Cross-Linking Affects Rearrangements in Acid Casein Gels: Evaluation of Acidification Conditions

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ABSTRACT

Acid casein was used as a simplified substrate for cross-linking experiments with microbial transglutaminase and subsequent acid-induced gelation. In solution, casein molecules were associated as particles with radii of approx. 11 nm that were probably internally cross-linked by the enzyme. This strongly affected both the gelation behaviour of casein and the properties of the final gels, which were also affected by acidification temperature. Highest gel stiffness was reached at an acidification temperature of 30 °C for casein cross-linked for 4 – 6 h.

INTRODUCTION

Microbial transglutaminase (mTGase) cross-links proteins between glutamine and lysine residues. It shows a high potential for application in the food industry, e. g. for enhancing texture and physical properties of acidified dairy products. The positive effects of enzymatic cross-linking on gel properties are reported in literature[1]. However, in case of a prolonged incubation time[2] or a high acidification rate[3], cross-linking of caseins may lead to lower stiffness of acid-induced gels.

The aim of our research is to shed more light on the molecular and microstructural phenomena causing the distinct gelation behaviour of cross-linked casein by using acid casein as a simplified substrate.

MATERIAL AND METHODS

Substrate

Acid casein (Sigma-Aldrich GmbH, Germany; or self-made according to IDF-Standard 114[4]) was dissolved in 0.1 mol L^{-1} phosphate buffer (pH 6.8) at 27 g kg^{-1} and stirred overnight at room temperature. Sodium azide (0.3 g kg^{-1}) was used to prevent microbial growth. MTGase (Activa MP, Ajinomoto Foods Europe SAS, France) was added in a concentration of 3 U per g casein and samples were incubated at 40 °C for defined periods of time. Enzyme inactivation was achieved by heat treatment (85 °C/10 min) and subsequent cooling in ice water. A reference (without enzyme) was treated the same way and is referred to as 0 h.

Dynamic Light Scattering

Particle size measurements with dynamic light scattering were carried out using DynaPro NanoStar (Wyatt Technology Europe GmbH, Germany). Casein solutions were filtered through 0.45 µm pore size cellulose filter, transferred to microcuvettes (Wyatt Technology Europe

GmbH, Germany) and subjected to laser light with a wavelength of λ = 663 nm. Scattering intensity was measured at an angle of 90° for acquisition times of 250 s. Temperature was controlled by a Peltier element and set to 40 °C, matching the temperature used for incubation with mTGase. The hydrodynamic radius was determined and the rayleigh spheres model was applied to obtain the mass weighted particle size distribution.

Gelation Experiments

Acid-induced gelation was monitored using a strain-controlled ARES RFS3 rheometer (TA Instruments, Germany) with a concentric cylinder geometry (d_i = 32 mm; d_o = 34 mm; h = 33.5 mm). The temperature equilibrated samples were mixed with 35, 40 or 45 g kg^{-1} glucono-δ-lactone as acidulant and immediately transferred into the rheometer. The storage modulus G' was recorded at a frequency of ω = 1.0 rad s^{-1} and a deformation of γ = 0.003; gelation temperature was kept constant at 20, 30 or 40 °C by a computer controlled circulator. From the curves, G'_{max} was extracted as an indicator of maximum gel stiffness. All results shown are mean values of duplicate measurements.

RESULTS AND DISCUSSION

The gelation behaviour of the self-made casein cross-linked by mTGase for 0, 3 or 24 h is shown in Fig. 1. The results are in line to those reported previously for commercial acid casein[2,5]: there is an increase of gel stiffness after moderate cross-linking (up to 3 h), that is counteracted again when incubation with mTGase was prolonged. Additionally, differences occurred between the samples concerning the decrease of G' after reaching the maximum. This phenomenon is frequently referred to as overacidification and can be attributed to the increase in electrostatic repulsion[6] and to the release of κ-casein

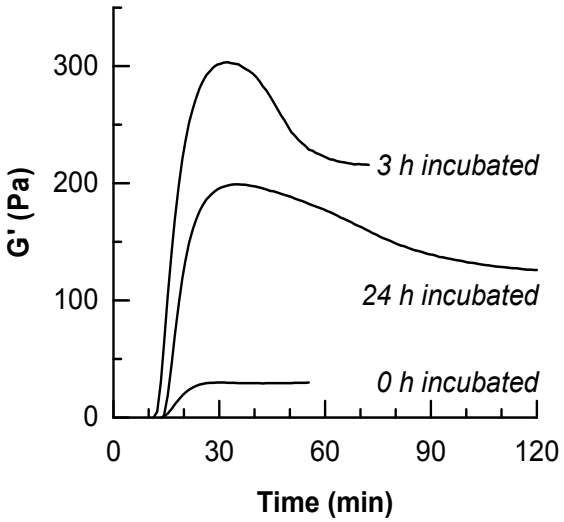

Figure 1. Acid-induced gelation (30 °C, 40 g kg^{-1} glucono-δ-lactone) of self-made acid casein treated with microbial transglutaminase (3 U per g casein, 40 °C) for distinct times.

