Carbohydrate Polymers 101 (2014) 544–553

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Understanding complex coacervation in serum albumin and pectin mixtures using a combination of the Boltzmann equation and Monte Carlo simulation

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ARTICLE INFO

Article history:
Received 5 February 2013
Received in revised form
10 September 2013
Accepted 17 September 2013
Available online 25 September 2013

Keywords:
Turbidimetric titration
Monte Carlo simulation
Sigmoidal Boltzmann equation
Protein
Polysaccharide
Complex coacervation
Binding

ABSTRACT

A combination of turbidimetric titration, a sigmoidal Boltzmann equation approach and Monte Carlo simulation has been used to study the complex coacervation in serum albumin and pectin mixtures. The effects of the mass ratio of protein to polysaccharide on the critical pH values, the probability of complex coacervation and the electrostatic interaction from charge patches in serum albumin were investigated. Turbidimetric titration results showed an optimum pH for complex coacervation (pH_m), which corresponded to the maximum turbidity in the protein/polysaccharide mixture. The pH_m monotonically decreased as the ratio decreased, and could be fitted using the sigmoidal Boltzmann equation. It suggests that pH_m could be a good ordering parameter to characterize the phase behavior associated with protein/polysaccharide complex coacervation. Qualitative understanding of pH_m by taking into account the minimization of electrostatic interaction, as well as quantitative matching of pH_m according to the concept of charge neutralization were both achieved. Our results suggest that the serum albumin/pectin complexes were ultimately neutralized by the partial charges originated from the titratable residues in protein and polysaccharide chains at pH_m. The Monte Carlo simulation provided consistent phase boundaries for complex coacervation in the same system, and the intermolecular association strength was determined to be several k_BT below the given ionic strength. The strongest binding site in the protein is convergent to the largest positive charge patch if pure electrostatic interaction was considered. Further inclusion of contribution from excluded volume resulted in the binding site distribution over five different positive charge patches at different protein/polysaccharide ratios and pH values.

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1. Introduction

Complex coacervation in protein and polysaccharide mixtures is an associative liquid–liquid phase separation. It has broad applications in microencapsulation, protein purification, and the stabilization of food emulsions etc (Dickinson, 2008; Liu, Low, & Nickerson, 2010; Xu, Mazzawi, Chen, Sun, & Dubin, 2011). Fundamental investigations of the complex coacervation in protein/polysaccharide mixtures from multi-length scales, including molecular interactions, binding, association of molecules and phase behaviors are critical not only for scientific research, but also for novel functional materials development.

Currently, various protein/polysaccharide mixtures have been used in food processing and products, pharmaceutical processing and controlled release etc., depending upon their chemical specificities, physical properties and biocompatibility (Dickinson, 2003). Understanding the interactions involved in the stabilization of protein/polysaccharide coacervate is not straightforward (Dickinson, 1998). The elementary interactions largely rely on extrinsic physicochemical conditions including temperature, pH, ionic strength, concentration, and protein/polysaccharide ratio etc., as well as the intrinsic characters of biomacromolecules such as charge density of polysaccharide chains and distribution of surface charge in proteins. The widely accepted major interaction contributed to complex coacervation is the electrostatic interaction (Gummel, Boue, Clemens, & Cousin, 2008) and sometimes the depletion force when neutral polysaccharide was included (Dickinson, 2008). Beyond the interactions, at tens of nanometer to micrometer scale, both the long range interaction network (association) and the steric stabilization (packing) play an important role during complex coacervation. A certain degree of packing and association strength is imperative to form visible phase domain, and to sustain the phase domain against thermo fluctuation. There are two limits in protein/polysaccharide mixtures (Bohidar, Dubin, Majhi, Tribet, & Jaeger, 2005): in the protein-rich mixture, polysaccharide chains were confined in a continuum of protein-rich domains; in

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0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.09.056

polysaccharide-rich mixture, proteins were dispersed in transient meshes constructed by polysaccharide chains in semi-dilute solutions. Under these limits, complex coacervation may be inhibited because of non-neutralized charges when one of the biopolymers is in excess (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Systematic study of the complex coacervation of well-known proteins and polysaccharides using a combination of computation simulation and experiments is a good approach to understand how the molecular association and microstructure change with complex coacervation. Our recent work on complex coacervation of bovine serum albumin (BSA) and pectin (Ru, Wang, Lee, Ding, & Huang, 2012) indicated that phase boundary (the critical pH where global phase separation occurred) shifted with the initial composition of BSA and pectin. We continue to work on the same system from different angles to reveal the determinant which contributed to the phase behaviors associated with protein and polysaccharide complex coacervation.

