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COMPARATIVE STUDY OF REPTILE EGG WHITE PROTEINS BY 2D-PAGE

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ABSTRACT

The egg white protein patterns of reptiles have not yet been fully studied. In this study, the egg white proteins of five reptile species were compared with chicken egg white by 2D-PAGE using nonlinear IPG strip pH3-10. The results showed that 35, 26, 45, 15, 33 and 36 protein spots were reproducible detected in Siamese crocodile (*Crocodylus siamensis*), soft-shelled turtle (*Trionyx sinensis tiwanese*), red-eared slider turtle (*Trachemys scripta elegans*), hawksbill turtle (*Eretmochelys imbricate*), green sea turtle (*Chelonia mydas*) and chicken *(Gallus gallus*) egg white, respectively. After protein pattern analysis, many spots corresponding to major chicken egg white proteins such as ovalbumin and ovotransferrin were found. In egg white of siamese crocodile, 12 isoforms of ovotransferrin like protein were found. The ovalbumin like proteins in reptiles showed different pIs and number of isoforms which are different from chicken egg white. Furthermore, minor proteins, molecular weights lower than 20 kDa were remarkably found in only red-eared slider turtle. The polymorphism of these proteins among reptile species might be served as the different biological function in each species. This study is the first report of proteomic pattern of Testudine egg white proteins.

KEYWORDS: reptile, 2D-PAGE, egg white protein, testudines, isoforms

1. INTRODUCTION

Egg white protein is a major raw material for food industry and pharmaceutical industry because of its biological properties especially forming and gelling. Some of the egg white proteins could be interesting for nonfood applications such as health applications (lysozyme and ovotransferrin). These proteins showed antimicrobial activities and antiviral activities [1]. To date, research has been focused on egg white proteins especially from the chicken because the chicken eggs constitute one of the major protein sources of our diet [2-3]. Egg white accounts for about 58% of entire egg mass and has a protein content of 10-12%, comprising mainly ovalbumin, ovotransferrin, ovomucoid, globulins and lysozyme [3-4]. The structure and functionality of major egg white proteins have been widely analyzed in various physicochemical conditions [4-7]. Electrophoretic methods have been used to study chicken egg white such as SDS-PAGE [3, 8-9], native-PAGE [8], isoelectric focusing [3, 8, 10], two-dimensional polyacrylamide gel electrophoresis

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(2D-PAGE) [7-10]. The 2D-PAGE is a high resolution technique for protein separation and this technique has been applied to the first proteomic investigation of hen egg white by Desert *et al*. [8]. Recently, Guerin-Dubiard *et al*. [7] have reported proteomic analysis of hen egg white. They have separated 69 protein spots and found new proteins. However, for the reptile egg white the information is limited. Some proteins of reptile egg white has been reported, i.e., acidic trypsin/subtilisin inhibitor [11], basic protein [12], alpha-macroglobulin and ovomacroglobulin [13], macroglobulin [14], ovalbumin-like protein [15], basic trypsin/subtilisin inhibitor [16] and lysozyme [17]. Some of reptile species for example crocodile, soft-shelled turtle and red-eared slider turtle are husbandry. The management of these species has been a focus of aquaculture practice. Moreover, hawksbill turtle and green turtle are endangered animal. Therefore, the analysis of the chemical composition of reptile egg and the physicochemical properties of its individual components can increase the potential applications in the food industry and can also enhance our understanding of various biological processes.

A comparative study of proteins from reptile egg white is important in order to define qualitative and quantitative differences among reptile species and chicken egg white. Inter-species variability may explain particular characteristic concerning functional and biological properties of egg proteins from different reptile origin. The aim of this study was to analyze and compare egg white proteins from different reptile species by 2D-PAGE using known chicken egg white proteins as standard for comparison.

2. MATERIALS AND METHODS

2.1 Egg white preparation

The reptile eggs, 2-3 days after being laid, were used. The egg white was manually separated from the egg yolk, mixed on magnetic stirrer and gently homogenized by Homoginizer (Polytron PT 30000, Kinematica AG, Switzerland).

2.2 Determination of protein concentration

Protein concentrations were determined by Bradford method [18] using bovine serum albumin as standard.

