The Study on the Localization of Acid Phosphatase Activity During Spermiogenesis in Chinese Mitten-Handed Crab, *Eriocheir sinensis*

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Abstract: Ultrastructural cytochemical techniques and electron microscopy were used for localization of acid phosphatase activity during spermiogenesis in *Eriocheir sinernsis.* The results showed that: Acid phosphatase was synthesized in the endoplasmic reticulum in the early spermatids. The acid phosphatase was found gradually in nucleus, the membrane of acrosomal vesicle, the cytoplasmic region and the acrosomal tubule. And then the reaction product particles became thicker during the spermiogenesis. In the mature sperm, acid phosphatase was localized in the percutor organ slightly, but it was massive and compact in the acrosomal tubule.

Keywords: Spermiogenesis; Acid phosphatase; *Eriocheir sinensis*

Introduction

Eriocheir sinensis belongs to Subphylum Crustacea, Class Malacostraca, Order Decapoda, Suborder Pleocyemata, Infraorder Brachyura, Superfamily Grapsoidea. The detailed studies on the sperm ultrastructure, acrosomal reaction, and fertilization have been reported [1-3]. But the report on the distribution of acid phosphatase in the reproductive cells during *E. sinensis* spermiogenesis has not been reported. Acid phosphatase (ACPase (EC.3.1.3.2)) is one of the most imporment enzymes which catalyze hydrolyzing of phosphate mono-ester and producing phosphoric acid. Acid phosphatase has a close relation with phosphorus metabolism and substance metabolism. It is also a marker enzyme of lysosome in cell [4]. During spermiogenesis, some important events occur, such as the change of organelles, the modifing and degradation of biomacromolecule. The acid phosphatase plays an important role in these events.

E. sinensis is a transitional form of in vitro fertilization and in vivo fertilization, with eggs adhering sperm in vivo and fertilizing in vitro. Therefore, the research on the localization of acid phosphatase during spermiogenesis of *E. sinensis* is meaningful. A preliminary study on the distribution of acid phosphatase during spermiogenesis of *E. sinensis* was reported in this paper, in order to offer basic data for the function of acid phosphatase duing spermiogenesis, and the appraisal of the sperm quality.

Received on June 5, 2006

1 Material and methord

1.1 Material

The male *E. sinensis* was bought from the Fuhe market in Baoting, held in fresh water for two days. The testes, vas deferens and seminal visicle were dissected out immediately .

1.2 Methord

Gomori reaction [5].

Fix: Small pieces of above three tissues about 0.5 mm^3 in diameter were fixed at 4 ℃ for 1 h in 2 % paraform and 2.5 % glutaraldehyde buffer in 0.1mol·L^{-1} Sodium Cacodylate and 8 % sucrose buffer at pH 7.3, and washed three times in 0.1 mol·L^{-1} Sodium Cacodylate containing 8 % sucrose buffer at pH 7.3.

Incubate: The pieces were incubated at 37 ℃ for about 40 min. In the experimental group, the incubation buffer containing β-Sodium Glycerophosphate served as substrate. On the contrary, the incubation buffer in the control group had no subtrate.

Postfix and embed: After several times of washing in 0.1 mol·L^{-1} Sodium Cacodylate containing 8 % sucrose buffer at pH 7.3, the samples were postfixed in 1 % osmium tetroxide in 0.1 mol·L^{-1} Sodium Cacodylate for 30 min. rinsed in 0.1 mol·L^{-1} Sodium Cacodylate containing 8 % sucrose buffer, dehydrated in a series of increasing concentration of ethyl alcohol, and finally embedded in Epon812. Ultramicrocutted, observed with JEM-100SX, taken pictures.

2 Results

2.1 The distribution change of acid phosphate during spermiogenesis

In the early spermatid: Acid phosphatase distributed on the membrane of endoplasmic reticulum vesicle irregularly. The Gomori reaction was negative in nucleus, indicating there was no acid phosphatase (Plate Ⅰ: 1, 2). The Gomori reaction was negative in the control group.

In the late spermatid: There was acid phosphatase in the nucleus, acrosomal tubules, the membrane of acrosomal vesicle and the cytoplasmic region. There were uniform and compact metal granules distributing in acrosomal tubules, the membrane of acrosomal vesicle and in the cytoplasmic region, but there were less in the percutor organ of the acrosomal tubules, and the granules were most refined and compact. The metal granules located in nucleus were uniform and sparse, and their diameter was large, There were no acid phosphase in the fibrous layer, middle layer and lamellar structure of the acrosomal vesicles (Plate Ⅰ: 3, 4). Acid phosphase did not appear in the control group.

