THE BIOCHEMICAL CHARACTERISTICS OF VITELLOGENIN IN THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* (HYMENTOPTERA: FORMICIDAE)

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ABSTRACT

The focus of our research has been to analyze the biochemical characteristics of vitellin (VN) and vitellogenin (VG) to fully understand the endocrine regulations of reproduction in *Solenopsis invicta.* Non-denaturing polyacrylamide gel electrophoresis (PAGE) was used to determine the native molecular weight of VN (Mr = 350 kDa). Under denaturing (SDS)-PAGE conditions, native VN separated into a heavy (= 182 kDa) and a light (=171 kDa) band. VG was present in the hemolymph of all female caste members and was not present in males. In alate, virgin females VG was evident 5 days following adult eclosion and increased steadily over the study period (= 54 days). In newly inseminated queens, VG increased over the first 3 weeks, reaching a peak at day 25. Following day 25, VG titers declined slightly until the emergence of the first workers (= minims) which occurred 5 weeks following dealation of the queen. A 1.2 kB *S. invicta* VG fragment has been cloned and will be used to investigate the endocrine regulations of VG gene expression in *S. invicta* queens.

INTRODUCTION

The objective of this research project is to develop novel means of controlling the imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), through basic research to identify hormones that regulate egg production and, hence, colony founding in newly mated queens. Identifying these endocrine mechanisms may lead to new, insect-specific chemicals that will disrupt reproduction and colony founding.

The focus of insect egg formation is vitellogenin (VG), the lipophosphoglycoprotein precursor for egg-yolk protein (vitellin = VN) (Engels, 1974, Hagedorn and Kunkel, 1979, Valle, 1993, Wyatt, 1997). VG is synthesized and released by the insect fat body, transported to the ovaries, taken up by oocytic VG receptors (Raikhel and Dhadialla, 1992, Sappington and Raikhel, 1998) and deposited in the oocyte to form VN (yolk protein) (Raikhel and Dhadialla, 1992). In most insect species, juvenile hormone stimulates VG synthesis by activation of the fat body VG gene (Engelmann, 1984, Harshman and James, 1998, Wyatt, 1988, Wyatt, 1991, Wyatt, 1997, Yin and Stoffolano, 1997). In some insect species, hormones such as the ecdysteroids and neurohormones also regulate VG synthesis (Bownes, *et al.*, 1996, Hagedorn, 1980, Hagedorn, 1983, Hagedorn, *et al.*, 1979, Pascual, *et al.*, 1992, Perriere, *et al.*, 1993, Shirk, *et al.*, 1990, Soller, *et al.*, 1997). Finally, neurohormones such as allatotropins and allatostatins that stimulate or inhibit corpora allata synthesis of juvenile hormone (Stay and Woodhead, 1993, Tobe, *et al.*, 1994, Unni, *et al.*, 1991), respectively, also influence VG synthesis.

In single queen (monogyne) fire ant colonies, mating is the first event associated with egg formation and colony founding. Alate (winged) virgin females and males swarm and undertake mating flights on warm days, usually following rain, and the females are inseminated (Vinson, 1997). The newly mated queens return to the ground, actively break off their wings (dealation) and initiate flight muscle histolysis and ovarian maturation. The newly mated queens then search for disturbed areas to form a primary nest. Once a new queen forms a primary nest, she deposits a few small eggs that produce tiny workers called minims. The minims feed and tend the young queen and assist her to build up metabolic reserves so that she is able to lay more, larger eggs that produce the large workers that will forage and tend her and the brood to produce the mature nest.

Alate females in multiple queen (polygyne) colonies apparently employ one of three strategies depending on their genotype (DeHeer, *et al.*, 1999; Goodisman, *et al.*, 2000). Alate females from polygyne colonies may use the same mating strategy employed by monogyne alate females. However, more typically, following mating the newly mated polygyne queens either return to their natal site or disperse and seek adoption in other polygyne nests. Presumably, similar physiological events related to flight muscle histolysis and ovarian maturation would follow the adoption.

