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Comparative Proteomic Analysis of Egg White Proteins under Various Storage Temperatures

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Supporting Information

ABSTRACT: Although the effect of storage temperature was suggested to be a more important factor than that of storage time on changes in unfertilized egg white proteins, no comprehensive analysis of the thermally induced egg white protein changes was carried out. This study presents a proteomic analysis of the changes in unfertilized egg white proteins after 15 days of storage at 4, 20, and 37 °C. Using two-dimensional electrophoresis followed by MALDI-TOF MS/MS, 32 protein spots representing 8 proteins were identified with significant differences in abundance when stored at different temperatures. An accelerated degradation of ovalbumin, possibly resulting from the reduction of antiprotease, was observed after the storage at higher temperature. In addition, an increase in the formation of ovalbumin complexes and a decrease in lipocalin family proteins were detected with increasing storage temperature, which may indicate a thermally promoted change in chicken eggs. The decrease of clusterin during the high-temperature storage was suggested to be an effective biomarker for egg quality evaluation. These findings will give insight into the effects of storage temperature on changes in unfertilized egg white proteins during storage and provide a better understanding of the thermally induced biochemical changes that may affect the egg deteriorative process.

KEYWORDS: *2-DE, egg white proteins, proteomics, MALDI-TOF MS/MS, storage temperature*

INTRODUCTION

The chicken egg white provides essential nutrients for embryonic development and contains various antimicrobial factors to protect the embryo against invading bacteria.[1] For humans, the chicken egg is one of the most versatile foods with high nutritive value. However, eggs are also a perishable food product that loses its quality rapidly during storage.[2] Many complex changes occur during egg storage, including thinning of the albumen, loss of water and carbon dioxide, increase in albumen pH, weakening and stretching of the vitelline membrane, and increasing water content in the yolk, as well as changes in protein conformation.[3−6] These changes, which influence the functional properties of egg white and yolk, can be affected by environmental conditions such as storage time, temperature, humidity, and gaseous environment. In developed countries, fresh eggs are typically stored at temperatures between 5 and 8 °C, whereas such is not the case in most developing countries. Changes in specific egg proteins during storage have been demonstrated, such as the conversion of ovalbumin to the more heat-stable *S*-ovalbumin,[7] and it has been shown that these changes are influenced more by the storage temperature than by storage time. Likewise, albumen and yolk pH were also found to increase more at higher storage temperatures than lower ones.[2] In a recent study, the anti-*Salmonella* activities of egg white were measured after storage at different temperatures for different lengths of time.[8] After a storage of 5 days, higher anti-*Salmonella* activity was detected in egg white stored at 37 °C when compared to those stored at 4 and 20 °C. Moreover, the degradation of ovalbumin and ovotransferrin was detected during long-term storage at 37 °C.[8] These changes in egg white proteins subjected to different storage temperatures were strongly suggested to be correlated to the reduction of antimicrobial activity and/or egg white thinning.

In the past few years, various proteomic techniques have been applied to the identification of novel proteins in chicken egg white.[9−12] These findings made it possible to investigate the changes in egg white proteins that occurred during storage and discover proteome markers for assessing egg qualities.[13] Recently, Omana et al. investigated changes in egg white proteins after different storage times using 2-DE and LC-MS.[14] Fourteen protein spots representing five egg white proteins were observed to change significantly ($p < 0.01$) in abundance during storage at an ambient temperature of 22 °C, and the maximum number of changes in protein abundance were detected after 20 days of storage, with fewer changes occurring upon further storage. Our previous proteomic study of egg white during the early phase of embryonic development revealed that most of the protein changes observed were affected by high temperature rather than the presence of an embryo.[15] To obtain a more comprehensive understanding of thermally induced changes in egg white proteins, unfertilized fresh chicken egg samples stored at three different temperatures (4, 20, and 37 °C) were collected and a comparative proteomic analysis was performed. The objective of the present study was to reveal the biological characteristics that play an important

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Table 1. Significantly Altered Unfertilized Chicken Egg White Proteins after Storage at Different Temperatures