2.3 2D-PAGE

Proteins were analyzed by two dimensional polyacrylamide gel electrophoresis (2D- PAGE) as described by O'Farrel [19] and modified method by Görg *et al*. [20] (on linear (3-10) and nonlinear (3-10) pH gradients). Protein samples, 100 μg/125 μl were solubilized in a focusing solution containing 7 M urea, 2 M thiourea, 0.3% DTT, 2% CHAPS, and 2% IPG buffer corresponding to the pH gradient used. The surfactant CHAPS and chaotropic thiourea were used throughout the isoelectric focalization to improve protein solubility and transfer to the second dimension [21]. Isoelectric focusing (IEF) was conducted with a Ettan IPGphor (Amersham Bioscience, USA) on 7 cm IPG strips using a gradient mode yielding 9,250 Vhr. After focalization, the gel strips were equilibrated for 15 min in an excess of buffer containing 50 mM Tris-HCl, pH 6.8, 6 M urea, 30% glycerol, 1% SDS, and 25 mM DTT. This step enabled the complete reduction of disulfide bridges and denaturation of polypeptides by SDS. A second 15 min equilibration step in the same solution containing 250 mM iodoacetamide instead of DTT was then performed in order to block SH groups according to Görg *et al*. [22]. Proteins were then subjected to SDS-PAGE according to Laemmli [23]. Strips were placed on top of second-dimension 12.5% acrylamide gels. The migration buffer used was Tris-glycine (pH 8.0). Electrophoresis was carried out at 10 mA/gel for 15 mins and at 20 mA/gel to finish the migration.

2.4 Protein detection

After migration, gels were fixed in 50% ethanol and 2% phosphoric acid and stained with 0.1% Coomassie brilliant blue G-250, 10% ammonium sulfate, 2% phosphoric acid and 20% methanol [24]. These gels were then washed with double destilled water until the background was clear. Protein spots were then analyzed by Image Master 2D Platinum Trail (Amersham Biosciences, USA).

3. RESULTS AND DISCUSSION

Soluble egg white protein patterns of five reptile species, i.e. crocodile, soft-shelled turtle, red-eared slider turtle, hawksbill turtle, and green sea turtle were studied and compared to that of chicken egg white using SDS-PAGE analysis. From the result, egg white of reptiles species showed several protein bands with molecular weights ranging from 14 - 250 kDa (Figure 1). The protein from reptiles showed different molecular weights and concentrations in albumen for example ovalbumin, ovotransferrin and lysozyme when comparing with hen egg white protein (Figure 1). The protein molecular weights lower than 20 kDa were easily observed in reptile egg white. However, the SDS-PAGE is limited in term of resolution. Figure 2 displayed the protein patterns of reptile egg white protein (9, 20, 16, 16, 13 and 13 protein spots) for Siamese crocodile, soft-shelled turtle, red-eared slider turtle, hawksbill turtle, green sea turtle and chicken, respectively, which were observed after Coomassie Brilliant blue staining. In this preliminary studied, the reptile egg white proteins did not resolve well in linear IPG strip (pH 3-10). For higher resolution, the procedure was changed by skipping 2D clean up step and egg white proteins were incubated in solubilizing reagent for 1 hr longer. In addition, protein separation by 2D-PAGE was performed using nonlinear IPG strip (pH 3-10). Figure 3 showed that the use of Coomassie Brilliant blue staining enables the visualization of 35, 26, 45, 15, 33 and 36 protein spots for Siamese crocodile, soft-shelled turtle, red-eared slider turtle, hawksbill turtle, green sea turtle and chicken, respectively. The protein patterns of each reptile species were compared and analyzed. Some protein spot from each species are separated from other egg white protein and identified by Image Master 2D Trial. Table 1 summarized proteins identification by pI and molecular weight on six reference gel. The well separated protein spots are known protein egg white such as ovalbumin, ovotransferrin and lysozyme.