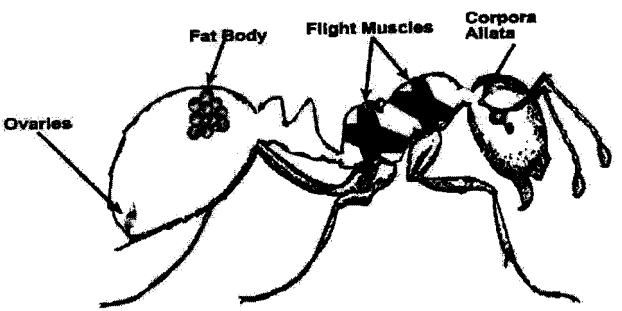

FIG. 1. Diagrammatic Representation of Imported Fire Ant Queen Showing Tissues Putatively Important for Ovarian Maturation

Mating initiates physiological changes related to ovarian maturation. Figure 1 shows a diagram of the internal tissues that are involved in ovarian maturation in a fire ant queen. It is postulated that a muscle-histolysis factor (MHF) is released in response to mating (Davis, *et al.*, 1989). This factor is heat-sensitive suggesting it might be a large protein, hence we speculate that MHF might be a proteinaceous neurohormone released in response to sensory and nervous stimuli during mating. Within two hours after mating and dealation, the flight muscles show evidence of histolysis (Jones, *et al.*, 1978). This process continues until the flight muscles are completely disintegrated. Presumably, protein released from the degenerating flight muscles is transferred to the fat body to serve as a source of amino acids for VG synthesis for the minim eggs. The corpora allata are an endocrine gland attached to the brain that secretes juvenile hormone. Evidence suggests that juvenile hormone regulates fire ant VG synthesis (Troisi and Riddiford, 1974, Vargo and Laurel, 1994). However, brain neurohormones are also essential for egg production since application of 100 μg JH to females lacking brain neurosecretory cells resulted in 50–70% fewer eggs than were produced by allatectomized females treated with 10 μg JH (Barker, 1978).

We speculate that there are several events related to reproduction and colony founding that are vulnerable to disruption for fire ant control. Identification of the MHF would suggest approaches to either promoting or inhibiting flight muscle degradation. Mimics of MHF would promote early muscle histoloysis and prevent mating flights; alternatively, inhibitors would suppress flight muscle breakdown and prevent reuse of the flight muscle proteins as a source of amino acids for early VG synthesis. Early studies suggested that juvenile hormone regulated flight muscle degradation and egg formation (Vargo and Laurel, 1994). Therefore, mimics or inhibitors of juvenile hormone might be effective to disrupt reproduction.

However, the action by juvenile hormone does not appear simple. VG is present in alate virgin females and workers, but eggs are not formed. Hence, synthesis of VG and its uptake by the ovaries and production of eggs are related, but separate, events. Whereas treatment with juvenile hormone and its analogs promotes dealation and egg formation, so too does anesthesia with CO_2 and treatment with acetone, the preferred solvent for juvenile hormone analogs. Therefore, the experiments to date have not unequivocally identified juvenile hormone as the main regulatory hormone for flight muscle degradation and the promotion of reproduction. It is our goal to apply the analytical power of molecular biology to study the natural patterns of gene expression for VG and the oocyte VG receptor and correlate these patterns with endocrine regulations both in vivo and, more importantly, in vitro – where confounding, interfering factors can be eliminated.

RESULTS AND DISCUSSION

As a first step to understanding reproduction in *S. invicta* queens, the biochemical characteristics of VG and VN were elucidated. Analysis of *S. invicta* soluble egg polypeptides using non-denaturing polyacrylamide gel electrophoresis (PAGE), identified two major bands (Fig. 2) (Lewis, *et al.*, 2001). The estimated Mr value of these two bands (VN1 and VN2) was 350 kDa and 175 kDa, respectively. The 175 kDa band was half the size of the larger polypeptide, suggesting that the *S. invicta* 350 kDa band represented *S. invicta* VN and was composed of two similarly sized polypeptides. Several species in the hymenopteran suborder Apocrita possess a native VN in the molecular weight range of 300-450 kDa that cleaves to form two homodimers (Harnish and White, 1982, Jensen and Borgesen, 1995, Nose, *et al.*, 1997, Wheeler and Kawooya, 1990). In the ant family, Ponerinae, three tribes also show this pattern, although two other tribes contain multiple subunits (Wheeler, *et al.*, 1999). We are currently unsure of the identity of the lower band evident in lanes 7 and 8. We speculate that it is either a degradation product of VN2 or may be a protein in the egg unrelated to VN.

