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Osmoregulation and the Gill $\text{Na}^+\text{-K}^+\text{-ATPase}$ Activity in *Eriocheir japonicus*

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Abstract

Osmoregulation of *Eriocheir japonicus* in media of varying salinities was investigated. The crabs exhibited a hyper- and hyposmoregulatory ability. The osmotic concentration of the hemolymph was isosmotic when they were acclimated to 80% seawater. The hemolymph sodium concentration showed the same change as did the hemolymph osmotic concentration. The specific activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ in the anterior gills was higher in crabs acclimated in 120% seawater than those maintained in freshwater, while the activity in the posterior gills was higher in freshwater crabs than in 120% seawater crabs. It was suggested that salt extrusive mechanism may be localized in the anterior gills and a salt absorptive mechanism in the posterior gills.

Many crab species that inhabit waters of changeable salinity have the ability more or less to regulate their hemolymph osmotic concentration. Sodium is one of the major osmotically active components in the hemolymph and its regulation is important for the osmoregulation. It has been known that crab gills are capable of active absorption¹⁾²⁾³⁾⁴⁾ or extrusion⁵⁾ of sodium ions, and that $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the gills plays an important role in the process of osmoregulation in crabs⁶⁾⁷⁾⁸⁾⁹⁾.

Since *Eriocheir japonicus* lives in freshwater and undergoes a dramatic osmotic change during the spawning migration from freshwater to seawater, this crab may be a strong osmoregulator. This study was designed to investigate the ability of osmoregulation in *E. japonicus*. The osmotic and sodium concentrations of hemolymph were analysed by acclimating the crabs to salt depleted or loaded conditions. Changes of the gill $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity were examined under the extreme osmotic conditions.

Materials and Methods

Intermolt crabs of *Eriocheir japonicus*, 20-40 mm in carapace width, were collected from rivers near Hakodate, Hokkaido, and maintained in freshwater at 15°C. Animals were exposed to a variety of concentrations of artificial seawater (35, 70, 80, 90, 100 and 120% SW) for 1 to 25 days. The experimental media were prepared by appropriate dilution or concentration of the van't Hoff's formula.

After exposure, hemolymph was collected from 3 to 9 animals at each time by cutting a walking leg and dripping hemolymph into a test tube. After crushing clotted hemolymph with a micro-spatula, it was centrifuged at $3000 \times g$ for 10 min.

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The resultant serum was used for the determinations of osmotic and sodium concentrations. The serum osmotic concentration was determined by the freezing-point method using an osmometer (Precision System, Model 2007). Sodium was measured by flame photometry using an atomic absorption spectrophotometer (Hitachi, Model 518).

The gill $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity was assayed with animals maintained in freshwater and those acclimated in 120% seawater for 20 days. The six pairs of gills, except the vestigial gills, were excised separately in three anterior and three posterior pairs. The gills were homogenized in 20 vol. (V/W) of 0.3M sucrose containing 5 mM EDTA·2Na and 100 mM imidazole (pH 7.2). The homogenate was centrifuged at $500\times g$ for 10 min and the supernatant was used for total and ouabain insensitive ATPase ($\text{Mg}^{2+}\text{-ATPase}$) assays. A 0.1 ml sample was added to 0.4 ml of the reaction medium which contained 100 mM NaCl, 20 mM KCl, 6 mM MgCl_2 , 6 mM ATP·2Na and 100 mM imidazole (pH 7.2), either with or without 1 mM ouabain, and preincubated at 37°C for 5 to 10 min before the sample was added. The reaction mixture was incubated at 37°C for 20 min. Enzymatic reaction was terminated by adding a 10 ml ice-cold iron-TCA reagent¹⁰. Inorganic phosphate (Pi) liberated from the substrate was measured by the method of Goldenberg and Fernandez¹⁰. $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity was calculated from the difference between total and $\text{Mg}^{2+}\text{-ATPase}$ activities. Protein of the sample was measured by the method of Lowry et al.¹¹ using bovine serum albumin as a standard.

