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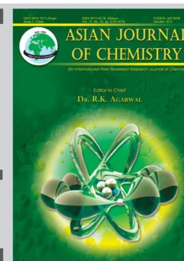

Effect of S-Configuration Transformation of Ovalbumin on its Molecular Characteristics and Emulsifying Properties

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The objective of this study was to investigate the changes of molecular characteristics and emulsibility after S-configuration transformation of ovalbumin and the relationship between molecular characteristics and emulsibility. N-Ovalbumin was purified by DEAE Sepharose CL-6B ion-exchange, S-ovalbumin obtained through thermal induction in alkalinity condition and morphous of emulsion was observed by microscope. After S-configuration transformation, the results showed that the increasing of free amino group, hydrophobicity and emulsifying activity were 54.69, 76.88 and 38.92 %, respectively and the decreasing of zeta potential, average droplet size of emulsion (d_{32}) and emulsifying stability were 7.1 mV, 2.07 μm and 51.07 %, respectively. Correlation analysis indicated that there was significant correlation between molecular characteristics and emulsify activity. Microscopic observation indicated that S-ovalbumin was prone to form smaller densely droplet emulsion. It was concluded that emulsify activity could be improved to a certain extent, but emulsifying stability declined after S-configuration transformation which is one of the reasons leading to quality deterioration of chicken egg albumen.

Key Words: Ovalbumin, S-Configuration transformation, Molecular characteristics, Emulsifying properties, Correlation.

INTRODUCTION

The egg white contains 9.7-12 % protein, of which 54-63 % is ovalbumin[1]. Ovalbumin is a monomer, globular phosphate glycoprotein with a relative molecular mass of 43.0 kDa and a isoelectric point of 4.5[2]. Masayuki Yamasaki *et al.*[3] found that the shape of ovalbumin was elliptic whose size was 70 Å × 45 Å × 50 Å and almost all the polypeptide chains were composed by known secondary structure according to 1.95 Å resolution ratio crystal structure determination. During storage or incubation period, natural ovalbumin (N-ovalbumin) converted spontaneously into S-ovalbumin, an irreversible extreme heat-stable form in comparison with N-ovalbumin, as determined by thermogram of the differential scanning calorimeter (DSC)[4-6]. Some researches indicated that S-ovalbumin emerged as an isomers of N-ovalbumin, rather than chemical modification derivative[7]. Both pH and temperature affecting the S-ovalbumin formation, the relative quantity of S-ovalbumin in the egg white can increase during storage period, from 5 % in fresh eggs to 81 % after six months of refrigerated storage[8,9]. Previous workers[10-12] reported S-ovalbumin could also be prepared when inducing N-ovalbumin at alkaline and heat condition and corresponding variation occurred in the characteristics and function of molecule during the preparation.

Certain functional properties of S-ovalbumin are different from that of N-ovalbumin, owing to a series of changes in the molecule and microscopic structure during the process of S-configuration transformation. Gelandsdal[13] reported that the heat-induced gel strength of S-ovalbumin was much lower than that of N-ovalbumin. In particular, S-ovalbumin is more susceptible to chymotrypsin and thermolysin than N-ovalbumin, with higher rate of degradation by porcine pancreatic elastase and subtilisin Carlsberg[6]. Due to the characteristic that S-ovalbumin information is independent from egg weight, hen age and nutritional status, shows high repeatability and low natural variability in fresh egg, it is a promising evaluation indicators for commercial shell egg freshness by determining its content in albumen[14].

The objective of present study was to investigate the correlation between the molecular characteristics and emulsifying properties of S-ovalbumin and to get the mechanism of how S-configuration transformation affected the molecular and emulsifying properties of ovalbumin. In order to accomplish this purpose, content of free amino group, zeta potential, hydrophobicity, emulsifying activity and average droplet size of emulsion (d_{32}) during S-ovalbumin information process were tested. Correlation analysis of above factors and microscopic observation of emulsion of S-ovalbumin were also conducted.

EXPERIMENTAL

Raw materials: Fresh eggs were provided by local poultry farm, available in the laboratory at 0-day from laying; DEAE Sepharose CL-6B, Ovalbumin (with a concentration more than 95 %), 1-aniline base naphthalene-8-sulfonic acid (ANS) and adjacent benzodiazepines formaldehyde (OPA) were purchased from Sigma company.