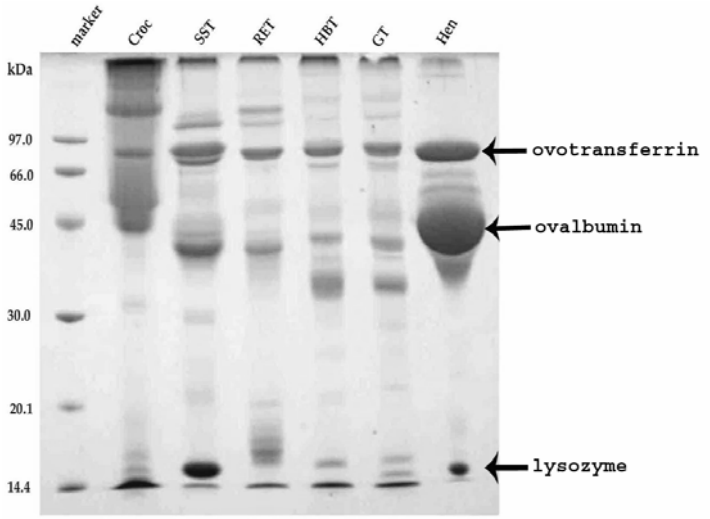

Figure 1 SDS-PAGE analysis of reptile egg white proteins. Whole reptile egg white proteins resolved on 13% acrylamide gel (1μg, lane marker: 15 μg lane croc-hen) and then stained with Coomassie Brilliant blue. Lane marker: molecular weight markers; Lane croc: Siamese crocodile egg white; Lane SST: soft-shelled turtle egg white; Lane RET: red-eared slider turtle egg white; Lane HBT: hawksbill turtle egg white; Lane GT: green sea turtle egg white and Lane hen: hen egg white

In reptile egg whites, ovotransferrin like protein was present in all species with different isoforms and molecular mass of approximately 70 kDa. The highest isoform (approximately 12 of ovotransferrin) were found in crocodile egg white (Figure 3A). The wider polymorphism of this protein could be explained by the degree of glycosylation [3, 7-9]. For ovalbumin, this protein is a major of hen egg white. On the other hand, 2D-PAGE of reptiles egg white revealed that ovalbumin is not a major protein and each species of reptile showed different pI from chicken egg white (Figure 3A-F). This result suggests that ovalbumin in reptile has different degree of modification such as glycosylation and phosphorylation (Table 1, Figure 3). Furthermore, this result might indicate the different biological function of ovalbumin in each reptile species. For lysozyme, soft-shelled turtle and green sea turtle had higher concentration than those of other species. Eventhough lysozyme has long been known to be present at a high concentration in egg white of avian but for reptile little information on the function of this protein are known. Minor proteins with molecular weight lower than 20 kDa were remarkably found in red-eared slider turtle (Figure 3C). These protein functions are under investigation. The protein spots with molecular weight about 21 kDa was detected in red-eared slider turtle, hawksbill turtle and green sea turtle. These protein spots might be used as protein markers for species specific. According to our knowledge, this study is the first proteomic study of reptile egg white.

