Electrophoretic Studies of Proteins in Egg and Albumen Gland of *Euhadra peliomphala**

By

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Summary

The soluble proteins in albumen gland and egg of *Euhadra peliomphala* were studied by disc electrophoresis, SDS-polyacrylamide gel electrophoresis and isoelectric focusing.

In feeding period, the proteins of albumen gland separated into 10 distinct bands on disc gel. In contrast, in both periods of the hibernation and estivation, the proteins separated into 16 to 20 minor bands.

The protein patterns of albumen gland revealed individual variation. Rm values of the protein bands of the egg are each identical to those of the albumen gland. It was assumed that the proteins of the egg are all derived from the albumen gland.

The protein bands of the egg were glycoproteins with molecular weights of 74,000, 94,000, 125,000, 156,000, 200,000 and 400,000 respectively. The soluble proteins of the egg were separated into 21 bands ranging from pIs of 3.5 to 9.0 by the gel isoelectric focusing. Only galactose was detected in HCl hydrolyzate of the extract from the albumen gland.

Key words : albumen gland, molecular weight of molluscan egg protein, SDS-PAGE, gel isoelectric focusing.

Introduction

Albumen gland is widely distributed in *Pulmonates* in mollusk and serves the egg with the nutritive substances.

These substances secreted from the albumen gland consist of galactogen, proteins, glycoproteins, amino acid and calcium. The biochemical properties of galactogen in albumen gland have been studied by many authors, although the protein constitution of albumen gland are little known. Recently, Morrill et al.¹⁾ reported that the soluble proteins isolated from the egg of *Lymnaea palustris* are derived from the albumen gland and their electrophoretic patterns

have species specific in nature.

This study deals with the disc and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as well as gel isoelectric focusing analyses of the soluble proteins of albumen gland and egg of *Euhadra peliomphala*.

Materials and Methods

1. *Materials* : Land snail, *Euhadra peliomphala* were used as all experimental materials collected from the farms in the vicinity of Koganei, Kodaira, and Fussa cities, Tokyo, Japan. Snails were bred with lettuce and potato in the laboratory and starved for two days before dissection. The albumen glands

* *Euhadra peliomphala* の卵と卵白腺の蛋白質の電気泳動的研究.

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extracted from the starved animals were homogenized with 20 volumes of 0.15 M NaCl. The homogenate was stirred at 4°C for 2 hours, and centrifuged at 12,000×g, at 4°C for 60 min. The supernatant was used as an original material. The soluble proteins from the egg were also prepared by the same procedure as that for albumen gland.

2. *Quantitative estimation of soluble proteins*: The protein concentration was measured by the method of Lowry et al.²⁹ with bovine serum albumin (Daiichi chemicals Co. Ltd.) as standard.

3. *Carbohydrate*: The total carbohydrate in the albumen gland was measured by the phenol-sulfuric acid method.³⁰

4. *Disc polyacrylamide gel electrophoresis*: Disc polyacrylamide gel electrophoresis (5% or 7%) was performed by the method of Davis using 0.05 M Boric-NaOH, pH 8.6 instead of Tris-HCl buffer. Electrophoresis was run at 3 mA/column at 4°C.⁴¹

5. *Detection of proteins on the gel*: Proteins were stained with coomassie brilliant blue R-250 and destained with 10% acetic acid containing 20% ethanol. The destained gel was scanned by Fuji autodensitometer FD-A IV at 600 nm.

6. *Detection of glycoproteins on the gel*: The gel was developed with Schiff's reagent and scanned by the above densitometer at 500 nm.

7. *Molecular weight*: The molecular weight of the protein was estimated by following two methods, (1) its molecular weight was measured by the Hedrick and Smith in gels of 5, 6, 7, 8, 9, and 10%, with myoglobin (M.W. 17,000), ovalbumin (M.W. 43,000), and bovine serum albumin (M.W. 68,000) for monomer, 130,000 for dimer, and 204,000 for trimer as standard,⁵¹ and (2) it was estimated with SDS-polyacrylamide gel electrophoresis by the method of Hayashi and Ohba in 5% gel with molecular weight makers ranged from 53,000 to 265,000 purchased from BDH Co. Ltd. as standard.⁶²

8. *Isoelectric point*: For the determination of pI, a gel isoelectric focusing was carried out by the method of Righetti and Drysdale with slight modification using 0.02 M phosphoric acid and 1 M NaOH as electrolytes.⁷² pI markers purchased from Oriental

Yeast Co. Ltd. were employed for the calibration of the pI curve.

9. *Polysaccharide*: Polysaccharide in albumen gland was extracted by the method of Goudsmit.⁸³ This extract deproteinized with 1 M trichloroacetic acid was hydrolyzed with 1 N HCl at 100°C for 5 hrs. The hydrolysate was chromatographed on Toyo filter paper No. 50 with a solvent comprising of N-buthanol/benzol/pyridine/water at 5/1/3/3. Detection of the polysaccharide on the paper was done by the method of Trevelyan.⁹²

Results

1. Seasonal variation in weight of albumen gland and its components

Figure 1 shows a seasonal variation of the wet weight of albumen gland obtained from December 1977 to December 1978 and some typical electrophoretic patterns of soluble proteins from the albumen gland at the different seasons.

