey proteins tend to cross-link less efficiently. Many researchers have reported that there was little or no cross-linking of pure α-lactalbumin through transglutaminase (Aboumahmoud & Savello, 1990; Traore & Meunier, 1992; Matsumura, Chanyongvorakul, Kumazawa, Ohtsuka, & Mori, 1996; Matsumura, Lee, & Mori, 2000). In a recent study on the cross-linking of whey proteins from a industrial whey protein isolate (WPI) and a β-lactoglobulin preparation, Faergemand et al. (1997) reported that β-lactoglobulin was unable to cross-link without reduction in its disulphide bonds, whereas α-lactalbumin could be cross-linked without the reduction.

α-Lactalbumin is the smallest of the milk protein family with the molecular weight of 14,174 Da (Fox & McSweeney, 1998). Bovine α-lactalbumin has eight cysteine residues that form four intramolecular disulphide bonds. Structurally, α-lactalbumin is similar to lysozyme but has no bactericidal effect. Biologically, it functions as a co-enzyme in the synthesis of lactose. In its native state, α-lactalbumin is completely folded, representing a nearly spherical molecule (Walstra, Geurts, Noomen, Jellema, & van Boekel, 1999). The ability of α-lactalbumin to form a soft curd in the stomach of an infant makes it easily digestible and as it is the principal protein component in human milk. One of the main applications of bovine α-lactalbumin is in infant milk formulas.

α-Lactalbumin binds one Ca^{2+} per mole at neutral pH. Due to the ability of α-lactalbumin to release the bound Ca^{2+}, it is described to possess a molten globule state. The molten globule state is characterised by the compact globular molecule with native-like secondary structure and disordered tertiary structure (Demarest, Boice, Fairman, & Raleigh, 1999; Matsumura et al., 1996). The Ca^{2+} bound to α-lactalbumin can be removed by calcium chelating agents, such as EDTA (Matsumura et al., 1996) or by lowering the pH below 5 (Fox & McSweeney, 1998). The transition from native to the molten globule state offers opportunity for greater cross-linking reactions by α-lactalbumin (Matsumura et al., 1996).

Previous studies on cross-linking behaviour of α-lactalbumin with transglutaminase have used pure α-lactalbumin obtained from chemical companies (Aboumahmoud & Savello, 1990; Traore & Meunier, 1992; Matsumura et al., 1996; Matsumura et al., 2000). Recently, however, many dairy companies have started manufacturing α-lactalbumin concentrate on industrial scale. With the availability of α-lactalbumin on large scale, no doubt more attention will be paid to its applications by the food industry. In this report, we present results on the cross-linking behaviour of an industrial α-lactalbumin concentrate. The tendency towards cross-linking has been investigated under various chemical and processing conditions that alter the protein structure.

2. Materials and methods

2.1. Materials

Industrial α-lactalbumin concentrate (PSDI-7200) was supplied by Arla Food Ingredients, Denmark. The protein powder contained 93% protein with $75 \pm 5\%$ of that being α-lactalbumin. The α-lactalbumin concentrate also contained β-lactoglobulin ($10 \pm 5\%$) and non-protein nitrogen [mainly glycomacropeptides (GMPs)-$<10\%$].

A Ca^{2+}-independent transglutaminase (Activa MP) was provided by Alsiano Ingredients, Denmark. The transglutaminase was manufactured by Ajinomoto Co., Ltd, Japan using a fermentation process and *Streptoverticillium mobaraense*. The enzyme preparation had an activity of $100\,U\,g^{-1}$ (hydroxamate method), and contained 1% enzyme. The enzyme was used in the original form without any further purification.

All other chemicals of analytical grade were obtained from Sigma Chemicals Co., Denmark.

2.2. Cross-linking of α-lactalbumin concentrate by transglutaminase

Cross-linking was carried out by incubating a solution of 0.5% (w/v) protein in a 20 mM imidazole buffer (pH 7.0) with the enzyme. To investigate the susceptibility of the industrial α-lactalbumin concentrate to cross-linking with transglutaminase, the interactions stabilising the secondary, tertiary or quaternary structures of the protein were altered either by changing processing conditions or by additives. Prior to addition of the enzyme, the protein solution was either heated in the temperature range 60–90°C for 5 min, pressure-treated at 3000 bar (30 min at room temperature), or modified by one of the following additives (for 1 h): 0.1% $w\,v^{-1}$ dithiothretol (DTT), 5 mM $CaCl_2$, 5 mM ethylenediaminetetraaceticacid (EDTA), 2% $w\,v^{-1}$ sodium dodecyl sulphate (SDS), 1 M NaCl or 1 M guanidine hydrochloride (GuHCl).

