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Formation mechanism of ovalbumin gel induced by alkali

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ABSTRACT

In this paper, the alkali-induced gelling behavior of ovalbumin, including its microstructure characteristics, intermolecular forces, and molecular structure changes, was investigated. The results showed that ovalbumin formed a three-dimensional gel with an ordered fibrous mesh structure under alkali conditions. The active force between gel molecules was maintained by a large number of ionic bonds (~85%), a small number of disulfide bonds (~5%), and very few hydrophobic interactions and hydrogen bonds. SDS-PAGE analysis showed that ovalbumin formed aggregates via ionic and disulfide bonds. ANS fluorescence spectroscopy analysis showed that strong alkali caused rapid denaturation of ovalbumin molecules to expose the hydrophobic core, thus greatly increasing the surface hydrophobicity. However, the hydrophobicity decreased during the gelation stage. FTIR analysis showed that strong alkali induced the secondary-level structure of ovalbumin molecules to interconvert, and most of them existed in a relatively stable α-folding structure. Endogenous fluorescence and UV spectroscopic analyses showed that the amino acid residues of ovalbumin in the gelation stage moved towards the polar environment. It was concluded that the secondary and tertiary structures of ovalbumin changed after treatment with strong alkali and that a crystal gel formed through the action of ionic and disulfide bonds. In addition, the long-term action of strong alkali gradually decreased the gelatinous property.

1. Introduction

Ovalbumin is a phosphoglycoprotein and is the most abundant protein in the white of eggs, accounting for 54%, 45%, and 40% of the total protein in the whites of hen, turkey, and duck eggs, respectively (Smith & Back, 1970; Weijers, Sagis, Veerman, Sperber, & Van Der Linden, 2002). By electrophoresis, three types of constituents, namely, A1, A2, and A3 (in 85:12:3 proportions), have been detected with different quantities of phosphate groups of molecules. A1 and A2 contain one and two phosphate groups, respectively, whereas A3 has no phosphate group (Guérin-Dubiard et al., 2006; Itoh, Sugawara, & Adachi, 1978). Ovalbumin has a relative molecular mass of 45 kDa and is a single peptide chain composed of 385 amino acids, more than 50% of which are hydrophobic amino acids (Mine, 1995).

Ovalbumin is characterized by its gelling, foaming, emulsification, and other functional properties; the gelling property is prominent (Doi, Shimizu, Oe, & Kitabatake, 1991; Mine, Noutomi, & Haga, 1991; Nieuwland, Bouwman, Pouvreau, Martin, & de Jongh, 2016; Relkin, Hagolle, Dalgleish, & Launay, 1999). Ovalbumin can form transparent, semitransparent, or opaque gels upon heating depending on the protein concentration, pH, and ionic strength of the environment, among which the effect of pH is the most significant on gel formation (Koseki, Kitabatake & Doi, 1989; Weijers et al., 2002). At pH 10, a fibrous, patchy polymer gel is induced by heat. However, at pH 5, the gel formed upon heating is clumpy, tufted, and grainy (Mine, Noutomi, & Haga, 1990; Veerman, de Schiffart, Sagis, & van der Linden, 2003).

Preserved egg is a traditional egg product originally created in China. Most consumers acknowledge its special flavor and high nutritional value (Wang & Fung, 1996; Zhao, Tu, Xu, Li, & Du, 2014). The processing technology for preserved eggs is unique. Without heat treatment, a highly elastic gel can be gradually formed in the presence of a strong base (Ganesan & Benjakul, 2014; Zhang, Jiang, Chen, Ockerman, & Chen, 2015; Zhao, Tu, Xu, et al., 2014). In the process of pickling preserved eggs, the dynamic penetration of an

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alkali solution induces moisture inside the egg to move outside and fixed in a gel system (Ganasen & Benjakul, 2011; Ganesan & Benjakul, 2010). A 3D network structure woven by loose, fine stands of linear fibers is typical of a polymer gel formed without heat induction. The proteins in egg whites are denatured by treatment with strong alkali, and then, they aggregate, crosslink, and react with solvent molecules, forming ionic bonds, disulfide bonds, and hydrophobic interactions. Among these, ionic and disulfide bonds both account for approximately 40% of the total interactions (Chen, 2015; Zhao, Tu, Xu, et al., 2014).

Our research on the white gel of preserved eggs and model of white gel formation under alkali conditions showed that ovalbumin plays dominant role in white gel formation under strong alkali conditions (Chen et al., 2015). Egg white protein is composed of many complex constituents, containing hundreds of proteins (Awade & Efstathiou, 1999; Mine, 2008). Thus, the molecular mechanism of gel formation under alkali conditions in the complex system of the white of preserved eggs is difficult to explain in detail. Therefore, ovalbumin, which plays an important role in the formation of the white gel of preserved eggs, was used as the object of study. This paper investigated the gelation behavior of ovalbumin, the microstructural characteristics of the gel, the aggregation forces within the gel, and the changes in the characteristics of the molecular structure in the presence of a strong alkali. The formation mechanism of the ovalbumin gel under strong alkali conditions is also discussed.