spot[a]	accession[c]	protein name	score[c]	matched peptides/% sequence coverage	exptl pI/M_r (kDa)	theor pI/M_r (kDa)
		Serpin Family				
23	129293	ovalbumin [*Gallus gallus*]	170	6/21	5.95/66.1	5.19/42.9
	126608	lysozyme C [*Gallus gallus*]	237	4/51	5.95/66.1	9.36/16.2
33	129293	ovalbumin [*Gallus gallus*]	104	7/24	5.86/170.1	5.19/42.9
34	129293	ovalbumin [*Gallus gallus*]	88	6/20	5.96/170.1	5.19/42.9
35	129293	ovalbumin [*Gallus gallus*]	299	9/32	5.28/125.8	5.19/42.9
36	129293	ovalbumin [*Gallus gallus*]	188	11/36	5.96/72.6	5.19/42.9
37	129293	ovalbumin [*Gallus gallus*]	256	7/24	5.37/125.8	5.19/42.9
53	129293	ovalbumin [*Gallus gallus*]	203	7/25	5.97/73.2	5.19/42.9
51	129293	ovalbumin [*Gallus gallus*]	181	9/28	5.74/73.2	5.19/42.9
54	129293	ovalbumin [*Gallus gallus*]	128	7/25	5.37/48.2	5.19/42.9
3	129293	ovalbumin [*Gallus gallus*]	154	5/19	4.54/41	5.19/42.9
15	129293	ovalbumin [*Gallus gallus*]	182	7/21	4.42/40	5.19/42.9
38	129293	ovalbumin [*Gallus gallus*]	193	6/21	5.68/73.2	5.19/42.9
14	129293	ovalbumin [*Gallus gallus*]	149	6/19	4.45/33.5	5.19/42.9
39	129293	ovalbumin [*Gallus gallus*]	264	6/21	4.52/28.9	6.85/77.8
40	129293	ovalbumin [*Gallus gallus*]	132	4/14	4.89/26.8	5.19/42.9
13	129293	ovalbumin [*Gallus gallus*]	143	7/24	4.61/26.0	5.19/42.9
41	129293	ovalbumin [*Gallus gallus*]	290	4/13	4.51/19.6	5.19/42.9
42	129293	ovalbumin [*Gallus gallus*]	261	6/22	4.49/18.1	5.19/42.9
43	1351295	ovalbumin [*Gallus gallus*]	134	4/18	4.80/25.6	5.19/42.9
44	129296	ovalbumin-related protein Y [*Gallus gallus*]	163	4/24	6.58/56.0	6.47/55.8
		Protease Inhibitors Kazal Family				
45	71895337	ovoinhibitor precursor [*Gallus gallus*]	129	10/25	6.16/67.2	6.16/54.4
57	71895337	ovoinhibitor precursor [*Gallus gallus*]	107	9/23	6.46/65.3	6.16/54.4
		Lipocalin Family				
55	45382221	extracellular fatty acid-binding protein precursor [*Gallus gallus*]	165	5/29	5.08/22.0	5.37/20.2
56	45382221	extracellular fatty acid-binding protein precursor [*Gallus gallus*]	223	9/51	5.63/19.8	5.56/20.3
5	45383612	prostaglandin D2 synthase, brain [*Gallus gallus*]	321	6/32	5.84/21	6.30/20.8
		Transferrin Family				
46	71274079	ovotransferrin BC type [*Gallus gallus*]	124	15/27	5.96/118.2	7.08/77.8
47	1351295	ovotransferrin [*Gallus gallus*]	186	11/20	6.61/63.7	6.70/77.5
52	1351295	ovotransferrin [*Gallus gallus*]	149	6/15	6.60/44.3	6.70/77.5
48	1351295	ovotransferrin [*Gallus gallus*]	174	6/17	6.44/38.1	6.70/77.5
49	1351295	ovotransferrin [*Gallus gallus*]	162	11/45	6.68/38.2	6.70/77.5
		Clusterin Family				
7	45382467	clusterin [*Gallus gallus*]	144	7/18	6.20/34	5.47/51.3
50	45382467	clusterin [*Gallus gallus*]	177	6/13	6.54/33	5.47/51.3

[a]Spot ID represents the protein spot number on the 2-DE gel image. [c]Accession numbers of matched proteins according to the NCBInr database. [c]MASCOT score. The Mascot threshold score for all of these identified proteins is 74.

role during egg storage to better understand the biochemical basis of the egg white deteriorative process.

MATERIALS AND METHODS

Egg White Sampling. Fresh chicken eggs (60 ± 0.5 g, average weight) from Lohmann White Single Comb White Leghorn hens (of the same flock, 40 weeks of age) laid within 24 h were collected from the Poultry Research Centre farm of Huazhong Agricultural University and used in this study. The eggs were stored at 4, 20, or 37 °C and 65% relative humidity for 15 days. Eggs were randomly selected for sampling, and egg white was carefully collected at the end of the storage period. Fresh eggs (0 days) were considered as control. To reduce variations, three biological replicates were performed during the following 2-DE analysis.

Protein Extraction. Egg white proteins were extracted according to the method previously described.[15] Briefly, egg white was separated from yolk and gently homogenized for 30 min with a magnetic stirrer. The egg whites of fresh eggs (control), as well as those stored at three different temperatures (4, 20, and 37 °C) for a period of 15 days, were then prepared for 2-DE analysis according to ref 15.