In all experiments, the enzymatic reaction was stopped by the addition of 1% *n*-ethylmaleimide (NEM). The extent of cross-linking was monitored by measuring the amount of ammonia produced during the enzymatic reaction (Matsumura et al., 1996).

2.3. Determination of ammonia

The transglutaminase-mediated release of ammonia was monitored using Boehringer Mannheim ammonia kit (Boehringer Mannheim GmbH, Mannheim, Germany) and the method described previously (Sharma et al., 2001).

2.4. SDS-PAGE

Protein samples, before and after cross-linking, were analysed by SDS-PAGE under reducing conditions using the method described by Faergemand et al. (1997). The separation of protein bands was carried out on a Phastgel Homogeneous 12.5% gel using Phastsystem^{TM} (Pharmacia, Allerød, Denmark). Semi-quantitative estimation of bands was carried out by analysing scanned images of the gels using Scion Image software, version 4.0.1 (Scion Corporation, Maryland, USA).

2.5. Reverse-phase HPLC

Protein samples, before and after cross-linking, were separated by RP-HPLC (Waters 600E System, Waters Corporation, Milford, Massachusetts, USA) on a 150×3.9 mm C_{18} column filled with 4 μm particles (Nova-Pak, Waters Corporation). A sample (20 μL) was injected into the column and the separation was carried out at 30°C with a flow rate of 1 mL min^{-1}. Elutant A contained 0.1% TFA and eluant B contained 0.8% TFA and 90% acetonitrile in water. The elution started with 90% eluant A and gradually (linearly) changed in 40 min to 90% eluant B. Eluted peaks were detected by UV detector (Waters 490E Multiwave detector) at 280 and 220 nm. The operation of the HPLC system and the integration of peaks were carried out using Waters Millennium 2010 software.

2.6. Circular dichroism

Circular dichroism (CD) measurements on the industrial α-lactalbumin concentrate and a Sigma α-lactalbumin were carried out in the far-UV (180–270 nm) at room temperature under constant nitrogen purge using a Jasco J-700 spectropolarimeter (Jasco UK Ltd, Great Dunmow, United Kingdom) with the cell length of 0.1 mm. The protein samples at the original pH were diluted with 1 M phosphate buffer, pH 7.4 to a final concentration of 1.0 mg mL^{-1}. Data were further processed for noise reduction, baseline correction and zero point adjustment.

3. Results and discussion

Previous studies on cross-linking behaviour of α-lactalbumin with transglutaminase have used pure α-lactalbumin obtained from chemical companies (Aboumahmoud & Savello, 1990; Traore & Meunier, 1992; Matsumura et al., 1996; Matsumura et al., 2000). Our study uses α-lactalbumin concentrate that is manufactured under large-scale industrial conditions. Due to the scale and the economics of manufacture, the industrial preparation is generally not as pure as the pure fraction obtained from a chemical company. Among other factors, the type and amount of impurity in the industrial preparation depend on the source of the whey used and the method followed for the fractionation of the protein.

As an industrial α-lactalbumin concentrate has not been previously used as a cross-linking substrate, the effect of conditions during the enzymatic reaction was first investigated.

3.1. Influence of environmental conditions during the enzymatic reaction

To determine the effect of environmental conditions on the transglutaminase reaction, the concentration of transglutaminase, reaction time, temperature and pH were varied as single variables at a protein concentration of 0.5% w v^{-1}. The protein solution prior to the addition of transglutaminase had approximately 0.2 mM ammonia. The influence of various environmental conditions during the enzymatic reaction on the net amount of ammonia produced is shown in Fig. 1. Increasing the temperature of reaction from 20°C to 50°C gradually increased the net amount of ammonia while further increase in the temperature to 60°C caused a decrease in the net amount of ammonia produced (Fig. 1). The maximum enzymatic activity at 50°C supports a previous study where transglutaminase from *Streptoverticillium mobaraense* was used (Motoki & Seguro, 1998). The increase in the incubation time caused a rapid increase in the amount of ammonia produced during the first 30 min of reaction, which was followed by a further, but a slower increase up to 5 h of incubation (Fig. 1). Incubation for longer than 5 h caused little change in the amount of ammonia produced. Using the same enzyme, rapid enzyme activity during the first 30 min was also shown for skim milk recently (Sharma et al., 2001). Increasing the enzyme concentration from 0 to 5 U g protein^{-1} rapidly increased the amount of ammonia produced (Fig. 1). At the enzyme concentration of 50 U g protein^{-1} the net

