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Specific effect of the linear charge density of the acid polysaccharide on thermal aggregation/disaggregation processes in complex carrageenan/lysozyme systems

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ABSTRACT: We study thermal aggregation and disaggregation processes in complex carrageenan/lysozyme systems with a different linear charge density of the sulphated polysaccharide. To this end, we determine the temperature dependency of the turbidity and the intensity size distribution functions in complex kappa-carrageenan/lysozyme (kCG/lys) and lambda-carrageenan/lysozyme (ICG/lys) systems. We demonstrate that increasing the temperature up to 80°C in complex kCG/lys systems results in a monotonous decrease of the turbidity and the average sizes of the complex particles. On the contrary, for ICG/lys systems these values considerably increase with temperature. This suggests that disruption of the intermacromolecular hydrogen bonds at high temperature is mainly responsible for the temperature induced disaggregation in kCG/lys systems, whereas enhancement of the hydrophobic forces in ICG/lys systems at high temperature is responsible for the intensification of its aggregation. SEM images of these systems confirm the strong difference in microstructure. DSC data also show that the temperature induced disaggregation in kCG/lys systems is in disagreement with a decreased thermal stability of lys in the presence of kCG.

Key words: Lysozyme, carrageenan, protein aggregation, protein complexes, structure.

1. Introduction

Protein aggregation plays a key role in the regulation of texture of food products (Zhou, Liu, & Labuza, 2008), reviewed in Doi, 1993). It can also entail several disadvantages such as limited shelf life, undesired immunogenic responses or loss of functionality in medicines, functional foods and cosmetic products (Blumlein and McManus 2013; De Groot and Scott 2007; Weiss, Young and Roberts 2009). Therefore, protein aggregation is a major topic in the field of food and health

science. Aggregates can either form directly after preparation, with their characteristics depending on the processing conditions including thermal treatments, but also storage can induce further aggregation (Promeyrat, Bax, Traore, Abry, Sante-Lhoutellier, & Gatellier, 2010); Sante-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008). Protein aggregates can vary widely in size and morphology as a result of the experimental conditions under which they were formed. For example, it has been shown that at pH values far away from the isoelectric point, amylin (Patil, Mehta, Jha, & Alexandrescu, 2011), hen egg white lysozyme (Amaudov & de Vries, 2005), and β -lactoglobulin (Arnaudov, de Vries, Ippel, & van Mierlo, 2003); Veerman, Ruis, Sagis, van der Linden, 2002) form long fibrillar aggregates. Near the isoelectric pH (Amaudov & de Vries, 2005), or at high salt concentrations (Amaudov & de Vries, 2006; Veerman, et al. 2002), spherical or amorphous aggregates are formed. Aggregate morphologies cannot always be categorized as either fibrillary or amorphous. Various morphologies can be formed depending on the maximum encountered temperature, heating rate and other factors (Bromley, Krebs, & Donald, 2006). It is also clear that net charge plays a major role in determining the aggregate morphology (Krebs, Devlin, & Donald, (2009).; Langton & Hermansson, 1992). Exposed hydrophobicity (Calamai, Taddei, Stefani, Ramponi, & Chiti, 2003) and formation of disulfide bonds naturally affect the aggregation and gelation propensity of proteins as these two forces are primarily driving the assembly process.

Complexation of proteins with natural and synthetic polyelectrolytes at room temperature can significantly affect aggregation of proteins within the complex (Tolstoguzov, 1998) due to changes of the net charge as well as the conformation and hydrophobicity of the protein within the complex. Depending on the structure of the interacting (bio)polymers, the composition of the system and the solvent, as well as the mixing conditions, complexation in such systems can lead to formation of large aggregates with various morphologies, or suppression of aggregation and increase in solubility of the complexes.

