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Characterization of Vitellin from the Fireflies, *Luciola unmunsana* and *L. lateralis*

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The vitellin of the fireflies, *Luciola unmunsana* and *L. lateralis* was characterized. The vitellin of *L. unmunsana* is composed of two subunits, designated Vn1 (195 kDa) and Vn2 (185 kDa) in SDS-polyacryamide gel electrophoresis. These two subunits of vitellin of *L. unmunsana* gradually decreased during embryogenesis. As expected, these protein bands were presented in female adult hemolymph and egg extracts, but not in male. The vitellin of *L. lateralis* is also composed of two subunits, designated Vn1 (195 kDa) and Vn2 (180 kDa) in SDS-PAGE, and these two protein bands gradually decreased during embryogenesis. Western blot analysis using each of polyclonal antiserum against vitellins of *L. unmunsana* and *L. lateralis* showed that two antisera strongly crossereacted with vitellin subunits of *L. unmunsana* and *L. lateralis*, suggesting that vitellins of *L. unmunsana* and *L. lateralis* have similarity with each other.

Key words : Vitellin, Firefly, *Luciola unmunsana, Luciola lateralis*, Embryogenesis

Introduction

During embryogenesis, the phospholipoglycoproteins are provided as a nutritional supply for the developing embryo in the insect. These proteins called vitellin as the major yolk protein are synthesized as single or multiple precursors, vitellogenin, in the insect fat body (Engelmann, 1979; Hagedorn and Kunkel, 1979). After its synthesis, vitellogenin is subsequently secreted into the hemolymph by vitellogenic follicles via receptor-mediated endocytosis (Telfer *et al.*, 1982; Raikhel and Dhadialla, 1992), and then selectively absorbed into oocytes by vitellogenin receptors as membrane-bound protein (Ferenz, 1993; Sappington and Raikhel, 1998), which now is termed vitellin. In most insects, it has been known that synthesis of vitellogenin occurs under the control of juvenile hormone in the fat body (Bownes, 1986; Socha *et al.*, 1991).

In most insects, vitellogenin is a large protein with the molecular weight ranging from 210-652 kDa and consists of a large subunit (150-190) and a small subunit (40-65 kDa) (Raikhel and Dhadialla, 1992). The vitellogenin and vitellin are identical in their immunological properties and similar in their physical and chemical characteristics, although there are differences in lipid content (Kunkel and Nordin, 1985; Raikhel and Dhadialla, 1992).

These studies on the vitellogenin and vitellin are extensively performed in diverse insect species. In firefly, luciferase genes have been studied deeply in some species, but developmental physiology is poorly understood yet. Although *Luciola unmunsana* and *L. lateralis*, are the abundant fireflies in Korea, furthermore, vitellin of the fireflies is not reported yet.

Therefore, this study was conducted to obtain physiological information of the fireflies, *L. unmunsana* and *L. lateralis*. In the present study, we have described the characteristics of vitellin in *L. unmunsana* and *L. lateralis*.

Materials and Methods

Insects

The firefly *Luciola unmunsana* adults were collected at

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Chungdo, Kyungsangnam Province in Korea, July 2000 and *L. lateralis* adults were collected at Posoung, Chollanam Province in Korea, July 2000. For the oviposition, the fireflies were transiently reared in a growth chamber at 21 ± 1°C with 60 ± 5% of relative humidity under a photoperiod of 12L : 12D.

Staged eggs

To examine development of the embryos, female adults were allowed to fertilize and oviposit in the moisten petri dish, and eggs were incubated at 21 ± 1°C with 60 ± 5% of relative humidity. Three eggs of *L. unmunsana* were collected from the dish every 3 days over 19 days post oviposition. Also, three eggs of *L. lateralis* were collected every 2 days over 16 days post oviposition. These eggs were punctured by a fine needle in 50 μl phosphate buffered saline (PBS; 120 mM NaCl, 2 mM KCl, 4.5 mM Na_2HPO_4, 1 mM KH_2PO_4, pH 7.4) containing 5 mM EDTA and 1 mM PMSF to obtain egg component. After the centrifugation of the component at 13,000 rpm for 20 min at 4°C, the supernatant was used for the SDS-polyacryamide gel electrophoresis (PAGE) analysis.

Electrophoresis

Native-PAGE was performed in 7.5% gel at 4°C according to the method of Davis (1964). SDS-PAGE was conducted on 10% gel at room temperature according to the method of Laemmli (1970). After electrophoresis, gels were fixed and stained with 0.1% Coomassie brilliant blue R-250. Molecular weight markers [myosin (200,000), β-galactosidase (116,000), phosphorylase b (97,000), bovine serum albumin (66,000), egg albumin (45,000) and carbonic anhydrase (31,000); Bio-Rad] were used as the standards.