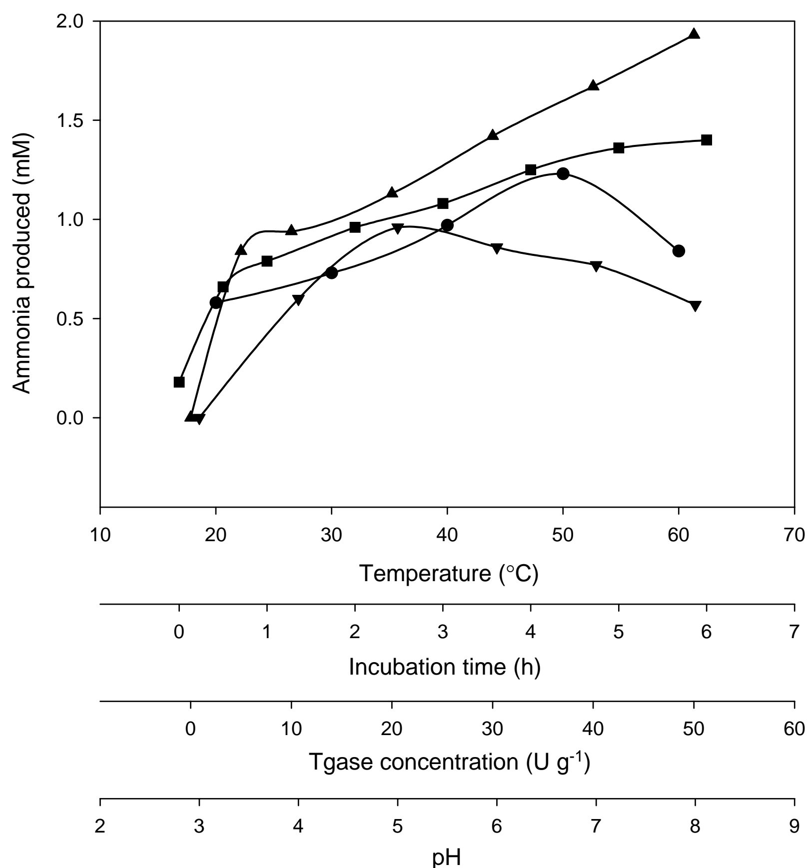

Fig. 1. Influence of reaction conditions on the amount of ammonia produced by cross-linking α-lactalbumin (0.5% w v^{-1}) and transglutaminase. The reaction conditions were varied as single variables and were: (●) incubation temperature at transglutaminase concentration of 10 U g^{-1} protein and incubation time of 4 h at pH 7.0; (■) incubation time at transglutaminase concentration of 10 U g^{-1} protein at 50°C and pH 7.0; (▲) enzyme concentration at incubation time of 4 h at 50°C and at pH 7.0; and (▼) pH at transglutaminase concentration of 10 U g^{-1} protein and incubation time of 4 h at 50°C.

amount of ammonia produced was approximately 1.9 mM, which appears to be more than that could possibly have been produced if all protein was just cross-linked. A simple calculation showed that there was a total of 1.8 mM of glutamine residue in the 0.5% protein solution. Assuming that all glutamine residues were cross-linked in the industrial α-lactalbumin concentrate, one would expect to have equivalent molar amounts of ammonia released (Ikura et al., 1980). However, as the net amount of ammonia produced at 50 U g^{-1} was 1.9 ± 0.01 mM, slight deamidation and/or amine incorporation could not be ruled out. As the pH was increased from 3 to 5, the net amount of ammonia produced increased from 0 to 1 mM followed by a decrease with the further increase in the pH (Fig. 1). Although maximum enzymatic activity occurred at pH 5, the protein solution was not completely stable at this pH. This would indicate that near the isoelectric point greater cross-linking could be introduced than at low or high pH values. Also, at pH 5, a considerable amount of bound Ca^{2+} would be removed from the protein possibly altering the conformation of the protein and making it easier to crosslink (Matsumura et al., 1996). At pH 3, although most of the calcium may have been removed from the protein the enzyme as such may not be active (Motoki & Seguro, 1998) thus showing no ammonia production in Fig. 1.