molecules from the gel network[7] when pH decreases below the isoelectric point (PI) of casein. Because pH development was not affected by mTGase treatment (results not shown), these differences indicate the influence of cross-linking on possible rearrangements of the caseins within the gel network. In the 0 h sample, where G' remained constant below the PI, casein monomers can easily rearrange to achieve an optimal network conformation, where hydrophobic bonds dominate over electrostatic repulsion forces. Furthermore, the release of κ-casein is probably also hindered because of the highly attractive hydrophobic environment. In mTGase treated samples, these rearrangements seem to be affected, allowing electrostatic repulsion to overcome hydrophobic interactions. This causes a release of casein molecules to the serum phase, which results in a decrease of G' after its maximum was reached. Interestingly, the kinetic of this decrease was also affected by incubation time; after excessive cross-linking for 24 h, the decrease was much slower compared to the 3 h sample, where still some monomers and small polymers are left, that could easily

be released from the network. In contrast to that, large and highly cross-linked polymers are present in the 24 h sample which need higher electrostatic repulsion at lower pH to be released from the network.

The increase in maximum gel stiffness after moderate cross-linking was recently linked to the total amount of covalent isopeptide bonds formed[8]. It was proposed that lower stiffness after prolonged incubation is caused by the presence of large casein polymers created from individual casein monomers, that act as a steric hindrance and thus inhibit the formation of a proper gel network[2,8]. However, current investigations of the casein solutions by dynamic light scattering revealed that a) casein appears not as monomers but as small particles with mean radii of approx. 11 nm, and b) particle size increases only marginally upon enzymatic cross-linking up to 24 h (Fig. 2).

The patterns of reducing SDS-PAGE of the samples are comparable to those presented by Jaros et al.[2] (results not shown), meaning that a polymerisation of

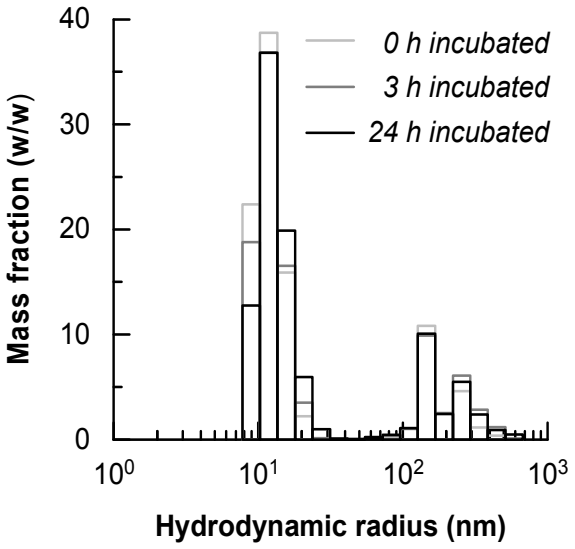

Figure 2. Particle size distribution of self-made acid casein incubated with microbial transglutaminase (3 U per g casein, 40 °C) for distinct times as obtained from dynamic light scattering at 40 °C.

the caseins because of intermolecular cross-linking occurred. But in combination with the results from "native" dynamic light scattering it can be proposed that cross-links are predominantly formed within distinct casein particles. This is already known for casein micelles[9], and for micellar assemblies of pure β- and κ-casein[10], but it has not been reported for mixed casein systems like acid casein or sodium caseinate yet. Determination of the particle size distribution uncovered the presence of large aggregates with radii between 100 and 500 nm, which had been also found by others (e. g. HadjSodok et al.[11]). The nature of these particles is still not clear[12].

