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Tick vitellin is dephosphorylated by a protein tyrosine phosphatase during egg development: Effect of dephosphorylation on VT proteolysis

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Abstract

Vitellin (VT) is a phospholipoglycoprotein that is the main component of arthropod egg yolk. Phosphorylation is a recurrent feature of every VT molecule described so far. However, the role played by such post-translational modification during egg development is not yet clear. In the eggs of the hard tick *Boophilus microplus*, VT is a phosphotyrosine-containing protein. VT–phosphotyrosine residues are gradually removed during tick embryogenesis due to the action of a 45 kDa egg tyrosine phosphatase. This enzyme is strongly inhibited by ammonium molybdate, sodium vanadate and cupric ion. The role of phosphotyrosine residues in VT proteolytic degradation was evaluated. Western blots probed with a monoclonal anti-phosphotyrosine antibody demonstrated that the high molecular mass VT subunits (VT 1 and VT 2) are the main targets of dephosphorylation during egg development. Both dephosphorylation and proteolysis of VT 1 and VT 2 are blocked by ammonium molybdate in total egg homogenates. When purified VT was dephosphorylated in vitro with lambda phosphatase and then incubated in the presence of bovine cathepsin D, VT proteolysis increased dramatically. Altogether, these data are the first to show that phosphotyrosine residues are present in a yolk protein, and that such residues might be involved in the regulation of VT breakdown during egg development.
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Keywords: *Boophilus microplus*; Yolk degradation; Tyrosine phosphatase; Tick egg development; Proteolysis; Dephosphorylation

1. Introduction

Ixodid ticks are recognized worldwide as vectors of arboviruses and parasitic protozoa (Taege, 2000). *Boophilus microplus* is a tick whose main hosts are bovine cattle. Once attached to its host this tick ingests a huge amount of blood. In the parasitic phase the larvae give rise to fully engorged adults. The females then fall to the ground and during this non-parasitic period egg laying occurs. Egg development takes around 3 weeks, and recently hatched larvae migrate to the upper part of the grass, where they wait for another vertebrate host (Sonenshine, 1991). *B. microplus* is considered the main parasite responsible for damage to the world livestock economy, for several reasons. First, it is the vector of *Babesia bovis*, which causes piroplasmosis, also known as "cattle sadness". This disease can cause a high mortality rate among cattle (Hunfeld and Brade, 2004). Second, *Boophilus* can ingest a very large amount of blood while attached to its host. In severely infested bovines the daily removal of blood is close to 100 ml. Blood loss leads to anemia and, consequently, loss of animal strength. Finally, tick feeding damages the hide and decreases the production of meat and milk.

One strategy to block disease transmission by ticks would be the identification of new molecular targets for the

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interruption of its life cycle. In this respect the non-parasitic phase, when the eggs are laid and embryonic development occurs, is tempting for two reasons. First, this phase occurs away from the host and, therefore, blocking strategies directed to the larvae will not affect the cattle. Second, embryonic development is strictly programmed. During development, an exclusive sequence of cellular and molecular processes occurs, most of which are not present in both larvae and adults.

The main biochemical event of egg development in oviparous organisms is the utilization of yolk components that were stored in the egg during oogenesis. Vitellogenin (VG) is a hemolymphatic phospholipoglycoprotein stored in growing oocytes. Once inside the oocytes, it is conventionally called VT and is the main component of yolk platelets (Sappington and Raikhel, 1998). After oocyte fertilization, several different VT processing mechanisms are triggered, and VT furnishes the building blocks for developing embryos. Several enzymes involved in this process have been characterized in the past few years in different biological models (Fagotto, 1990; Fialho et al., 2005; Hunfeld and Brade, 2004; Sappington and Raikhel, 1998; Sonenshine, 1991; Taege, 2000; Yamamoto and Takahashi, 1993). Such studies have addressed the identification, purification and/or cloning and characterization of VT proteolytic systems in developing eggs of arthropods. However, they do not provide information about the susceptibility of VT itself to proteolytic attack.