Along with the development of sperm, the metal granules congregated to uniform and large granules located in nucleus and acrosomal tubules. There was little granule in the percutor organ all the time. The

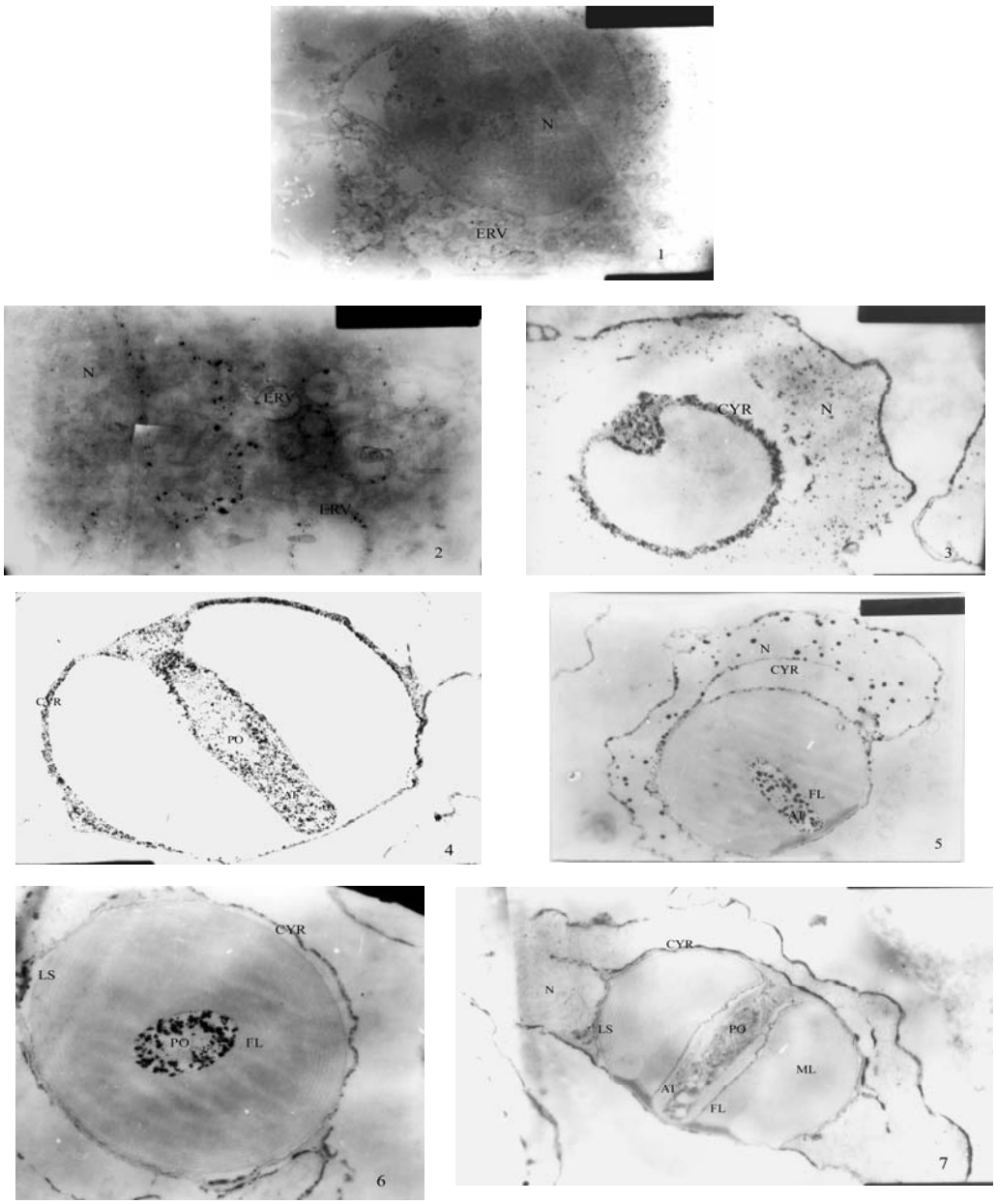

Plate Ⅰ Location of acid phophatase during spermiogenesis of *Eriocheir sinensis*