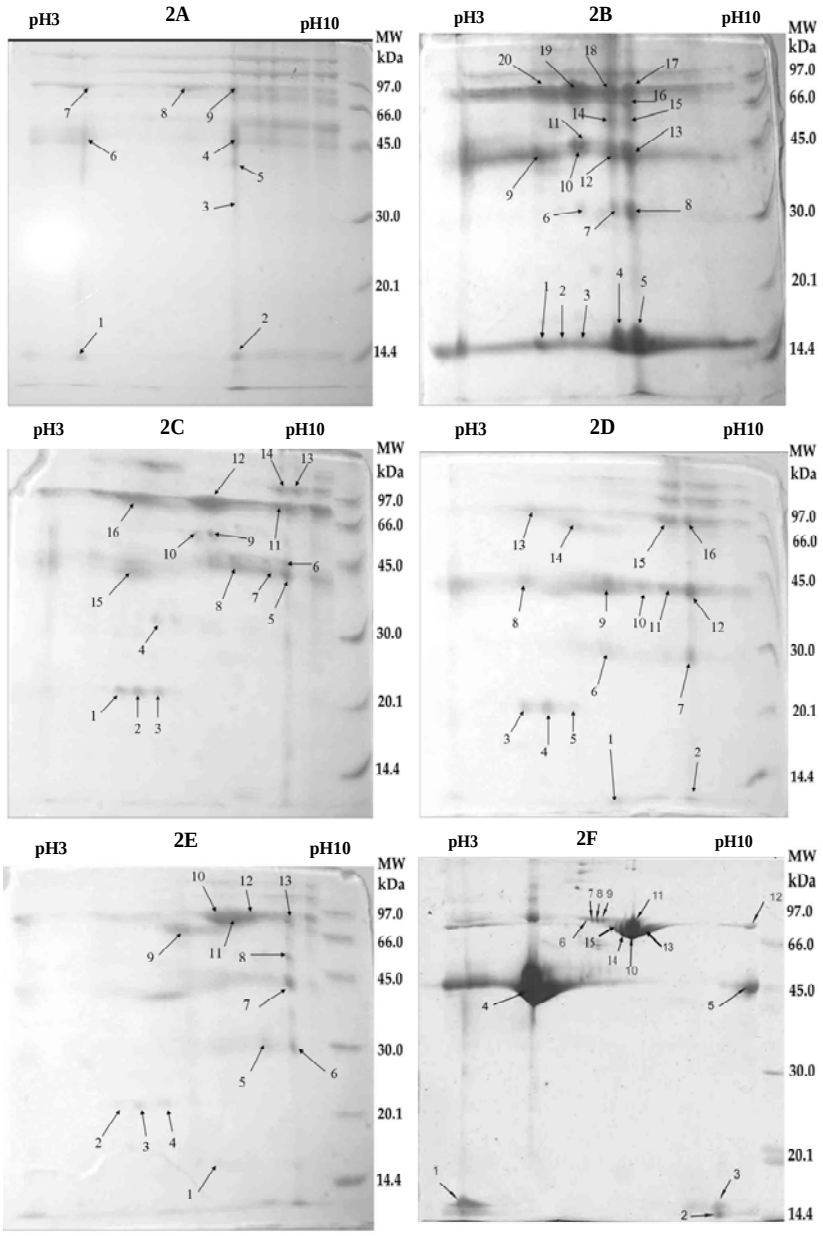

Figure 2 Two-dimensional electrophoresis of reptile and chicken egg white proteins. The amount of 125 μg of total protein was loaded on gel A, B, C, D, E and F, respectively and pH 3-10 L IPG strip. (A) Siamese crocodile, (B) soft-shelled turtle, (C) red-eared slider turtle, (D) hawksbill turtle, (E) green sea turtle and (F) chicken egg white proteins. Gels were stained with Coomassie Brilliant blue G-250.

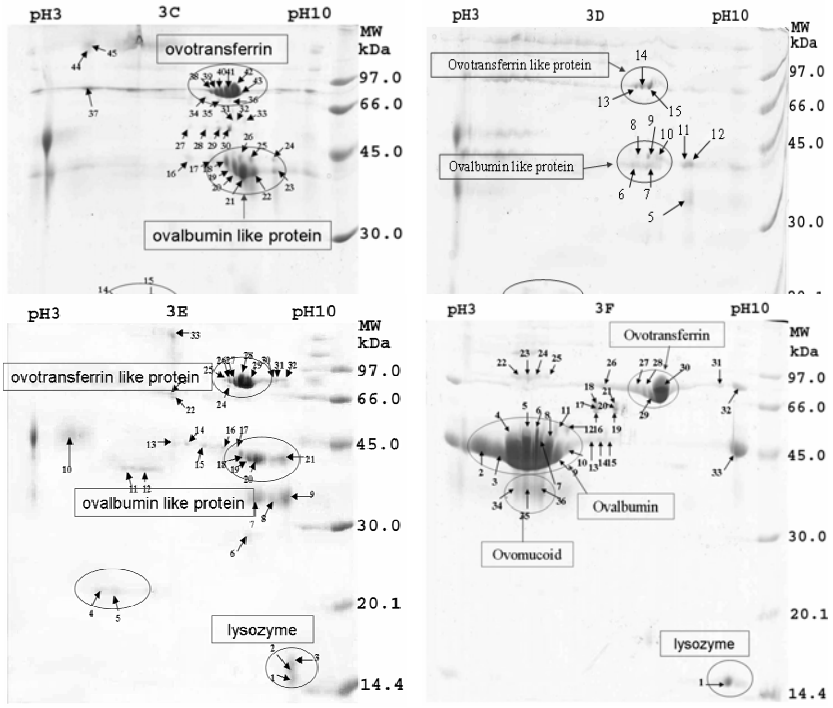

Figure 3 Two-dimensional electrophoresis of reptile and chicken egg white proteins. The amount of 100 μg of total protein was loaded on gel A, B, C, D, E and F, respectively and pH 3-10 NL IPG strip. (A) Siamese crocodile, (B) soft-shelled turtle, (C) red-eared slider turtle, (D) hawksbill turtle, (E) green sea turtle and (F) chicken egg white proteins. Gels were stained with Coomassie Brilliant blue G-250.