Results

Time course changes of hemolymph osmotic concentrations after transfer to media of various salinities are illustrated in Fig. 1. The hemolymph osmotic concentration of freshwater crabs was 658 mOsm/kg. When the crabs were transferred to 35% seawater, it immediately reached a stable level of 695–732 mOsm/kg that was considerably hyperosmotic to the medium. In 70% seawater, the hemolymph value appeared to become stable on and after day 10, measuring 733–800 mOsm/kg, somewhat hyperosmotic to the medium. The hemolymph value was isosmotic to the medium in 80% seawater after day 20 and measured 813–816 mOsm/kg.

In 90% seawater, the hemolymph osmotic concentration was stabilized after day 10 and measured 855–913 mOsm/kg which was isosmotic or slightly hyposmotic to the medium. In 100% seawater, it measured 914–959 mOsm/kg after day 15, an apparently lower level than that of the medium. The hemolymph concentration hyposmotic to the medium was also observed in 120% seawater. The final level was between 1003 and 1059 mOsm/kg after 20 to 25 days.

It was shown that hemolymph of *E. japonicus* was isosmotic to the medium in 80% seawater, hyperosmotic in more diluted seawaters and hyposmotic in those more concentrated, after acclimation. The period required for acclimation to media took at most 20 days.

Changes of the sodium concentration of hemolymph in relation to that of the media are shown in Fig. 2. Generally, the level of hemolymph sodium paralleled the changes of the osmotic concentration. In 35% seawater, the hemolymph

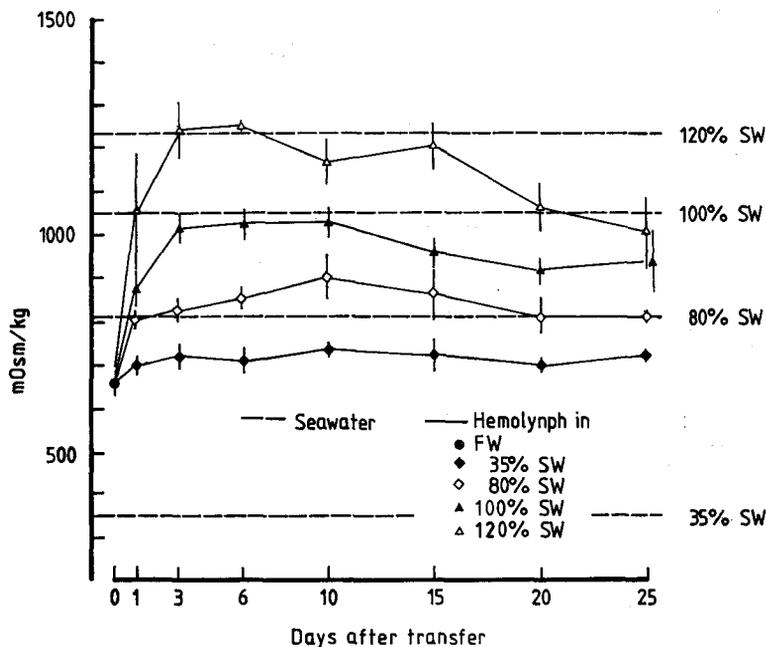


Fig. 1. Changes of the hemolymph osmotic concentration of freshwater crabs after transfer to various salinities. Bars represent standard deviations for 3–9 samples. Dashed lines represent the level of each experimental medium.

consistently showed a constant level of about 350 mEq/l that was considerably hyperionic to the medium. In 70% and 80% seawater, hemolymph sodium finally reached nearly isoionic levels after 15 or 20 days, measuring 349–359 mEq/l and 392–432 mEq/l, respectively. The hemolymph was hypoionic to the media in 90%, 100% and 120% seawater, measuring 396–452 mEq/l after day 10, 432–472 mEq/l after day 15 and 490–536 mEq/l after day 20, respectively. Similar to the case of osmotic concentration, it also took about 20 days at most for the hemolymph sodium concentration to adjust to a stable level.