2-DE Analysis. 2-DE analysis was performed using the Ettan IPGphor 3 System (GE Healthcare, Piscataway, NJ, USA) for the first-dimension isoelectric focusing (IEF) and the Ettan DALTSix System (GE Healthcare) for SDS-PAGE in the second dimension. IEF was performed using DryStrip IPG strips (24 cm; pH 4−7) with 125 μL of the sample (100 μg of protein) diluted in rehydration buffer (Bio-Rad, Hercules, CA, USA), using the same conditions previously described[15] (20 °C; step 1, 300 V for 0.5 h; step 2, 700 V for 0.5 h; step 3, 1500 V for 1.5 h; step 4, 9000 V for 3 h; step 5, 9000 V for 5 h, for a total of 64000 Vh). The individual strips were then equilibrated to resolubilize proteins and reduce disulfide bonds. The second-dimension electrophoresis was performed using a 12.5% SDS−polyacrylamide gel. The gels were run at 2 W per gel for 45 min followed by 17 W per gel for around 4.5 h, until the dye front reached the bottom of the gel.[16] The protein spots on analytical and preparative 2-DE gels were visualized by silver and Coomassie Brilliant Blue staining, respectively. Gel evaluation and data analysis were carried out using the ImageMaster v

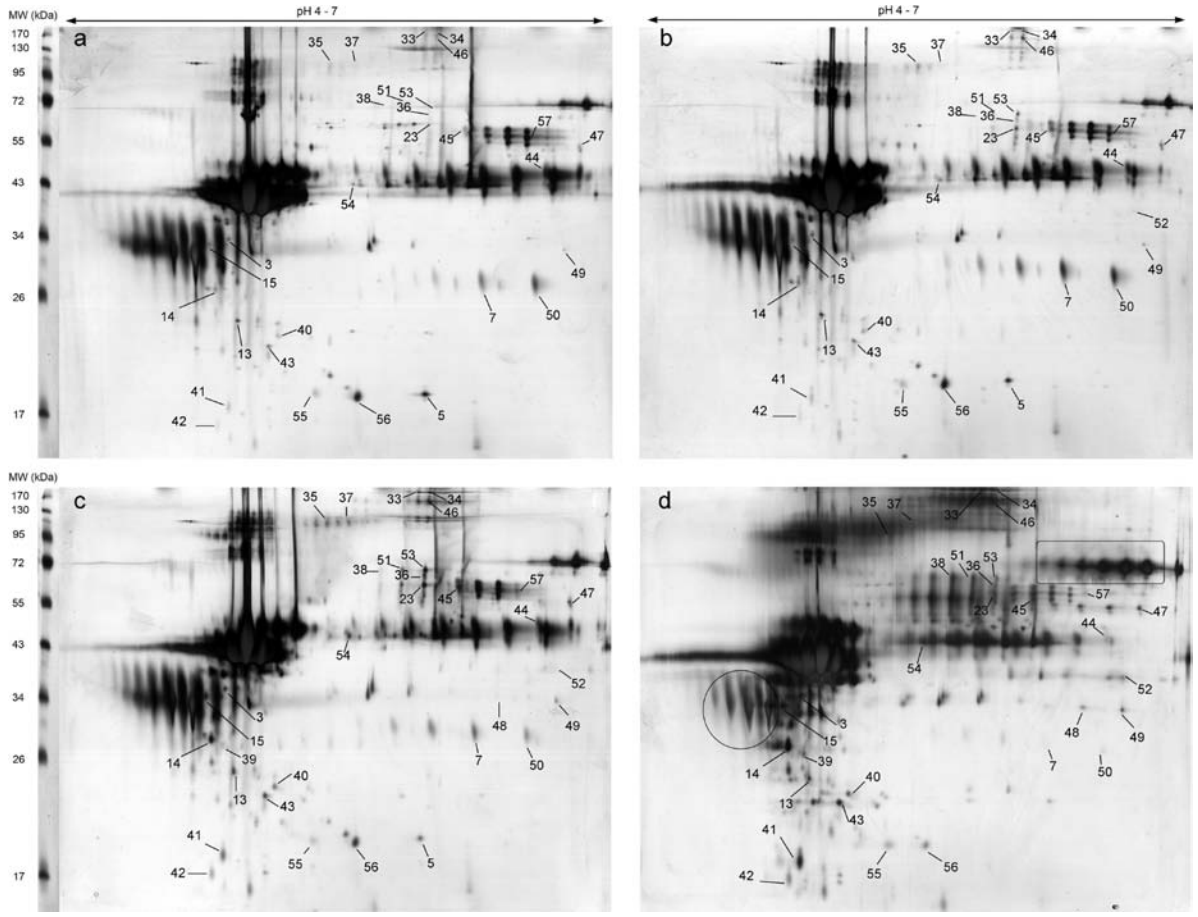

Figure 1. Representative 2-DE gel images of the proteins from unfertilized chicken egg white prepared by IEF/SDS-PAGE separation and subsequently silver stained: (a) 2-DE gel image with egg white proteins of control (fresh egg white, 0 days); (b–d) 2-DE gel image of egg white proteins after 15 days of storage at 4, 20, and 37 °C, respectively. Spots that significantly ($p < 0.01$) changed in abundance after 15 days of storage are indicated by numbers and arrows. The molecular weights (MW) and p*I* scales are indicated. Each gel is representative of three independent replicates.