1, Acid phophatase located in the endoplastic reticulum vesicles of early spermatid×15 000;

2, A magnified part of early spermatid, showing endoplastic reticulum vesicles×25 000;

3, 4, 5, The location of acid phophatase of late spermatid×20 000 ×20 000 ×15 000;

6, The location of acid phophatase of mature sperm×15 000; 7, Mature sperm in the control group×15 000;

AT - acrosomal tubule; CYR - cytoplasmic region; ERV - endoplasmic reticulum vesic;

FL - fibrous layer; LS - lamellar structure; N - nucleus; PO - percutor organ

Gomori reaction was still negative in the fibrous layer, middle layer and lamellar structure of the acrosomal vesicle (Plate Ⅰ: 5). There was no metal granule in the control group either.

2.2 Distribution of acid phosphatase in mature sperm

Refer to Fig. 1 [6] which displays the mature sperm. There was little cytoplasm in the sperm. Organelles have also degenerated, and some even disappeared. Only a thin cytoplasmic region left between the nucleus and acrosome. There were no acid phosphatases in the cytoplase region.

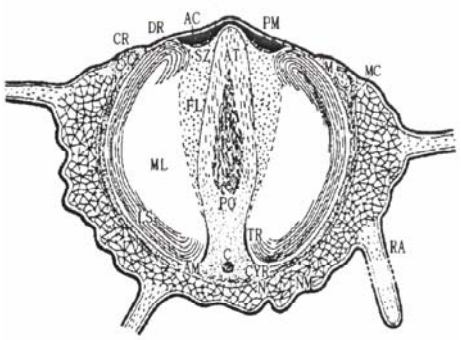

Fig. 1 Diagram of longitudinal section of the *E. sinensis* spermatozoa

AC. apical cap, AM. acrosomal membrane, AT. acrosomal tubules, C. centriole, CR. Convex ring, CYR. cytoplasmic region, DR. ditch ring, FL. Fibrous layer, LS. lamellar structure, M. mitochondria, MC. membrane complex, ML. middle layer, N. nucleus, NM. nuclear membrane, PM. plasma membrane, PO. percutor organ, RA. radial arm, SZ. subcap zone, TR. thickened ring.（Du.1999）

The main part of the sperm was occupied by the nucleus and acrosome. The shape of nucleus was unique and named nuclear cup, located in the behind part of sperm. Acid phosphatase was not observed in the nucleus.

Acrosomal vesicle was situated in the nuclear cup. It consisted of apical cap, acrosomal tubules and acrosomal vesicle.

a) Apical cap Apical cap is the head part with high election density. And there was no distribution of acid phosphatase.

b) Acrosomal tubule Acrosomal tubule was formed by the prolongation of the invaginated retral membrane of the acrosomal vesicle. It was filled with thread-like material. The percutor organ is in the middle part of the acrosomal tubule, formed by high election density canaliculus. The experiment result indicated that there were sporadic and thin metal granules in the percutor organ. All these showed that there was little acid phosphatase in the acrosomal tubule. There were many large and dense metal granules. That is to say, there was acid phosphatase in the other part of acrosomal tubule.

c) Acrosomal vesicle From inside to outside, the matter circling acrosomal tubules in the

acrosomal vesicle was divided into three parts: fibrous layer, middle layer and lamellar structure. Our experiment showed that there was no acid phosphatase in the three parts of acrosomal vesicle in mature sperm. (Plate Ⅰ: 6). There was not acid phosphatase in the control group (Plate Ⅰ: 7).

3 Discussion

3.1 Productive and operational mechanism of acid phosphatase

Pochon-Masson in 1983 pointed out: It is the main character of Decapoda Crustacea sperm that the rough endoplasmic retidulum replaces the Golgi apparatus to form the acrosome [7]. In the early period of *E. sinensis* spermiogenesis, Gomori reaction was positive in endoplasmic reticulum vesicles, which indicated acid phosphatase might be synthesized in the endoplasmic reticulum vesicle. Fernandes and Báo studied in 1999 the distribution of acid phosphatase in phytophagous bug sperm. They pointed out that acid phosphatase wasn't observed in the proacrosomal vesicle, they were absorbed in nucleus by the shrink of some vesicles and increased gradually. In the mature sperm, Gomori reaction products redistributed, and finally located in the acrosomal vesicle in a concentrated way. But the mechanism of acid phosphatase synthesis was different. It was synthesized in the Golgi apparatus [8]. The result revealed that acid phosphatase was synthesized in the endoplasmic reticulum. The endoplasmic reticulum was vesicle-shaped throughout the spermiogenesis of *E. sinensis.* And the pyknotic round granules, which were produced by rough endoplasmic reticulum vesicles in early spermatid, participated in forming the acrosome [9].