Despite the importance of proteins in food technology, thermal aggregation of food proteins within complexes with polysaccharides has received limited attention. Our recent work (Antonov, Zhuravleva, Ruth, Moldenaers, 2015) on complexation of dextran sulphate with lysozyme (lys) showed a spectacular stabilization of lys within the complexes against thermal aggregation at a high dextran sulphate/lys weight ratio (q) equal to 5.0 whereas a weak enhancement of the thermal aggregation of lysozyme within the complex occurred at $q=0.3$, corresponding to maximal binding

in the system. The thermal aggregation of β -lactoglobulin and dextran sulfate was studied by Vardhanabhuti, Yucel, Coupland and Foegeding (2009). However, this study was performed for concentrated systems, for which the concentrations were in the region of segregative phase separation.

In the present study we examine the effect of the linear charge density of the acid polysaccharide on the direction and intensity of thermal aggregation and disaggregation processes in complex polysaccharide/protein systems consisting of carrageenan and lysozyme. To this aim, we have chosen lysozyme and carrageenan concentrations low enough to be far from the region of phase separation. This allows us to single out information on the initial interaction and aggregation processes between the two types of macromolecules, well-separated from the aggregation process induced by association of the single-type macromolecules. We determine the temperature dependencies of the turbidity and the intensity size distribution functions in complex kappa-carrageenan/lys (kCG/lys) and lambda-carrageenan/lys (ICG/lys) systems, containing sulphated polysaccharides that differ in linear charge density. kCG has only one negative charge per disaccharide unit and ICG bears on average 2.7 charges per disaccharide unit. SEM images of these systems allowed to visualize the differences in structure.

2. Experimental section

2.1. Materials.

2.1.1. Chemicals. Lys from chicken egg white (dialyzed, lyophilized powder) was purchased from Sigma-Aldrich and used without further purification. The kCG sample (382 sulphated groups per mole of kCG, 94% dry weight, 17.03% ash, 5.28% Na⁺; 0.33% K⁺, 0.006% Ca⁺⁺, 0.009% Mg⁺⁺, sulphate content $\cong$ 1.0 eq. H⁺/mol dissacharide, Cl⁻ not detected; dn/dc = 0.115 cm³/g; M_w = 567 kD; M_n = 356 kDa in 0.1 M LiNO₃ at 60°C) was supplied by Sanofi Bio-Industrie (France). The ICG sample (1000 sulphated groups per mole ICG) was a commercial product from Sigma. The viscosity average molecular weight of the ICG sample (520 kDa $\pm$ 50) was estimated from the intrinsic viscosity in 0.1 M NaCl solution, using the Mark-Houwink parameters given by Rochas, Rinaudo, and Landry (1990):

$$[\eta] = 3.1 \times 10^{-5} \times M^{0.95} \text{ cm}^3 \text{ g}^{-1} \quad (1)$$

Milli-Q ultrapure water was used throughout the experiments.

2.1.2. *Preparation of the binary solutions and carrageenan/protein mixtures.* Lys solutions were prepared by dispersing the protein in a mono/bisphosphate ($\text{NaH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$) buffer with $\text{pH}=7.0$ and $I=0.01$ under stirring for 1 h at 23°C . The resulting solutions of lys were centrifuged at $50,000g$ and 23°C for 1 h to remove insoluble particles. The lysozyme content in the stock solution was determined by means of UV absorption using the extinction coefficient for highly purified lysozyme which is $2.64 \text{ ml mg}^{-1} \text{ cm}^{-1}$ at 281.5 nm in 0.1 M potassium chloride (Aune, & Tanford, 1969). kCG and ICG stock solutions were prepared by dispersing the gum in mono/bisphosphate ($\text{NaH}_2\text{PO}_4 + \text{Na}_2\text{HPO}_4$) buffer with $I=0.01$, followed by strong stirring for 40 min at room temperature and subsequently 20 min stirring at 85°C . After cooling the kCG or ICG solution to room temperature the pH of the solutions was adjusted using a 0.1 M NaOH solution. Centrifugation to remove insoluble particles was performed in a similar way as for lysozyme. The concentration of carrageenan in the stock solutions was determined by drying at 104°C . The carrageenan concentration in all solutions was sufficiently low to avoid gelation. Solutions of the proteins and polysaccharides were kept at least overnight in the fridge to allow for full hydration of the molecules. To prepare mixed solutions of lys and kCG or ICG with the required concentrations, weighed amounts of the lys stock solution were added to a polysaccharide solution at 23°C , followed by addition of a weighed amount of the phosphate buffer and stirring for 1 h.