Preparation of hemolymph and egg extracts

Hemolymph of *L. unmunsana* was collected by puncturing pterothorax of adults with a fine needle. This was subsequently diluted into PBS containing 5 mM EDTA and 1 mM PMSF. The hemolymph was centrifuged at 13,000 rpm for 20 min at 4°C to remove hemocytes and cell debris. The supernatant was stored -70°C until use. Eggs of *L. unmunsana* and *L. lateralis* were homogenized in PBS containing 5 mM EDTA and 1 mM PMSF. The mixture was then centrifuged at 13,000 rpm for 20 min at 4°C and the supernatant was stored at -70 until use.

Preparation of polyclonal antibody

Egg proteins of *L. unmunsana* and *L. lateralis* were separated on the native PAGE gel, respectively. After staining the gel with Comassie blue, these Vn bands were cut off, and were eluted using dialysis tube (Bio-Rad) at 100 V for 60 min, respectively. The eluted Vn was mixed with equal volume of Freunds complete adjuvant (a total of 200 μl) and injected into Balb/c mice. Three successive injections were performed with one-week interval beginning a week after the first injection with antigens mixed with equal volume of Freunds incomplete adjuvant (a total of 200 μl). Bloods were collected 3 days after the last injection and centrifuged at 13,000 rpm for 5min. The supernatant antisera were stored at -70°C until use.

Western blot analysis

For Western blot analysis, SDS-PAGE was carried out as described above. Proteins were blotted to a sheet of nitrocellulose membrane (Sigma, 0.45 μm of pore size) (Towbin *et al.*, 1979). The blotting was performed in transfer buffer (25 mM Tris and 192 mM glycine in 20% methanol) at 30 volt overnight at 4°C. After blotting, the membrane was blocked by incubation in 1% BSA solution for 2 hr at room temperature. The blocked membrane was incubated with Vn antiserum solution (1:500 v/v) for 1 hr at room temperature and washed in TBST (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$) containing NBT (nitro-blue tetrazolium) and BCIP (5-bromo-4-chloroindolyl phosphate) was added. The reaction was quenched with distilled water.

Results and Discussion

Female adult of *L. unmunsana* and *L. lateralis* was collected from the fields and the female was oviposited in the laboratory (Fig. 1A and C). The shape and size of *L. unmunsana* egg were similar to those of *L. lateralis* (Fig. 1B and D).

To elucidate vitellin in *L. unmunsana*, eggs and adult hemolymph were characterized by native- and SDS-PAGE analysis (Fig. 2). In native-PAGE analysis, major protein considered as vitellin was detected in egg (Fig. 2A). The major protein was considered as a dimer in SDS-PAGE analysis, as shown in lane 3 of Fig. 2B. The sex-limited proteins appeared in the female adult hemolymph and were also detected in egg with a same electrophoretic mobility (Fig. 2B). The results showed that the vitellin of *L. unmunsana* is composed of two subunits, designated Vn1 (195 kDa) and Vn2 (185 kDa) in SDS-PAGE.

To confirm the vitellin of *L. unmunsana*, the staged eggs were analyzed by SDS-PAGE (Fig. 3). The gel revealed two subunits of vitellin with the molecular weights of 195 kDa and 185 kDa. These two proteins clearly represent subunits of vitellin as they are gradually used up during embryogenesis.