7.0 program (GE Healthcare). The differences of 2-DE data were evaluated by a one-way ANOVA and a Tukey's significance test ($p < 0.01$) using SPSS 13.0 (SPSS, Chicago, IL, USA).

Gel Analysis and Protein Identification. Stained gels were scanned and calibrated via a PowerLook 1100 scanner (Umax Technologies, Dallas, TX, USA), followed by protein spot analysis using ImageMaster v 7.0 (GE Healthcare). Only those spots that showed significant ($p < 0.01$) and reproducible changes after storage at any of the three temperatures when compared to the control (fresh egg white, 0 days) were considered to be differentially accumulated proteins in relative abundance. The target protein spots were excised manually from the stained gels and then washed and digested with sequencing-grade trypsin (Promega, Madison, WI, USA).[17] The samples mixed with an equivalent matrix solution (HCCA) were applied for further MALDI-TOF MS/MS analysis using a fuzzy logic feedback control system (Ultraflex MALDI-TOF-TOF mass spectrometer (Bruker, Karlsruhe, Germany).[15] Proteins were unambiguously identified by searching against the nonredundant sequence database (NCBInr) via the MASCOT program (http://www.matrixscience.com).

RESULTS

In the present study, the effect of different temperatures (4, 20, and 37 °C) on changes in chicken egg white proteins after 15 days of storage was investigated by 2-DE analysis. Over 120 protein spots were detected, of which 38 spots were found to show significant ($p < 0.01$) changes in intensities, and 32 spots were further identified by MALDI-TOF MS/MS analysis (Table 1 and Supplementary Table S1 in the Supporting Information) as indicated in Figure 1. Most of these protein spots increased in abundance when the eggs were stored at higher temperatures (Figures 2 and 3). In contrast, some proteins, such as those in spots 5, 7, 44, 50, 56, and 57, decreased in abundance after storage at higher temperatures (Figure 3).

Among these altered proteins, 19 protein spots were identified as being ovalbumin, which is the most abundant egg white protein (comprising 54% of the total protein in egg white).[18] Some spots representing ovalbumin, such as spots 27–31, showed a much lower molecular weight than the theoretical value (42.9 kDa) (Table 1). Meanwhile, five ovalbumin spots (spots 23, 36, 53, 51, and 38) showed a higher molecular weight than the theoretical value, and spot 23 was identified as a lysozyme–ovalbumin mixture. However, we were unable to detect the presence of additional proteins in the other four ovalbumin spots. Another four of the ovalbumin spots were observed to be 3 (spots 35 and 37) or 4 (spots 33 and 34) times the theoretical molecular weight of ovalbumin, indicating possible ovalbumin polymers. The abundances of the 19 ovalbumin spots detected were found to increase with increasing storage temperature (Figures 1 and 2). After storage for 15 days at 37 °C, spots 51, 38, and 41 showed sharp