The seasonal variation of the wet weight of the albumen gland was roughly divided into four periods as hibernation, feeding, estivation and egg laying as indicated in Fig. 1. In the feeding period, the wet weight of the albumen gland showed the highest value of around 400 mg. Disc electrophoresis of the soluble protein from the albumen gland at that period showed 10 bands which were tentatively named as A₁ through A₁₀ (see Fig. 2). The wet weight of the albumen glands taken at the hibernation and the estivation was below 50 mg and the analysis of the protein by the disc electrophoresis disclosed rather different patterns from those of the feeding period, comprising 16 to 20 faint bands. In egg laying period, the wet weight of the albumen gland came up to around 200 mg and then it gradually fell down to around 50 mg as shown in Fig. 1.

Electrophoresis of the protein at this period disclosed that there were differences in patterns before and after oviposition. The protein pattern before oviposition revealed similarity to that of the feeding period, showing 10 distinct major bands in the gel, while after oviposition the pattern changed rapidly to that of the hibernation.

Fig. 1. Seasonal variation of wet weight of albumen gland and disc electrophoretic patterns of soluble protein from albumen gland.

Preparations of soluble protein from albumen gland was submitted to disc electrophoresis as described under the materials and methods. Upper graph shows the seasonal variation of wet weight of albumen gland. Four distinct periods could be divided from protein patterns of albumen gland, tentatively named hibernation, feeding, estivation and egg laying. Photographs show the disc electrophoretic patterns of soluble proteins in albumen gland. a) and b) in egg laying are the disc electrophoretic patterns of protein before and after oviposition, respectively.

The individual variation of the protein constitution of the albumen gland was also noted (Fig. 2). It was assumed that the individual variation is likely adopted Hardy-Weinberg's law. The details of the latter result will be reported elsewhere.

2. Comparison of proteins from egg and albumen gland

As shown in Fig. 2, the soluble proteins of the egg in the 2nd days of oviposition separated into 9 bands. These bands were tentatively named as E_1 through E_9 . The amount of E_3 to E_7 showed the individual variation.

Rm (relative mobility) values of the each protein band were calculated for both egg and albumen gland proteins. It was found that each Rm of E_1 through E_9 corresponded to that of A_2 through A_{10} . Results might indicate that the 9 protein bands observed in egg were all derived from albumen gland. Any minor bands of the soluble proteins from the albumen gland could not be identified in the egg proteins.

3. Polysaccharids in albumen gland

In order to identify polysaccharids presented in the albumen gland, HCl hydrolysate of the albumen gland was analyzed by the method of Trevelyan.⁹⁾ Only galactose was identified in the hydrolysate. This indicated that the free polysaccharide in the albumen gland was mainly galactogen.

4. Glycoprotein of albumen gland and egg

As shown in Fig. 3, all protein bands stained with coomassie brilliant blue were found to be PAS positive. To measure the carbohydrate content of the PAS positive protein bands, the gel was sliced in 3 mm thick and each slice was then analyzed by Phenol-sulfuric acid method. The carbohydrate content of each E_1 , E_2 , E_3 , E_4 - E_5 mixture, E_6 , E_8 and E_9 were found to be 6, 8, 5, 8, 8, 13, and 90%, respectively.

5. Molecular weight of soluble proteins of egg

As shown in Fig. 4-a, the molecular weight of the egg proteins determined by the method of Hedrick and Smith⁵⁾ were 74,000 for E_1 , 94,000 for E_2 , 125,000 for E_3 , 156,000 for E_4 - E_5 mixture, 200,000 for E_6 and 400,000 for E_8 , respectively.

Fig. 2. Densitograms and disc electrophoretic patterns of soluble protein in albumen gland and egg.

Left : Densitograms and disc electrophoretic patterns of albumen gland (upper) and egg (lower).

Right : Individual variations of proteins in albumen gland (A.g.) and egg (E) from the same individual. Three pairs of A.g. and E are derived from three different individuals.

To confirm the molecular weight obtained by the method of Hedrick and Smith, each protein band was extracted from the sliced gel and was analyzed by SDS-polyacrylamide gel electrophoresis. As shown in Fig. 4-b, the molecular weights of those bands were 75,000 for E₁, 100,000 for E₂, 25,000 for E₃, 100,000 and 105,000 for E₄-E₅ mixture, 130,000 for E₆ and 68,000 for E₈, respectively.

It was found that the molecular weights of E₁ and E₂ determined by the Hedrick and Smith method were almost identical to those by the SDS-polyacrylamide gel electrophoresis. While, the values of the E₃, E₆ and E₈ by the SDS-polyacrylamide gel electrophoresis showed much smaller values compared to those by the method of Hedrick and Smith.⁵⁾

Fig. 3. Glycoproteins of albumen gland and egg.

