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Transglutaminase cross-linking kinetics of sodium caseinate is changed after emulsification

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ABSTRACT

Enzymatic cross-linking is an important method of modifying the structure of food products to control their texture and stability. In this paper we look at the effect that adsorption to the oil–water interface of triglyceride oil-in-water emulsion has on rates of cross-linking of sodium caseinate by microbial transglutaminase. The kinetics of cross-linking has also been assessed for the individual casein proteins within the caseinate. In solution the rates were α_{s2}-casein > β-casein > α_{s1}-casein > κ-casein. This order is not as expected given the rheomorphic nature of the proteins and the number of glutamine and lysine residues in each protein. In particular, the α_{s1}-casein was cross-linked much more slowly than expected. When sodium caseinate was adsorbed to an emulsion the rates for all constituent caseins were decreased but the cross-linking rate for α_{s1}-casein was markedly reduced, indicating the most significant change in accessibility following adsorption. This knowledge will facilitate optimal production of cross-linked emulsions for use in future studies aimed at engineering emulsions with improved nutritional quality.

1. Introduction

Foods are multi-component materials with complex structures and textures that provide the sensory properties desired by consumers. A major task of modern food technology is to generate new engineered structures that have acceptable characteristics from a limited range of "natural" ingredients (Blanshard & Mitchell, 1988). Proteins are one of the main building blocks that are available to food technologist for conferring semisolid textural attributes, and the cross-linking and aggregation of protein molecules into three-dimensional gels is one of the most important mechanisms for developing structures with desirable mechanical properties (Foegeding, 2005). Among the many traditional food textures that are based predominantly on protein gels are those of cheese, yoghurt, sausage and surimi (fish meat gel). The main processing techniques that are currently used to make milk protein gels are enzyme action (renneting), acidification and heating (van Vliet, Lakemond, & Visschers, 2004).

There are a number of enzymes that are used in the formation of protein structures. Their use generally falls into two categories; those that cleave proteins such as the chymosin (rennin) used in cheese production, which hydrolyses the peptide bond between phenylalanine and methionine in κ-casein; and those that catalyse the formation of covalent bonds between peptides, such as transglutaminase or tyrosinase. For example, in milk products, transglutaminase has been used to improve the texture in yoghurt and ice cream by increasing the breaking strength in low and full fat yoghurt (Jaros, Partschefeld, Henle, & Rohm, 2006). In addition laccase has been used to modify the gel structure of myofibrillar systems to improve thermal stability (Lantto, Puolanne, Kalkkinen, Buchert, & Autio, 2005).

The enzyme transglutaminase catalyses the acyl-transfer reaction between the γ-carboxamide group of peptide-bound glutamine and the ε-amino group of peptide-bound lysine, resulting in a ε-(γ-glutamyl)lysine isopeptide bond (Griffin, Casadio, & Bergamini, 2002). The rate of cross-linking by transglutaminase is dependent on the macromolecular structure of each protein substrate. Reactive glutamine residues tend to reside in flexible regions of the polypeptide chains, so the flexible caseins are therefore good substrates (Huppertz & De Kruif, 2007; Liu & Damodaran, 1999; Roos, Lorenzen, Sick, Schrezenmeir, & Schlimme, 2003; Vasbinder, Rollema, Bot, & De Kruif, 2003). In contrast, the more rigid and compact globular whey proteins cannot be cross-linked in their native state (Han & Damodaran,

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1996), although after heat or chemical denaturing, which increases molecular flexibility, they become a suitable substrate (Eissa, Bisram, & Khan, 2004; Murray & Faergemand, 1998; O'Sullivan, Kelly, & Fox, 2002; Tang & Ma, 2007).

The effectiveness of transglutaminase-catalysed covalent cross-linking to enhance functional properties is well known (Dickinson, 1997; Dickinson, Ritzoulis, Yamamoto, & Logan, 1999). Cross-linking β-casein has been shown to improve emulsion stability (Liu & Damodaran, 1999) and it has been postulated that in heat-treated milk the thermal unfolding of whey proteins leads to them being cross-linked to caseins, resulting in milk that is more stable to heat coagulation (O'Sullivan et al., 2002). However, in these studies the effect of the interfacial protein adsorption onto the lipid droplets on the rates of cross-linking was not investigated. As mentioned above, the rate of reaction will depend on the substrate structure and there are significant differences in the primary structures of the four bovine casein species that will affect the rates of cross-linking. Indeed, the difference in cross-linking rates of β and α_{s1} caseins has already been investigated (Menendez, Schwarzenbolz, Partschefeld, & Henle, 2009). Thus, the number of lysine residues in β-, α_{s1}-, α_{s2}- and κ-casein are 11, 14, 24 and 9 respectively, while the number of glutamines is 21 in β-casein and 15 in each of the three other caseins (Swaisgood, 1982). The secondary and tertiary structures of the four proteins are difficult to assess because of their flexible nature. However, they all have a certain amount of organised structure (especially κ-casein: 31% β-sheet, 23% α-helix; (Kinsella, Whitehead, Brady, & Bringe, 1982)), and κ- and α_{s2}-casein have one disulphide bond. In addition, κ-casein has only one phosphoserine (S149) and carbohydrate moieties attached to threonine residues. As a result it does not tend to bind calcium and behaves rather differently to the other caseins in terms of its association. The food ingredient sodium caseinate contains the four main caseins; β-, α_{s1}-, α_{s2}- and κ- in the ratios 3:4:1:1, respectively (Farrell et al., 2004). Both β-casein and α_{s1}-casein are good emulsifiers but the steric stability provided by the highly phosphorylated β-casein (Dickinson, 1999) with its charged N-terminal region produces more stable emulsions. The κ-casein is the smallest of the caseins and only half of the molecule has the sort of rheomorphic structure that so typifies the other caseins.