BSA is a well-known model protein in complex coacervation study. Dubin and others have published a number of papers in the complex formation of BSA with poly(diallyldimethylammonium chloride) (PDADMAC)-based polyelectrolytes (Antonov, Mazzawi, & Dubin, 2010; Bohidar et al., 2005; Grymonpre, Staggemeier, Dubin, & Mattison, 2001; Kayitmazer, Strand, Tribet, Jaeger, & Dubin, 2007; Li, Mattison, Dubin, Havel, & Edwards, 1996; Seyrek, Dubin, Tribet, & Gamble, 2003; Xia, Dubin, & Dautzenberg, 1993; Xu et al., 2011). Phase boundaries, binding affinity and the structure in these complexes have been systematically investigated. They introduced the concept of charge patches, which were defined as a part of the protein providing strong binding to a given polyelectrolyte, to facilitate the understanding of binding "on the wrong side of p*I*", with p*I* the isoelectric point of a protein. As a classic model protein, BSA has been intensively studied, and the charge patch concept is extremely helpful in understanding the versatile capabilities of BSA to form complexes with various polyelectrolytes. For example, Laos et al. found that both BSA and β-lactoglobulin carried an average net charge of the same sign as the furcellaran when they formed complexes (Laos, Brownsey, & Ring, 2007). Recently, we used an alternative definition of charge patches, which consist of spatially neighbored titratable residues (i.e., solvent accessible and charged residues) of the same sign of charges. Our approach was inspired by the concept of binding sites in proteins which provide topological pocket to bind given molecules (Del Carpio, Takahashi, & Sasaki, 1993), also provided meaningful details of BSA complex coacervation with a polycation through Monte Carlo simulations (Li, Shi, Huang, & An, 2012). Furthermore, Chodankar, Aswal, Kohlbrecher, Vavrin, and Wagh (2008) studied the BSA/sodium polystyrene sulfonate (NaPSS) complexes using small-angle neutron scattering (SANS), and found equilibrium distributions of polyelectrolyte chains in saturated (charge neutralized) or free bound proteins as well as complexes in supernatants or in coacervates. BSA has also been studied in a wide range of food related areas including emulsion stability (Dickinson, 2003; Tolstoguzov, 2003), encapsulation and release (Burova et al., 1999; de Kruif, Weinbreck, & de Vries, 2004), surface adsorption (Brzozowska, de Keizer, Norde, Detrembleur, & Stuart, 2010; Brzozowska, Zhang, de Keizer, Norde, & Stuart, 2010; Wang, Ho, & Huang, 2007), multiple layer assemble for biosensors (Caruso & Mohwald, 1999), macro-encapsulation for scaffold development (Toh, Ho, Zhou, Hutmacher, & Yu, 2005) etc. Therefore, fundamental studies of the protein/polysaccharide complex coacervation in BSA solutions have broad impacts.

Pectin, a polysaccharide extracted from fruits, is traditionally used as a gelling agent. It has also received considerable attentions because of its capability to form complex coacervate. Jones et al. prepared nanoparticles with different structures and aggregation proneness using complex coacervates of β-lactoglobulin and pectin through different thermal treatments (Jones, Decker, & McClements, 2010). Schmidt et al. used SANS to study lysozyme/pectin complex, and found that increasing charges in pectin chains could enhance the packing of proteins (Schmidt, Cousin, Huchon, Boue, & Axelos, 2009). Bedie et al. studied the encapsulation of thiamine using whey protein isolate/pectin complexes, and found that the complexes had optimum entrapment efficiency at pH 3.5 (Bedie, Turgeon, & Makhlouf, 2008). Wang et al. have studied the effects of salt concentration and protein/polysaccharide ratios on the assembly, structure and rheological properties of β-lactoglobulin/pectin complexes (Wang, Wang, Ruengruglikit, & Huang, 2007; Wang, Lee, Wang, & Huang, 2007). Researches related to the complex coacervation using pectin and proteins were also found in several comprehensive reviews (Dickinson, 1998, 2003; Tolstoguzov, 2003). Although many researches related to pectin-based complex coacervation have been reported, thorough understanding of the role of pectin in the coacervation process is far from clear.

In this work, we used a combination of turbidimetric titration, and theoretical approaches particularly Monte Carlo simulation to study the complex coacervation in serum albumin/pectin mixtures. A set of turbidimetric titration experiments, and the Monte Carlo coarse grained model based on the specified charge distribution in serum albumin and polysaccharide (PS), pectin in this work were first described. Subsequently, we presented the effect of the mass ratios of protein/PS on titration curves and the optimum pH for complex coacervation. A tentative theoretical understanding of this effect by taking into account of energy minimization and charge neutralization was provided. In the simulation part, phase boundaries associated with percolation concepts and simulation configurations were determined and compared with experimental observation. The binding affinity of charge patches in protein and the structure in the mixtures were also thoroughly studied. Finally, findings obtained from this work and results related to the effects of protein/PS ratio on complex coacervation were also discussed in detail.

2. Materials and methods

2.1. Turbidimetric titration experiments

Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (Lot: 058K0726, St. Louis, MO). LM-Pectin with 31% esterification (Danisco A/S, Denmark) was purified by dialysis (MWCO = 12,000) followed by freeze-drying, similar to the procedure used in our previous works (Ru et al., 2012; Wang, Wang, et al., 2007; Wang, Lee, et al., 2007). Sodium chloride (NaCl), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Fisher Scientific (Pittsburgh, PA). All solutions for the titration experiments were prepared using Milli-Q water.

Solutions of BSA to pectin mass ratios (α) ranging from 9:1 to 1:9 were titrated whereas the total concentrations of BSA and pectin mixtures were kept at a constant of 3.0 mg/mL, and the salt (NaCl) concentration was fixed at 0.1 M. At this salt concentration, the small amount of ions released from either protein or polysaccharide became negligible. In the control titration of pure BSA or pectin saline solutions, the concentration of biopolymer was fixed at 2.5 mg/mL with the same salt concentration. The pH meter (Thomas Scientific 8025) was calibrated with two buffers of pH 4.0 and 7.0, respectively, and the turbidity detector (Brinkman PC 910 colorimeter equipped with a 1 cm path length optical probe and a 420 nm filter) was calibrated by Milli-Q water. The turbidity, which was defined as $(100 - 100 * I_1/I_0)\%$, was used to detect the aggregation and the phase separation in samples. Here I_0 and I_1 are the intensities of the incident and transmitted light, respectively. All solutions (except two solutions in control titrations which were

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