Food Hydrocolloids 70 (2017) 8–13

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

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

Yurij A. Antonov [a, *], Irina L. Zhuravleva [a], Ruth Cardinaels [b, c], Paula Moldenaers [b]

[a] N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Kosygin Str. 4., 119334 Moscow, Russia
[b] Soft Matter Rheology and Technology, Department of Chemical Engineering, KU Leuven, Celestijnenlaan 200f, Box 2424, B-3001 Leuven, Belgium
[c] Polymer Technology, Department of Mechanical Engineering, TU Eindhoven, Box 513, 5600 MB Eindhoven, The Netherlands

ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form
16 February 2017
Accepted 14 March 2017
Available online 18 March 2017

Keywords:
Lysozyme
Carrageenan
Protein aggregation
Protein complexes
Structure

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 (lCG/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 lCG/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 lCG/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.

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 & McManus, 2013; De Groot & Scott, 2007; Weiss, Young, & 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 et al., 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 (Arnaudov & 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 (Arnaudov & de Vries, 2005), or at high salt concentrations (Arnaudov & 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, and 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.

* Corresponding author.
E-mail address: chehonter@yandex.ru (Y.A. Antonov).

http://dx.doi.org/10.1016/j.foodhyd.2017.03.017
0268-005X/

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 sulphate 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 (lCG/lys) systems, containing sulphated polysaccharides that differ in linear charge density. kCG has only one negative charge per disaccharide unit and lCG 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 ≅1.0 eq. H+/mol dissacharide, Cl^- not detected; dn/dc = 0.115 cm^3/g; M_w = 567 kD; M_n = 356 kDa in 0.1 M $LiNO_3$ at 60 °C) was supplied by Sanofi Bio-Industrie (France). The lCG sample (1000 sulphated groups per mole lCG) was a commercial product from Sigma. The viscosity average molecular weight of the lCG sample (520 kDa ± 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):

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

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 (NaH_2PO_4+Na_2HPO_4) buffer with pH = 7.0 and I = 0.01 under stirring for 1 h at 23°C. The resulting solutions of lys were centrifuged at 50,000*g* 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 ml mg^{-1} cm^{-1} at 281.5 nm in 0.1 M potassium chloride (Aune & Tanford, 1969). kCG and lCG stock solutions were prepared by dispersing the gum in mono/bisphosphate (NaH_2PO_4+Na_2HPO_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 lCG 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 lCG 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 lCG/lys mixtures with a lys concentration of 0.005 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%.

2.2.2. Dynamic light scattering

Determination of the intensity-size distribution functions, as well as zeta potentials of lysozyme, kCG, lCG as well as kCG + lys and lCG + 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 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 μm) before the measurements. The carrageenan was centrifuged at 50,000*g* 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 lCG/lys mixtures containing 0.01 wt% lys were subjected to complete drying at 60 °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 lCG was monitored with a highly