High-performance liquid chromatography (Agilent1100, American); differential scanning calorimeter (DSC 204 F1, Naichi instrument manufacturing company of Germany); UV/visible spectrophotometer (DU 700, beckman Kurt Co., Ltd. of American); Malvern Zeta potential size locator (Zetasizer Nano ZS, Malvern Instruments Ltd of British); fluorescence spectrophotometer (F-4500, Hitachi of Japan).

Purification of N-ovalbumin: N-Ovalbumin was isolated from fresh chicken egg white using the following improved semi-large-scale procedure[9]. Egg white and egg yolk of 0-day fresh hen eggs were separated by hand. 600 mL of 50 mmol/L *tris*-HCl buffer (pH 7.5, containing 10 mmol/L β-mercaptoethanol) was added to the total egg white fraction (about 300 mL) and stirred for 24 h at 4 ºC. Subsequently, the solution was centrifuged for 0.5 h at 14, 000 g, 4 ºC. Then, the pellet was discarded and the supernatant was added with 1800 mL of 50 mmol/L *tris*-HCl (pH 7.5). After 0.5 h gentle stirring, the solution was filtered by a paper filter. The filtrate was mixed with 500 g of DEAE Sepharose CL-6B, followed by overnight incubation at 4 ºC with gentle stirring. Next, the solution was filtered by a glass filter (G2) and washed with 10 L demineralized water and 5 L of 0.1 mol/L NaCl, successively. The protein was eluted with a series concentrations of 0.10, 0.15, 0.20, 0.25 and 0.35 mol/L 1 L NaCl. The elutions of 0.15 and 0.20 mol/L NaCl were collected and concentrated using ultrafiltration unit (30 kDa molecular weight cut-off membrane). The concentrated solution was dialyzed extensively against demineralized water and afterwards lyophilized. The freeze-dried N-ovalbumin had been stored at -40 ºC till use.

SDS-PAGE electrophoresis: SDS polyacrylamide gel electrophoresis was conducted using 12 % acrylamide running gel and 5 % stacking gel. Sample powders were added to buffer solution to reach the concentration of 1 mg/mL and heated about 3-5 min in boiling water. 25 and 40 mA of electric current were used when samples in satcking gel and in running gel, respectively[15].

RP-HPLC: The RP-HPLC analysis was determined according to Nau method[16]. Briefly, 10 µL sample was injected directly into HPLC using Zorbox300SB C_{18} (4.6 mm × 250 mm) column at 25 ºC. 7-70 % acetonitrile containing 0.05 % TFA was used as mobile phase of gradient elution and wavelength of UV detector was 280 nm.

Preparation of S-ovalbumin: S-Ovalbumin was prepared from ovalbumin according to the method described by Smith and Back[10] & Groot and Jongh[17]. 2.5 g N-ovalbumin was dissolved in 60 mL of demineralized water and then adjust the pH to 9.9. Then, the solution was incubated at 55 ºC for 72 h. Subsequently, the solution was dialyzed extensively against demineralized water at 4 ºC. After dialysis, the pH of the solution was adjusted to 6.7 and the protein was lyophilized. The final material had been stored at -40 ºC until use.

Differential scanning calorimetry (DSC) analysis: After sample being induced 0, 24 and 72 h, respectively, phosphoric acid buffer solution (50 mmo1/L, pH 7.4) contained 2 mg/mL ovalbumin was heated from 25-120 ºC at a speed of 1 ºC/min, degassed for 10 min before calorimeter analysis.

Determination of free amino content: Orthophaladetyde (OPA) method was used. The calculation formula of the relative content of free amino was: free amino content (%) = $A_t/A_0 \times 100$ %. (At represents the light-sucking value of samples induced by different time, A_0 represents that of the untreated sample).

Determination of hydrophobicity: The concentration of ovalbumin was diluted to 1-5 mg/mL by adding phosphoric acid buffer solution (20 mmo1/L, pH 7.4). And 20 µL ANS solution as fluorescence probe was added into 4 mL diluted samples with different concentration, followed by kept for 1 h at room temperature. Then fluorescence intensity was determined using a fluorescence spectrophotometer at the excitation wavelength of 370 nm and emission wavelength of 470 nm. The surface hydrophobicity of protein molecule was defined as the rate of curves of the function of protein concentration and fluorescence intensity.