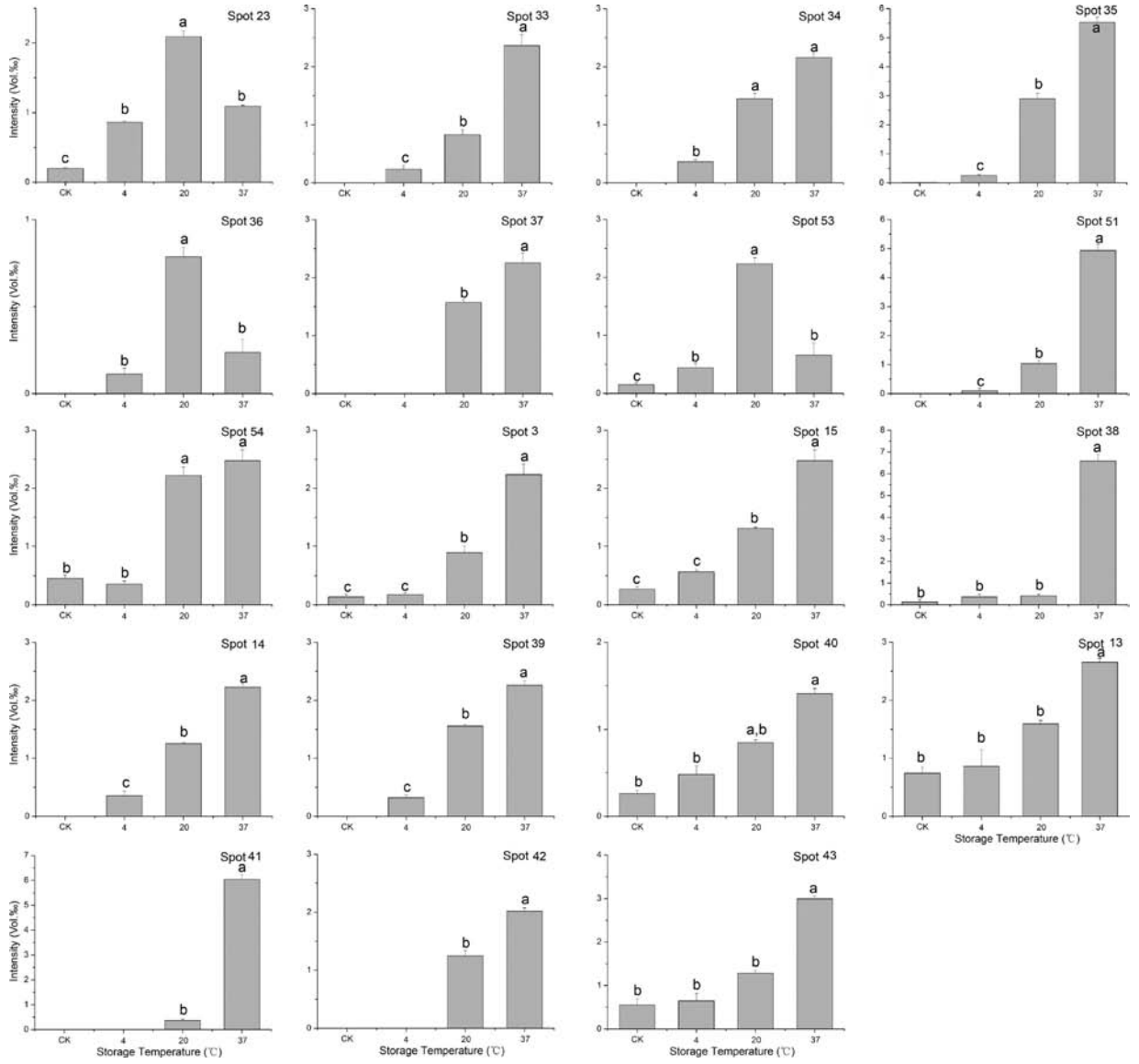

Figure 2. Changes in the intensity of egg white ovalbumin after the 15 day storage period at 4, 20, and 37 °C. Column profiles of ovalbumin abundance are shown as bar charts. Only spots that indicate statistically significant ($p < 0.01$) change from control (CK, fresh egg white, 0 days) are shown. The data represent the mean intensity values (±SD) from three replicates at each temperature. The bars with no common letter (a–c) indicate significant difference ($p < 0.01$).

increases in intensity, >5 times that observed when stored at lower temperatures. Nevertheless, three ovalbumin spots (spots 23, 36, and 53) showed their highest intensities when stored at 20 °C.

Along with ovalbumin, seven other proteins exhibiting changes in abundance were identified, including ovoinhibitor precursor (spots 45 and 57), ovalbumin-related protein X (spot 44), ovotransferrin (spots 47–49 and 52), ovotransferrin BC type (spot 46), extracellular fatty acid-binding protein precursor (Ex-FABP, spots 55 and 56), clusterin (spots 7 and 50), and prostaglandin D2 synthase (PG D2 synthase, spot 5) (Table 1 and Figure 1). The observed increases or decreases in protein abundance for these spots can be seen in Figure 3. As shown in Table 1, four ovotransferrin spots were identified with much lower molecular weight than the theoretical value, and the spot intensities increased dramatically after storage at 37 °C (Figures 1 and 3). A similar increase was also observed for spot 46, representing BC type ovotransferrin, but it had a higher molecular weight (118.2 kDa) than the theoretical value (77.8 kDa). A novel ovoinhibitor spot (spot 45) detected in this study showed the highest intensity after storage at 20 °C. Most of the other protein spots exhibited decreases in abundance when stored at high temperatures (Figures 1 and 3).

■ DISCUSSION

Proteomic methods have been widely used to search for biomarkers indicating quality changes during the storage or processing of foods (e.g., meat and milk),[13] as well as in the study concerning protein stability of clinical samples during storage.[19] Physicochemical changes in egg white during storage have been well-documented, and the effect of elevated temperature was found to be greater than that of increased storage time.[20] Thus, the effect of storage temperature on the change of egg white proteins at the proteomic level is of great interest. In the present study, chicken eggs were stored at three different temperatures (4, 20, and 37 °C) for a period of 15