The correlation between osmotic concentrations of the medium and of the hemolymph 20 days after transfer is shown in Fig. 3. The curve crosses with the isosmotic line at point 816 mOsm/kg, that is equivalent to 80% seawater. Below or above this point, the hemolymph turns hyper- or hyposmotic to the medium. The same correlation is also found for sodium concentrations (Fig. 4). This indicates that *E. japonicus* is a hyper- and hyposmoregulator and that osmoregulation largely depends on the sodium regulatory mechanism.

The specific activity of gill $\text{Na}^+\text{-K}^+\text{-ATPase}$ was determined with animals maintained in freshwater and those acclimated in 120% seawater for 20 days (Fig. 5). In freshwater crabs, the enzyme activity of the posterior gills ($7.27 \mu\text{M Pi/mg protein/hr}$) was twice as much as that of the anterior ($3.34 \mu\text{M Pi/mg protein/hr}$). In 120% seawater acclimated crabs, however, no difference was found between

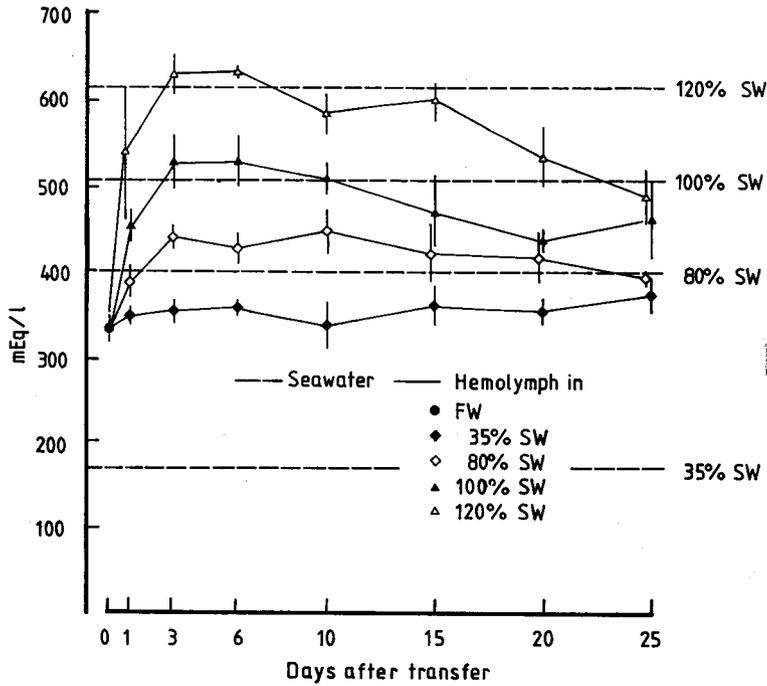


Fig. 2. Changes of the hemolymph sodium concentration of freshwater crabs after transfer to various salinities. Bars represent standard deviations for 3-9 samples. Dashed lines represent the level of each experimental medium.

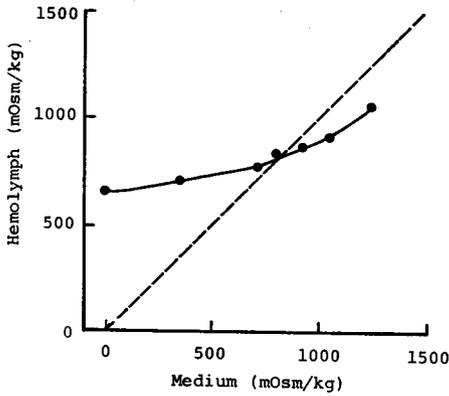


Fig. 3. Correlation between the osmotic concentrations of the hemolymph and the medium 20 days after transfer. Dashed line represents the isosmotic line.