In this paper we assess the effect that presenting the protein in an adsorbed form has on the rate of cross-linking catalysed by transglutaminase. By using a food grade protein ingredient (sodium caseinate) we have also been able to assess whether the constituent proteins are involved in the cross-linking to a greater or lesser extent.

2. Materials and methods

2.1. Materials

Food-grade sodium caseinate (Na-Cas; 90% protein) was obtained from DMV International (The Netherlands). The individual β-, α- and κ-caseins were obtained as ≥98%, approx. 85% and ≥80% pure according to the supplier (Sigma, Poole, UK; C6905, C6780 and C0406, respectively,). Microbial transglutaminase (TG) was fractionated free of maltodextrin from the commercial TG product Activa WM (Vesantti Oy, Helsinki, Finland) by cation-exchange chromatography as outlined previously in Lantto et al. (2005), using sodium acetate buffer as an eluent (30–500 mM gradient, pH 5.5). The enzyme activity was assayed according to Folk (1970) using 0.03 M N-carbobenzoxy-L-glutaminylglycine and hydroxylamine as substrates at pH 6.0. The measured activity of the TG stock solution was 11,000 nkat/mL (1 nkat is defined as the amount of enzyme activity that converts 1 nmol of the substrate per second in the assay conditions (Lantto et al., 2005)). The solution was stored at −20 °C prior to use. N-ethylmaleimide used as a TG inhibitor (Josten, Meusel, & Spener, 1998) was purchased from Sigma. Medium-chain triglyceride oil (MCT, Miglyol 812S) was obtained from Sasol GmbH (Germany). The fatty acid composition of the oil was: 59.1% C8:0; 39.8% C10:0; 0.6% C12:0 and 0.5% comprising C6:0, C16:0 and C18:1. Prior to use, the oil was cleaned with Florisil (Sigma, F9127; 2:1 w/w, 140 rpm, 30 min) to remove any possible surface-active impurities, filtered and stored under nitrogen. The buffers and reagents used in this work were prepared with ultra pure water.

2.2. Na-Cas solutions and Na-Cas stabilized emulsions

Na-Cas was dissolved with gentle stirring at room temperature in phosphate buffer (10 mM NaH_2PO_4, 150 mM NaCl, 0.01% (w/v) NaN_3, pH 6.5) to give a concentration of 1.11, 5.56 or 11.11 mg/mL. These stock solutions were used in cross-linking studies in solution. Three different final concentrations of Na-Cas: 1, 5 and 10 mg/mL were used, as described further in the text. For emulsion preparations, 1.67 and 8.33 mg/mL protein stock solutions were used. The oil-in-water (O/W) emulsions (25% MCT oil, w/w; Na-Cas:oil ratio 5×10^{-3} or 2.5×10^{-2} w/w) were produced by passing a premix of oil and Na-Cas solution through an EmulsiFlex-B3 valve homogenizer (Avestin Inc., Ottawa, Canada). Four or six passes were made at 68.9 kPa for the higher or the lower protein concentration, respectively. The oil droplet size was determined using a LS-230 particle sizer (Beckman Coulter Ltd., High Wycombe, UK). The measurements were made for at least eight replicate emulsions and the results are presented as an average. The proportion of protein adsorbed was determined after centrifuging the emulsion at 13,000 rpm (11,600 × *g*) for 10 min and assaying the amount of non-adsorbed protein in the clear subnatant formed below the emulsion cream layer. The subnatant was carefully removed using a syringe and needle, and the concentration of non-adsorbed protein was determined by the OPA method (Church, Swaisgood, Porter, & Catignani, 1983). The OPA reagent was prepared daily by combining the following reagents and diluting to a final volume of 100 mL with water: 3.81 g sodium tetraborate (BDH Chemicals Ltd, Poole, UK), 100 mg SDS (Sigma), 80 mg OPA (Sigma; dissolved in 2 mL ethanol) and 88 mg DTT (Amersham Biosciences, Uppsala, Sweden). The assay involved adding a small aliquot (100 μL) of the emulsion subnatant to 3 mL of the OPA reagent. The solution was thoroughly mixed and incubated for 3 min at room temperature. The absorbance was measured at 340 nm. The amount of the adsorbed protein in the emulsion was calculated by subtracting the measured amount of non-adsorbed protein in the subnatant from the original total amount of protein in the emulsion. After dilution, the emulsions were used for the experiments on cross-linking with TG. Table 1 shows basic characteristics of the emulsions.

2.3. Enzymatic cross-linking of Na-Cas

Na-Cas solutions and Na-Cas-stabilized emulsions were diluted with the phosphate buffer and after 10-min incubation at 40 °C aliquots of TG solution (prepared in the phosphate buffer) were

Table 1
Characteristics of the Na-Cas stabilized emulsions.

$C_{Na\text{-}Cas}$ (mg/mL)	$d_{(4,3)}$ (μm)	$d_{(3,2)}$ (μm)	SSA (cm^2/mL)	% Adsorption[a]
1.0	5.71 ± 0.38	3.78 ± 0.20	12729 ± 575	95.6 ± 0.5
5.0	3.69 ± 0.17	2.95 ± 0.18	16308 ± 658	37.2 ± 1.2

$d_{(4,3)}$, volume mean droplet diameter; $d_{(3,2)}$, surface area mean droplet diameter; SSA, specific surface area.
[a] Based on the OPA method.