

ELECTROPHORESIS OF TSAIYA DUCK EGG SHELL PROTEINS AND THEIR CROSS-REACTION WITH HEN'S ANTI-OVOCLEIDIN 17 ANTIBODY

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ABSTRACT

This study was aimed to understand the electrophoretic profiles of Tsaiya duck eggshell proteins and their cross-reaction with hen's anti-ovocleidin-17 antibody. The electrophoretic profiles of Tsaiya duck eggshell showed that the prominent expression of proteins was located at 15 and 18 kDa, followed by medium expression of 28, 35, 40 and 80 kDa. Other trace bands were also observed. When eggshell proteins were detected with hen's anti-ovocleidin-17 antibody by Western blotting, two proteins were cross-reacted. While N-terminal sequencing was performed on these two proteins, only the 15 kDa protein was successfully sequenced and eight amino acid residues of NKYPKGWL were obtained. A search of NCBI data revealed that the 15-kDa eggshell protein had a very high homology with the goose eggshell protein ansocalcin. The role of this 15-kDa protein playing on the eggshell formation needs further studies.

KEY WORDS: Ansocalcin, Anti-ovocleidin-17 antibody, Eggshell proteins, Tsaiya duck.

INTRODUCTION

The eggshell organic matrix only accounts for 2-3% of the eggshell weight, whereas it plays an important role on eggshell formation. Among the organic matrix, eggshell proteins are divided into three classes based on the locations where they are observed. Firstly, the proteins have already found in egg white, such as ovalbumin, ovotransferrin, and lysozyme. Secondly, the proteins are present in the bone and other hard tissues, such as osteopontin. The proteins in the third class are eggshell-specific, like ovocleidin-17 (OC-17), OC-116, ovocalyxin-32, ansocalcin, etc (review see Nys *et al.*, 1999; Lakshminarayanan *et al.*, 2002; 2003). Although eggshell organic matrix has been widely studied in the hen, goose, ostrich, etc, it has only limited study in the Tsaiya duck (Chen, 2000). This study aimed to understand the electrophoretic profiles of Tsaiya duck eggshell proteins and their

cross-reaction with hen's anti-OC-17 antibody.

MATERIALS AND METHODS

Extraction of Organic Matrix

For extraction of organic matrix in the powder, 50% acetic acid was employed to dissolve eggshell at 4 °C. The dissolved solution was transferred into a pleated dialysis tubing with cutoff of 3.5 kDa (SnakeSkin™, Product # 68035, Pierce Chemical Company, U. S. A) to demineralize it against dH₂O. The solution in the tube was lyophilized to obtain the organic matrix (Chen, 2000). Lyophilized organic matrix was stored at -20 °C for further electrophoresis of proteins.

SDS-PAGE Electrophoresis of Eggshell Proteins

Organic matrix from duck eggshell were dissolved in SDS-PAGE sample buffer (0.05M Tris-HCl pH6.8, 4% SDS, 2% β-MSH, 12% glycerol, 0.01% BPB) and separated by SDS-PAGE in a 12.5% polyacrylamide gel. After electrophoresis, protein bands were observed by staining the gel with coomassie brilliant blue R-350.

Western Blotting of Eggshell Proteins

After electrophoresis was finished, the proteins on the gel were electro-blotted (100 V) to a polyvinylidene difluoride (PVDF) membrane in transfer buffer for one hr. The procedures of western blotting were modified from the method of Panheleux *et al.* (1999).

N-terminal Sequencing of Eggshell Proteins

The eggshell proteins cross-reacted with anti-OC-17 antibody were cut from the PVDF membrane and submitted to the Institute of Biological Chemistry, Academia Sinica for N-terminal sequencing.