For subsequent experiments, the transglutaminase reaction was carried out at 50°C and at pH 7 using 0.5% w v^{-1} protein.

3.2. *Extent of cross-linking under various conditions*

The extent of cross-linking under modified environmental conditions affecting the substrate was investigated. The modifications to the environmental conditions were carried out prior to the addition of the enzyme. The modifications, carried out independently were: preheating of the protein solution in the range 60–90°C for 5 min, addition of DTT (0.1% w v^{-1}), calcium chloride (5 mM), EDTA (5 mM), SDS (2% w v^{-1}), NaCl (1 M), GuHCl (1 M) and by subjecting the protein solution to a high hydrostatic pressure at room temperature (3000 bar for 30 min). Cross-linking was

carried out at 50°C for 4 h at pH 7 using 0.5% protein and $10\,U\,g^{-1}$ enzyme. The extent of cross-linking was measured using three methods: by the estimation of ammonia, by separation of proteins on reduced SDS gels and by the reverse-phase HPLC.

3.2.1. Ammonia

Compared with control, the addition of EDTA and SDS showed a major increase in the amount of ammonia produced (Fig. 2). Preheating, the addition of NaCl and GuHCl and the high hydrostatic pressure also caused slight increase in the amount of ammonia produced whereas there was slight decrease in ammonia when the protein solution contained DTT or calcium chloride.

Our results on the effect of EDTA on cross-linking of industrial α-lactalbumin are similar to those of Matsumura et al. (1996) who used a laboratory-made α-lactalbumin. The increased susceptibility of α-lactalbumin to cross-linking in the presence of EDTA is attributed to the change in the tertiary structure of the protein. Addition of EDTA causes removal of Ca^{2+} from α-lactalbumin that alters its structure and induces a transition to the molten globule state. In α-lactalbumin, Ca^{2+} is reported to be bound in a pocket in which calcium is surrounded by seven legands: the carboxyl group of Asp 82, Asp 87 and Asp 88, the carbonyl oxygens of Lys 79 and Asp 84 and two water molecules (Fox, 1989). The removal of Ca^{2+} from the tertiary structure of α-lactalbumin probably frees up the Lys 79 residue, which may subsequently be available for cross-linking. The increase in the amount of cross-linking in the SDS-treated protein solution could mean that the hydrophobic forces play an important role in stabilising protein in the industrial α-lactalbumin concentrate. Heating the protein solution in the range 60–90°C caused a small increase in the amount of cross-linking but there was no correlation between the preheating temperature and the amount of ammonia. In general, increasing temperature progressively disorders both protein and water by disrupting the hydrogen bonding which stabilises the protein structure and causes unfolding (Kinsella, Whitehead, Brady, & Bringe, 1989). This unfolding can lead to protein–protein interaction and thus may have a mixed effect on the availability of transglutaminase reactive sites, i.e. some sites may be exposed while some may be buried. However, the net exposure of reactive sites appears to be somewhat increased by heating the protein solution. Breaking of ionic and hydrogen bonds (by the addition of NaCl and GuHCl, respectively) also appear to slightly enhance the susceptibility of α-lactalbumin concentrate to cross-linking but the effect was smaller when compared with the effect of EDTA or SDS (Fig. 2). Although breaking of disulphide bonds by the addition of DTT has been previously suggested as a means of enhancing cross-linking in α-lactalbumin (Aboumahmoud & Savello, 1990; Faergemand et al., 1997; Motoki, Nio, & Takinami, 1984), our study did not support results of the earlier studies. The main reason for this difference appears to be in the type of substrate as discussed later in the paper. The addition of Ca^{2+} (i.e. calcium chloride) also did not enhance the amount of cross-linking in α-lactalbumin concentrate. Binding of Ca^{2+} stabilises the conformation of α-lactalbumin and reduces its hydrophobicity.

