Larval Salt Gland of *Artemia salina* Nauplii

Regulation of Protein Synthesis by Environmental Salinity

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Summary. Naupliar brine shrimp (*Artemia salina*) have been used to study the salt-dependent regulation of protein synthesis. Measurement of the *in vivo* rates of protein synthesis was found to be very complex and dependent upon the leucine concentration of the external medium, rate of leucine entry and time of equilibration between various internal pools of leucine. Techniques were developed which permitted the measurement of rates of incorporation of \(^{14}\)C-L-leucine into naupliar protein at various salinities under conditions that provided the organisms with a constant internal specific activity. It was found that salinities over 0.25 M NaCl caused decreased rates of protein biosynthesis. A comparison of the rate of protein synthesis in the presence of chloramphenicol, cycloheximide and puromycin indicated that qualitative as well as quantitative changes in synthesis of proteins was directed by the external salinity. A feed-back mechanism based on the partitioning of available energy (ATP) between ion transport and protein synthesis is hypothesized.

Introduction

Young nauplii of the brine shrimp, *Artemia salina*, possess a salt gland which has been shown to regulate the sodium and potassium levels of the internal body fluids (Conte et al., 1972). The larval salt gland is morphologically distinct (Hootman et al., 1972) and consists primarily of two types of cells. One cell type ("dark" cells) is characterized by high electron densities and is relatively non-differentiated as regards its fine-structure. The less-electron dense cell, which has been termed a "light" cell, is ultrastructurally characterized by an extensive membranous complex intimately associated with mitochondria and gives the appearance of being functionally adapted for unidirectional secretion of electrolytes.

Differentiation of salt secretory cells (halocytogenesis) within the larval salt gland constitutes a major developmental requirement during these early stages of embryogenesis. Little is known of the biochemical events underlying halocytogenesis but through the use of cellular inhibitors a little insight has been gained. Ewing et al. (1972) studying the effects of several kinds of cellular inhibitors on survival of nauplii observed that inhibitors of protein synthesis and cytoplasmic ribonucleic acid (RNA)
synthesis had a marked dependency upon the external sodium chloride concentrations (NaCl). In contrast, inhibition of nuclear RNA synthesis which drastically alters naupliar development did not show a differential salt sensitivity. These data imply that halocytophogenesis in Artemia must be dependent upon protein and nucleic acid synthesis. The present study is an attempt to further elucidate this phenomenon through a quantitative evaluation of protein synthesis occurring in nauplii reared under various salinities as measured by the in vivo incorporation of $^{14}$C-leucine into naupliar protein.

**Materials and Methods**

1. **Source and Treatment of Nauplii**
   
a) **Incubation of Embryos.** Dried brine shrimp “eggs” (gastrulae) from Great Salt Lake, Utah, were purchased from Long Life Products, Harrison, New Jersey, and stored at $-20^\circ$C. Preparations of sterile cysts were obtained by treatment of cysts with antiformin as described by Finamore and Clegg (1968). Incubation of 10 grams of sterilized cysts with 500 ml of autoclaved artificial sea water (Instant Ocean Sea Salts containing 100 units of penicillin per ml) produced cultures of free-swimming nauplii free from bacterial and fungal contamination. The incubation of cysts was accomplished by placing the suspensions of cysts in Fernbach culture flasks and shaking at a constant temperature of $26^\circ$C for 33 hours. Newly hatched nauplii were harvested from empty shells by the method of Finamore and Clegg (1968).

   b) **Salt Acclimation of Nauplii.** Newly hatched nauplii (33–35 hr) were harvested from rearing flasks and filtered on Miracloth filters to remove excess incubation media. Nauplii were briefly washed with distilled water, weighed and added to flasks containing acclimation media of varying salinities in the ratio of 1 g wet weight of nauplii to 30 ml of acclimation medium. The acclimation media were prepared from artificial seawater that already contained 0.5 M NaCl and it was either diluted with distilled water or fortified with additional NaCl until the desired concentration of NaCl was obtained. Nauplii were then reincubated for an additional four hours in the acclimation medium at $26^\circ$C with shaking.

2. **In vivo Uptake of $^{14}$C-L-Leucine by Nauplii**
   
a) **Incubation and Assaying Method for Specific Radioactivity of Free Internal Leucine.** Acclimated nauplii (37–39 hrs) were again filtered as described earlier to remove excess acclimation media. Approximately 0.2 g of acclimated nauplii were added to a series of small disposable petri dishes, each containing 5.0 ml of acclimation medium and a pre-determined amount of $^{14}$C-L-leucine. Following a 10 minute period of adjustment, 0.5 $\mu$C of $^{14}$C-L-leucine (Sp.Act. 2 C/mg) was added to each dish. At various times, nauplii from individual petri dishes were transferred to a filtering apparatus to remove the $^{14}$C-labeling media and were washed quickly with 10.0 ml of 0.5 mM $^{14}$C-leucine solution, followed by 10.0 ml of distilled water. Nauplii were eluted from the Miracloth filter into a Potter-Elvehjem homogenizer with 5.0 ml of 0.5 M perchloric acid solution (HClO$_4$) and homogenized. The suspension was transferred to 15 ml Correx centrifuge tubes and centrifuged at 10000 $\times$ g for 10 minutes. The pellet was saved for $^{14}$C-radioactivity and protein