RESULTS AND DISCUSSION

Electrophoresis of Eggshell Proteins

The electrophoretic profiles of Tsaiya duck eggshell proteins showed that the prominent expression of proteins was located at 15 and 18 kDa, followed by medium expression of 28, 35, 40 and 80 kDa. Other trace bands were also observed (Figure 1). Some corresponding bands were also noted in the electrophoretic profiles of uterine fluid proteins (Huang, 2004). The electrophoretic profiles of Tsaiya duck eggshell proteins were similar to those of Pekin ducks (Panheleux *et al.*, 1999). It has also been noted that electrophoretic profiles of soluble eggshell matrix of different avian species were specific within groups of birds (a: laying hen, breeder hen, quail, pheasant, and possibly turkey; b: guinea fowl; c: duck and goose), but some of the protein bands were common to all groups (Panheleux *et al.*, 1999).

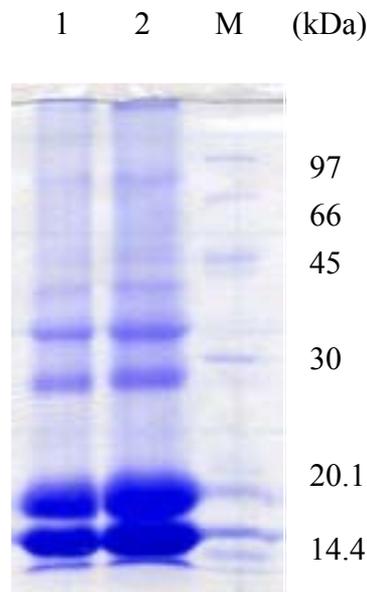


Figure 1. SDS-PAGE electrophoretic profiles of duck eggshell protein.

Lane 1: 100 μ g eggshell organic matrix; Lane 2: 200 μ g eggshell organic matrix;
M : molecular weight marker.

Western Blotting and N-terminal Sequencing of Eggshell Proteins

When eggshell proteins were detected with anti-OC-17 antibody by Western blotting, two proteins were cross-reacted (Figure 2). When N-terminal sequencing was performed on these two proteins, only the 15 kDa protein was successfully sequenced and eight amino acid residues of NKYPKGWL were obtained. A search of NCBI data revealed that it had very high identity with the goose eggshell protein ansocalcin having N-terminal sequence of NKCPKGWL (Lakshminarayanan *et al.*, 2003). Only the third amino acid residue was different between these two proteins. Later cDNA study confirmed that these eight amino acid residues (NKCPKGWL) were the same between ansocalcin and the major protein in Tsaiya duck eggshell (Huang *et al.*, 2004).

Morphology of calcium carbonate crystal has been altered by the addition of ansocalcin *in vitro*, and this morphology was changed with increased ansocalcin concentrations (Lakshminarayanan *et al.*, 2002). In addition, OC-17 content in the older hen eggshell was higher than that in the younger one (Panheleux *et al.*, 2000). Furthermore, the corresponding 15 kDa band in the uterine fluid tended to increase with eggshell formation (Huang, 2004). It has also been indicated that amino acid sequence of ansocalcin has 36% identity with that of OC-17 (Lakshminarayanan *et al.*, 2003). Therefore, this study gives us an inspiration that this 15 kDa Tsaiya duck eggshell protein may also affect CaCO₃ crystal morphology and play an important role on eggshell mineralization.

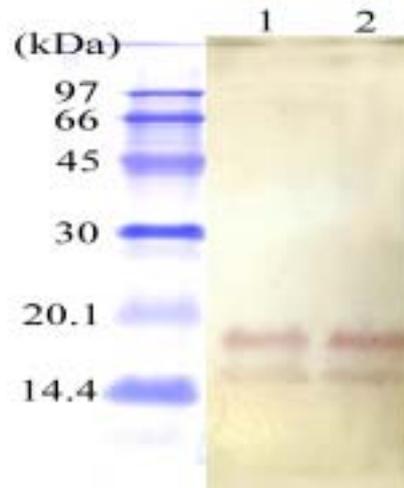


Figure 2. Western blotting of Tsaiya duck eggshell proteins against anti-OC-17 antibody. Eggshell protein of 5 μ g was applied in both Lane 1 and 2 and 15% SDS-PAGE was performed.

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