2. Materials and method

2.1. Gel preparation

Ovalbumin and ultrapure water were used to prepare a 10% (m/v) ovalbumin solution, and a 2.0% (m/v) NaOH solution was prepared at the same time. Then, the two solutions were mixed in a beaker or a centrifuge tube at a volumetric ratio of 1:2. After the two solutions were mixed by slight oscillation, the mixture was placed at 25 °C to observe the gel formation process (Zhao, Tu, Li, et al., 2014). Samples were taken at 0 min, 5 min, 15 min, 30 min, 1 h, 3 h, and 5 h to analyze the properties and microstructure of the gel, the interaction among gel molecules, and the changing law of the molecular structure of the protein.

2.2. Determination of gel strength

A TEE-32 texture analyzer (Stable Micro System, Surrey, UK) was used to determine the strength of the ovalbumin gel in single compression cycle mode. The gels formed from mixing 10 mL of an ovalbumin solution with 20 mL of a NaOH solution in 100-ml beakers were measured directly. The measurement parameters were as follows: pretest speed of 2 mm/s, test speed of 1 mm/s, post-test speed of 2 mm/s, target distance of 6 mm, compression ratio of 70%, and trigger point load of 5 g. The probe used was a P/0.5 cylindrical probe. Data acquisition and analysis were completed using Texture Loader software.

2.3. Determination of LF-NMR spin–spin relaxation time (T_2)

An ovalbumin sample was placed in a sample tube that was then placed in the center of a radiofrequency coil of a low-field nuclear magnetic resonance apparatus (0.3T, Jiangxin Corporation, Ningbo City, China) according to the methods of He et al. (2013) with slight modifications. A CPMG pulse train was used to scan and determine the spin–spin relaxation time (T_2) of the sample. The parameters used in the experiment were as follows: sampling point, TD = 16,384; echo number, $C_0 = 30$; number of scans, NS = 10; and relaxation decay time, $D_0 = 7000$ ms. After sampling, a T_2-CPMG fitting procedure was used to calculate T_2. Each sample was scanned three times. The average spin–spin relaxation time (T_2) was calculated.

2.4. Scanning electron microscopy (SEM)

The ovalbumin gel microstructure was examined using environmental SEM (ESEM, Quanta-200F, FEI, Ltd., The Netherlands) according to the method described in our previously published work (Chen et al., 2015) with some modifications. Ovalbumin gels were fixed in 2.5% (v/v) glutaraldehyde for approximately 24 h at room temperature and then rinsed with 0.1 M phosphate buffer (pH 7.2) three times for approximately 15 min. The fixed gel samples were freeze-dried using a freeze-dryer (Alpha1-2, Martin Christ, Germany) and then observed by ESEM with an acceleration voltage of 10 kV in low vacuum mode.

2.5. Measurement of the free sulfhydryl (SH) group and total sulfhydryl groups

Approximately 3.0 g of each ovalbumin gel sample was placed into a 50-mL centrifuge tube, and 27 mL of phosphate buffer (100 mM, pH 8.0) was added. An Ultra Turrax homogenizer (IKA T18 digital, IKA Works Guangzhou Co., Ltd., China) was used to homogenize the sample at 12,000 r/min for 2 min, and then, the mixture was centrifuged at 8000 r/min for 20 min. The supernatant was taken for later use. The biuret method was used to determine the concentration of the supernatant.

Free sulfhydryls were detected as follows: 2.8 mL of a Tris-Gly buffer solution (0.1 M Tris, 0.1 M glycine, 4 mM EDTA, pH 8.0) and 0.02 mL of Ellman's reagent (4 mg/mL DTNB dissolved in 0.1 M, pH 8.0 Tris-glycine buffer solution) were added to 0.2 mL of the supernatant collected as described above. Then, the solution was placed in a water bath at 40 °C for 15 min for development. After cooling, a T6 spectrophotometer (Persee Co., Ltd., Beijing, China) was used to determine the absorption of the sample at 412 nm, and a molar extinction coefficient of $1.36 \times 10^4\ M^{-1}\ cm^{-1}$ was used to calculate the sulfhydryl content (Ellman, 1959). A phosphoric acid buffer solution was used as the blank control.

The total sulfhydryl groups were detected as follows: 2.8 mL of 0.5% SDS in an 8 M urea-Tris-Gly buffer solution (0.1 M Tris, 0.1 M glycine, 4 mM EDTA, pH 8.0) and 0.02 mL of Ellman's reagent (4 mg/mL DTNB dissolved in 0.1 M, pH 8.0 Tris-glycine buffer solution) were added to 0.2 mL of the supernatant collected as described above. The solution was placed in a water bath at 40 °C for 15 min for development. The photometric absorbance reading was taken as described for free sulfhydryls.

The sulfhydryl content was calculated using the following:

$$\mu M\ SH/g\ pro = 73.53 \cdot A_{412} \cdot D\ /C$$

where 73.53 was calculated from unit conversion ($10^6/1.36 \times 10^4\ M^{-1}\ cm^{-1}$), A_{412} is the measured UV absorption value at 412 nm, D refers to the dilution factor (15.01 in this study), and C is the concentration of protein in the supernatant (mg/mL) (Beveridge, Toma, & Nakai, 1974).

2.6. Selective protein solubility

The solubility of the ovalbumin gel protein was analyzed according to the method described by Pérez-Mateos et al. (Pérez-Mateos, Lourenço, Montero, & Borderías, 1997) with some modifications. Ovalbumin gels were successively solubilized in four solvents: 0.6 M sodium chloride (S1); 0.6 M sodium chloride + 1.5 M