

Effect of Transglutaminase-Induced Crosslinking of Sodium Caseinate on Gel Formation Kinetics and Solubility Upon Acidification to Low pH

Martina Lille¹, Riitta Partanen¹, Johanna Buchert¹, and Kaisa Poutanen¹

¹VTT Technical Research Centre of Finland, Espoo, Finland

ABSTRACT

Crosslinking of sodium caseinate with transglutaminase may change its aggregation behaviour at pH-values typical for human stomach and thereby its digestibility in the gastrointestinal tract. In this work the effect of crosslinking on solubility of sodium caseinate was indirectly assessed during acidification by small deformation rheological measurements and multiple light scattering.

INTRODUCTION

The enzyme transglutaminase (TG) forms covalent bonds between glutamine and lysine residues in proteins. Caseins, the main milk proteins, are readily crosslinked with TG since glutamine residues are easily accessible in the flexible casein molecule.

It is unknown how TG-induced crosslinking affects the digestion of caseins in the human gastrointestinal tract. The reported differences in digestion of the major milk proteins, caseins and whey proteins, are attributed to differences in their behaviour at the acidic conditions prevailing in the stomach. It is assumed that when a neutral solution of these proteins enter stomach (pH ~2), native whey proteins stay soluble, whereas caseins aggregate and form clots. The behaviour of casein may, however, be more complicated than that due to the buffering capacity of casein, which can result in an increase in the gastric pH shortly after a casein solution has entered the stomach. If the pH rises to above the pI

of casein (~ 4.6) it is possible that aggregation is delayed, which may affect the rate of protein digestion, gastric emptying and the rate at which proteins are absorbed in the small intestine.

The aim of this work was to elucidate how TG-induced crosslinking alters the solubility of sodium caseinate at pH-values typically found in stomach. The sodium caseinate solution was slowly acidified to a final pH of 3.3 by the addition of glucono δ -lactone. The effect of crosslinking on the solubility of sodium caseinate was indirectly assessed during acidification by small deformation rheological measurements and multiple light scattering.

MATERIALS

Sodium caseinate (EM 7) was from DMV International (The Netherlands) and D-(+)-gluconic acid δ -lactone (GDL) from Sigma. Transglutaminase (TG) was fractionated free of maltodextrin from the commercial TG product Activa WM (Ajinomoto, Japan).

METHODS

A sodium caseinate solution containing 3% protein was prepared in MilliQ-water. The crosslinking was performed by adding 500 nkat (30 U) TG per g sodium caseinate and incubating the mixture for 24 h at room temperature. The non-crosslinked or crosslinked sodium caseinate solutions were acidified by adding 3% GDL to the protein

solution and incubating for 24 h at room temperature.

The structure formation and breakdown of the sodium caseinate solution during acidification was followed with a stress-controlled rheometer (StressTech, Reologica Instruments AB, Lund, Sweden) in dynamic oscillation mode. Samples were placed into the concentric cylinder measuring system (CC 25) shortly after GDL addition. Silicon oil was applied on the sample surface to avoid drying. All measurements were carried out at 20 °C for up to 24 h at a frequency of 0.1 Hz. Strain was controlled at 0.01.

Changes in transmission and backscattering in the sodium caseinate samples during acidification were measured with multiple light scattering in a Turbiscan Lab Expert (Formulation, France).

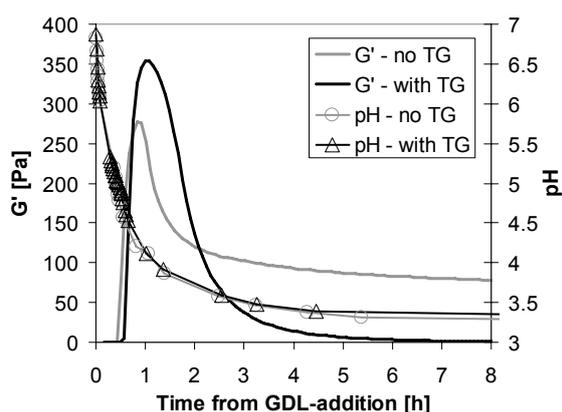


Figure 1. Effect of TG-induced crosslinking on gel formation and breakdown of a sodium caseinate solution during chemical acidification with GDL.

