International Journal of Biological Macromolecules 70 (2014) 167–173

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Phase separation in mixtures of ovalbumin and konjac glucomannan: Physicochemical and microscopic investigations

Bin Zhou [a,b], Jinjin Liu [a,b], Ting Ye [a,b], Yuntao Wang [a,b], Wei Xu [a,b], Bin Li [a,b,*]

[a] College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[b] Key Laboratory of Environment Correlative Dietology, Huazhong Agricultural University, Ministry of Education, China

ARTICLE INFO

Article history:
Received 3 April 2014
Received in revised form 3 June 2014
Accepted 11 June 2014
Available online 24 June 2014

Keywords:
Phase separation
Ovalbumin
Konjac glucomannan
Aggregate
Confocal laser scanning microscopy

ABSTRACT

The phase behavior and microstructure of ovalbumin (OVA)/konjac glucomannan (KGM) mixtures were studied at pH 7.0. Phase diagrams were established by centrifugation and visual observation. Micro-phase separation of the OVA/KGM mixtures was quantified by measuring the turbidity. The microstructures of the phase separated mixtures were studied by measuring rheological property and confocal laser scanning microscopy (CLSM). The phase behavior of OVA/KGM mixtures appeared to be one single phase or two separated phases depending on the content of OVA and KGM. OVA had a pronounced effect on turbidity of OVA/KGM mixtures. The particle size of mixtures increased with increasing OVA and KGM concentration, which was the largest (119.1 μm) at 0.25 wt.% KGM and 5 wt.% OVA. The G′ and G″ cross-over at a mixture of 0.20 wt.% KGM and 4 wt.% OVA demonstrated the buildup of microstructure during phase separation. The association of OVA aggregates could be observed under CLSM.

1. Introduction

Ovalbumin (OVA) is the main globular protein component in egg white and constitutes over half of the egg white proteins by weight [1,2]. It is a monomeric phosphoglycoprotein with a molecular weight of 45 g/mmol and an isoelectric point of 4.5 [1,3]. It has been reported by Nicolai et al. that a well-defined iso-scattering point during heat-induced aggregation and gelation of globular proteins (OVA) indicated micro-phase separation [4]. Furthermore, the natural macromolecule, OVA, was used to produce nanogels by self-assembly for cosmetic and pharmaceutical applications [5–7]. Here we studied the phase behavior of proteins and polysaccharides (konjac glucomannan) using industrially important globular proteins: OVA.

Konjac glucomannan (KGM) is a high molecular weight water-soluble neutral polysaccharide extracted from the tubers of *Amorphophallus konjac* C. Koch [6,8,9]. The polymer backbone contains both (1→4)-β-D-glucopyranose and β-D-mannopyranose, having glucose and mannose units in a molar ratio of 1:1.6 with a low degree of acetyl group sat the C-6 position [10–12]. To our knowledge, it has been generally used in food industry [13,14], medical application [15], chemical engineering [16], and other fields because of its unique physical and chemical properties.

Mixtures of food biopolymers in aqueous solution are generally unstable [17]. The phase separation in globular protein and polysaccharide mixtures has been widely studied because of some practical considerations: for example, products with desired structure and texture can be designed by controlling the phase separation of protein and polysaccharide [18,19]. The phase separation observed in the protein–polysaccharide mixtures may occur due to coacervation or thermodynamic incompatibility [20,21]. It has been reported that native globular proteins and polysaccharides with the same charge may phase-separate from each other at relatively high volume fractions of the components [22]. Recently, turbidimetric method was used by Ako et al. to study the phase separation in gelling mixtures of β-lactoglobulin and κ-carrageenan [23]. Moreover, it is worth mentioning that confocal laser scanning microscopy (CLSM) method has also been successfully used to observe the state of phase separation in the mixtures of proteins and polysaccharides [17,24]. In previous works, there are relatively few studies on investigation of the phase behavior of OVA/KGM/water systems. Besides, statistical correlations with microscopic investigations or other physicochemical properties are not yet available up to date.

As a model glycoprotein, OVA played an important role in egg food process, which has long been exploited to produce foods with different structural and textural characteristics. The objective of this study was to exploit these features to investigate the influence

* Corresponding author at: College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China. Tel.: +86 13296507469; fax: +86 27 87282966.
E-mail address: libinfood@mail.hzau.edu.cn (B. Li).

http://dx.doi.org/10.1016/j.ijbiomac.2014.06.030
0141-8130/

of phase separation and microstructures on the OVA/KGM mixtures. In particular, the OVA/KGM/water system was shown to be a suitable model system to study the texture development in food biopolymer mixtures. Now the mixtures were characterized by means of phase diagrams, turbidity, size distribution, rheology and CSLM.

2. Materials and methods

2.1. Materials

OVA from chicken egg white were provided by Sigma Co. (A-5253, St. Louis, MO, USA) with a protein content of 62–88%. KGM was purchased from Shiyan Huaxianzi Konjac Productions Co., Ltd. (Hubei, China), which was used without further purification. The dye for CLSM was rhodamine B (Sigma–Aldrich Chemie Gmbh, Munich, Germany). All chemicals used in this study were of analytical grade reagents (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China).

