The intracellular fluid isosmotic regulation in yellow clam *Sunetta scripta* L. (Mollusca: Bivalvia) acclimated to different salinities

P. J. George & R. Damodaran*

Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science & Technology, Cochin 682 016, Kerala, India

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*Sunetta scripta* is an osmoconformer and relies heavily on intracellular fluid isosmotic regulation. A conspicuous reduction in the intracellular ninhydrin positive substances (NPS) is noticed in the bivalves exposed to hypoosmotic stress. The role of NPS in the hemolymph osmolarity is negligible whereas it contributes substantially to the intracellular medium. Taurine, glycine, proline, alanine, glutamate and aspartate are not only the dominant solutes in the cytoplasm but also the most varied ones in the isosmotic regulation. The nitrogenous osmolytes acts as compatible over wide variations in their concentration and hence the euryhalinity of the bivalve depends upon these osmoprotectants. The presence of arginine, lysine, serine and threonine is observed but they seem to have comparatively limited role in the process. Significant qualitative difference is noticed between tissues but the variation is quantitative among size groups. The principal osmolyte is glycine in adductor muscle whereas it is taurine in mantle tissue and foot muscle. Overall taurine is found to be the most abundant and varied osmolyte. Consequently, *S. scripta* subjected to hyperosmotic stress is a potential source of taurine.

Marine intertidal bivalves are well known osmoconformers. Since the hemolymph osmolarity is not subjected to considerable regulation, the cells of the bivalves have to cope with the variation in the external milieu. This necessitates regulation of osmotic effectors in the cells concomitant with osmotic variation in the hemolymph to maintain a more or less constant cell volume. Thus regulation of intracellular osmolytes is the mechanism that underlies the salinity tolerance of euryhaline bivalves.

In marine bivalves, the osmotic constituents in the hemolymph are mainly inorganic ions whereas the free aminoacids and their derivates form the major osmotic effectors in the cells. These metabolically expensive nitrogenous osmolytes are the most varied ones during cellular adaptation to osmotic stress. In fact, the ability of the cell to function over wide variations in the concentration of these aminoacids or their derivates suggests that the intracellular microenvironment is not substantially altered by the gradation. Hence the ability of the euryhaline bivalves to conform osmotically depends upon the limit of tolerance and mobilization of compatible solutes by the intracellular system.

Even though a number of works have been conducted on euryhaline bivalves in India so far no attempt has been made to study the role of intracellular organic osmolytes in their salinity tolerance. Previous studies on the pattern of osmolytes accumulation have shown considerable variation between age groups and tissues of a species. Therefore, the role of NPS in the intracellular fluid isosmotic regulation and the qualitative and quantitative nature of the components are investigated in different tissues of various size groups of *S. scripta* acclimated to 15 and 35×10⁶ salinity.

Yellow clam, *Sunetta scripta* L. (Bivalvia: Veneridae) specimens were collected from the natural population near Cochin coastline. The samples were sorted out into small (20±2 mm), medium (30±2 mm) and large (40±2 mm) size groups based on their shell length. The groups were acclimated for four weeks to defined environmental and nutritive conditions (control). (sal=35×10⁶, temp=28±0.5°C, pH=7.85±0.05, dissolved oxygen=4.5 ml l⁻¹, diet= cyanobacterium, *Synechocystis salina*). The bivalves were then gradually exposed to 15×10³ salinity and maintained for a period of four weeks (test). The environmental and nutritive conditions were the same in control and test except salinity. Even though the bivalve is capable of tolerating an ambient salinity range of 5 to 40×10³, the scope for growth is found to be well within a range of 15 to 35×10³ and hence the salinity levels are selected for the study.

* Correspondent author
Samples of adductor muscle, foot muscle, mantle tissue and hemolymph were collected from test and control specimens. The excised tissues were quickly blotted, weighed and transferred to test tubes containing 80% ethanol, heated at 85-90°C for 15 min. and then homogenized. The homogenates were centrifuged at 20,000 g for 30 min. and the supernatants were saved. Hemolymph was also deproteinised (80% ethanol), centrifuged and collected as the supernatant. Methyl ethyl ketone containing 5% 6 N HCl was added to the extracts to precipitate inorganic ions. The supernatants collected after centrifugation were lyophilized and the resultant residues were dissolved in appropriate volumes of distilled water. Aliquots of the samples were estimated for NPS.