For identification of vitellin in *L. lateralis*, furthermore,

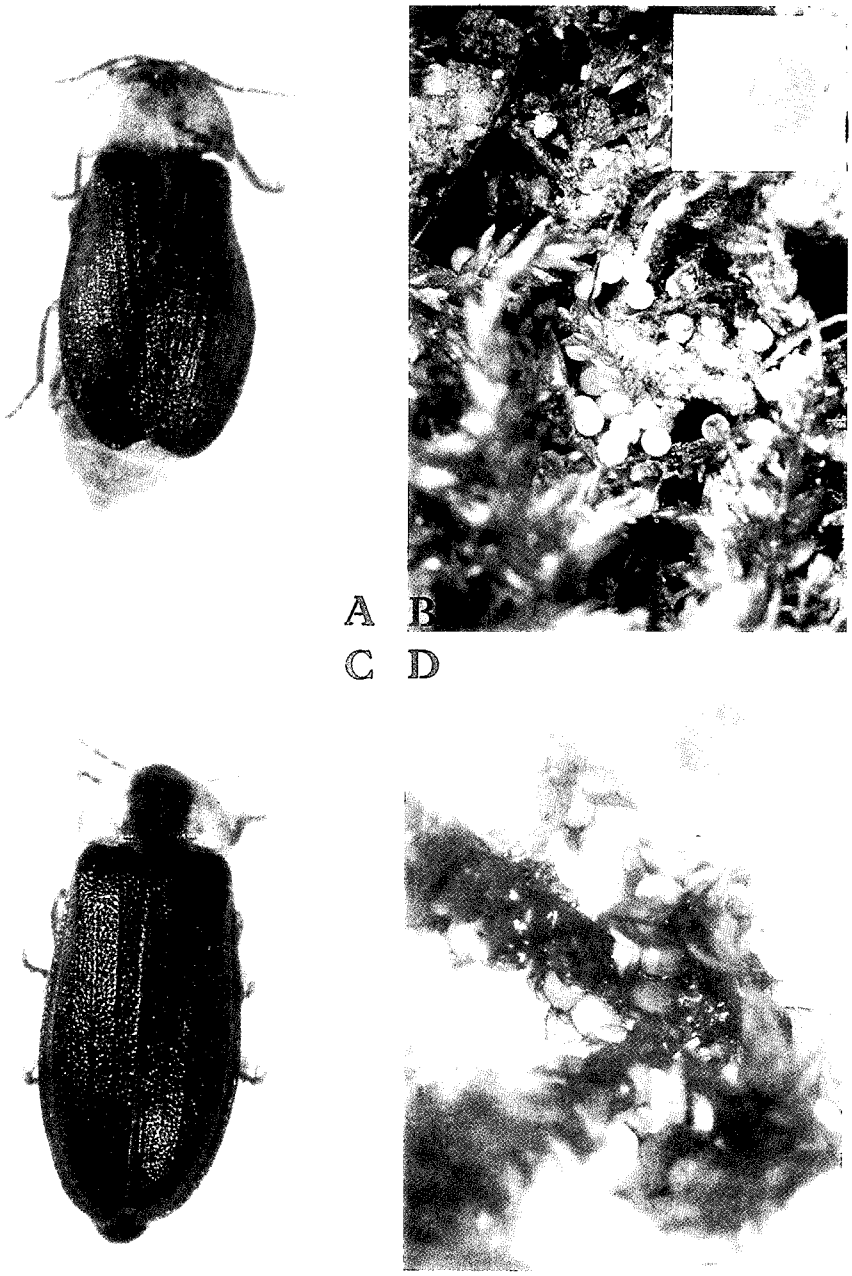

Fig. 1. Photograph of female adult (A and C, × 10) and eggs (B and D, × 12.5) of the fireflies, *L. unmunsana* (A and B) and *L. lateralis* (C and D).

eggs were characterized by native- and SDS-PAGE analysis (Fig. 4). In native-PAGE analysis, major protein considered as vitellin was detected in egg (Fig. 4A) and the major protein was considered as a dimer in SDS-PAGE analysis, as shown in lane 1 of Fig. 4B. The vitellin of *L. lateralis* is also composed of two subunits, designated Vn1 (195 kDa) and Vn2 (180 kDa). From the SDS-PAGE analysis of the staged eggs it is confirmed that two subunits clearly represent vitellin as they are gradually decreased during embryogenesis.

To verify the similarity between two vitellins of *L. unmunsana* and *L. lateralis*, we have prepared vitellin band from eggs by extracting from native-APGE gels and produced vitellin antibody from mice. Western blot analysis (Fig. 5) using each of polyclonal antiserum against vitellin of *L. unmunsana* or *L. lateralis* showed that two antisera strongly crossereacted with vitellin subunits of *L. unmunsana* and *L. lateralis*, respectively. This observation suggests that vitellin of *L. unmunsana* and *L. lateralis* have similarity, at least in part, with each other.

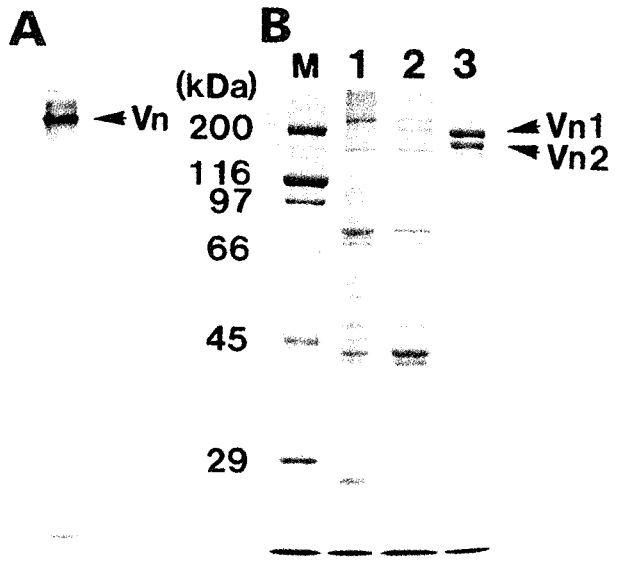

Fig. 2. Identification of vitellin of *L. unmunsana* using native- (A) and SDS-PAGE (B). Panel A, egg extracts. Panel B, Lane 1, adult male hemolymph; Lane 2, adult female hemolymph; Lane 3, egg extracts. Subunits (Vn1 for a large subunit and Vn2 for a small subunit) of vitellin (Vn) are represented on the right of each panel. Molecular weight markers (M) are indicated.

