Ultrasonics Sonochemistry 31 (2016) 302–309

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

# High intensity ultrasound modified ovalbumin: Structure, interface and gelation properties

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ARTICLE INFO

*Article history:*
Received 15 December 2015
Received in revised form 12 January 2016
Accepted 13 January 2016
Available online 15 January 2016

*Keywords:*
High intensity ultrasound
Structure
Emulsifying activity
Foaming ability
Gelation
Ovalbumin

ABSTRACT

Influence of high intensity ultrasound (HIUS) on the structure and properties of ovalbumin (OVA) were investigated. It was found that the subunits and secondary structure of OVA did not change significantly with HIUS treatment from the electrophoretic patterns and circular dichroism (CD) spectrum. The amount of free sulfhydryl groups increased and intrinsic fluorescence spectra analysis indicated changes in the tertiary structure and partial unfold of OVA after sonication increased. Compared with the untreated OVA, HIUS treatment increased the emulsifying activity and foaming ability, and decreased interface tension (oil–water and air–water interface), which due to the increased surface hydrophobicity and decreased the surface net charge in OVA, while the emulsifying and foaming stability had no remarkable differences. The increased particle size may be attributed to formation of protein aggregates. Moreover, the gelation temperatures of HIUS-treated samples were higher than the untreated OVA according to the temperature sweep model rheology, and this effect was consistent with the increased in surface hydrophobicity for ultrasound treated OVA. These changes in functional properties of OVA would promote its application in food industry.



## 1. Introduction

With the development of modern food industry, one of the constant challenges is looking for innovative technologies to enhance the processing efficiency, and to reduce the energy consumption [36]. Concerning this, ultrasound as an innovative green technology has been widely studied in recent years. In general, depending on the ultrasonic frequency and intensity, ultrasound can be divided into low- and high-intensity approaches in food industry. Low-intensity approaches utilize small amplitude ultrasonic waves at high frequency (>1 MHz) that do not damage the materials. These approaches are normally used for analytical applications, such as the determination of composition, structure, and physical state [22]. High-intensity ultrasound (HIUS) typically uses acoustic frequencies between 20 and 100 kHz, and can alter the physicochemical properties or structure of a material due to cavitation [22].

For this reason, in order to get much better functional properties of food protein, many researchers used HIUS to alter their molecular characteristics. For example, Guülseren et al. [9] reported the application of HIUS in changing the structure and function of bovine serum albumin (BSA). It was found that surface activity, surface hydrophobicity and surface charge of ultrasonicated BSA increased, and the free sulfhydryl group content decreased, also the secondary structure of proteins became more ordered. These changes may alter BSA bulk functionality. On the other hand, it was proved that HIUS can improve emulsion activity of soy protein [13], dairy proteins [32] and peanut protein isolate [40], and change the physical properties of soy protein [11], black bean protein isolates [18] and milk protein concentrate [27,39]. Furthermore, by treating the egg white protein (EWP) with HIUS, it was found that ultrasound-induction increases the surface hydrophobicity, with no effect on the total sulfhydryl content. The apparent viscosity decreased the stability of foams and emulsions thus increased the size aggregation. All of the changes showed that HIUS could improve some functional properties of EWP [3].

Ovalbumin (OVA) is the major protein present in egg white, and its behavior predominantly affects the functional properties of

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http://dx.doi.org/10.1016/j.ultsonch.2016.01.014
1350-4177/

EWP [31]. Despite the excellent functional properties displayed by OVA, however, in order to meet the complex needs of manufactured food products (for example, bakery products, meringues, mayonnaise, meat products and cookies). Several methods for improving functionalities of OVA were developed long ago, e.g., dry-heating phosphorylated to improve functional properties [26,28], pH-induced to the molten globule state [4,10,25], Maillard reaction to generate protein–polysaccharide conjugates [20,21,34,1,8]. Moreover, high-pressure pulsation [35,7] and high hydrostatic pressures were used to modify the solubility and gelling ability of OVA [12].

However, there is scare literature that focuses on the effect of HIUS on the structure and properties of OVA. Therefore, in this study, we used HIUS to treat with OVA, with the aim to research the impact of HIUS on the emulsifying, foaming and gelation properties, diameter, zeta-potential, surface hydrophobicity and sulfhydryl content of OVA, and monitor the structural changes of ultrasonicated OVA, which will be helpful for the further understanding of the relationship between the structure and function properties.

## 2. Materials and methods

### 2.1. Materials

Ovalbumin (OVA, A5378, >90% pure by agarose electrophoresis, and a molecular weight of 45 kDa) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB) and 8-anilinonaphthalene-sulfonic acid (ANS) were purchased from Aladdin Reagent Company (Shanghai, China). Other reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents were analytical grade unless otherwise stated.