In the interpretation of the above results, it is important to distinguish between the effect of the modifications on the substrate and that on the enzyme activity. Pre-heating and high hydrostatic pressure clearly has effect on the protein rather than the enzyme, as these conditions during the cross-linking were constant. Modifications that affect the calcium, such as EDTA and the addition of calcium chloride, are also substrate-related effects, as the microbial transglutaminase used in the present study was a calcium-independent enzyme. For other modifications, i.e. DTT, NaCl and GuHCl, some effects on the enzyme activity cannot be ruled out, however; the effects are unclear.

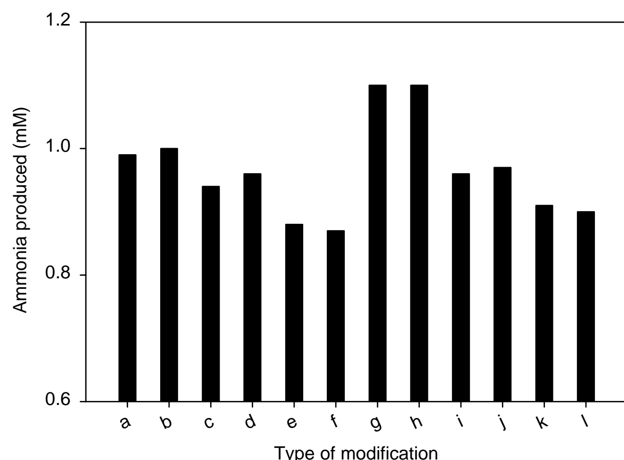

Fig. 2. Effect of modification with heating, additive or hydrostatic pressure on the amount of ammonia produced by cross-linking α-lactalbumin concentrate with transglutaminase. Cross-linking was carried out at the protein concentration of 0.5% $w\,v^{-1}$, transglutaminase concentration of $10\,U\,g^{-1}$ protein at 50°C and at pH 7.0 for 4 h. The modifications to protein were carried out prior to the addition of transglutaminase and the modifications were: heating for 5 min at (a) 60°C, (b) 70°C, (c) 80°C or (d) 90°C or (e) addition of DTT (0.1%), (f) $CaCl_2$ (5 mM), (g) EDTA (5 mM), (h) SDS (2%), (i) NaCl (1 M), (j) GuHCl (1 M), or (k) hydrostatic pressure (3000 bar, 30 min at room tempearture). The control sample (l) had no modification made prior to the cross-linking with the enzyme.

3.2.2. SDS-PAGE

The SDS-gel electrophoretic patterns obtained after cross-linking of the industrial α-lactalbumin concentrate that was modified prior to the addition of the enzyme

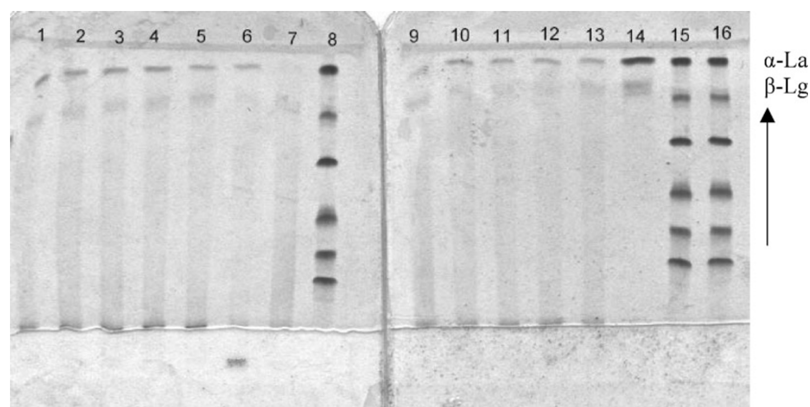

Fig. 3. SDS-PAGE patterns of transglutaminase-treated α-lactalbumin concentrate after modification with heating, additive or hydrostatic pressure. Modifications to protein were carried out prior to the addition of the enzyme. Lane 1: heating at 60°C for 10 min; lane 2: heating at 70°C for 10 min; lane 3: heating at 80°C for 10 min; lane 4: heating at 90°C for 10 min; lane 5: addition of 0.1% DTT; lane 6: addition of 5 mM $CaCl_2$; lane 7: addition of 5 mM EDTA; lane 8: molecular mass standards (from top to bottom: 14000, 20100, 30000, 43000, 67000 and 94000 Da, respectively); lane 9: addition of 2% SDS; lane 10: 1 M NaCl; lane 11: 1 M guanidine HCl; lane 12: hydrostatic pressure 3000 bar; lane 13: protein with transglutaminase; lane 14: protein without transglutaminase; lane 15 and 16: molecular mass standards. The cross-linking was carried out at the protein concentration of 0.5% (w/v), transglutaminase concentration of $10\,U\,g^{-1}$ protein at pH 7.0 and at 50°C for 4 h.

