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Sequence and the developmental and tissue-specific regulation of the first complete vitellogenin messenger RNA from ticks responsible for heme sequestration

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Abstract

The first full-length mRNA for vitellogenin (Vg) from ticks was sequenced. This also represents the first complete sequence of Vg from the Chelicerata and of a heme binding Vg. The Vg cDNA from the American dog tick, *Dermacentor variabilis* was 5744 nt in length (GenBank Accession number AY885250), which coded for a protein of 1843 aa with a calculated molecular weight of 208 kD. This protein had an 18 aa signal sequence, a single RXXR cleavage signal that would generate two subunits (49.5 and 157 K in molecular weight) and lipoprotein N-terminal and carboxy von Willebrand factor type D domains. Tryptic digest MS analysis of vitellin protein confirmed the function of the cDNA as the tick yolk protein. Apparently, vitellin in *D. variabilis* is oligomeric (possibly dimeric) and is comprised of a mixture of the uncleaved monomer and subunits that were predicted from the single RXXR cleavage signal. The highly conserved GL/ICG motif close to the C-terminus in insect Vg genes was different in the tick Vg message, i.e., GLCS. This variant was also present in a partial sequence of Vg from *Boophilus microplus*. Phylogenic analysis showed that the full length Vg cDNA from *D. variabilis* and the partial cDNA from *B. microplus* were distinct from insects and Crustacea. The Vg message was not found in whole body RNA from unfed or fed males or in unfed and partially fed (virgin) females as determined by Northern blotting. The message was found in replete (mated) pre-ovipositional females, increased to higher levels in ovipositing females and was absent after egg laying was complete. The endocrine regulation of the Vg mRNA is discussed. The tissue sources of the Vg message are both the gut and fat body. Tryptic digest MS fingerprinting suggests that a second Vg mRNA might be present in the American dog tick, which needs further study.

Keywords: Vitellogenin; Vitellin; Ticks; Acari; American dog tick; Dermacentor variabilis; Heme

1. Introduction

Vitellogenin (Vg) is the major yolk protein of eggs in both vertebrate and invertebrate animals and shares a common ancestry (Chen et al., 1997). Once the protein is synthesized in insects, the signal sequence is cleaved, and Vg protein is exported from the cell into the hemolymph. Vg is further processed into smaller subunits and transferred to developing oocytes as vitellin (Vn), the egg storage protein (reviewed in Raikhel and Dhadialla, 1992). Numerous complete Vg cDNAs have been sequenced in the Mandibulata. The insect mRNAs are large, ranging from 5441 nt for the honey bee, *Apis mellifera* (Piulachs et al., 2003) to 6654 nt for the wasp, *Encarsia formosa* (Donnell, 2004). Crustacean vitellogenin mRNAs range from 7782 to 8012 nt for the prawn, *Macrobrachium rosenbergii* (Yang et al., 2000) and the sand shrimp, *Mentapenaeus ensis* (Tsang et al., 2003), respectively. The fat body has long been accepted as the primary site of Vg RNA synthesis in the Insecta (see for example, Kokoza et al. 2001; Tufail and Takeda, 2002; reviewed in Melo et al., 2000; Raikhel et al., 2002), although the ovaries may be a secondary producer of Vg RNA in Coleoptera (Zhai et al., 1984), the higher

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Diptera (Brennan et al., 1982; Issac and Bownes, 1982) and *Rhodnius prolixus* (Melo et al., 2000). The site of Vg RNA synthesis in the Crustacea is in dispute. In some Custacea, the main Vg source is the hepatopancreas (Tseng et al., 2001; Lee and Chang, 1999; Okuno et al., 2000) while in others the ovary also produces vitellogenin (Serrano-Pinto et al., 2004; Avarre et al., 2003; Pateraki and Stratakis, 2000). In the shrimp, *Metapenaeus ensis*, Vg is encoded by at least two genes, one expressed in both the hepatopancreas and ovary and the other in the hepatopancreas only (Tsang et al., 2003). Evidence for multiple insect Vgs has also been reported (e.g., Hirai et al., 1998).

