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Identification and Partial Characterization of vitellin and vitellogenin from *Scolopendra cingulata* LATREILLE (Myriapoda Chilopoda)

by

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Abstract: Vitellin was identified from mature *Scolopendra cingulata*. After homogenization of ovaries in 0.05 M Tris-HCl pH 7.4, 0.15 M NaCl buffer and centrifugation, the supernatant (soluble oocyte proteins), analysed by polyacrylamide gradient gel electrophoresis (PAGE), showed two major protein bands with apparent molecular weights of about 380 and 450 kilodaltons, respectively. These compounds were both glycolipoproteins and are defined as vitellins. Hemolymph samples of both vitellogenic females and males were also taken up and diluted in the same buffer. The same protein bands, defined as vitellogenins were present in the hemolymph of females and, at far lower levels, in males.

1. Introduction:

Recent work on oogenesis in Myriapoda Chilopoda concerned firstly the ultrastructure of the germinal cells and has been mostly related to *Lithobius forficatus* (HERBAUT 1972 - 1974). There is cytological evidence for a heterosynthetic mechanism of vitellogenesis, yolk proteins entering the oocyte by pinocytosis. The only other studies concerned, to our knowledge, the nucleolar ultrastructure of *Scolopendra* (BEAMS & SEKHON 1967), and *Scutigera* oocytes (BEAMS & SEKHON 1968). Other experimental studies have related mainly to endocrine control of oogenesis (HERBAUT 1975, 1976, 1977).

There is not molecular data concerning vitellogenin and vitellin in the oogenetic cycle of the Chilopoda. In this paper preliminary findings concerning vitellin and vitellogenin in the Scolopendromorph *Scolopendra cingulata* LATREILLE are presented.

2. Material and Methods:

Animals: *Scolopendra cingulata* were collected in Southern France, in the garrigues near Frontignan. The animals were kept in the laboratory at room temperature (16 - 20° C), and fed regularly (three times a week) until use.

Preparation of samples: Ovaries were dissected from mature females and homogenized in 0.05 M Tris-HCl, pH 7.4 0.15 M NaCl buffer. After centrifugation (5000 rpm, 10 mn), the supernatant, defined as soluble oocyte proteins was analysed by electrophoresis. Hemolymph was also collected, both from males and females. Each sample was diluted in the same buffer.

Polyacrylamide gel electrophoresis:

A) Electrophoresis was conducted in non-denaturing 5 - 25 % polyacrylamide gradient slab gels as described by SLATER (1969), using the buffer system of DAVIS (1964). The gels were then stained for proteins using Coomassie Brilliant Blue R250, for glycoproteins using periodic acid/Schiff (PAS) reagent and for lipoproteins

using Sudan Black B. Standard molecular weight markers used were thyroglobulin (Mr 669000), ferritin (Mr 440000), catalase (Mr 232000), albumin dimer (Mr 134000) and albumin monomer (Mr 67000).

B) Sodium dodecyl sulphate/polyacrylamide denaturing gels were prepared according to the method of LAEMMLI (1970), except that the separating gel consisted of a 5 - 25 % polyacrylamide gradient slab gel. Standard molecular weight markers were phosphorylase B (Mr 94000), albumin (Mr 67000), ovalbumin (Mr 46000), carbonic anhydrase (Mr 30000), trypsin inhibitor (Mr 20000) and α -lactalbumin (Mr 14400).

C) For the two-dimensional electrophoresis, a cut-off gel strip of a 5 - 25 % polyacrylamide gradient gel (0.75 mm thick) was equilibrated for 20 mn in 2.2 % SDS, 1 % 2-mercaptoethanol, 10 % glycerol and 125 mM Tris-HCl, pH 6.8, loaded on top of the SDS electrophoresis gel (1 mm thick) and then sealed in place with 1 % (w/v) agarose in SDS equilibration solution.

3. Results:

The non-denaturing electrophoretic analysis of soluble oocyte proteins showed two major protein bands with apparent mol. wt. of 380 and 450 kilodaltons (= kd), respectively (Fig. 1a). These two proteins also stained strongly both with Sudan Black B for lipids (Fig. 1 b) and with PAS reagent for carbohydrates (Fig. 1 c). So, being both lipoglycoproteins and major components of oocytes, they were defined as *Scolopendra cingulata* vitellins.

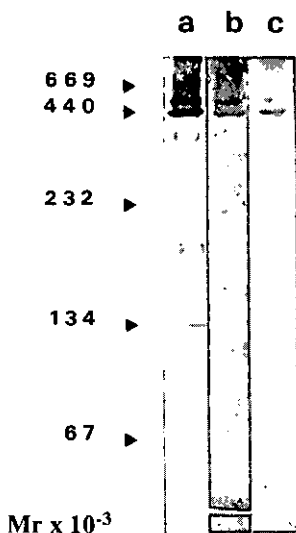


Fig. 1: Electrophoretic separation of native oocyte proteins from *Scolopendra cingulata* on a 5 - 25 % polyacrylamide gradient gel. (a) Gel stained with Coomassie blue; (b) gel stained with Sudan black B; (c) gel stained with periodic acid/Schiff reagent. Mr of standards are indicated on the left.

Hemolymph electrophoretic analysis of a vitellogenic female in a non-denaturing system also showed two major protein bands of about 380 and 450 kd, respectively (Fig. 2 a). Both stained with Sudan Black B and PAS reagent (Figs 2 b, c) and consequently are lipoglycoproteins. They were defined as vitellogenins. Hemolymph from males showed the same components, but at far lower levels than in females (Fig. 3). A two dimensional electrophoresis of vitellins (Fig. 4) showed that each of them was made of a major polypeptide of about 145 kd.