Determination of zeta potential: *Tris*-HCl buffer solution (pH 7.4, 20 mmo1/L) was added with a suitable quantity of 10 mmo1/L NaCl and followed by degassed. Then, ovalbumins induced with different time were added and dissolved, filtrated using 0.45 µm membrane. Zeta potential was determined by Malvern Zeta potential size locator at 25 ºC and each sample was tested in triplicate.

Determination of the average droplet size: The average droplet size of emulsion was measured using Malvern Zeta potential size locator. Optical parameters were as follows: the refractive index of corn oil and water, 1.4673 and 1.33, respectively; absorb parameter 0.001.

Determination of emulsification activity and stability: Ovalbumin induced with different time was dissolved in phosphate buffer solution (pH 7.4, 0.1 mo1/L) and 30 mL of sample were added with 10 mL of corn oil. And then the mixture was homogenized for 1 min at a speed of 10,000 r/min at room temperature. Then 100 µL of homogenate was added into 5 mL of 0.1 % SDS, absorbance at 500 nm was monitored and the obtained absorbance value (A_1) was defined as emulsification activity (E_A). The again (15 min later) obtained absorbance value was defined as A_2 and emulsification stability could be expressed by the following equation: $E_S = A_1 \times t/(A_1\text{-}A_2)$.

Correlation analysis: Free amino content, Zeta potential, average droplet size, hydrophobicity and emulsification activity were selected and analyzed with SPSS for Windows, v. 15.0 (SPSS, Chicago IL, USA).

Microstructure observation of emulsion: One drop of emulsion was put onto a slide and covered with coerslip, then observed under a microscope with an amplification of 200 times.

RESULTS AND DISCUSSION

Determination of N-ovalbumin purity: The result of the SDS-PAGE electrophoresis of N-ovalbumin was showed in Fig. 1. According to the result, there was only a single belt of

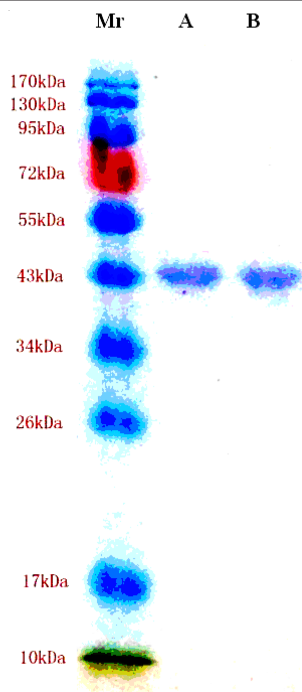


Fig. 1. SDS-PAGE profile of N-ovalbumin (lane A is the purification product of 0.15 mol/L NaCl batch and lane B is that of 0.20 mol/L NaCl batch)

N-ovalbumin which was concordant with standard protein. The result of RP-HPLC analysis (Fig. 2) showed that the two obtained purity of N-ovalbumin are 98.32 and 98.03 %, respectively, which indicate that the purification effect was perfect.