Using denaturing (SDS)-PAGE analysis, *S. invicta* VN separated into a 182 kDa band (= heavy subunit) and a 171 kDa band (= light subunit) (Fig. 3) (Lewis, *et al.*, 2001) suggesting that the two subunits were not identical. Furthermore, the presence of these two bands in females appears to correlate with their reproductive status. One or both VGs were present in the hemolymph of all female caste members of *S. invicta* (Fig. 3). In mated queens, the heavy and light bands were present in both the hemolymph and the eggs; whereas in alate, virgin, females and workers, only the heavy band was present in the hemolymph. Thus it is unclear whether the two subunits are products from two different genes that are differentially expressed after mating; two cleavage products from a single gene; or the product of a single gene that has undergone differential post-translational processing.

Reproduction in social insects is usually highly regulated by the presence of a queen pheromone (Wilson, 1971), so it is not surprising that VG synthesis/uptake is regulated. In worker bees of *Apis mellifera* Linnaeus (Hymenoptera: Apidae), VG is

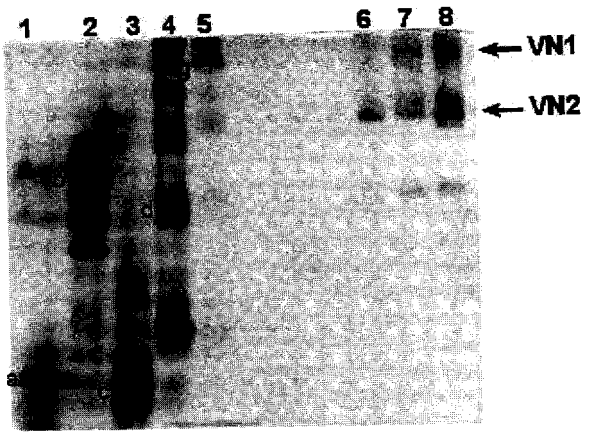

FIG. 2. Native PAGE (7.5% acrylamide) of soluble egg polypeptides (lane 6-8). Molecular weights of dimeric (~350 kDa; VN1) and monomeric (~175 kDa; VN2) native VNs were calculated from a standard line derived from Ferguson plots of non-denatured molecular weight standards (lanes 1-5) separated on native gels of various acrylamide concentrations. The non-denatured standards migrate in relation to molecular size as well as charge and include (a) α-lactalbumin (14.2 kDa), (b) carbonic anhydrase (29 kDa), (c) chicken egg albumin (45 kDa), (d) bovine serum albumin dimer (132 kDa), (e) bovine serum albumin monomer (66 kDa), (f) urease hexamer (545 kDa) and (g) urease trimer

produced only during the nurse stage and is present as a minor fraction in haploid drones (Trenczek and Engels, 1986, Trenczek, *et al.*, 1989). In workers of the ant species *Camponotus festinatus* Buckley (Hymenoptera: Formicidae), VG increases in the absence of the queen, but decreases within 6-7 weeks following eclosion of a reproductive queen (Martinez and Wheeler, 1991a, Martinez and Wheeler, 1991b). In alate, virgin females of *S. invicta*, circulating VG is observed, but without apparent oocyte maturation (Lewis, *et al.*, 2001, Vargo and Laurel, 1994). High VG titers without egg maturation were also reported for *A. mellifera* (Engels, 1974). To determine the onset of VG synthesis in alate, virgin females of *S. invicta*, we monitored VG titers over a two-month period. VG was present in the hemolymph of alate, virgin queens by 5 days following adult eclosion and increased steadily during the study (Fig. 4) (Lewis, *et al.*, 2001). Ovarian maturation was not observed until day 54 suggesting that VG uptake, not synthesis, may be the critical step in the onset of reproduction. The presence of ovary-specific VG receptors (Sappington and Raikhel, 1998) suggests that synthesis or activity of the receptor may be the regulated event for egg maturation.

