クルマエビ, Penaeus japonicus Bateを空気中に露出した場合の鰓弁の微細構造の変化
Ultrastructural Observations on the Gill Filaments of Kuruma Prawn, *Penaeus japonicus* Bate Exposed to Air

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Abstract

The fine structure of the gill filaments of the Kuruma prawn, *Penaeus japonicus*, exposed to air were studied with transmission electron microscopy, and were compared to normal gill filaments. After 12 h of 14 °C air exposure, electron microscopic examinations of the gill filaments did not show any changes except in the configuration of the mitochondria. Contrasting with normal mitochondria, which show well developed cristae and an electron dense matrix, mitochondria in the air exposed prawns had collapsed and rearranged cristae. The normal mitochondria were called energized and the modified, non energized. During aerial exposure, oxygen supply to the metabolically active mitochondria decreased in the collapsed gills, electron transfer and ATP formation could not be harnessed efficiently, thus resulting in the modified mitochondria.

Many studies have dealt with the relationships between mortality and the various environmental conditions under which prawns are transported. Although no observations have been made on the histological changes occurring in prawn organs during live transportation, the gill is assumed to be the main site of respiration even in air. In cold air, prawn withstand long periods without any apparent damage. Despite anatomical works on the general description of white shrimp *P. setiferus*\(^1\), the branchial organ of the Kuruma prawn\(^2\), and histology and fine sturcture of the gills of various penaeid species (Pink shrimp *P. duorarum*\(^3\) and Brown shrimp *P. aztecus*\(^4\,5\)) few studies have been done on the fine structure of the gills of the kuruma prawn *P. japonicus*.

The purpose of this study was examine the ultrastructural changes in the gill filaments of the Kuruma prawn when exposed to air, which is practically an important condition in live transportation. Kuruma prawns are presently transported live in chilled sawdust without water. During this study, electron microscopy observations were made on the branchial filament tissues of normal and air exposed Kuruma prawn.

Materials and Methods

Animals

Kuruma prawns *P. japonicus* were obtained from Aira Prawn Farming of Iwaseki Industry Co., Kagoshima. They had been reared in field ponds at 20–32 °C from the early summer to autumn. Prawns were acclimated in a 200 l indoor tank equipped with a central pipe, sand and gravel filter at the bottom, and fed with prawn pellets (2.5–3 % body weight per day) for one month. The water temperature was controlled at 24 °C.

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with a cooling equipment (Rei-Sea cooler RZ-140) with a precision ± 0.4 °C.

Transmission electron microscopy (TEM)

Five individual prawns (10.95 ± 0.97 g) at the intermolt stage were exposed to 14 °C air (75-85% relative humidity) in an incubator for 12 h. After this, gill samples were excised and fixed in ice-cooled 5% glutaraldehyde buffered with 0.1 M 7-collidine (pH 7.7) for 2 h. Samples were repeatedly rinsed with 0.2 M 7-collidine and left overnight in a refrigerator. The next day, they were post fixed in 1% osmium tetroxide buffered with the same solution for 2 h. Osmolarities of the fixative and buffer were adjusted to approximate that to the prawn gills by the addition of sucrose (6.5%). After dehydration through an ethanol series, the samples were embedded in Epon 812. Thin sections of the tissues, cut by an ultramicrotome (ULTROTOME III 8200) using glass knives, were double stained with uranyl acetate and lead citrate. They were observed under a Hitachi H-700 H electron microscope and compared with normal gills of individuals not exposed to air. Normal tissues were obtained from 5 similarly-sized intermolt individuals (11.08 ± 0.68 g). Study of the ultrastructure of the normal gills was completed prior to the examination of the gills of air-exposed prawns.

Results

Ultrastructural observation of normal gill filaments

The gills of the normal Kuruma prawn were of the dendrobranchiate type, consisting of a central axis that bore primary gill filaments which in turn branch into finer gill filaments. The Kuruma prawn has 18 pairs of gills in the branchial chamber, that is, six wall-gills, five anterior joint-gills, six posterior joint-gills and a foot-gill.

The focus of the present study was the distal filaments of the gills, which are the smallest functional anatomical units of the gill system visible to the unaided eye.

In the histological sections, normal filaments consisted of a cytoplasmic sheet derived from cells that are mainly medial in position. These cells and their extended cytoplasmas made up the gill epithelium and are shown in Figs. 1 and 2. Each cytoplasmic process contains a basal nucleus, rough endoplasmic reticulum (er), golgi apparatus (G) and numerous round to elongate mitochondria (m) (Fig. 1). Ribosomal particles appeared to be grouped freely throughout the cytoplasm of the cell. The nucleus of the epithelial cell (N) lies in the septum. Its cytoplasm ranges from light to dense (Fig. 1), conspicuous, and ranges in shape from round to multi-lobed (Figs. 1 and 2b).