Preliminary separation of amino acids from the samples was carried out by ion-exchange chromatography using strong cation exchange resin (Dowex 50x8% DVB 200-400 mesh H+ form, SIGMA) equilibrated with 0.2 M citrate buffer pH 2.2. The ion exchange column (60x0.9 cm) was connected to a gradient generating device and eluant fractions were collected in 1 ml aliquots using an automatic fraction collector. The fractions were analyzed, quantitatively using ninhydrin and an elution graph was prepared. The quantification was done using internal standard technique. The purity of the peaks were tested using two dimensional thin layer chromatography employing silica gel coated plates.

NPS content is very high in the adductor, foot and mantle whereas, it is negligible in the hemolymph. A conspicuous reduction in the intracellular NPS is noticed in various size groups of Sunetta scripta subjected to hypoosmotic stress. Comparatively, NPS is not only high in small size group but also has a greater variation during the cellular adaptation (Table 1).

In different tissues of small, medium and large size groups of S. Scripta, (Table 2) the major osmolytes which constitute NPS pool are taurine, glycine, alanine, proline, asparatate and glutamate. These free amino acids are not only the abundant osmoprotectants in the cell but also the most varied ones in intracellular fluid osmotic regulation. The presence of other amino acids like arginine, lysine, serine and threonine is also noticed but they seem to have comparatively limited role in the process.

The similarities among the osmotic system outweigh the variation noticed between size groups and tissues. Overall, high concentration of glycine is noticed in the adductor whereas, taurine is the most abundant one in the foot and mantle tissues.

The intertidal bivalve S. scripta is usually exposed to wide variations in ambient salinity for prolonged periods and must have adaptations to cope with it. The species is an osmoconformer and hence the long term survival in any salinity must depend on intracellular fluid osmotic regulation. The high NPS content in the cell and its conspicuous reduction in the bivalves subjected to hypoosmotic stress indicates its significant role in the intracellular fluid osmotic regulation. However, the role of NPS in maintaining hemolymph osmolarity is very negligible (Table 1). These observations indicate that the osmotic constituens of the two systems are different and the nitrogenous solutes are preferentially selected for maintaining a constant cell volume. Previous studies on euryhaline bivalves support the conclusion.

In S. scripta, higher salinity tolerance has been noticed in small size groups. The higher NPS content of the small size group may facilitate more expendable intracellular solutes as salinity drops from 35 to 15×10^-3 (Table 1). This may be helping the smaller size groups to maintain their cellular volume more effectively using compatible nitrogenous osmolytes. It suggests that for a species living in defined environmental, nutritive and physiological conditions, the size of the NPS pool is also significant in its salinity tolerance.

The NPS content recorded a reduction of 46-56% in S. scripta acclimated to 15×10^-3 salinity (Table 1). This point to the fact that the intracellular milieu is maintained without serious impediment for various life supporting molecular interactions over the change in concentration. Hence it is likely that every component in the NPS pool is subjected to scrupulous selection in order to make a compatible solute microenvironment in the cytoplasm. Study on qualitative nature of the nitrogenous solute system revealed that free amino acids including taurine as major intracellular osmolytes (Table 2). Quantitatively taurine, glycine, alanine, proline, glutamate and aspartate are the dominant solutes. As far as S. scripta is concerned, the

| Table 1—NPS content of Sunetta scripta acclimated to 15 and 35×10^-3 salinity (μ moles/g wet weight or ml) |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Salinity Tissues | Small 15 | Medium 15 | Large 15 | Small 15 | Medium 15 | Large 15 |
| Adductor | 201.5±24.6 | 104.1±27.2 | 186.5±31.7 | 96.1±28.3 | 174.3±25.6 | 94.3±18.6 |
| Foot | 164.1±26.2 | 84.8±24.3 | 156.3±27.2 | 79.4±16.4 | 156.2±19.7 | 74.5±15.6 |
| Mantle | 159.1±31.5 | 69.4±13.4 | 162.7±23.2 | 74.5±15.4 | 149.5±13.2 | 68.2±16.4 |
| Hemolymph | 0.17±0.06 | 0.19±0.04 | 0.18±0.02 | 0.18±0.04 | 0.09±0.02 | 0.12±0.03 |
above solutes are also the most varied ones during the acclimation to osmotic stress. Hence these osmoprotectants are offering a compatible solute microenvironment to the cellular biochemical processes even over a wide range in concentration. Therefore, these osmoprotectants are serving a significant role in the euryhalinity of S. scripta. Compatibility of such solute system in marine molluscs and the nonperturbable environment they offer over wide variations in concentration had been already discussed. The presence of arginine, lysine, serine and threonine is observed but they seem to have comparatively limited role as intracellular osmotic pressure effectors.