In comparison to the many complete cDNAs sequenced from the Mandibulata, no complete Vg cDNA has been sequenced from the Chelicerata. In ticks, incomplete sequences have been reported from *B. microplus* (GenBank accession number U49934; Tellam et al., 2002), *Amblyomma americanum* (GenBank accession number BI27356; Bior et al., 2002) and *Dermacentor variabilis* (*Dv*, GenBank accession number AY885250; Thompson et al., 2005). The site of synthesis of Vg in ticks is also in question. Chinzei and Yano (1985) identified the fat body as the source of vitellogenin in the soft tick, *Ornithodoros moubata*. However, Rosell and Coons (1992) and Coons et al. (1989) concluded that the *D. variabilis* midgut may play a role in vitellogenin production (reviewed in Sonenshine, 1991). The tick vitellogenin also appears to have a unique function unlike previous yolk proteins sequenced. Ticks are unable to synthesize heme and have an absolute requirement for heme in their diet (Braz et al., 1999). The Vg of ticks sequesters heme and transfers this heme to the egg (Gudderra et al., 2002a,b; Logullo et al., 2002). This unusual heme-binding characteristic of Vg appears to be critical to embryo development and the unique life strategy of ticks where they have lost the ability to synthesize heme and are obligate blood-feeders. This current paper reports the first full-length sequence of Vg from any chelicerate and the first heme binding yolk protein, resolves the issue of whether there are multiple tissue sources of Vg in ticks which has been in dispute for many years, examines the developmental regulation of this message during adult development, and provides evidence of possible multiple tick Vg genes.

2. Experimental procedures

2.1. Ticks

A pathogen-free line of the American dog tick, *D. variabilis*, was reared as described previously (Sonenshine, 1993). Adult ticks were confined within plastic capsules attached to New Zealand white rabbits (*Oryctolagus cuniculus*) and allowed to feed and mate as required. Rearing conditions were $26 \pm 1\,^{\circ}C$, $92 \pm 6\%$ relative humidity and 14:10 (L:D). All use of animals was conducted at Old Dominion University and approved by the Institutional Animal Care and Use Committee.

2.2. Tissue dissection, egg and hemolymph collection

Ovaries, midguts and fat bodies from replete (mated, pre-ovipositing) females were dissected, washed in ice-cold phosphate-buffered saline (PBS; pH 7.0, 0.010 M NaH_2P0_4, 0.014 M Na_2HP0_4, 0.15 M NaCl) and immediately stored in RNAlater (Ambion, Austin, TX) at –80 °C until used for RNA isolation. Hemolymph was collected as described by Johns et al. (1998). Briefly, ticks were immobilized on slides, ventral side up on double-sided tape and one or two forelegs amputated with microdissecting scissors. While applying gentle pressure, clear hemolymph exuded from the opening to the body cavity was collected with a glass Drummond micropipette, transferred to an Eppendorf tube, and diluted 1:1 in PBS. Newly oviposited eggs were collected and stored at –80 °C. Eggs ($n = 25$) were rinsed once with Dulbecco's phosphate buffer (Pierce, Rockford, IL) with Tween 20 (0.05% v/v) and homogenized in 500 µl of the same buffer with a ceramic mortar and pestle. The homogenate was clarified by centrifugation at 960*g* at 4 °C for 10 min. Diluted hemolymph and egg homogenate were stored at –80 °C until needed for further analysis.

2.3. RNA isolation and 5′ RACE

Total RNA was isolated from either whole bodies at different adult developmental stages (see results) or dissected tissues (described above) using TRI Reagent (Sigma, Saint Louis, MI) according to the manufacturer's recommendations except that RNA pellets were dissolved in water containing 10 µM aurin trichloroacetic acid to prevent degradation (Hallick et al., 1977). Samples were assayed for RNA content using a Molecular Devices Corporation Spectromax 384 Plus plate reader (Sunnyvale, CA) and then stored at –80 °C until needed for further analysis.

The initial Vg cDNA fragment from *D. variabilis* was obtained from a cDNA library made from the fat body of replete (mated, vitellogenic) females as described in Thompson et al. (2005). To obtain the remainder of the 5′ region of the Vg cDNA, 5′RACE was performed using SMART RACE (Clontech, Palo Alto, CA). First-strand Vg cDNA was synthesized from total fat body RNA isolated from replete (mated, pre-ovipositional) females in the presence of SMART IV oligonucleotide and Vg specific reverse primer. The resulting cDNA fragments were amplified using the Advantage 2 PCR Kit (Clontech). Several 5′RACE rounds were needed to obtain multiple fragments in the 5′ region of the Vg cDNA, which were compiled using ContigExpress (Vector NTI) to obtain the full Vg cDNA sequence. PCR amplification products were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and sent for sequencing in the Nucleic Acid Research Facilities at the Virginia Commonwealth University (Richmond, VA).