2.2. Methods

2.2.1. *Turbidity measurements.* Turbidity values of aqueous Lys solutions and complex kCG/lys and ICG/lys mixtures with a lys concentration of $0.005 \text{ wt}\%$ as a function of temperature were determined at 500 nm using a CARY 300 Bio UV/VIS spectrometer. The lys and complex mixtures were heated inside the spectrometer cell from 20°C to 80°C with steps of either 5° or 10° . All spectra were determined 10 min after attainment of the desired temperature. The error of the absorption measurements is typically about 2% – 3% .

2.2.1. *Dynamic light scattering.* Determination of the intensity-size distribution functions, as well as zeta potentials of lysozyme, kCG, ICG as well as kCG+lys and ICG+lys particles was performed with a Malvern Zetasizer Nano-ZS (ZEN3600) instrument (England), using a rectangular quartz cell with a width of 1 cm . The concentration of lys in the water – lysozyme – carrageenan mixtures was kept constant at $0.0025 \text{ wt}\%$. For each sample the measurement was repeated 7 times. The lys solutions were filtered through DISMIC-25cs (cellulose acetate) filters (hole size of $0.22 \mu\text{m}$)

before the measurements. The carrageenan was centrifuged at 50,000g and 23°C for 1 h to remove insoluble particles. Mixed complex solutions of lys and carrageenan were used without filtration.

2.2.3. Scanning electron microscopy (SEM). Microstructural investigation was performed with a JEOL JSM-840 scanning electron microscope. kCG/lys and ICG/lys mixtures containing 0.01 wt% lys were subjected to complete drying at 40°C. The SEM images were recorded multiple times and on multiple samples to ensure that representative images are presented. Images were captured within 5 min after inserting the sample in the SEM chamber.

2.2.4. High-Sensitivity DSC.

Thermal denaturation of Lys in aqueous solutions in the absence and in the presence of kCG and ICG was monitored with a highly sensitive differential scanning calorimeter (DASM-4 M, Puschino, Russia). Thermograms were obtained between 10°C and 90°C, at a scan rate of 2 °C/min. For all measurements, the pH was 7.0. Degassing during the calorimetric experiments was prevented by application of an additional constant pressure of 172.25 kPa over the liquids in the cells. The data were sampled and processed using the service program WSCAL Version 2.0 based on the principles described by Filimonov, Potekhin, Matveev, & Privalov (1982). Effective (van't Hoff) enthalpy of denaturation was calculated according to (Velicelebi & Sturtevant, 1979):

$$\Delta H_{vH} = 4RT_m^2 \Delta C_m / Q_m \quad (1)$$

where T_m is the thermal midpoint, ΔC_m the peak height (in JK^{-1}), and Q_m the area under the peak (in J). The lys concentration used for DSC was 0.25 wt% for the lys solution and 0.417 wt% for the mixtures with kCG and ICG. This is outside the dilute region, which was required to obtain significant heat signal.