The similarities among the osmotic system outweigh the variations noticed between size groups and tissues. Glycine is an important osmoprotectant in the intertidal bivalve studied. It was already pointed out that the relatively high content of glycine may be characteristic of bivalves of littoral area. Though the abundance of the simplest amino acid is noticed, its contribution to different tissues is not uniform. The importance of glycine as a principal osmolyte is noticed in adductor muscle, whereas, taurine claims the status in mantle and foot. Similar observation in marine bivalves had been reported.

Overall, taurine is found to be the most abundant and varied osmolyte in intracellular fluid isosmotic regulation of S. scripta. It seems that taurine exerts a sparing effect on the use of essential amino acids as intracellular osmolytes. High content of taurine points to the fact that high salinity acclimated ones can be used as a potential source of this bioactive substance. A substance present in such abundance inherently suggests an integral role in the physiological process. Taurine reported to be implicated in diverse functions in many vertebrates and invertebrates, they include osmoregulation, neuroinhibition, cardiac rhythm control, membrane stabilization, nutrition, thermoregulation, detoxification, antioxidation, abolition of cell swelling and increased cell viability. This suggests a potential field for studies on taurine in marine molluscs.

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**References**


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**Table 2** - Amino acid content of *S. scripta* (small, medium and large size) acclimated to 35 and 15 × 10^{-3} salinity (μ moles/g wet weight)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Adductor</th>
<th>Foot</th>
<th>Mantle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salinity</strong></td>
<td><strong>35</strong></td>
<td><strong>15</strong></td>
<td><strong>35</strong></td>
</tr>
<tr>
<td><strong>S. scripta (small size)</strong></td>
<td><strong>S. scripta (medium size)</strong></td>
<td><strong>S. scripta (large size)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Alanine</strong></td>
<td>21.2</td>
<td>9.7</td>
<td>18.1</td>
</tr>
<tr>
<td><strong>Arginine</strong></td>
<td>2.1</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td><strong>Aspartate</strong></td>
<td>9.4</td>
<td>1.6</td>
<td>7.5</td>
</tr>
<tr>
<td><strong>Glutamate</strong></td>
<td>12.5</td>
<td>6.8</td>
<td>15.6</td>
</tr>
<tr>
<td><strong>Glycine</strong></td>
<td>36.6</td>
<td>29.1</td>
<td>29.3</td>
</tr>
<tr>
<td><strong>Lysine</strong></td>
<td>3.1</td>
<td>2.7</td>
<td>nil</td>
</tr>
<tr>
<td><strong>Proline</strong></td>
<td>16.2</td>
<td>7.5</td>
<td>12.9</td>
</tr>
<tr>
<td><strong>Serine</strong></td>
<td>3.2</td>
<td>2.3</td>
<td>3.1</td>
</tr>
<tr>
<td><strong>Threonine</strong></td>
<td>5.1</td>
<td>3.2</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>Taurine</strong></td>
<td>34.2</td>
<td>11.4</td>
<td>51.9</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td>30.5</td>
<td>18.1</td>
<td>27.2</td>
</tr>
</tbody>
</table>

**Footnotes**

- 18. Though the abundance of the simplest amino acid is noticed, its contribution to different tissues is not uniform. The importance of glycine as a principal osmolyte is noticed in adductor muscle, whereas, taurine claims the status in mantle and foot. Similar observation in marine bivalves had been reported.
- Overall, taurine is found to be the most abundant and varied osmolyte in intracellular fluid isosmotic regulation of *S. scripta*. It seems that taurine exerts a sparing effect on the use of essential amino acids as intracellular osmolytes. High content of taurine points to the fact that high salinity acclimated ones can be used as a potential source of this bioactive substance. A substance present in such abundance inherently suggests an integral role in the physiological process. Taurine reported to be implicated in diverse functions in many vertebrates and invertebrates, they include osmoregulation, neuroinhibition, cardiac rhythm control, membrane stabilization, nutrition, thermoregulation, detoxification, antioxidation, abolition of cell swelling and increased cell viability. This suggests a potential field for studies on taurine in marine molluscs.

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