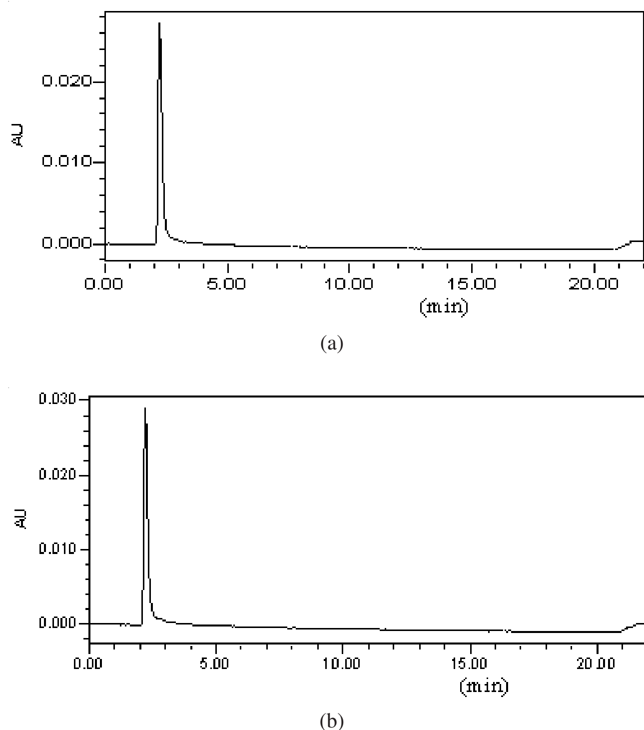


Fig. 2. RP-HPLC profile of N-ovalbumin (Fig. (a) is the purification product of 0.15 mol/L NaCl batch, Fig. (b) is that of 0.20 mol/L NaCl batch)

DSC analysis of S-ovalbumin formation: The results of the T_m value of induced samples were presented in Fig. 3, which showed that the T_m value of un-induced N-ovalbumin

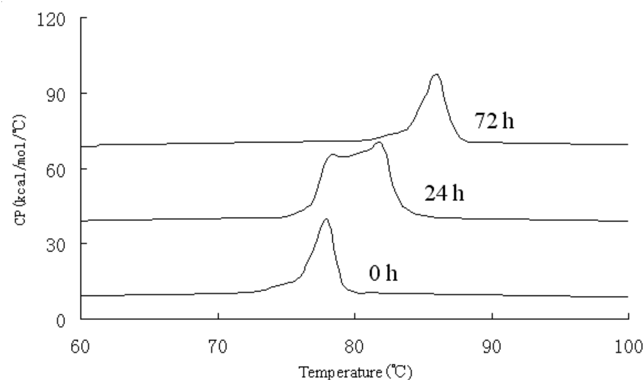


Fig. 3. DSC profile of ovalbumin induced with different time

was 78 ºC, S-ovalbumin induced with 72 h was 86 ºC and there were two peaks (78 and 83 ºC) of ovalbumin induced with 24 h. The above results indicated that thermal stability of S-ovalbumin was obtained successfully and intermediate product (I-ovalbumin) existed during S-ovalbumin formation.

Effects of S-configuration on the hydrophobicity and Zeta potential of ovalbumin: Fig. 4 showed the effects of S-configuration on the hydrophobicity and Zeta potential of ovalbumin. Hydrophobicity of S-ovalbumin increased with the extension of induction time and it increased about 76.88 % in 72 h. Hydrophobic groups which mostly distributed in internal molecular exposed during S-configuration, resulting in a increase of the hydrophobicity[18,19]. Surh *et al.*[20] and Matemu *et al.*[21] reported that protein surface charge density change could be characterized by Zeta potential and demonstrated that ovalbumin was negatively charged because their isoelectric point (4.5) was less than the pH value of buffer solution (7.4). In contrast with hydrophobicity, the Zeta potential decreased correspondingly with prolonging induced time, it considerably reduced 7.1 mV from -10.9 mV at 0 h to -18.0 mV 72 h later, which manifested that the surface negative charge density of S-ovalbumin significantly increased and the structure of S-ovalbumin enhanced.

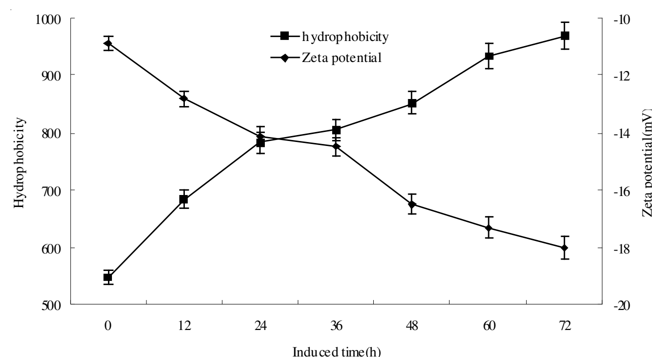


Fig. 4. Effect of S-configuration transformation on hydrophobicity and Zeta potential of ovalbumin