2.2. Preparation of sample

2.2.1. Stock solutions

KGM was dissolved in phosphate buffer solution (0.5 wt.%) at room temperature for 2.5 h to obtain a homogenous solution. 10 wt.% OVA solution was obtained by suspending OVA powders in phosphate buffer solution (0.01 M, pH 7) and stirring for 3 h.

2.2.2. OVA/KGM mixtures

The mixed solutions with the different OVA and KGM concentrations were prepared by adding different amounts of 0.5 wt.% KGM solution and phosphate buffer solution (0.01 M, pH 7) into 10 wt.% OVA solutions and stirring 2 h for thoroughly mixing. Based on the various concentrations of OVA/KGM, the final analyses were categorized into nine groups coded as A (0.05 wt.% KGM, 1 wt.% OVA), B (0.10 wt.% KGM, 2 wt.% OVA), C (0.15 wt.% KGM, 3 wt.% OVA), D (0.20 wt.% KGM, 4 wt.% OVA), E (0.25 wt.% KGM, 5 wt.% OVA), F (0.05 wt.% KGM, 3 wt.% OVA), G (0.10 wt.% KGM, 3 wt.% OVA), H (0.20 wt.% KGM, 3 wt.% OVA), I (0.25 wt.% KGM, 3 wt.% OVA) respectively (Fig. 1b). The homogenous mixtures were characterized using particle size, rheology and CLSM measurements to obtain information about samples.

2.3. Determination of phase diagrams

Phase diagrams were established based on centrifugation combined with visual observation [25]. A series of solutions with the different OVA concentration (1–6 wt.%) and KGM concentrations (0.05–0.30 wt.%) were prepared by adding different amounts of 0.5 wt.% KGM solution and phosphate buffer solution (pH 7) into 10 wt.% OVA solutions. After stirring for 2 h at room temperature, these solutions were centrifuged at 6000 rpm for 20 min at 4 °C. The phase separation of the mixtures was obtained based on the visual observation. The phase diagram was performed using Origin 8.5.1 software (Microcal, USA).

2.4. Turbidity measurements

Turbidity measurements were carried out using a UV–vis spectrophotometer (Mapada Instruments Co. Ltd., Shanghai, China) according to the method developed by Ako et al. [23]. The turbidity was determined at 680 nm. All samples were measured in triplicate and data were reported as mean with standard deviation.

2.5. Viscosity measurements

Viscosities of the mixed solutions were measured using a rotational concentric cylinder viscometer (NDJ-8S, Shanghai Cany Precision Instrument Co., Ltd., Shanghai, China) at 25 °C. For the measurement of viscosity, the 1st rotator was selected, and rotation speed was 60 rpm [26].

2.6. Determination of particle size and size distribution

A Malvern MasterSizer 2000 (Malvern Instruments Co. Ltd., Worcestershire, UK) was used to determine the droplet size distribution and average diameter of samples [27]. The refractive index and adsorption of the dispersed phase were set as 1.414 and 0.001, respectively. The mixed solutions in the sample chamber were diluted 1000-fold by deionized water. Volume weighted average diameter ($d_{4,3}$, μm), was calculated as follow:

$$d_{4,3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}$$

where n_i is the number of particles with the same diameter d_i; d_i is the particle size.

The relative volume distribution of particles and other size distribution parameters were calculated by Malvern optical model from the light scattering data assuming an equivalent sphere [28]. The measurement was obtained from the average of ten readings for each sample.

2.7. Determination of dynamic moduli

Rheological properties were performed according to the Zhu's method with minor modification [29]. The storage (G′) and loss (G″) moduli were measured by oscillatory shear measurements using a stress controlled rheometer (AR2000, TA Instruments). Parallel plate geometries were used (diameter 40 mm, gap 1 mm). Measurements were made at 0.2% strain and 1 Hz frequency, which was within the linear viscoelastic range (LVR). The measurement temperature was fixed at 25 ± 0.1 °C and stabilized in less than 1 min. Time sweeps were carried out over the range of 0–5000 s. The sample solutions were poured onto the flat plane of the rheometer directly after preparation (i.e. before the onset of phase separation) and covered with a thin layer of silicone oil to prevent evaporation. All the experimental dynamic rheological data were obtained directly from the TA Rheology Advantage Data Analysis software.

2.8. Confocal laser scanning microscopy

The OVA/KGM mixed samples were dyed by fluorochrome rhodamine B under magnetic stirring for 90 min [30]. Different samples were prepared as described in Section 2.2, and poured between a concave slide and a cover slip before hermetically sealed. CLSM was used in the fluorescence mode. Observations were made with the LSM 510 META (Carl Zeiss, Jena, Germany) and a 40× objective lens. Rhodamine B was excited using a helium–neon laser with a wavelength of 543 nm and the fluorescence was detected with a photomultiplier. Averages over several scans were taken to reduce the effect of signal fluctuations. The measurements were done in an air conditioned room at 25 °C.

2.9. Statistical analysis

Statistical analysis of results was performed using Statistical Analysis System (SAS 9.0, SAS Institute Inc., Cary, NC). The data of each treatment was analyzed for statistical significance using analysis of variance (ANOVA) function. Significant differences between