3. Results and discussion

Preliminary experiments showed that lys interacts with kappa and lambda carrageenan at pH 7.0 and ionic strength 0.01, leading to the formation of carrageenan/lys water insoluble complexes with a maximal yield (Y_{Max}) at a carrageenan/lys weight ratio (q_{Max}) = 0.3 wt/wt or $7.56\text{-}8.25 \cdot 10^{-3}$ mole/mole for both relatively weak and highly charged carrageenan (data not presented). Therefore we examine the effect of the net charge of carrageenan on the thermal aggregation and disaggregation processes in the complex systems at two selected carrageenan/lys weight ratios namely $q_{\text{Max}} = 0.3$ wt/wt (or $7.56\text{-}8.25 \cdot 10^{-3}$ mole/mole ratio depending on the type of carrageenan)

corresponding to maximal yield and $q = 5.0$ wt/wt (or 0.126-0.137 mole/mole) corresponding to considerable solubilisation of the water insoluble complexes. First, the temperature dependency of the turbidity at 500 nm (τ_{500}) in complex kappa-carrageenan/lys and lambda-carrageenan/lys systems was determined. The data are presented in Figure 1a,b. The τ_{500} value for 0.04 wt% solutions of Lys, kCG and ICG is $\cong 0.002$ (data not shown), i.e. all the binary biopolymer solutions are optically transparent. Increasing the temperature of a dilute binary lys solution (0.005 wt%) leads to a monotonous increase of the turbidity from 35°C onwards, which is at first limited and becomes more pronounced above 50°C (Fig. 1a). Further increasing the temperature up to 80°C leads to a considerable increase of the turbidity, which is partially irreversible. After keeping the Lys solution 10 min at 80°C, a subsequent decrease of the temperature to 23°C leads to a reduction of the turbidity but the values remain higher than that of the unheated lys solution. The complex kCG/lys(0.005wt%) system at $q=0.3$ wt/wt or $7.56-8.25 \cdot 10^{-3}$ mole/mole and room temperature is characterized by an appreciable τ_{500} value (0.172) due to the presence of water insoluble complexes. Increasing the temperature of this complex system up to 80°C results in a significant monotonous decrease of the turbidity values. In other words, the water insoluble kCG/lys complexes become less aggregated at high temperature. The process of temperature induced disaggregation of the complexes is to a large extent reversible. After cooling the system the τ_{500} values increase again up to ~ 0.15 (data not shown). Whereas initial complex formation in kCG/lys systems is caused by electrostatic interactions between the oppositely charged polyions, subsequent aggregation of the formed complexes is driven by secondary forces. The peculiarities of the thermal behavior of the water insoluble kCG/lys complexes point to the key role of hydrogen bonds in the stabilization of the water insoluble kCG/lys complexes during heating because only hydrogen bonds are depressed with increasing temperature. In the system with $q=5.0$ wt/wt or 0.126-0.137 mole/mole the τ_{500} values at room temperature are close to that determined for the lys solution alone, indicating that the complexes formed are water soluble ones. Increasing the temperature up to 80°C does not cause aggregation of these complexes. These facts allow to conclude that in the case of water soluble kCG/lys complexes, formed with an excess of kCG, the key factor stipulating their insensitivity to thermal aggregation is their high negative charge (~ -23 mV).

FIGURE 1

A completely different thermal behavior was observed for ICG/lys systems containing carrageenan with a large negative charge (Fig. 1b). Similar to the kCG/lys system (at $q=0.3$ wt/wt or $7.56 \cdot 10^{-3}$ mole/mole) the complex ICG/lys system at $q=0.3$ wt/wt or $8.25 \cdot 10^{-3}$ mole/mole and room temperature is characterized by an appreciable τ_{500} value (0.190) due to the formation of water insoluble complexes. However, increasing the temperature leads to a sharp increase of the τ_{500} values, and at temperatures above 65°C , the system becomes unstable and undergoes phase separation. In other words, the water insoluble ICG/Lys complexes become more aggregated at high temperatures. This process of temperature induced aggregation of the complexes is largely irreversible. After cooling the system the turbidity values do not decrease (data not shown). These peculiarities of the thermal behavior of the water insoluble ICG/lys complexes suggest the key role of hydrophobic interactions in the intensification of aggregation of the water insoluble ICG/lys complexes during heating since these interactions increase with temperature. For the system with an excess of ICG (at $q=5.0$ wt/wt or 0.137 mole/mole) the τ_{500} values at room temperature are close to those determined for the lys solution alone, because the complexes formed are mainly water soluble ones. Increasing the temperature up to 80°C leads to a monotonous increase of the aggregation of the system although to a lesser degree than for the system at $q=0.3$ wt/wt or $8.25 \cdot 10^{-3}$ mole/mole. Unexpectedly, the temperature dependence of the turbidity of this system is the same as for a binary lys solution. These facts allow to hypothesize that in the case of water soluble ICG/lys complexes formed in an excess of ICG in the system, the high negative charge of the ICG/lys system at $q=5.0$ wt/wt or 0.137 mole/mole interferes with the hydrophobic interactions when increasing the temperature.