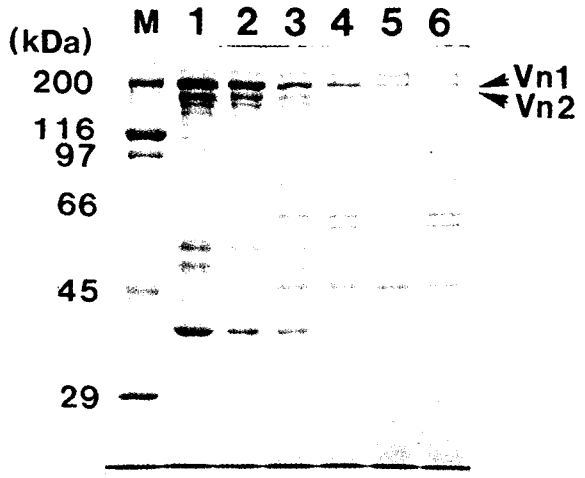

Fig. 3. SDS-PAGE analysis of the staged eggs of *L. unmunsana*. Lane 1, 2 day-old egg; Lane 2, 7 day-old egg; Lane 3, 10 day-old egg; Lane 4, 13 day-old egg; Lane 5, 16 day-old egg; Lane 6, 19 day-old egg. Subunits (Vn1 for a large subunit and Vn2 for a small subunit) of vitellin are represented on the right of panel. Molecular weight markers (M) are indicated.

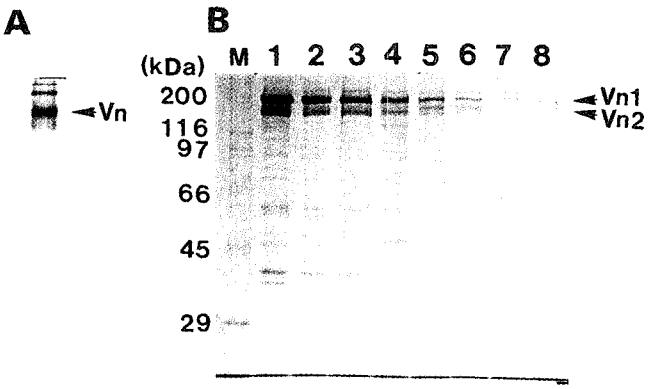

Fig. 4. Native- (A) and SDS-PAGE (B) analyses of the staged eggs of *L. lateralis*. Panel A, 2 day-old egg. Panel B, Lane 1, 2 day-old egg; Lane 2, 4 day-old egg; Lane 3, 6 day-old egg; Lane 4, 8 day-old egg; Lane 5, 10 day-old egg; Lane 6, 12 day-old egg; Lane 7, 14 day-old egg; Lane 8, 16 day-old egg. Subunits (Vn1 for a large subunit and Vn2 for a small subunit) of vitellin (Vn) are represented on the right of each panel. Molecular weight markers (M) are indicated.

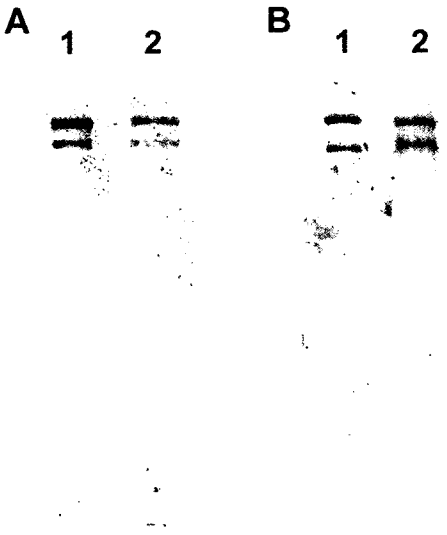

Fig. 5. Western blot analysis of the egg extracts from *L. unmusana* (A) and *L. lateralis* (B). The egg extracts were subjected to 10% SDS-PAGE, electroblotted and incubated with antiserum against vitellin of *L. unmusana* (Lane 1) or *L. lateralis* (Lane 2).

Insect vitellin probably possesses an important biological role during embryogenesis, because this protein is generally found most abundantly in adult females (Kunkel and Nordin, 1985). Therefore, SDS-PAGE analysis of the staged eggs is a effective way to confirm subunits of vitellogenin and/or vitellin in insects (Kim *et al.*, 2000a, b)

In conclusion, these results have shown the vitellin profiles in *L. unmunsana* and *L. lateralis*. A detailed study of the vitellins of *L. unmunsana* and *L. lateralis* would provide a further information for the developmental physiology.

Acknowledgments

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