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Molecular migration of konjac glucomannan and gum Arabic phase separation and its application in oil-water interfaces

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ABSTRACT

Molecular migration of gum Arabic (GA) fragments was investigated, when thermodynamic phase separation occurred in mixtures of konjac glucomannan (KGM) and GA at different concentrations. It has been quantitatively analyzed by GPC-MALLS. After reaching phase equilibrium, GA with the small molecular weight migrated into KGM-rich phase, resulting in a decrease of its relative content (from 13.58% to 0.05%) in GA-rich phase. GA was dyed with fluorochrome, and aggregations of GA in separated phase were recorded using fluorescence microscopy. When KGM content was fixed at 0.4 wt%, increasing GA concentration led to the simultaneous formation of larger aggregates of GA. In the process of phase separation, the amount of coexisted KGM in GA-rich phase decreased as a function of time. To collect the separated phases, the fractionated GA was named as FGA. FGA showed better interfacial properties than original GA, resulting in decreasing of oil-water interfacial tension and increasing of the moduli of interfacial adsorption layer. The proposed reason was that fractions of GA maintained in GA-rich phase, which has proved to possess larger molecular weight, contributed to the better emulsifying capacity. So FGA was the concentrated sample of large molecular fractions that has high emulsifying capacity. Optimized FGA-stabilized emulsions presented small droplet size (around 20 μm), high surface charge at neutral pH (about −30 mV) and high stabilities under various pH range or NaCl concentration. Taken together, this paper provides a flexible method for separating and concentrating GA with high emulsifying capability and has great potentials to be applied in in developing new food ingredients.

1. Introduction

Thermodynamic phase separation is commonly observed when two biopolymers are mixed in the concentrated solutions. Mixtures are often separated into two layers when standing for a long time until phase equilibrium (Loret, Schumm, Pudney, Frith, & Fryer, 2005). To understand the complicity of multi-components food systems and to develop new food ingredients based on natural food biopolymers, studies of phase behavior with mixed various biopolymers is a primary work (Chun et al., 2014). Besides, phase behavior of those biopolymers is associated with micro-network of products, and has potentials to regulate textural or rheological properties and to prolong the storage stability (Jia et al., 2014).

Segregative phase separation, also called thermodynamic incompatibility, takes place generally for biopolymer mixtures in a good solvent and when there is non-specific interaction among biopolymers. Mixtures of different biopolymers tend to separate because the losing entropy is small with respect to reduction in enthalpy. Thus, repulsive interactions between two polymers lead to the formation of different phases. Each phase is enriched in one of the two biopolymers (Grinberg & Tolstoguzov, 1997). In food system, the commonly used solvent is water. Under this condition, such systems are often named aqueous two-phase systems or water-water interface separating systems. Segregative phase separation of biopolymers led to the formation of a phase-separated protein network structure containing less polysaccharide molecules (Abhyankar, Mulvihill, Fenelon, & Auty, 2010). However, the fact is that biopolymers have broad molecular weight distribution or multiple segments. During the phase separation, different segments of biopolymers move and aggregate with various speeds, resulting in a phenomenon of molecular fractionation (Aoki, Al-

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Assaf, Katayama, & Phillips, 2007).

Konjac glucomannan (KGM), a neutral polysaccharide, has the β-(1–4) linked linear backbone containing β-D-glucose and β-D-mannose sugar residues in an approximate ratio of 1:1.6 (Jacon, Rao, Cooley, & Walter, 1993; Li & Xie, 2006). KGM solution possesses high viscosity at the low concentration due to its high water absorption capacity, large molecular weight (around 1000 kDa) and dense entangled networks (Jin et al., 2014). So mixtures containing KGM are more inclined to segregative phase separation, such as KGM/gelatin and KGM/β-lactoglobulin mixtures (Harrington & Morris, 2009; Tomczyńska-Mleko, Brenner, Nishinari, Mleko, & Kramek, 2014). Systems reached final phase equilibrium when two separated phases were formed. One of phases is KGM-rich one that is consisted of concentrated KGM molecular network. The majority of the other biopolymers are always excluded from the dense KGM molecular network to form a separated layer. The process of network reconstruction of KGM and molecular fractionation of the second biopolymers are dynamic. And it is strongly influenced by initial biopolymer concentrations, molecular weight, and external conditions such as ionic strength or temperature (Bhargava, Wang, & Koenig, 1999; Chen & Santore, 2014; Hashimoto, Shibayama, & Kawai, 1983). Phase separation will be inhibited if the polymer concentration is low or molecular weight is small. This dynamic and spontaneous process of network construction and molecular migration can be understood as a kind of molecular sieve, which would be applied to purify multi-segmental biopolymers without any high-energy input.