Effects of S-configuration on the free amino content and average size of emulsion of ovalbumin: According to the results of Fig. 5, free amino content increased with the prolonging of inducing time, 20.57 % free amino increased after 36 h inducing and 54.69 % free amino increased 72 h later. The average droplet size (d_{32}) of emulsion, which reflects

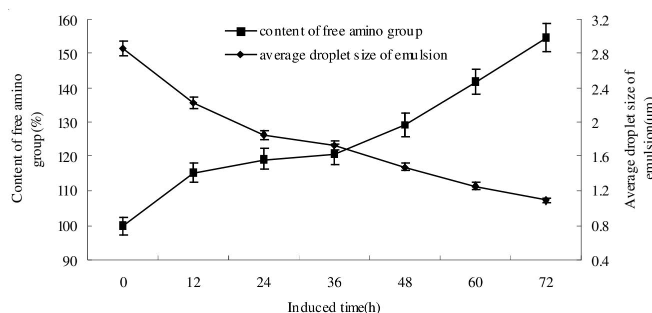


Fig. 5. Effect of S-configuration transformation on content of free amino group and average droplet size of emulsion of ovalbumin

emulsified characteristics from microscopic aspects, were studied to analysis the variation of emulsification activity of ovalbumin during S-ovalbumin inducing process. Results showed that d_{32} was continuously lower with time prolonging, reducing from 2.96-0.89 μm at 72 h which falling off 2.07 μm.

Effects of S-configuration on the emulsified activity and stability of ovalbumin: Emulsification characteristics of S-ovalbumin were reflected from macroscopic aspects by investigating simultaneously the changes of their emulsified activity and stability during S-configuration transformation. As shown in Fig. 6, the emulsified activity of S-ovalbumin continuously increased with prolonging of inducing time, increased from 1.485-1.743 in 0-24 h. Then there was a linear increase in 36-72 h and emulsified activity increased to 2.063 72 h later, which was 38.92 % higher than N-ovalbumin. Emulsification activity increased due to that smaller emulsion droplet could make transmittancy drop and S-configuration could make hydrophobic groups expose[22]. Compared with emulsification activity, there was a decline in emulsification stability. A significant reduction of 51.07 % was obtained from 0-72 h (emulsification stability was 1627.5 at 0 h and 831.2 at 72 h). This decline phenomenon could be explained from the fact that protein molecule gradually polymerizes again owing to the synergetic effect of hydrophobic and electrostatic forces[23].

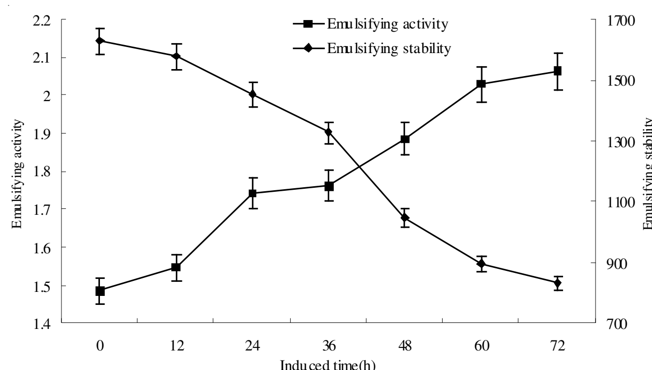
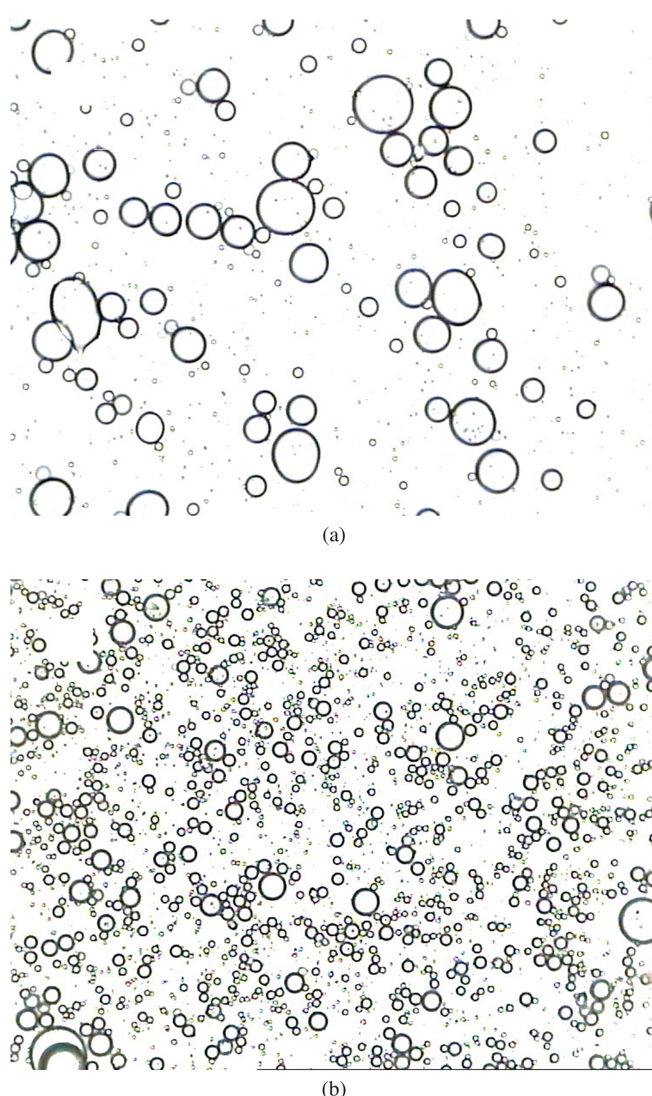