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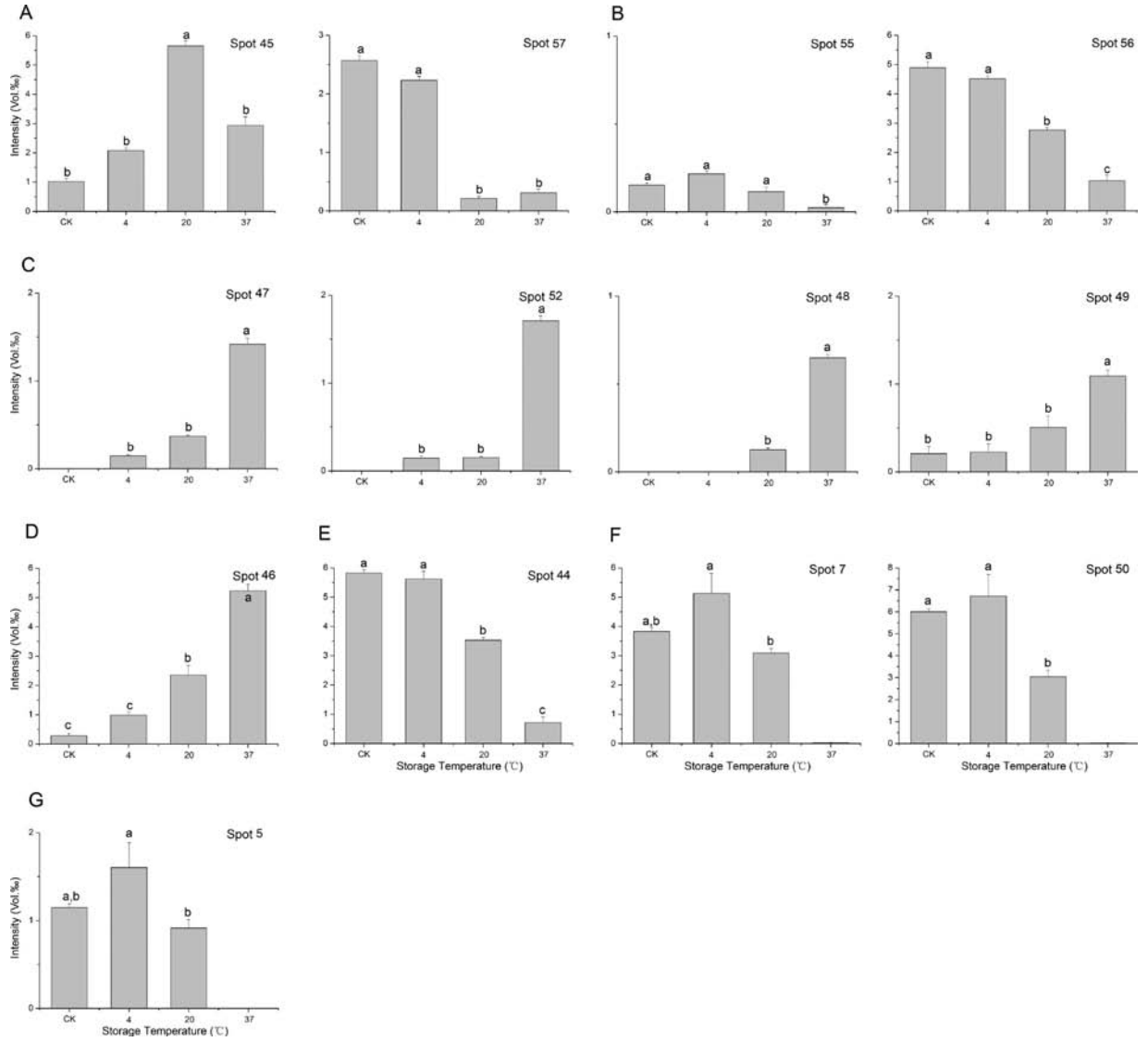

Figure 3. Changes in the intensity of egg white proteins after the 15 day storage period at 4, 20, and 37 °C: (A) ovoinhibitor precursor; (B) extracellular fatty acid-binding protein precursor; (C) ovotransferrin; (D) ovotransferrin BC type; (E) ovalbumin-related protein X; (F) clusterin; (G) prostaglandin D2 synthase, brain. Column profiles of these proteins are shown as bar charts. Only spots that indicate statistically significant ($p < 0.01$) change from control (CK, fresh egg white, 0 days) are shown. The data represent the mean intensity values (±SD) from three replicates at each temperature. The bars with no common letter (a−c) indicate significant difference ($p < 0.01$).

days, and 32 significantly altered egg white protein spots were identified through proteome analysis. These identified proteins were further clustered into six families: serpin (ovalbumin and ovalbumin-related protein X), clusterin (clusterin), transferrin (ovotransferrin and ovotransferrin BC type), lipocalin (PG D2 synthase and Ex-FABP), kazal-type protease inhibitors (ovoinhibitor precursor), and lys (lysozyme C).

A diversity of ovalbumin isoforms emerged after the storage, especially at higher temperatures. Similar to the multiple forms of α-amylase in human saliva,[21] the ovalbumin spots differed largely by both apparent MW (ranging from 18.1 to 170.1 kDa) and p*I* (ranging between 4.42 and 5.97) on gel. A marked increase in intensity was observed at 37 °C, especially for spots 51 and 38, which were suggested as ovalbumin isoforms through modifications and/or protein interactions (Figures 1 and 2). As a mixture of lysozyme and ovalbumin was detected in spot 23, which indicates a possible protein−protein interaction, the adjacent spots (spots 36 and 53) may also consist of ovalbumin complexes with other egg white proteins unidentified in this study. These hypothetical interactions were suggested to be sensitively regulated by storage temperature (Figures 1 and 2). The interaction of lysozyme and ovalbumin has been previously reported in vitro,[22−24] and this spot (spot 23 in Figures 1 and 2) was also detected in our former study,[15] but only lysozyme was identified through MALDI-TOF MS/MS. Interestingly, the abundance of this ovalbumin−lysozyme complex continued to increase during the early incubation period at 38 °C in fertilized eggs, but remained stable after 2 days of storage in unfertilized eggs.[15] Still, such interactions need to be verified, and the relationship between these interactions and the antimicrobial activity of egg white has yet to be determined. Meanwhile, nine ovalbumin spots (spots 3, 13−15, and 39−43 in Figure 1 and Table 1) showing an apparent MW lower than that of the native protein were observed increasing in abundance at higher storage temperatures. The apparent p*I* (ranging between 4.42 and 4.89) of