VG profiles were also monitored in newly inseminated queens starting from the day of dealation (day 0) through production of the first workers (= minims). VG increased steadily over the first 3 weeks, was highest on day 25, then declined slightly until the emergence of the first minims (Fig. 5) (Lewis, *et al.*, 2001). VG titers dropped after minim emergence. By 10 days following minim emergence, a significant number of other protein bands were evident in the hemolymph. This was likely due to renewed protein synthesis in response to resumed food consumption by the queen.

In aged, alate, virgin females and newly inseminated queens, an 80 kDa protein was present (Figs. 4,5). The 80 kDa protein decreased as VG titers increased in aged, alate alate, virgin females. This same protein declined as the eggs from newly inseminated

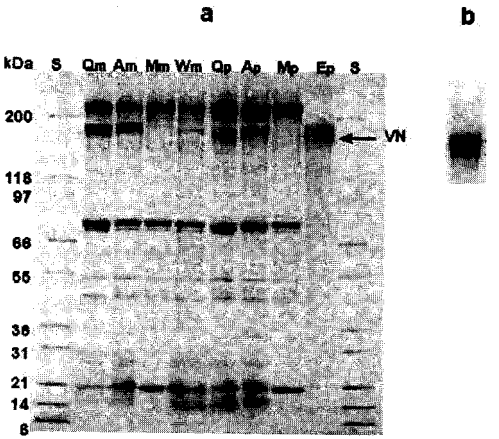

FIG. 3. SDS-PAGE (exponential gradient consisting of 6-16% acrylamide and 0.16%-0.85% bis-acrylamide) comparing hemolymph polypeptides from castes of monogyne and polygyne *S. invicta*. Each lane represents 8 μg of soluble polypeptides from the hemolymph of a monogyne queen (Qm), monogyne alate virgin female (Am), monogyne male (Mm), monogyne worker (Wm), polygyne queen (Qp), polygyne alate virgin female (Ap), polygyne male (Mp) and soluble egg polypeptides from a polygyne queen (Ep). Each hemolymph sample consists of 4 animals except for the monogyne queen in which only 1 animal was used. VG/VN (arrow) and the molecular weight standards (S) are indicated. (b) SDS-PAGE (exponential gradient consisting of 6-9% acrylamide and 0.16% - 0.42% bis-acrylamide) of egg protein showing the region of vitellogenins. The lane contained 4 μg of egg protein (Reprinted with permission from Lewis *et al.*, 2001).

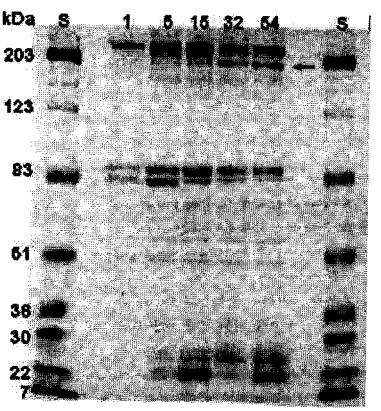

FIG. 4. Age effects on hemolymph polypeptides in alate virgin females of *S. invicta*. Polygyne, alate, virgin females were monitored from ages 0-54 days. Hemolymph samples were collected on the day following eclosion (Day 1) and at intervals through day 54. Each lane represents 8 μg hemolymph polypeptides pooled from 3 animals and separated by SDS-PAGE (exponential gradient consisting of 6-16% acrylamide and 0.16%-0.85% bis-acrylamide). VG (arrow) and the molecular weight standards (S) are indicated (Reprinted with permission from: Lewis *et al.*, 2001).

queens neared minim emergence. Storage proteins accumulate in alate, virgin females prior to mating in *C. festinatus* (Martinez and Wheeler, 1994), but decline during colony founding in *C. festinatus* and *Crematogaster opuntiae* Buren (Hymenoptera: Formicidae) (Martinez and Wheeler, 1994, Wheeler and Buck, 1995). Thus, the 80 kDa polypeptide observed in both aged, alate virgin females and newly inseminated queens is likely a putative storage protein and may be reciprocally related to VG synthesis.

