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Rheology and structure of mixtures of ι-carrageenan and sodium caseinate

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ABSTRACT

Mixtures of ι-carrageenan (IC) and sodium caseinate (SC) were investigated and the results are compared with a similar study of mixtures of κ-carrageenan (KC) and SC. Segregative phase separation was observed at high biopolymer concentrations and the binodal was determined. At low IC concentrations, SC formed aggregates involving a very small amount of IC that were characterized with light scattering. The influence of adding SC on the gelation of IC during cooling and the shear modulus of the gels was studied in the presence of NaCl or KCl. The main conclusion of this work is that SC binds to both IC and KC, in the coil conformation as well as in the helix conformation, but that its effect on the rheology is much weaker for IC than for KC.

1. Introduction

Carrageenan is a food grade polysaccharide derived from algae and is used as a texturing agent in processed food among which dairy products (Piculell, 2006; Therkelsen, 1993). For this reason the influence of adding carrageenan on the behavior of aqueous solutions of milk proteins has been widely investigated. There exists a variety of carrageenans with slightly different structures, the most common of which are κ- and ι-carrageenan. The difference between these variants is that κ-carrageenan (KC) contains one sulfate groups per sugar unit and ι-carrageenan (IC) two. Both variants show a coil–helix transition when the temperature is reduced below a critical temperature (T_c). T_c decreases with increasing ionic strength, and for KC also depends strongly on the type of salt (Rochas & Rinaudo, 1980). For pure IC, T_c depends on the valence, but little on the type of ion (Piculell, Nilsson, & Muhrbeck, 1992). In the helix conformation the carrageenan chains have a tendency to aggregate and gel. The elastic modulus of the gel increases with increasing polymer concentration. It depends on the salt concentration and, especially for KC, on the type of salt. The coil–helix transition can be reversed by heating and leads to melting of the gels. The temperature at which this happens (T_h) is most often larger than T_c for KC, but T_c and T_h are almost the same for IC.

Casein is the main protein component of milk and is a mixture of mainly four types of caseins (α_1-, α_2-, β- and κ-casein) (Fox, 2003). In milk it is present in the form of spherical complexes with a radius of approximately 100 nm that are called casein micelles. Casein micelles are stabilized by colloidal calcium phosphate (CCP), which can be removed by precipitation and washing at pH 4. The precipitate can be redissolved in the form of sodium caseinate (SC) by adding NaOH (Mulvihill & Fox, 1989). SC associates into small particles with a hydrodynamic radius (R_h) that depends somewhat on the temperature, the ionic strength and the pH ($R_h = 11$ nm at 20 °C, 0.1 M NaCl and pH 7) (HadjSadok, Pitkowski, Benyahia, Nicolai, & Moulai-Mostefa, 2007).

The effect of adding KC or IC on skimmed milk or casein micelle solutions has been studied quite extensively (Arltoft, Ipsen, Madsen, & De Vries, 2007; Bourriot, Garnier, & Doublier, 1999; Dalgleish & Morris, 1988; Drohan, Tziboula, McNulty, & Horne, 1997; Hemar, Hall, Munro, & Singh, 2002; Ji, Corredig, & Goff, 2008; Langendorff et al., 1999, 2000; Puvanenthiran, Goddard, McKinnon, & Augustin, 2003; Rodd, Davis, Dunstan, Forrest, & Boger, 2000; Schorsch, Jones, & Norton, 2000; Spagnuolo, Dalgleish, Goff, & Morris, 2005; Trckova, Stetina, & Kansky, 2004; Tziboula & Horne, 1998). It appears that both carrageenans have a specific interaction with casein which leads to the formation of weak gels at low carrageenan concentrations at which pure carrageenan solutions remain liquid. On the basis of investigations of mixtures with individual caseins it was concluded that κ-casein binds specifically to the carrageenans (Lynch & Mulvihill, 1994a, 1994b, 1996; Snoeren, Both, & Schmidt, 1976; Snoeren, Payens, Jeunink, & Both, 1975). In comparison, mixtures with sodium caseinate have attracted relatively little attention in the past (Lynch & Mulvihill, 1994a, 1994b, 1996; Oakenfull, Miyoshi, Nishinari, & Scott, 1999).

Recently, we reported on a study of the structure and the rheology of mixtures of KC and SC at pH 7 (Nono, Lalouette, Durand, & Nicolai, 2011; Nono, Nicolai, & Durand, 2011). For KC in the coil conformation, macroscopic phase separation was observed at higher polymer concentrations between a phase rich in protein and a phase

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rich in polysaccharides. In homogeneous mixtures caseinate aggregates were formed that contained a very small amount of KC. With increasing KC concentration an increasing fraction of these aggregates formed micron size particles. These particles could be observed with confocal laser scanning microscopy (CLSM) and had a tendency to associate into large flocs that precipitated. The fraction of proteins that precipitated increased with increasing KC concentration, but was independent of the SC concentration. It increased strongly with decreasing pH and at pH 5.0 almost all proteins precipitated in 6 g/L KC. Weak gels formed by KC in the helix conformation were strongly reinforced by the presence of SC. It was concluded that a mixed network was formed in the mixtures with both pure KC junctions and bonds that involved KC as well as SC.

Here we complement this investigation of KC/SC mixtures with a study of IC/SC mixtures and we compare the results obtained with the two types of carrageenan. We will show that even though SC binds to both KC and IC, the effect of SC on the rheology is much weaker for IC than for KC.