3.2 Acid phosphatase and nucleus

In the nucleus of middle and late spermatid during spermiogenesis of *E. sinensis*, Gomori reaction was positive. We conjectured that the acid phosphatase proenzyme was synthesized in the endoplasmic reticulum in the early spermatid stage, and transported from the cytoplasm to the nucleus, and then acid phosphatase zymogens were activated by a specific signal. The regulating action of enzyme to gene was usually fulfilled by phosphorylation and dephosphorylation. Acid phosphatase in the nucleus could regulate gene expression through activating or inhibiting some key enzymes related with the gene expression.

Furthermore, the mature sperm nucleus was nonconcentrated except *Nephrops* and *Homarus* in Decapoda. Researches proved that the genetic material in the nucleus was not integrated with basic proteins (histone or protamine) [10]. Our study discovered that there were basic proteins in prophase and metaphase, but not in the mature sperm nucleus during *E. sinensis* spermateteosis. It was reckoned that there was a nucleus-cytoplasm transfer of basic proteins. In 1967, Chevaillier studied the basic proteins during spermiogenesis of *Nephrops norvegicus*, *Eupagurus bernhardus* and *Carcinus maenas*. His results showed that histone, which filled in the nucleus during spermiogenesis, appeared in the mature sperm cytoplasm. These histones were free histones, rich in arginine in some part [11]. The acid phosphatase, appearing in middle and late spermatid nucleus during *E. sinensis* spermiogenesis, was maybe concerned with the transfer of basic proteins in the nucleus during spermiogenesis. This function was fulfilled by the

action of phosphorylation and dephosphorylation on basic proteins.

3.3 Acid phosphatase and acrosmal reaction

When the egg is still in the female *E. sinensis*, the acrosome of sperm clinging on the egg elongates, the apical cap heaves and the front of it dehisces also [2]. The acrosmal reaction of *E. sinensis* is approximately divided into two phases. The first phase, the acrosomal vesicle evaginates, and the inside material is released. After ovulation, the nuclear cup shrinks and oppresses the acrosomal vesicle, the middle part of fibrous layer, middle layer and lamellar structure evaginate in turn, and form the evagination vesicle. In succession, the membrane of acrosome and plasmic membrane disrupt, the inclusion of evagination vesicle, like egg membrane lysin, releases in order to dissolve the egg membrane. The second phase, the acrosmal tubule extends. After the two phases, the shape of *E. sinensis* sperm is the simplest. After the sperm gets into the egg, the acroamal tubule extends sequentially, contacts with the egg membrane, and the sperm and the egg membrane fuse at last. The acrosomal tubule brings the nucleus material into the egg, and the acrosomal reaction before fertilization is finished [3, 12, 13].

In the first phase, the fibrous layer goes through the slit pores of acrosomal membrane and plasmic membrane, forming a pore, and the material in the middle layer is released to lyze the egg membrane. In the second phase, the percutor organ elongates, contacts and fuses with the egg membrane, and spurs the cytoplasm band and nuclear material into the egg. It is reckoned that the acid phosphatase occupying the acrosomal tubule, is related with the lysis of egg membrane, the formation of masnulonucleus, and egg activation.

Acknowledgements

The project was supported by the National Natural Science Fund (30371115) and the Hebei Province Natural Science Fund (303118).

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中华绒螯蟹精子形成过程酸性磷酸酶的分布特征

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摘 要：将金属盐法与光镜、电镜技术相结合，研究中华绒螯蟹精子形成过程中酸性磷酸酶分布的变化。研究表明，在精子形成的早期，酸性磷酸酶由细胞质中的内质网小泡上产生，随后出现在细胞核内、顶体囊膜上及顶体管内，并且反应产物逐渐由分布均匀的细密颗粒聚集成分布均匀的较大的颗粒；当精子成熟后酸性磷酸酶均匀分布在顶体管中，反应产物颗粒比较大，穿孔器部分发现有少量酸性磷酸酶分布。

关键词：生物化学；精子发生；酸性磷酸酶；中华绒螯蟹