are shown in Fig. 3. Comparison of the electrophoretic pattern for the protein without the enzyme (lane 14) with that containing the transglutaminase (lane 13) clearly indicates that considerable cross-linking of proteins had occurred without any of the modifications applied to the protein solution. After transglutaminase cross-linking, both α-lactalbumin and β-lactoglobulin showed a decrease in their intensities in the gel. Since the cross-linked polymer material was retained at the boundary between the stacking and resolving gel did not move into the resolving gel it was difficult to determine its molecular weight. Matsumura et al. (1996) using gel permeation chromatography and SDS-PAGE, showed that α-lactalbumin in the molten globule state, cross-linked with a microbial transglutaminase, formed dimers, trimers and oligomers larger than tertramers. Similarly, Han and Srinivasan (1996) found that cross-linking of a pure α-lactalbumin with a pig liver transglutaminase caused formation of homologous dimer, trimer and polymer. In our SDS gels, the absence of dimmer, trimer and polymer suggests that either these were not present or the amount of protein loaded was too little to shows the presence of these components.

The industrial α-lactalbumin concentrate contained almost 10% β-lactoglobulin and from the SDS-gel electrophoretic patterns it can be seen that some of the β-lactoglobulin also took part in the cross-linking reaction as shown by the decrease in the intensity of the second band on the gel. These findings are in agreement with those of Faergemand et al. (1997). In agreement with the results obtained using the ammonia assay above, of the modifications studied, the most profound effects were obtained from the addition of EDTA and SDS (Fig. 3, lanes 7 and 9). The addition of EDTA to α-lactalbumin concentrate, prior to the enzyme addition, led to a near complete polymerisation of α-lactalbumin whereas the effect on β-lactoglobulin was limited (Fig. 3, lane 7). Of the other modifications studied, the addition of NaCl, GuHCl and high hydrostatic pressure showed little effect on the amount of cross-linking (Fig. 3, lanes 10, 11 and 12, respectively). The addition of $CaCl_2$ led to the formation of a polymer that resolved in the stacking gel. It is likely that this high molecular weigh aggregate was formed by the Ca-induced aggregation of β-lactoglobulin rather than by the enzymatic cross-linking of α-lactalbumin as the β-lactoglobulin band in the gel decreased considerably.

Densitometry of the bands indicated that nearly two-thirds of α-lactalbumin in the industrial protein concentrate was cross-linked by transglutaminase without any modification to the protein (densitograms not shown). The addition of EDTA left no measurable α-lactalbumin while the addition of SDS led to nearly 95% cross-linking of α-lactalbumin.

3.2.3. RP-HPLC

The RP-HPLC profiles in Fig. 4 showed that the major peak in the transglutaminase-treated sample eluted at the same position as that in the untreated sample. The peak area before and after cross-linking was similar, suggesting that cross-linking did not alter the average hydrophobicity of the major peak. However, as a consequence of cross-linking, there was a decrease in the height and some broadening of the peak. Among the variables investigated only EDTA and SDS showed some influence on the RP-HPLC profiles of transglutaminase-treated α-lactalbumin, supporting the results from ammonia and SDS-PAGE (RP-HPLC profiles not shown).