VT of most organisms contains covalently bound phosphate (Byrne and Gruber, 1989; Masuda and Oliveira, 1985; Sappington and Raikhel, 1998; Silva-Neto et al., 2002). Despite its wide distribution, the role of phosphorylation during egg development in blood-sucking arthropods is not clear. Usually, maternally derived phosphoproteins found in developing arthropod eggs contain only phosphoserine residues (Sappington and Raikhel, 1998). In the present study, we demonstrate that *B. microplus* VT is a phosphotyrosine-containing protein. We also demonstrate the presence of a protein, tyrosine phosphatase, able to dephosphorylate tick VT. Inhibition of this enzyme blocks VT proteolytic degradation by egg proteases. Furthermore, we show that in vitro dephosphorylation of tick VT increases the rate of its degradation by commercial aspartyl protease.

2. Materials and methods

2.1. Chemicals

Molecular mass standards, glycine, acrylamide, bis-acrylamide, TEMED, *p*-nitrophenylphosphate (pNPP), pCMB, dithiotreithol (DTT), tris, tetramisole, bovine serum albumin (BSA) and sodium acetate were purchased from Sigma Chemical Company (St. Louis, MO, USA). Phenylarsine oxide (PAO), okadaic acid (OKA) and *o*-vanadate were purchased from Calbiochem–Novabiochem Corp. (La Jolla, CA, USA). Mouse monoclonal anti-phosphotyrosine PY-99 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A set of pre-stained molecular mass standards (SeeBlue®) and rabbit polyclonal anti-phosphoserine were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Ammonium molybdate and dimethylsulfoxide (DMSO) were purchased from Merck (Gibbstown, NJ, USA). ECL system was purchased from Amersham. All other chemicals were of analytical grade, from Brazilian suppliers.

2.2. Ticks and eggs

Ticks were obtained from a colony maintained at the Faculdade de Veterinária at the Universidade Federal do Rio Grande do Sul, Brazil. *B. microplus* of the Porto Alegre strain, free of *Babesia* sp., were reared on calves obtained from a tick-free area. Engorged adult females were kept in Petri dishes at 28 °C and 80% relative humidity until completion of oviposition. Eggs were pooled and kept for the desired length of time under the same conditions. To obtain samples during the days after oviposition, a group of eggs was collected every day at the same time and stored at −18 °C for further analysis. A control group of eggs was allowed to complete development in order to determine the exact day of hatching. Eggs were homogenized with a plastic pestle in 0.2 M acetate buffer pH 5.0 and, when indicated, in the presence of a cocktail of protease inhibitors (AEBSF 1.04 mM, aprotinin 0.8 μM, leupeptin 20 μM, bestatin 40 μM, pepstatin A 15 μM and E-64 14 μM) from Sigma Fine Chemicals (St. Louis, MO, USA). This preparation is referred to as egg homogenate. Protein content of egg homogenate was determined by the method described by Lowry et al. (1951) using BSA as standard.

2.3. VT dephosphorylation and proteolytic assays

VT was purified as described previously in the presence of 1 mM ammonium molybdate, using eggs obtained on the first day after oviposition (Logullo et al., 2002). Before use, VT was dialyzed to remove ammonium molybdate. VT dephosphorylation was carried out routinely as follows. Purified VT (80 μg) was incubated in the presence or in the absence of 200 U of recombinant lambda phosphatase from Upstate Biotechnology (Waltham, MA, USA) at 37 °C according to the manufacturer's instructions. Aliquots were removed at times indicated in figure legends. When dephosphorylated VT was used for proteolysis assays, lambda phosphatase was removed after the dephosphorylation reaction by centrifugation in a Centricon-100 device (Millipore, USA). The extent of dephosphorylation was evaluated by SDS-polyacrylamide gel electrophoresis (PAGE) followed by Western blotting. In the experiments where VT proteolysis was evaluated, samples were dephosphorylated for only 1.5 h. Lambda phosphatase was

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