The most abundant organelles found in the cytoplasm were mitochondria and rough endoplasmic reticulum (er) (Fig. 1). Abundant mitochondria in the cytoplasm of epithelial cells in the filament suggest the need to produce large quantities of energy probably related to regulatory functions of the epithelial cells and their extended cytoplasmic sheets. The commonly encountered type were normal in appearance, having well developed cristae and a uniformly electron dense matrix (Fig. 2a).

Ultrastructural observation of gill filaments of prawn exposed to air

After air exposure, all the gill tissue cellular organelles retained their normal ultrastructure with the exception of the mitochondria regardless of their location. Most profiles revealed their size to be some what larger than those of the normal gill tissue. Primary degenerative changes found in mitochondria were indicated by and irregularity in shape. They were packed with tubular cristae more largely spaced than those usually found in normal mitochondria and had a matrix of low electron density (Figs. 2b and 2c). In some mitochondria, the cristae were poorly developed and often found only near the periphery of the matrix (indicated by arrows in Fig. 2b).

Discussion

In air exposed prawns, most of the cells of the gill filament did not loose their structural integrity and organization. Only fine structural changes in the mitochondria with a complete rearrangement of the cristae were observed. These changes were attributed as effects of air exposure. The cristae of the inner membrane of the mitochondria were considered to exist in two configurational states: 1) energized (found in
Fig. 1. Longitudinal section of normal (a) and air exposed (b) gill filament of *Penaeus japonicus*. N, nucleus of epithelial cell in the septum; m, mitochondrion; er, rough endoplasmic reticulum; G, golgi apparatus. ×7,000.

Fig. 2. Epithelial cell and cytoplasmic extensions showing the mitochondria and nucleus. (a) Normal gill mitochondrion (note the well developed cristae and electron dense matrix). ×36,000; (b) Mitochondria of the epithelial cell when exposed to air (asterisk notes the mitochondrion with collapsed and rearranged cristae; also cristae were found near the periphery of the matrix as indicated by the arrows). ×12,000; (c) High magnification of the collapsed mitochondrion indicated by the asterisk in b. ×36,000.
normal gills, Fig. 2a), and 2) non energized (found in air-exposed gills, Fig. 2c).

Mitochondria are known as the site of metabolic activity, constantly providing the framework for electron-transfer processes that produce ATP. Electron transfer and hydrolysis of ATP are the two enzymic means of generating the energized state7. On the non-energized state, the membrane of the cristae were observed to be collapsed probably due to a lack of oxygen and lower respiration rates. Larger sized prawns show a significant fall in ATP concentrations and a rise in AMP concentrations in abdominal muscle when stored in air at 17°C for 24 hours89. On the energized state (as in normal under water respiration) cristae retain their tubular characteristic. Oxygen uptake measurements from prawns in air showed that prawns are initially able to consume adequate amounts of oxygen to maintain energy metabolism (i.e. ATP turnover) and this ability decreases markedly with lapse of time99. Taylor and Weateley91 say that hypoventilation occurs when crustaceans leave the water (their gill baiers more rapidly) and despite this the oxygen level in their blood decreases. After long term air exposure, oxygen supply to the metabolically active mitochondria decrease and the oxidation reactions in the cristae could not be completed. Unsufficient molecular O2 in the hemolymph due to air exposure decreases the rate of oxidation reactions and phosphorylation. The resulting lack of ATP could not maintain the mitochondria in the energized state and the cristae collapse. Hence, the degree of gill collapse would depend upon the extent of gill filament dysfunction which in turn depends upon the extent of changes in the mitochondria of the cells. It is suggested that configurational changes are relevant to mitochondrial work performances coupled with ATP synthesis.

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References

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空気中に露出したクルマエビおよび海水中に静置したクルマエビの鰓弁の微細構造を透過型電子顕微鏡で比較観察した。海水中に静置したクルマエビの鰓弁のクリスチでは、内部のクリスチが互いに接触するくらい微密に配列していたが、24℃の飼育から14℃の空気に露出後12時間目のクルマエビ鰓弁のミトコンドリアでは、クリスチが崩壊し配列もまばらであった。これより、空気への露出による鰓の変形で酸素が十分供給されず、ミトコンドリア内の電子伝達およびそれに伴う ATP の生産が減退することが推察された。