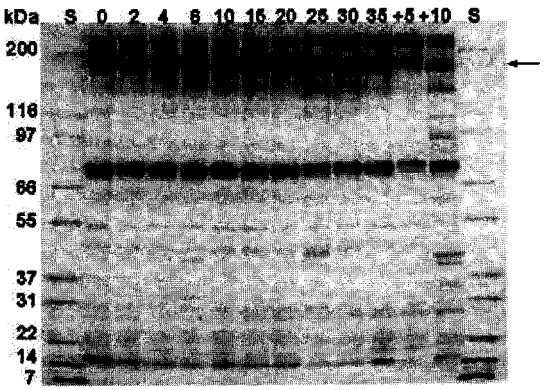

FIG. 5. Age effects on hemolymph polypeptides in field-collected, newly inseminated queens of *S. invicta*. Hemolymph polypeptide samples (8 μg) were collected at dealation (Day 0) and at intervals until 10 days following nanitic emergence ($^{+}$10) and separated by SDS-PAGE (exponential gradient consisting of 6-16% acrylamide and 0.16%-0.85% bis-acrylamide. Each time point represents 7-10 pooled samples. VG (arrow) and the molecular weight standards (S) are indicated (Reprinted with permission from: Lewis *et al.*, 2001).

To fully understand the events associated with reproduction in *S. invicta*, VG gene expression by the fat body is being investigated. A 1.2 kB *S. invicta* VG fragment was cloned and sequenced using reverse transcription-polymerase chain reaction (RT-PCR). The RT reaction was carried out using an oligo-dT_{16} primer. Degenerate primers were used for PCR amplification. Degenerate primer design was based on published VG gene sequences from related insects. The 1.2 kB fragment showed strong homology with other VG genes available through the National Center for Biotechnology Information BLAST e-mail server. Rapid Amplification of cDNA Ends (RACE) was used to determine the remaining sequence. Figure 6 shows a comparison of the first 60 amino acids from the *S. invicta* VG gene with the VG genes of *Pimpla nipponica* Uchida (Hymenoptera: Ichneumonidae) and *Athalia rosae ruficornis* Jakovlev (Hymenoptera: Tenthredinidae). VG gene expression studies are in progress to determine the endocrine regulators that ultimately influence reproduction.

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Pn MWCPLFLVLLAGAAT AEHLQAWKTDTEYQY AVRGRTLSALHDVAD QYSGIIMRALLTIQP
Ar MWSPLLLCLLVGIAS AD-QHAWKTGQEYTY QVRGRTLAALHQVAD QYTGIALKAQLKCQP
Si MWYLAFLLIIGAYAA DH---AWETGNEYHY LIESRTLTVLDKLSQ QFSGIVIKGGLTIQV
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FIG. 6. Partial amino acid sequence alignment of the vitellogenin gene from *Solenopsis invicta* (Si) compared with two Hymenopteran species, *Pimpla nipponica* (Pn) and *Athalia rosae* (Ar). The (+) indicates identity between all three species. The (-) indicates homology between two of the three species.

The data illustrate that *S. invicta* VG is a large, female-specific polypeptide with an estimated native Mr of 350 kDa. Presently, it is unclear whether the two subunits observed (heavy = 182 kDa, light = 171 kDa) under denaturing conditions represent a single gene that is differentially modified during the reproductive phase, or the products of two genes. VG synthesis in alate, virgin females is time-dependent and does not ultimately control ovarian maturation since ovarian maturation was not evident in the presence of VG. Future studies will target the endocrine events that trigger reproduction and investigate the significance of the two subunits.

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