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these ovalbumin spots did not surpass the p*I* scale of the native protein (Figure 1 and Table 1). Among these nine spots, six (spots 3, 13−15, 39, and 40) share the same N-terminal peptide (^{105}LYAEER) and mostly bear the C-terminal part of native ovalbumin comprising 386 amino acids (Figure 1, Table 1, and Supplementary Table S1 in the Supporting Information). For the other three ovalbumin spots presenting an even lower MW, a larger extent of truncation in the N-terminal was proposed, especially for spot 41 (the matched N-terminal peptide started from ^{264}L). Perhaps the most intriguing situation corresponded to spot 42, which displayed the lowest apparent MW (18.1 kDa) among the identified ovalbumin spots on gel. The calculated mass (using the "Compute pI/M$_w$" ExPASy tool http://web.expasy.org/compute_pi/) of spot 42 (from the matched N-terminal ^{127}G to C-terminal ^{382}R) was 28.7 kDa, visibly higher than the apparent MW (Figure 1, Table 1, and Supplementary Table S1 in the Supporting Information), thus suggesting that certain internal parts might be absent in the sequence of this spot. An alternative hypothesis for this unexpected situation could be the cleavage of ovalbumin into peptides and subsequent association.[21] These nine ovalbumin spots were not detected in the previous study,[9] indicating that they are not original ovalbumin isoforms in the fresh chicken egg white. By comparison of the 2-DE gels (Figures 1 and 2), most of these spots were found to emerge after the storage and increase in intensities at higher storage temperature, suggesting the accelerated degradation of ovalbumin under the thermal storage. Furthermore, MS/MS data analysis suggested that these degradations would involve very specific processes, affecting only the N terminus, to different but limited extents, and in a way excluding degradation of the C-terminal part (Supporting Information, Table S1).

Clusterin is a widely expressed secretory glycoprotein belonging to the chaperone protein family. Previous studies have reported that clusterin was able to interact and stabilize unfolded or partly folded proteins, significantly inhibiting their aggregation or precipitation.[9] Spots 7 and 50, which were identified as clusterin (α/β heterodimer), both decreased significantly ($p < 0.01$) during the storage under high temperatures (Table 1 and Figures 1 and 3). These clusterin spots remained at a stable level of abundance after storage at 4 °C but almost disappeared following storage at 37 °C (Figure 3). The disappearance of clusterin spots at high-temperature storage conditions may be caused by interaction with other proteins or degradation at high temperatures. As clusterin plays an important role in the stabilization of egg white proteins, protein aggregation and precipitation would be expected to increase in the absence of clusterin during high-temperature storage. Thus, clusterin could serve as a marker for evaluating egg quality and freshness.

Ovotransferrin (OTf) is another important protein in high abundance in the egg white. OTf was reported to have antimicrobial activity against a wide range of bacteria (including Gram-positive and Gram-negative).[25] Moreover, it has been found that certain ovotransferrin peptides (such as OTAP-92 located within the N-lobe of OTf) exert antimicrobial ability via interaction with bacterial membranes.[26] In this study, four spots (spots 47−49 and 52) were identified as degraded protein fragments of OTf (Figure 1 and Table 1). Only modest changes in protein abundance were observed for these four spots after storage at 4 and 20 °C, but a marked increase in the intensities of these OTf spots was detected after the 15 day storage period at 37 °C (Figures 1 and 3). This indicated that OTf degradation increased after storage at 37 °C, which is in accordance with the results previously reported.[26] The characteristics of these OTf fragments, such as their antimicrobial activity, will require further investigation. Spot 46 was identified as ovotransferrin BC type but displayed a higher MW than that reported previously by Omana et al.[14] The observed MW of spot 46 was close to the sum of the theoretical value of ovotransferrin BC type and ovalbumin, suggesting a possible OTf−ovalbumin interaction. The appearance of such hypothetical egg white protein interactions during storage, especially at high temperatures, will require further validation and functional analyses.