RESULTS AND DISCUSSION

When milk or as in our case a sodium caseinate solution (at a high enough concentration) is slowly acidified to a pH close to its isoelectric point (4.6) a gel is formed. It is well known that the strength of the milk or caseinate gel can be considerably increased by crosslinking caseins with TG prior to or during acidification^{1,2}.

In our work, the pH of a sodium caseinate solution was gradually reduced to 3.3, a value far below the isoelectric point. Non-crosslinked sodium caseinate started to form a gel network 25 min after the GDL-addition at a pH of about 5 (Fig. 1). G' reached a maximum of 277 Pa 51 min after GDL-addition at a pH of 4.2 after which it started to decrease. The decrease in G' is attributed to a gradual increase in solubility of sodium caseinate upon decreasing the pH below the pI. After 24 h acidification at a pH of 3.2 G' had levelled off to a value of 68 Pa, which suggests that the some sodium caseinate was still insoluble at this pH.

TG-crosslinking slightly delayed the start of gel formation of the sodium caseinate solution – G' started to increase 34 min after GDL-addition at a pH of 4.7 (Fig. 1). The maximum in G' was higher for the crosslinked sodium caseinate (354 Pa at pH 4.1) than for the non-crosslinked one (277 Pa at pH 4.2). The gel structure was, however, almost completely lost in the crosslinked system 8 h after GDL-addition when G' dropped to below 1 Pa at a pH of about 3.3, which indicates that TG-crosslinked sodium caseinate is highly soluble at this pH.

The results of the Turbiscan measurements were in accordance with the rheological results. Transmission started to decrease very soon after GDL-addition and reached zero at the point where G' started to increase (Fig. 2A). At the point where G' for the crosslinked sodium caseinate solution dropped below zero, transmittance started to increase (Fig. 2B). The evolution of backscattering in the caseinate samples during acidification is shown in Fig. 3. Backscattering could be followed as long as transmission was below 0.1. At first backscattering increased for a short while for both samples, reached a maximum and subsequently started to decrease in a similar manner than G' .

Flanagan et al.³ also reported improved solubility of sodium caseinate in the pH range 3-6 after TG-induced crosslinking.

The solubility was determined by measuring the protein content in the supernatant after adjustment of pH by HCl and centrifugation.

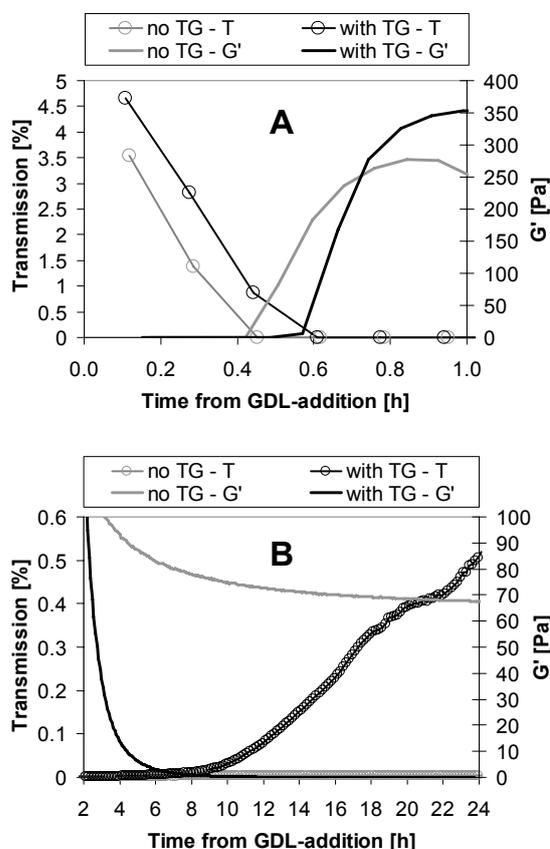


Figure 2. Transmission as function of acidification time for non-crosslinked and TG-crosslinked sodium caseinate solution during A) the initial phase of structure formation and B) during structure breakdown.