FIGURE 2

To obtain information about the sizes of carrageenan and the complex carrageenan/lys particles at different temperatures, DLS measurements of the same systems were performed. The scattering intensity size distribution functions for kCG (0.005-0.03wt%) and ICG (0.005-0.03 wt%) solutions as well as kCG/Lys and ICG/lys mixtures at a constant concentration of the protein (0.0025 wt%) are presented in Figure 2a,b, and Figure 3a,b respectively. Since the interaction of Lys with kCG in the range of $0.05 < q < 4$ wt/wt or $1.26 \cdot 10^{-4}$ - 0.1 mole/mole leads to formation of too large ($>5 \mu\text{m}$) and quickly aggregating complex particles, and in the range of $q > 5$ wt/wt or 0.126

mole/mole the sizes of the particles were too close to those for the kCG alone, the mixtures with $q=5.0$ wt/wt or 0.126 mole/mole having a size below 1 μm , were used for DLS measurements. The intensity autocorrelation functions indicated that the data is good enough to calculate the mean size of the particles. As can be seen in Fig. 2a, an increase of the temperature from 20°C to 80°C leads to a considerable shift of the dominant peak of kCG from an average radius of ~ 300 nm to 71 nm. Taking into account that in this temperature range kCG undergoes a conformation transition from the ordered conformation to the coil conformation, such decrease in the average radius of the kCG molecules can be mainly attributed to this conformation transition. Complexation of kCG with lys at $q=5.0$ wt/wt or 0.126 mole/mole and 20°C results in the formation of two groups of particles with an average radius of 300 and ~ 25 nm. The first peak belongs to both free kCG and kCG/lys water soluble complex particles, whereas the second peak refers to lys associates. The sizes of lys molecules (1.9 nm, Fig 2b)) are much smaller those of kCG, and kCG/lys complexes. The heating of this complex system up to 80°C leads to a considerable shift of the dominant peak from an average radius ~ 300 nm to 71 nm, and an insignificant shift of the minor peak from 25 nm to 14 nm. Thus, the DLS data are in accordance with the turbidimetry data. The decrease in kCG particle size due to its conformation transition mainly occurs in the temperature range of 50°C to 80 °C whereas the particle size of kCG/lys complexes is already reduced at lower temperatures. Moreover, a continuous decrease of the turbidity over the whole temperature range (20°C-80°C) is observed in lys/kCG mixtures. This suggests that the conformation transition of kCG has a limited effect on the thermal disaggregation of the complexes.

FIGURE 3

The scattering intensity size distribution functions for the ICG (0.005-0.03wt%) solutions and ICG/lys mixtures with 0.0025 wt% lys are presented in Figures 3a,b. Similar to the data obtained for kCG (Fig. 2a), an increase of the temperature from 20°C to 80°C leads to an appreciable shift of the dominant peak of ICG from an average radius ~ 200 nm to 95 nm. The decrease in the average size is less than for kCG and can not be explained by a conformation transition because ICG molecules do not possess an ordered conformation. Berth and coworkers (Berth, Vukovic, Lechner, 2008) studied the physicochemical behavior of kCG, iota carrageenan, and ICG at different temperatures. At room temperature they established aggregation for all carrageenan types. There was a strong tendency for a concentration-dependent aggregation in the order $\lambda < \kappa <$