4. CONCLUSIONS

Reptile egg white proteins have been well separated by nonlinear IPG strip pH 3-10. The 2D-PAGE patterns of reptile egg white revealed ovalbumin like proteins, ovotransferrin like protein with different pI and number of isoforms when compared with chicken egg white. The polymorphism of these proteins among reptile species might be served as different biological function in each species. The further work is needed in a number of areas to achieve its potential use in food application. Ultimately, the separation and purification of each reptile egg white protein are promising to be able to characterize their functional properties, and identify those with improved or alter properties.

Table 1 Summarized protein identification by pI and molecular weight

Reptile species and Chicken/type of proteins	pI		MW (kDa)	
	Experimental	Theory	Experimental	Theory
Siamese crocodile				
-Ovotransferrin	5.91	6.38 [25]	53	78.043 [25]
	6.07		52	
	6.25		50	
	6.50		48	
	6.71		48	
	6.92		47	
	7.22		49	
-Ovotransferrin	5.94	6.38 [25]	88	78.043[25]
	6.01		87	
	6.09		85	
	6.18		84	
	6.32		83	
	6.47		81	
	6.64		81	
	6.75		81	
	6.90		83	
	7.05		84	
	7.24		84	
	7.45		84	
Soft-shelled turtle				
-Lysozyme	9.42	9.41 [7]	14	14.159 [7]
	8.80	9.41 [7]	14	14.159 [7]
-Ovalbumin	3.61	5.19 [7]	41	42.750 [7]
	4.07		41	
	4.53		41	
-Ovotransferrin	5.79	6.69 [7]	89	75.828 [7]
	5.88		85	
	5.95		91	
	6.08		88	
	6.20		87	
	6.38		88	
	6.52		88	
	6.96		85	
	7.13		85	
Red-eared turtle				
-Ovalbumin like protein	6.75	5.19 [7]	44	42.750 [7]
	6.82		42	
	6.98		42	
	7.37		41	
	7.79		41	
-Ovotransferrin	6.38	6.97 [26]	85	74.916 [26]
	6.52		83	
	6.64		83	
	6.79		82	
	6.98		86	
	7.26		82	

Table 1 Summarized protein identification by pI and molecular weight (cont.)

Reptile species and Chicken/type of proteins	pI		MW (kDa)	
	Experimental	Theory	Experimental	Theory
Hawksbill turtle				
-Ovalbumin like protein	6.23	5.19 [7]	41	42.750 [7]
	6.58		41	
	6.32		43	
	6.80		42	
	6.90		42	
-Ovotransferrin like protein	6.29	6.69 [7]	86	75.828 [7]
	6.44		86	
	6.63		85	
Green turtle				
-Ovalbumin like protein	7.60	5.19 [7]	42	42.750 [7]
	8.03		42	
	8.41		42	
	9.52		42	
-Ovotransferrin like protein	6.88	6.69 [7]	82	75.828 [7]
	7.00		82	
	7.24		80	
	7.52		81	
	7.91		79	
-Lysozyme	9.96	9.41[7]	15	14.159 [7]
	9.89	9.41[7]	15	14.159 [7]
	9.92	9.41[7]	16	14.159 [7]
Chicken				
-Ovalbumin	3.39	5.19 [7]	48	42.750 [7]
	4.13		45	
	4.46		48	
	4.83		46	
	5.03		48	
	5.15		47	
	5.29		46	
	5.38		45	
	5.51		48	
-Ovalbumin Y	5.73	5.20 [7]	50	43.772 [7]
	5.84		51	
-Ovoinhibitor	5.91		51	
	5.80	6.07 [7]	64	49.405 [7]
	5.80		66	
	5.80		71	
-Ovotransferrin	6.01		63	
	6.01		65	
	6.01		70	
	6.34	6.69 [7]	83	75.828 [7]
	6.54		79	
	6.70		76	
	6.94		82	
-Ovomucoid	4.56	4.82 [7]	40	20.098 [7]
	4.79		38	
	5.17		38	
-Lysozyme	9.83	9.41[7]	15	14.159 [7]

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