### 2.2. High intensity ultrasound (HIUS) treatment

OVA powder was dispersed in deionized water at 5% (w/v) total solids and the solution was slowly stirred for 4 h at ambient temperature prior to overnight storage at 4 °C. A 20 kHz horn-transducer ultrasound processor (Fisher Scientific, FB705, USA) with a 12 mm titanium probe was used to process 100 mL of OVA dispersions in 100 mL flat bottom cylindricality flasks which were immersed in an ice-water bath. Samples were treated at different levels of amplitude (0%, 60%, 90%) for 20 min and 40 min (pulse duration of on-time 5 s and off-time 2 s). After ultrasound treatment, all samples were lyophilized and then stored at room temperature in air tight containers until analyzed.

### 2.3. Acoustic energy determination

The acoustic energy was determined by measuring the temperature rise of the sample as a function of treatment time, under adiabatic conditions at room temperature. The acoustic energy, P (W), was calculated as follows [29]:

$$P = m \cdot c_p \cdot \left(\frac{dT}{dt}\right) \quad (1)$$

where m is the mass of the sonicated sample (g), $c_p$ (J $g^{-1}$ $K^{-1}$) is the specific heat of medium at a constant pressure dependent on composition and volume of medium, $dT/dt$ (K/s) is the slope at the origin of the curve of a plot of temperature against time. In this study, we used the distill water to estimate the acoustic energy and expressed in W/$cm^2$. Ultrasonic intensity was measured by calorimetry using thermocouple (model TASI-8530, Suzhou, China). Ultrasound treatment with the 20 kHz probe at various amplitude (60%, 90%) generated ultrasonic intensities of 34–36 and 45–48 W/$cm^2$, respectively.

### 2.4. Measurement of emulsifying properties

The determination of emulsifying properties was followed by the method of Pearce and Kinsella [33]. To prepare emulsion, 15 mL OVA solution (1% (w/v) protein in phosphate buffer (PBS, 10 mM, pH7.4)) was mixed with 5 mL soybean oil, the mixture was stirred with a homogenizer (T25, IKA) at 8000 rpm for 1 min at 25 °C. A 100 μL emulsion sample was taken from the bottom of the container at the intervals of 0 and 10 min, and then diluted with 5 mL 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance of the diluted emulsion was measured at 500 nm by UV–vis spectrophotometer (UV-1100, MAPDA). The emulsifying activity was determined by the absorbance measured immediately after emulsion formation (0 min), and the emulsion stability was estimated by measuring the absorbance in 10 min.

### 2.5. Measurement of foaming properties

The foaming properties were determined by the method of An et al. [1] with slight modification. 50 mL OVA fresh solution (5%, w/v) after sonication was whipped with a homogenizer (T25, IKA) at 8000 rpm for 1 min at 25 °C. Foaming ability (FA) was measured by comparing the foam volume at 2 min with the initial liquid volume of sample. Foam stability (FS) was determined by comparing the foam volume at 30 min with the initial foam volume of sample.

$$FA\ (\%) = V_0/50 \times 100 \quad (2)$$

$$FS\ (\%) = V_{30}/V_0 \times 100 \quad (3)$$

where $V_0$ is the foam volume at 2 min, $V_{30}$ is the foam volume at 30 min.

### 2.6. Measurement of surface hydrophobicity ($H_0$)

The surface hydrophobicity of OVA was determined by a fluorescence spectrum assay using 8-anilino-1-naphthalenesulfonic acid (ANSA) as a fluorescent probe [16]. The OVA and ANSA were dissolved in 10 mM PBS (pH 7.0) to get the 1 g/L and 2.4 mM solution, respectively. And then aliquots of 50 μL ANSA solutions were added into 5 mL OVA solution. The solution was excited at 385 nm, and the emission spectrum was measured from 400 to 650 nm using fluorescence spectrophotometer (SHIMADZU RF-5310PC). The emission and excitation slits were set to 5 nm, and the measurements were performed at 25 °C. The area of the fluorescence spectrum was corrected with the area of the buffer, and the relative exposed hydrophobicity was expressed as the area of the sample.

### 2.7. Surface sulfhydryl groups determination (SH)

The number of free sulfhydryl groups was determined by the method of Ellman's reagent [5]. Briefly, the Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid), DTNB) and protein sample were dissolved in Tris–HCl buffer (86 mM Tris, 90 mM glycine and 4 mM ethylenediamine tetraacetic acid (EDTA), pH8.0) to get 0.4% (w/w) and 0.5% (w/w) solution, respectively. 50 μL DTNB was added to 5 mL OVA solution. The solution was mixed and reacted for 15 min at 25 °C, and then the absorbance of solution was measured at 412 nm. The buffer was used as the reagent blank. An extinction coefficient of sulfhydryl groups of 13,600 $M^{-1}$ $cm^{-1}$ was used to calculate the micromoles of SH/gram of protein.