Salts alter the charge distribution of proteins and may thereby affect their solubility at a certain pH. Gastric juice contains electrolytes such as sodium, potassium and chloride⁴, so it's important to consider the effect of salt concentration on protein solubility when protein digestion is investigated.

The behaviour of sodium caseinate was radically changed in the presence of 100 mM NaCl (Fig. 4). TG-crosslinked sodium caseinate, which was completely solubilised in the absence of NaCl, formed in the presence of NaCl a rather strong gel at

the final pH of 3.2. A slightly weaker gel was formed with the non-crosslinked sodium caseinate in the presence of NaCl.

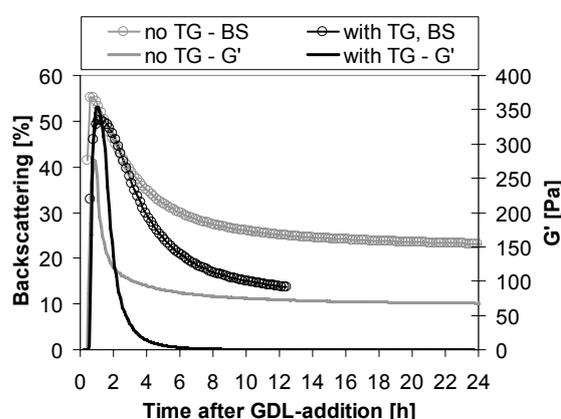


Figure 3. Backscattering as function of acidification time for non-crosslinked and TG-crosslinked sodium caseinate solution during gel formation and breakdown.

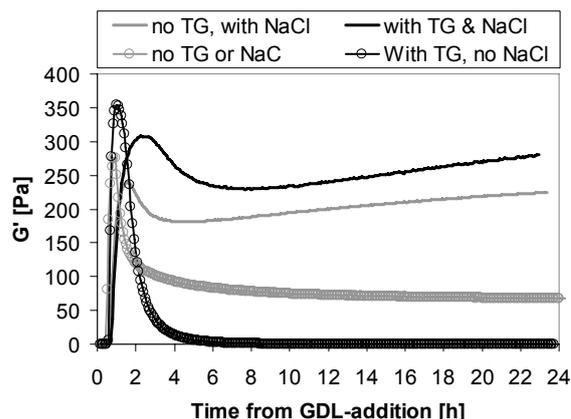


Figure 4. Effect of TG-induced crosslinking and NaCl (100 mM) on gel formation and breakdown of a sodium caseinate solution during chemical acidification with GDL.

CONCLUSIONS

The solubility of sodium caseinate in the pH range 3.3 to 7 can be improved by TG-induced crosslinking. Upon acidification, a delay is observed in the gel formation of the crosslinked sodium caseinate, which indicates that the crosslinked sodium caseinate stays soluble down to a lower pH than the non-crosslinked caseinate. Upon further

acidification the gel formed with the crosslinked sodium caseinate breaks down sooner than the one formed from non-crosslinked caseinate, which means that the crosslinked caseinate becomes soluble again at a higher pH than the non-crosslinked one. The solubility changes radically in the presence of 100 mM NaCl. Both caseinates show a gel structure, i.e. are insoluble to a certain extent in the pH range 3-5.

The observed effects of transglutaminase-induced crosslinking and NaCl on solubility of sodium caseinate in the pH range 3-7 may be of importance for the digestibility of the protein in the human gastrointestinal tract.

ACKNOWLEDGMENTS

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