Fig. 6. Effect of S-configuration transformation on emulsifying activity and stability of ovalbumin

Correlation analysis: Correlation analysis (Table-1) showed that there was highly significant correlation between emulsification activity and all molecular characteristics (the correlation coefficient R was all more than 0.9500, $p < 0.001$). It could indicate that the researched factors were the key points of emulsifying properties. This study laid a solid theoretical foundation for the further analysis of the relationship between molecule characteristics and emulsified properties.

Microstructure observation of emulsion: Emulsion microscopic images could be analyzed from the size and the intensity of droplet[24]. As presented in Fig. 7, the droplets of N-ovalbumin were bigger and scattered sparsely, while that of S-ovalbumin obtained 72 h later were smaller and spread densely. The results confirmed that the ovalbumin emulsified activity can be improved through S-configuration transformation to a certain extent.



(a)

(b)

Fig. 7. Microscopic images of droplets of emulsions of N-ovalbumin (a) and S-ovalbumin (b) (× 200)

TABLE-1
CORRELATION ANALYSIS BETWEEN MOLECULAR CHARACTERISTICS AND EMULSIFYING ACTIVITY OF S-OVALBUMIN

	Zeta potential	Content of free amino group	Hydrophobicity	Average droplet size
Emulsifying activity	-0.98496***	0.95491***	0.97585***	-0.96644***

*Significant at p ≤ 0.05, ***Significant at p ≤ 0.001.

Conclusion

In the present study, N-ovalbumin was isolated from fresh eggs by ion-exchange. The RP-HPLC analysis showed that the samples were pure through purification. DSC analysis indicated that S-ovalbumin was successfully obtained and intermediate product (I-ovalbumin) existed during S-ovalbumin forming. Hydrophobicity of S-ovalbumin increased with the extension of induction time and it increased about 76.88 % in 72 h; Zeta potential declined, 7.1 mV lost in 72 h; free amino content continuously increased, 54.69 % free amino increased after inducing 72 h. The average droplet size (d_{32}) of emulsion reduced from 2.96-0.89 μm at 72 h. There was an increase of 38.92 % of emulsified activity and a decrease of 51.07 % of emulsifying stability of S-ovalbumin induced 72 h. There was highly significant correlation between emulsification activity and molecular characteristics and the droplets of N-ovalbumin were bigger and scattered sparsely while that of S-ovalbumin were smaller and spread densely. The results from the present investigation suggested that emulsify activity of ovalbumin was improved to a limited extent by the formation of S-ovalbumin, however, emulsifying stability descended. Therefore, it could be concluded that S-configuration transformation of ovalbumin is one of the reasons leading to quality deterioration of commercial chicken egg. Further research is needed to fully explain the mechanism of S-configuration transformation and its effect on the functional characteristics of ovalbumin.

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