Lipocalins are transporters for small hydrophobic molecules and play a particular role in chicken embryo development, including the process of endochondral bone formation.[27] Two of the proteins identified included PG D2 synthase (spot 5) and Ex-FABP (spots 55 and 56), which belong to the lipocalin family. PG D2 synthase was reported to play a key function in reproduction.[27,28] Ex-FABP, also known as Ch21 protein, is a developmentally regulated low molecular weight protein existing in chick embryo skeletal tissues.[29,30] In previous studies, no significant change in PG D2 synthase was observed after 20 days of storage at 22 °C, but the abundance significantly decreased after prolonged storage.[14] These three lipocalin family protein spots (spots 5, 55, and 56) were found here to clearly decrease in abundance after storage at 37 °C. Such decreases may be related to the selective binding and transport of long-chain unsaturated fatty acids, which still needs to be validated.

Although the increased degradation of ovalbumin and OTf during storage at high temperatures has been shown previously[14] as well as in this study, some of the proteolytic fragments may also have come from the solubilized peptides of the eggshell or vitelline membrane. The activation of certain proteases or reduction in antiprotease activity at physiological temperatures might play an important role in these proteolytic degradations. The analysis of thermally induced protein changes in egg white could help to elucidate the key roles of certain proteins in the deteriorative process and reveal potential biomarkers associated with inferior quality.[31] The reduction of some pivotal proteins, such as clusterin, may directly stimulate the breakdown of the firm egg white system, leading to the accelerated deterioration process. Besides protein modifications, including phosphorylation, glycosylation, and proteolysis, the formation of protein complexes, which was clearly observed during storage at 37 °C, was presumed as another reason leading to the loss in some key protein functionalities such as the antimicrobial activity of lysozyme and OTf. In a previous study,[8] large differences in anti-*Salmonella* activity of egg white began to emerge at 15 days of storage at 4, 20, and 37 °C, respectively. The egg white anti-*Salmonella* activity decreased rapidly under storage at 37 °C but remained at the highest level when stored at 20 °C. Image analysis of the present work revealed three adjacent ovalbumin isoforms (spots 23, 36, and 53 in Figures 1 and 2) and one ovoinhibitor isoform (spot 45 in Figures 1 and 3), showing their highest intensities in the sample of eggs stored at 20 °C for 15 days. The increase in abundance of these protein isoforms might, at least partially, contribute to the higher anti-*Salmonella* activity level. For spot 23, lysozyme peptides were detected together with those of ovalbumin, which was strongly suggested as an ovalbumin−lysozyme complex. By comparison with other ovalbumin isoforms, these three adjacent ovalbumin spots presented a different pattern in

stability during storage under different temperatures. Stronger stability was suggested for these three hypothetical protein complexes at ambient storage temperature of 20 °C than at 4 or 37 °C. Spot 45 is a newly found ovoinhibitor isoform in addition to the nine ovoinhibitor spots previously identified.[9] The inhibiting ability of this ovoinhibitor isoform to bacterial proteases that are secreted by pathogens during the host colonization process still needs further analysis.

To give a detailed view of most major egg white proteins, the p*I* range of 4−7 was selected in this study. Due to the limitation of this method, most medium- to low-abundance egg white proteins were not included; neither were some well-known proteins such as lysozyme C and VMO-1.[9] The bioactive roles of the minor protein components and their changes during storage and embryogenesis are currently under investigation in our laboratory using combinatorial peptide ligand libraries. Even though the degradation percentage of the high-abundance egg white proteins could not be quantificationally calculated in this study because of the overstaining of these proteins, the increase in abundance of the five isobaric OTf differing by p*I* (boxed area in Figure 1d) and the decrease of ovomucoid abundance (circled area in Figure 1d) could be obviously observed during storage of 37 °C. Protease−antiprotease balance in egg white is thought to be involved in embryogenesis and antimicrobial defense.[32] Such a thermally induced decrease in the abundance of major egg white antiproteases (e.g., ovomucoid and ovoinhibitor) identified in this study was supposed to be one of the major reasons causing the accelerated protein degradations as well as the reduced antimicrobial ability at higher storage temperature.

In conclusion, a 2-DE-based proteomic analysis was performed to investigate the effect of storage temperature on changes in albumen proteins. In total, 32 protein spots representing 8 different proteins were detected, showing significant changes in abundance during storage at different temperatures. Degradation of ovalbumin was more prominent at higher temperatures, and the formation of ovalbumin complexes appeared to increase with increasing storage temperature. Notably, a lysozyme−ovalbumin complex was detected, which showed its highest abundance after the 15 day storage period at 20 °C. Meanwhile, a sharp increase in ovotransferrin degradation was observed after storage at 37 °C when compared to lower temperatures. Of particular interest was the disappearance of clusterin protein spots when the eggs were stored at high temperature (37 °C), which may indicate an important biomarker for evaluating egg quality. These findings not only give a fundamental understanding of the influence of storage temperature on changes in egg white proteins during storage but also provide important knowledge for the quality control of egg storage and processing.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

PG, prostaglandin; HCCA, α-cyano-4-hydroxycinnamic acid; MW, molecular weight; Ex-FABP, extracellular fatty acid-binding protein precursor; OTf, ovotransferrin.

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