Coomassie : Protein patterns of albumen gland and egg stained with coomassie brilliant blue R-250.

PAS : Glycoproteins of albumen gland and egg stained with PAS reagent.

6. Isoelectric point of the egg proteins

Figure 5 shows the isoelectric focusing patterns of egg proteins and pH gradient determined by the use of marker proteins. E₁ and E₃ are the extracts obtained from the sliced gel. As can be seen in Fig. 5, the egg proteins separated into 21 fractions ranging from pIs of 3.5 to 9.0. The E₁ protein itself separated into 4 fractions of which pIs were 3.7, 4.0, 4.2 and 4.5, respectively. Similarly, the E₃ protein also separated into fractions with 6 different pI bands. The values were 4.5, 4.9, 5.2, 5.5, 5.8 and 6.0, respectively.

Discussion

Recently, Morill reported that the soluble proteins of albumen gland of *Lymnaea palustris* separated into 4 to 11 bands on electrophoresis and that the egg proteins were derived from the albumen gland.¹⁾ In the present study, it was also clearly

Fig. 4. Estimation of molecular weight of egg proteins.

- a): A plot of molecular weight versus slope obtained by the method of Hedrick and Smith.
- b): A plot of molecular weight versus $\log(100 \times R_m)$ obtained by SDS-polyacrylamide gel electrophoresis.

MYO: myoglobin (M.W. 17,000), OVA: ovalbumin (M.W. 43,000), BSA: bovine serum albumin (M.W. 68,000 for monomer, 130,000 for dimer, and 204,000 for trimer).

shown that the most of the soluble proteins of the albumen gland in *Euhadra peliomphala* are transferred to egg and that the electrophoretic patterns and the protein constitutions of the albumen gland have the seasonal variation as well as individual variation. Concerning the seasonal variation of the protein patterns and the differences in wet weight of the albumen gland among the seasons, the present study disclosed that the increase of the wet weight

Fig. 5. Schematic drawing of gel isoelectric focusing of whole, E₁ and E₈ bands (right). pH gradient determined by marker proteins (left).

The height of the protein bands in gels (left) directly correspond to the distance of the graph (left). Nothing is described for the figure present in left side of the Fig. 5.

in the feeding and egg laying periods are due to accumulation of proteins which will be later on transferred to egg. This finding is compatible with that reported by Morill¹¹ and by Bayne.^{10,11} It has been known that the albumen gland of *Plumonates* contains galactogen, a galactose polymer. Goudsmit has reported that the galactogen is also synthesized in the albumen gland of *Helix pomatia* from UDP-d-galactose.⁹ In this report it was shown that all protein bands of the albumen gland and egg separated by Disc electrophoresis have characteristics of glycoprotein, and that the HCl hydrolysate of the albumen gland of *Euhadra peliomphala* also contains galactogen.

The molecular weight of galactogen in *Helix pomatia* and *Lymnaea stagnalis* have been reported by Horstmann to be 4×10^6 and 2.2×10^6 , respectively. However, the molecular weight of the glycoprotein in egg has not yet reported. The molecular weights of E₁ (25,000) and E₂ (100,000) determined by SDS-polyacrylamide gel electrophoresis were not much different from those by the method of Hedrick and Smith, indicating the E₁ and E₂ would have no subunit structure. On the other hand, the molecular weights of the other bands determined by the SDS-polyacrylamide gel electrophoresis showed

much smaller values compared to those by the other method. The latter findings indicate that proteins other than E_1 and E_2 might have subunit structures.

Isoelectric point of the soluble proteins of egg from *Euhadra* is not yet definitely reported. It was disclosed that the soluble proteins of egg consisted of 21 bands with pIs from 3.7 to 9.0. E_1 and E_3 which were extracted from the sliced gel were further divided into 4 and 6 bands by the gel isoelectric focusing. A precise mechanism of the transfer of proteins from the albumen gland to egg is almost unknown. Studies concerning the transfer mechanism in *Euhadra* are now in progress.

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要 旨

日本産ミスジマイマイ *Euhadra peliomphala* の卵白腺と卵の可溶性蛋白質をディスク・SDSおよびゲル等電点電気泳動法で研究した。

摂餌期では、卵白腺の蛋白質はディスク電気泳動法で明瞭な 10 band に分画されたが、冬眠期と夏眠期の両期では微量な 16~20 band に分画された。卵白腺蛋白質パターンは季節変化と個体変異を示した。

卵白腺と卵の蛋白質は Rm 値が共に等しく、卵蛋白質は卵白腺から由来したものと考えられる。

卵蛋白質は分子量を 74,000, 94,000, 125,000, 156,000, 200,000, 400,000 をもつ糖蛋白質で、その蛋白質はゲル等電点電気泳動法で pI 3.5~9.0 の 21 band に分画された。

卵白腺の HCl 加水分解物中の糖は galactose のみ検出された。