iota. Only at 60°C, all samples were molecularly dispersed. Based on the results of Berth et al. (2008), we can conclude that the decrease in the average sizes of ICG with increasing temperature is the result of the suppression of the self-association of ICG molecules. Complexation of ICG and lys at $q = 5.0$ wt/wt or 0.137 mol/mol and 20°C results in formation of complex water soluble particles with an average radius of 265 nm. The heating of this complex system up to 80°C leads to enhanced aggregation of the particles and a considerable shift of the main peak from an average radius ~265 nm at 20°C to 413 nm at 80°C. In other words, the DLS data are in accordance with the turbidimetry data.

The turbidimetry and DLS results show a qualitative difference in the sign and extent of the heat induced aggregation/disaggregation processes for kCG and ICG. The structures of the kCG/lys and ICG/lys complexes at $q = 5.0$ wt/wt or 0.126-0.137 mol/mol after preheating up to 60°C were visualized using SEM. In order to do that, complex solutions containing lys and carrageenan (kCG and ICG) were subjected to drying under heating at 60°C before visualization by SEM. The obtained images are presented in Figure 4a,b. Qualitative differences occur in the thermal aggregation/disaggregation processes for complex systems containing carrageenan with on average either one or 2.7 charges per disaccharide unit. In the first system preheating does not lead to aggregation of the complex particles (Fig. 4a), as can be deduced from the fact that the complex particles are separately distributed in the imaged sample. In the second system containing ICG pronounced aggregation of the complex particles can be observed (Fig. 4b). Moreover, although SEM images can not uncover the exact origin of the binding forces, a comparison of the two images allows to propose that there is a strong difference in the character of the binding forces in these systems. In the kCG/lys system a thin cobweb between lys and the body of kCG reminiscent of hydrogen bonds can be seen whereas the structure of the ICG/lys system is reminiscent of the structure of isoelectrical protein precipitates.

FIGURE 4

Finally, DSC was employed to collect information about the thermal stability of the protein within the complexes. Thermodynamic parameters obtained from DSC scans of lys alone and in the presence of carrageenans at pH 7.0 and $q=0.3$ wt/wt or $7.56\cdot 10^{-3}$ mole/mole are presented in Table 1. The free lysozyme exhibits an almost reversible thermal unfolding. The DSC thermogram of lysozyme could be fitted using one single transition with a melting temperature (T_m) of

approximately 74.58°C, overall unfolding enthalpy of 514 kJ/mole, and ΔS_{cal} 1479 Jmol⁻¹K⁻¹. The ratio of the calorimetric and van't Hoff enthalpy was 1.10, suggesting that, at first approximation, lysozyme is a single cooperative unit. The thermal behavior of lysozyme in the complex kCG/Lys system differs from that of lys alone. The DSC thermogram of the kCG/Lys system could also be fitted using one single transition with a melting temperature (T_m) of 71.4°C and overall unfolding enthalpy of 445 kJ/mole. These values are lower compared with those of lys alone, especially the unfolding enthalpy. More considerable changes of the thermodynamic parameters of denaturation were observed for the ICG/lys system. The melting temperature, overall unfolding enthalpy and entropy decreased considerably to 67.26°C, 363 kJ mol⁻¹ and 1039 Jmol⁻¹K⁻¹ respectively.

Table 1. Thermodynamic parameters obtained from DSC scans of lys alone and in the presence of carrageenans at pH 7.0 and $q=0.3$ wt/wt or $7.56-8.25 \cdot 10^{-3}$ mole/mole, with T_m the melting temperature, $\Delta T_{1/2}$ the peak width at half height, ΔH_{cal} and ΔS_{cal} the calorimetric unfolding enthalpy and entropy, ΔH_{vH} the van 't Hoff enthalpy and η the ratio of the calorimetric to the van 't Hoff enthalpy.