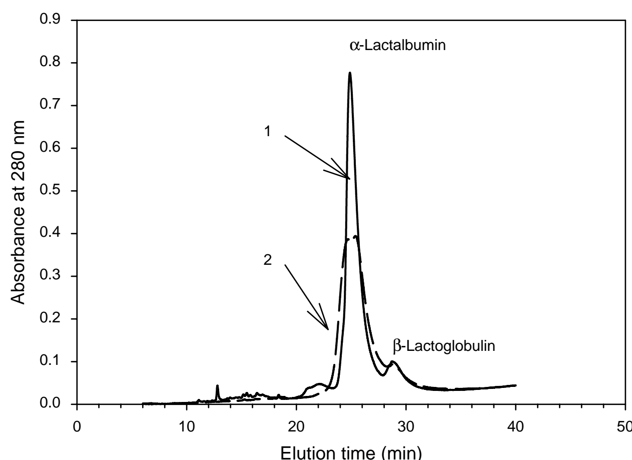

Fig. 4. RP-HPLC profiles of α-lactalbumin concentrate before (1) and after (2) cross-linking with transglutaminase. The cross-linking was carried out at the protein concentration of 0.5% (w/v), transglutaminase concentration of $10\,U\,g^{-1}$ protein at 50°C and at pH 7.0 for 4 h.

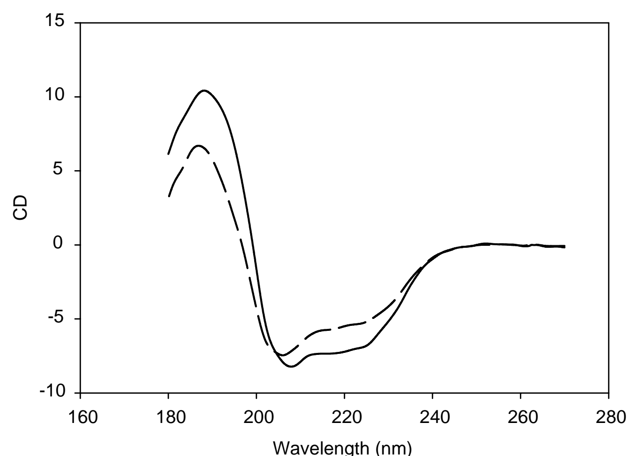

Fig. 5. Circular dicroism spectra of industrial α-lactalbumin concentrate (dashed line) and Sigma α-lactalbumin (solid line). The protein solutions were diluted with 1 M phosphate buffer, pH 7.4, to a final concentration of $1.0\,mg\,mL^{-1}$.

From the above results it can also be suggested that protein in the industrial α-lactalbumin concentrate is considerably cross-linked by transglutaminase even without the reduction of the disulphide bonds. This finding differs from the observations made by Traore and Meunier (1992) and Aboumahmoud and Savello (1990) for pure α-lactalbumin fractions. Traore and Meunier (1992) used a human placental factor $XIII_a$ while Aboumahmoud and Savello (1990) used a guinea pig transglutaminase and both suggested that a reduction of disulphide bond was essential for cross-linking by α-lactalbumin. Our results, however, are in agreement with those of Faergemand et al. (1997) who used an industrial WPI and found a considerable cross-linking of α-lactalbumin without reduction of disulphide bonds.

To investigate further on the reasons for differences between the cross-linking behaviour of the industrial α-lactalbumin concentrate and pure α-lactalbumin, structural differences between the industrial α-lactalbumin concentrate and a pure protein fraction obtained from Sigma Chemical Company were determined using CD. Fig. 5 shows the far-UV CD spectra of the two proteins. The lower ellipticity in the industrial α-lactalbumin compared with that in the Sigma protein around 222 nm suggests that the commercial α-lactalbumin had more altered secondary structure than the Sigma α-lactalbumin. During processing, especially spray drying, considerable unfolding and denaturation of α-lactalbumin cannot be ruled out. Such processing would therefore have exposed more sites for transglutaminase-specific reaction leading to a different behaviour than a pure α-lactalbumin as noticed by others (Aboumahmoud & Savello, 1990; Traore & Meunier, 1992; Matsumura et al., 1996; Matsumura et al., 2000).

4. Conclusions

Industrial α-lactalbumin concentrate was considerably susceptible to cross-linking by transglutaminase. It is likely that considerable structural modifications occurred during the manufacture of the industrial α-lactalbumin concentrate thereby exposing sites for transglutaminase reaction. The cross-linking of α-lactalbumin in industrial protein concentrate was further enhanced by chelation of Ca^{2+} and by breaking hydrophobic bonds. The consequences of this are that, contrary to previous assumptions based on results using α-lactalbumin from small-scale purifications; industrial α-lactalbumin can be functionally modified for dairy products with transglutaminase. This opens up the possibility for improved functionality of dairy ingredients containing α-lactalbumin.

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