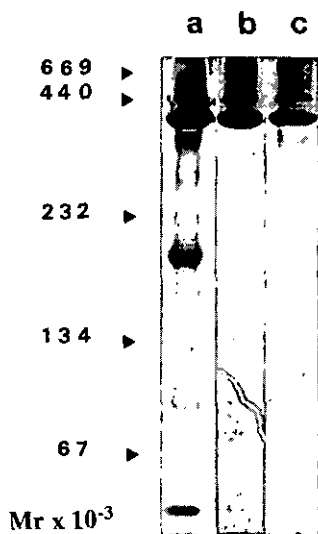


Fig. 2

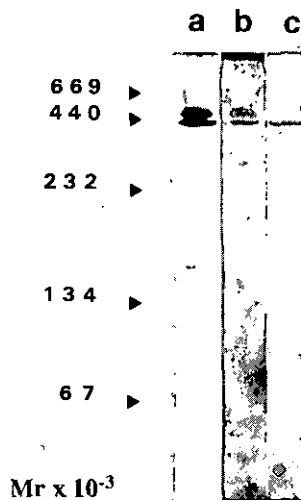


Fig. 3

Fig. 2: Electrophoretic separation of native hemolymph proteins from a vitellogenic female on a 5 - 25 % polyacrylamide gradient gel. (a) Gel stained with Coomassie blue; (b) gel stained with Sudan black B; (c) gel stained with periodic acid/Schiff reagent. Mr of standards are indicated on the left.

Fig. 3: Electrophoretic separation of native hemolymph proteins from a male on a 5 - 25 % polyacrylamide gradient gel. (a) Gel stained with Coomassie blue; (b) gel stained with Sudan black B; (c) gel stained with periodic acid/Schiff reagent. Mr of standards are indicated on the left.

4. Discussion:

The results reported here are the first molecular data concerning vitellins from the Chilopoda; so it is not possible to compare them to other data from the same zoological group. In *Scolopendra cingulata* we report the presence of two vitellins and vitellogenins (about 380 and 450 kd, respectively), both being constituted most presumably by the same major polypeptide of 145 kd, but further investigations are needed to confirm this.

Concerning vitellogenins and vitellins, if their apparent molecular weights are comparable, no further conclusions can be drawn concerning their molecular identity.

It must be noted 1) that in Insects, most native vitellins ranged between 200 and 450 kd (ENGELMANN 1986) and 2) that the major polypeptide compound has a high molecular weight (> 100 kd). In Insects, two vitellins are also found, in amongst other species, *Tenebrio molitor* (380 and 470 kd; DELSWORTH et al. 1982), *Thermobia domestica* (300 and 430 kd; ROUSSET et al. 1988), whereas only one vitellin was found in other species such as *Aedes aegypti* (170 kd; BO-ROVSKY et al. 1987) or *Pieris rapae* (380 kd; KIM et al. 1988).

Natural presence of vitellin in males is also reported in Insects (Lepidoptera, *Rhodnius*, for review see LAMY 1984; *Acheta domesticus*, RENUCCI et al. 1987). In *Scolopendra* the same protein bands found in female hemolymph exist in males, but the demonstration of the possible uptake of these vitellogenins by growing oocytes has not been carried out. In another chilopod, *Lithobius forficatus*, it was shown (HERBAUT 1974 b) that implanted ovaries in males generally do not degenerate, but vitellogenesis does not proceed. It must be remembered also that in a natural case of intersexuality described in *L. forficatus*, vitellogenic oocytes were not observed (DESCAMPS & HERBAUT 1971). So, the occurrence in *L. forficatus* male hemolymph of the same protein bands

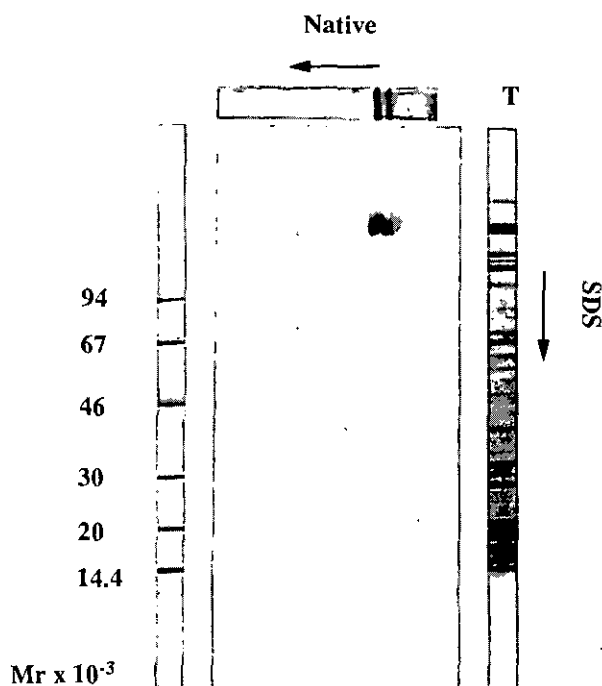


Fig. 4: Two-dimensional gel electrophoresis of proteins from oocytes. Proteins were separated by native PAGGE (5 - 25 %) in the first dimension and then by SDS PAGGE (5 - 25 %) in the second dimension. T: total SDS PAGGE (5 - 25 %) of the oocyte soluble proteins. Mr of standards are indicated on the left.

as in female hemolymph (DESCAMPS & FABRE, unpublished observations) does not mean the ability for the "male" vitellogenins to be correctly processed.

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