Gum Arabic (GA) is an unique polysaccharide has wide applications in food industry due to its excellent emulsifying properties and low viscosity (Buffo, Reineccius, & Oehlert, 2001; Mothe & Rao, 1999). It is a heterogeneous polymer and commonly regarded to consist of three broad components, which are arabinogalactan-protein complex (AGP), arabinogalactan (AG) and glycoprotein (GP), respectively. Among those fractions, AGP has high molecular weight, which corresponds to the protein rich factions and represents about 10% of the total mass. It usually acted as an active component for emulsification. The structure of this fraction has been reported to have a 'wattle blossom-type' structure where blocks of polysaccharides with molecular mass around 250,000 are linked to a common polypeptide chain (Phillips & Williams, 2009). However, AG, the major component that represents 90% of whole GA, is hard to absorb on oil-water interface. GP has high protein content but its proportion is only 1%. Therefore, it is a promising work in the food industry to obtain fine-tuning and simple fractionation procedures to prepare AGP-concentrated GA.

The objective of this study was to investigate phase behaviors of KGM and GA systems in the aqueous state, to find the principle of molecular sieve, and to apply it in separating and concentrating fractionated GA (FGA). The interfacial properties of FGA were evaluated using dynamic interfacial analysis. Preparation and characterization of FGA-stabilized emulsions were studied to evaluate their practical applications.

2. Materials and methods

2.1. Materials

KGM was purchased from Huaxianzi Konjac Productions Co., Ltd. (Hubei, China). The commercial GA powder (FT powder LOT 24686) provided by TIC GUMS (Belcamp, MD, USA) contains 2 wt% of the protein. All the raw materials were used without further purification. The fluorescence dye for microstructural observation was rhodamine B. Medium-chain triglyceride (MCT) is of food grade ($\rho = 0.9436$ kg/m^3). All other chemicals used are of analytical grade.

2.2. Preparation of stock solutions

Stock solutions of KGM (1 wt%) and GA (20 wt%) were prepared by dispersing dry powders in Milli-Q water. To ensure complete dissolution of biopolymers, solutions were gently dispersed at room temperature under mechanical stirring for 2 h and 24 h respectively. Sodium azide (0.005 wt%) was added to stock solutions in order to prevent bacteria growth. Then centrifugation was performed at 4500 rpm for 10 min at 25 °C to remove any insoluble fractions of GA.

2.3. Phase diagrams

KGM/GA mixtures were prepared in a beaker at appropriate ratios and then kept under thorough stirring at 25 °C for 1 h. The concentrations of KGM and GA were controlled in the range of 0.1–0.5 wt% and 2.5–6.0 wt% respectively. All the final biopolymer concentration was below 7.0 wt%. The fixed volume of mixtures was placed in 10 mL tube quiescently at room temperature for 24 h. The phase diagrams were determined by the visual observation and phase-volume-ratio method (Polyakov, Grinberg, & Tolstoguzov, 1980).

2.4. Gel permeation chromatography multi-angle laser light scattering (GPC-MALLS)

The molecular distribution of FGA after phase separation was measured using GPC-MALLS (Mao et al., 2013). The transparent FGA solutions were collected carefully and appropriately diluted with the eluent. Then the samples were centrifuged at 4500 rpm for 10 min and filtered through 0.45 μm pore size filters before loading on to the analytical GPC-MALLS at 25 °C. The GPC was equipped with the column of OHpak SB-806 HQ and connected to the DAWN HELEOS multi-angle light scattering detector operated at a wavelength of 658 nm and an Optilab rEX refractometer. The eluent (0.1 M aqueous NaCl solution) was filtered through 0.22 μm Millipore filter before usage. The refractive index increment ($d\eta/dc$) used is 0.141 mL/g (Al-Assaf, Phillips, & Williams, 2005).

2.5. Fluorescence microscopy

Fluorescence microscope (ECLIPSE 80i, NIKON) was used to observe the micro-network structures of separated phases. The GA was dyed by rhodamine B (0.2 wt% DMSO solution) under magnetic stirring for 10 min in order to fluorescently label the protein of GA. The samples were gently transferred and compressed between a microscope slide and a cover slip.

2.6. Interfacial rheological

The solutions of FGA were prepared by re-dissolving freeze-dried FGA powders at the concentration of 10 wt%. Oil-water interfacial tension (γ) was measured by an automated drop tensiometer (Tracker Teclis-IT Concept, France). Measurement of interfacial tension was based on axisymmetric oil drop shape analysis in aqueous solution. A droplet volume was set 5 μL and measurement time lasted for 10^4 s until a stable adsorption layer at oil droplet was formed. The characteristic time t and the adsorption kinetic parameter were determined by fitting experimental curves with an exponential decay function. The oscillations were produced by a position-encoded motor and transmitted through a piston coupled to the syringe carrying the capillary. For the sinusoidal volume fluctuations, the surface amplitude was chosen in the linear domain and fixed at 10%. The frequency ranged from 0.01 to 0.1 Hz. All measurements were performed at 25 °C and repeated three to five