System	$T_m, ^\circ\text{C}$	$\Delta T_{1/2}, ^\circ\text{C}$	$\Delta H_{cal}, \text{kJ mol}^{-1}$	$\Delta H_{vH}, \text{kJ mol}^{-1}$	η	$\Delta S_{cal} \text{Jmol}^{-1}\text{K}^{-1}$
lys	74.58±0.8	8.2±0.2	514±15	461	1.10	1479±50
kCG/lys	71.40±0.8	8.1±0.2	445±17	488	0.91	1292±50
ICG/lys	67.26±0.8	6.9±0.2	363±17	426	1.08	1039±50

The DSC results described above show that binding with ICG more significantly decreases the stability of lysozyme against heat-induced denaturation than binding with kCG. Moreover, these results indicate that thermal disaggregation of the complex particles in the kCG/lys system is not correlated with a decrease of the thermal stability of lys in the presence of kCG.

4. Conclusion

The use of polyelectrolytes to affect the thermal aggregation of proteins is a topic of great scientific and technological interest in food science and industry. We have shown that interpolymer

complexes formed by lysozyme and relatively weakly charged kappa-carrageenan undergo disaggregation during heating, whereas complexes of lysozyme with a highly charged lambda-carrageenan exhibit an increasing tendency to aggregate with increasing temperature. The data suggest that the dominant mechanism for the temperature induced disaggregation in kCG/lys systems is disruption of the hydrogen bonds stabilizing water insoluble complexes at room temperature, whereas the temperature induced aggregation in ICG/lys systems can be attributed to enhanced hydrophobic forces at high temperature, that are mainly responsible for the formation of water insoluble complexes in the given system. The question why different secondary forces are dominating the formation of water insoluble complexes in different carrageenan/lys systems is still open. However, we can hypothesize that a stronger complexation in the system comprising ICG with a higher linear charge leads to formation of water insoluble complexes with a higher hydrophobicity as compared to the system comprising kCG. Hence, optimizing the charge density of the polyelectrolyte and the degree of hydrophobicity of the particles will possibly allow to tailor the temperature induced aggregation/disaggregation processes in complex biopolymer systems. The results obtained can be used in technologies for production of cheese, milk cocktails, ice cream, and confectionery (to decrease syneresis during storage and to increase the storage time), including mixtures for infants at concentrations up to 1 g per litre. In more dilute solutions these biopolymer mixtures can be used for medical treatment of for example nonspecific pulmonary diseases (<https://ru.wikipedia.org>).

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Caption to figures

Figure 1. The turbidity values at 500 nm (τ_{500}) as a function of temperature for lys solution, kCG/lys systems (a) and ICG/lys systems (b) at a lys concentration of 0.005 wt%, $I=0.01$, pH 7 and 23°C.

Figure 2. The intensity size distribution functions at different temperatures for (a) kCG (0.005-0.03 wt%) and (b) kCG /lys systems with a lys concentration of 0.0025 wt% at $q=5.0$ wt/wt or 0.126 mol/mol, pH 7.0, $I=0.01$ and 23°C.

Figure 3. The intensity size distribution functions at different temperatures for (a) ICG (0.005 wt%) and (b) ICG /lys systems with a lys concentration of 0.0025 wt% at $q=5.0$ wt/wt or 0.137 mol/mol, pH 7.0, $I=0.01$ and 23°C.

Figure 4. SEM images of the kCG/lys and ICG/lys systems with a lys concentration of 0.01 wt% at $q=5.0$ wt/wt or 0.126-0.137 mol/mol, pH 7.0, $I=0.01$ and 23°C.

Highlights (for review)

- kCG efficiently decreases thermal aggregation of lys within kCG/lys complexes
- Decrease in thermal aggregation is more pronounced at large kCG/lys weight ratio (q).
- ICG increases thermal aggregation of lys within ICG/lys complexes.
- At $q=q_{\text{Max}}$ ICG does not significantly affect the thermal aggregation of lys.

Figure 1

Figure 2

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500 Figure 3

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Figure 4