2. Materials and methods

2.1. Materials

The sodium caseinate powder, provided by DMV International (Veghel, Netherlands), was dissolved in deionized water (Millipore) containing 3 mM sodium azide as a bacteriostatic agent. The sample contained 1.5 g/100 g Na, 56 mg/100 g Ca and 10 mg/100 g K. The pH was adjusted by slow addition of 0.01 M HCl or 0.01 M NaOH under continuous stirring and was set at 7 unless otherwise indicated. About 100 mL solution was dialyzed against about 4 L of solvent for a period of 24 h renewing the solvent several times. The solution was then centrifuged (Beckman Coulter, Allegra 64R Centrifuge) at $6 \times 10^4 g$ for 2 h after which both a sediment and a turbid top layer appeared. After removal of the turbid layer, the clear supernatant was collected and filtered through 0.45 μm pore size filters. The SC concentration (C_{SC}) was determined after filtration using UV absorption with an extinction coefficient of 0.81 L/g cm. The amount of protein that was eliminated by this purification method was less than 5%.

The sodium ι-carrageenan used for this study was an alkali treated extract from *Eucheuma cottonii* and was kindly provided by Cargill (Baupte, France). The sample contained 5.5 g/100 g Na, 56 mg/100 g Ca and 300 mg/100 g K. NMR showed that it contained about 5% KC. The IC powder was dissolved in Milli-Q water containing 3 mM sodium azide by stirring a few hours at 70 °C. The solution was extensively dialyzed against the solvent at pH7 and subsequently filtered through 0.45 μm pore size Anatope filters. The concentration (C_{IC}) was determined by measuring the refractive index using refractive index increment 0.145 g/mL.

2.2. Rheology

Rheology oscillatory shear measurements were done using a stress controlled rheometer (AR2000, TA Instruments). Both parallel plates and cone and plate geometries were used. The applied stress was varied and the results shown here were obtained with low enough stresses so that the response was linear. After loading the sample the geometry was covered with a thin layer of mineral oil in order to avoid evaporation. The system was presheared in the liquid state at 80 °C at a shear rate of 100 s^{-1} for a few minutes.

2.3. Confocal scanning laser microscopy

Confocal scanning laser microscopy was used in the fluorescence mode. Observations were made with a Leica TCS-SP2 (Leica Microsystems Heidelberg, Germany). A water immersion objective lens was used (HCx PL APO 63× NA = 1.2) with theoretical resolution 0.3 μm in the x–y plane. SC was labeled with the fluorochrome rhodamine B isothiocyanate (Rho), by adding a small amount of a concentrated rhodamine solution to the SC solutions before heat treatment. Rho was exited using a helium–neon laser with wavelength 543 nm and the fluorescence was detected with a photomultiplier.

2.4. Light scattering

Light scattering measurements were done using commercial static and dynamic light scattering equipment (ALV-Langen, Germany) equipped with an He–Ne laser emitting vertically polarized light at $\lambda = 632$ nm. The temperature was set at 20 °C and controlled by a thermo-stat bath to within ±0.1 °C. Measurements were made at angles of observation (θ) between 12 and 150 degrees. The relative excess scattering intensity (I_r) was calculated as the intensity minus the solvent scattering divided by the scattering intensity of toluene. In dilute solutions I_r is related to the weight average molar mass (M_w) and the structure factor ($S(q)$) of the solute (Brown, 1996; Nicolai, 2007):

$$I_r = KCM_wS(q) \tag{1}$$

with K a constant that depends on the refractive index increment of the solute. $S(q)$ is a function of the scattering wave vector: $q = 4\pi n \cdot \sin(\theta/2)/\lambda$, with n the refractive index of the solution.

3. Results

3.1. Pure IC solutions

The gelation of pure IC has already been investigated before (Hossain, Miyanaga, Maeda, & Nemoto, 2001; Piculell, 2006) and here we only show a few measurements that are pertinent for the present investigation on the effect of adding SC. It was shown elsewhere that gelation of KC can be very slow close to T_c (Meunier, Nicolai, Durand, & Parker, 1999) and it was found here that gelation of IC is even slower than that of KC at the same value of $T_c - T$. This is shown for a solution of 2 g/L IC at 0.1 M NaCl in Fig. 1a where G' at 1 Hz is plotted as a function of time after cooling the system to different temperatures starting from 80 °C. This system gelled between 40 and 35 °C, but it took considerable time to approach the steady state values of G' and G'' even at 20 °C. We note that true steady state was not observed at any temperature within a period of 1.5 h, but the evolution was very slow after this delay unless T was within a few degrees of T_c. Equilibration during heating was also slow. Fig. 1b shows the evolution of G' after heating the system in steps starting from 2 °C. The gel fully disintegrated between 35 and 40 °C.

The implication is that for IC cooling and heating ramps need to be done at prohibitively slow rates in order to be representative of the steady state. For KC, results obtained at a rate of 1 °C/min were the same as those obtained at even slower rates for $(T_c - T) > 3$ °C, but for IC this was not the case and therefore we opted for a different approach. We rapidly cooled the system from 80 °C to a given temperature or heated it from 2 °C. Subsequently, we let the sample evolve for 90 min as is illustrated in Fig. 1. For this particular system a slow increase of G' was observed during cooling at 35 °C, while a gel formed at 2 °C melted within a few minutes at 40 °C.

The frequency (f) dependence of G' and G'' was determined 90 min after heating or cooling. G' was independent of f and larger than G'' indicative of gels, except very close to T_c where the systems behaved like viscoelastic liquids. Thus, except close to T_c, G' obtained at 1 Hz may be taken as the elastic modulus of the gels.