Cross-linking within the particles probably leads to a fixation of casein molecules within their particle conformation, thus reducing their ability to reorganise upon temperature and pH changes and hence altering their gelation behaviour. Therefore, a detailed evaluation of the effects of acidification conditions was carried out with commercial acid casein. Increasing temperature or glucono-δ-lactone concentration increases the acidification rate through accelerating the hydrolysis reaction or releasing more gluconic acid per time, respectively[13]. However, temperature has an additional effect on noncovalent interactions between the casein molecules. From Fig. 3a it is evident that acidification rate had almost no impact on stiffness of gels from casein solutions incubated for 0 – 2 h. After longer incubation, higher stiffness was reached when acidification was slower, which is in line with previous results[2,5]. However, it seems that there was rather an effect on the absolute value of G'_{max} than an alteration of the qualitative interrelation between gel stiffness and incubation time. In contrast, gelation temperature strongly affected the gelation behaviour of the samples (Fig. 3b). This suggests that acidification rate itself plays a minor role for gel stiffness. For gelation at 40 °C, which corresponds to the incubation temperature,

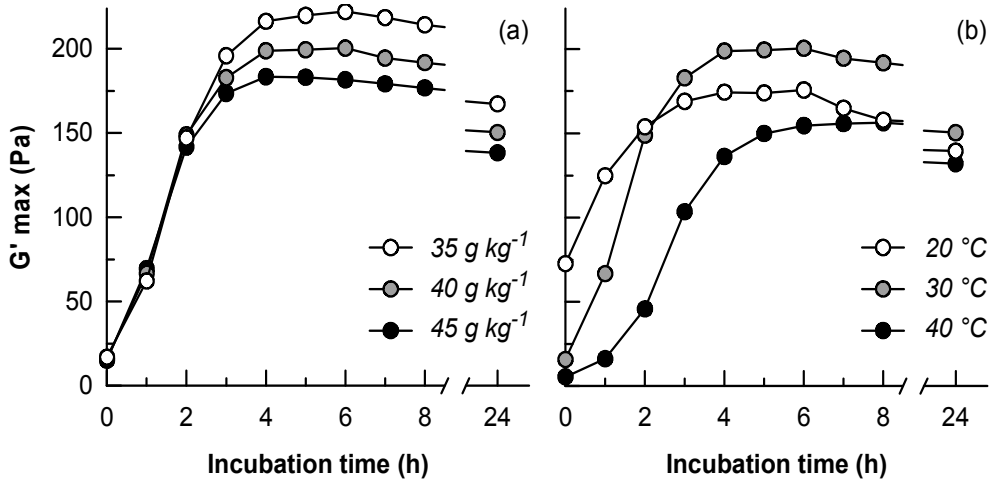

Figure 3. Maximum stiffness (G'_{max}) of acid-induced gels from commercial acid casein incubated with microbial transglutaminase (3 U per g casein, 40 °C) for different periods of time. (a) glucono-δ-lactone concentration was varied at 30 °C; (b) gelation temperature was varied at 40 g kg^{-1} glucono-δ-lactone.

there was a sigmoidal increase of gel stiffness with incubation up to 8 h. Decreasing the temperature to 30 °C led to an increase of gel stiffness with a maximum after 4 – 6 h of incubation. At 20 °C stiffness of gels from 0 – 2 h cross-linked caseins further increased, but stiffness of gels from 3 – 8 h incubated samples decreased. HadjSadok et al.[11] reported for casein solutions that decreasing the temperature reduces the number of casein monomers per particle, resulting in a higher number of particles within the sample. In the case of cross-linked casein, the molecules may be partially fixed within each particle, thus allowing only monomers and smaller aggregates to dissociate when the temperature is decreased to 30 °C. These smaller particles could then act as fillers during gel formation and thus strengthen the network further. This might also happen in gelation experiments at 20 °C, but at this temperature hydrophobic interactions are probably not strong enough to result in high gel stiffness. However, moderately cross-linked casein particles, as they are present in samples incubated for up to 2 h are possibly still capable of dissociation upon cooling, leading to a higher number of particles and hence to a larger contact area, which could form a dense and strong gel network even at 20 °C. Gels from extensively cross-linked samples (24 h) showed the same temperature dependency as moderately treated ones, but to a lesser extent, suggesting that they are less sensitive to temperature changes.

CONCLUSIONS

It has been shown, that acid casein forms particles in solution, which seem to be mainly internally cross-linked by microbial transglutaminase. This would strongly affect the ability of the particles to reorganise, especially upon temperature changes. The results clearly demonstrate that temperature plays an outstanding role for acid-induced gelation, which is probably because of the restriction of temperature dependent dissociation phenomena and the balance between hydrophobic bonding and electrostatic repulsion. However, further investigations of the dynamic behaviour of differently cross-linked casein particles using dynamic light scattering and other techniques are still under progress.

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