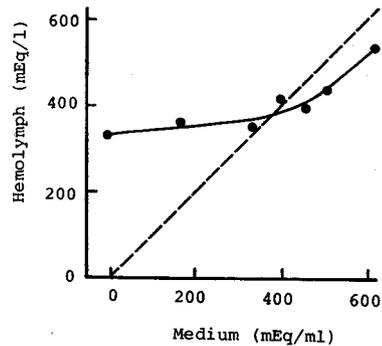


Fig. 4. Correlation between the sodium concentrations of the hemolymph and the medium 20 days after transfer. Dashed line represents the isoionic line.

the anterior and posterior gills. The specific activity of the anterior gills was significantly higher in 120‰ seawater crabs ($4.36 \mu\text{M Pi/mg protein/hr}$) than in freshwater ones. However, the enzyme activity of the posterior gills was significantly higher in freshwater crabs than in 120‰ ones ($5.08 \mu\text{M Pi/mg protein/hr}$).

Discussion

E. japonicus exhibited an ability of hyper- and hyposmotic regulation over a wide range of salinities investigated. This type of osmoregulation has been reported in *Artemia salina*¹²⁾, *Palaemonetes varians*¹³⁾, *Pachygrapsus crassipes*¹⁴⁾, *E. sinensis*¹⁵⁾ and some other crustaceans¹⁶⁾. Most of these animals live in environments of fluctuating salinity such as intertidal zones and inland saline waters. Although *E. japonicus* does not consistently live in such environments, especially in high salinity, they do undergo a fluctuation of salinity during the spawning and larval periods.

The higher $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity of the posterior gills in freshwater crabs compared to that in 120‰ seawater crabs suggests that a sodium absorptive mechanism may be located in these gills. An elevation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the posterior gills at low environmental salinities was reported in *Carcinus maenas*¹⁷⁾, *E. sinensis*⁸⁾ and *Thalamita crenata*⁹⁾. Towle et al.⁷⁾ found an increase in the $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in the whole gills of *Callinectes sapidus* when transferred to diluted seawater. This may be due to an increase of the enzyme activity in the posterior gills. *Metopograpsus thukuhar*, though it is a hyper- and hyposmoregulator, exceptionally showed no change of the gill $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity through a range of 25–100‰ seawater⁹⁾.

Active absorption of sodium was demonstrated in the gills of *C. sapidus*²⁾⁴⁾ and in the 3 pairs of posterior gills of *E. sinensis*¹⁾³⁾. Furthermore, salt absorbing cells were morphologically identified in the posterior gills of *C. sapidus*¹⁸⁾ and *Gecarcinus lateralis*¹⁹⁾.

On the other hand, the higher activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ of the anterior gills in 120‰ seawater crabs may suggest the localization of sodium extrusive ability. Augenfeld²⁰⁾ found that $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity in *A. salina* increased with the elevation of environmental salinity.

Sodium extrusion from crustacean gills was shown in *Uca pugilator*⁵⁾, *U. pugnax*⁵⁾, *A. salina*²¹⁾ and *Parartemia zietziana*²²⁾. Copeland²³⁾ and Flemister²⁴⁾ demonstrated morphologically the presence of salt secreting cells in the gills of *A.*

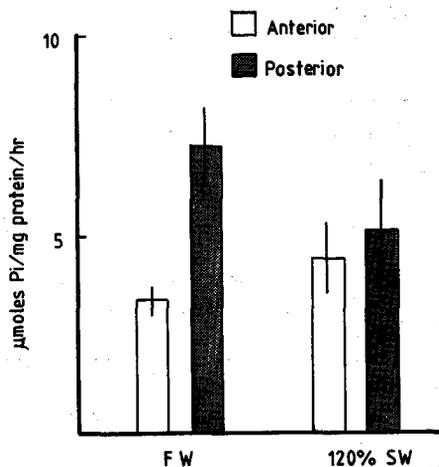


Fig. 5. $\text{Na}^+\text{-K}^+\text{-ATPase}$ activities of the anterior and posterior gills of crabs acclimated to freshwater or 120‰ seawater. Bars represent standard deviations for 5–9 samples.

salina and *Ocypode albicans*. In contrast to these, Dall²⁵) claimed that in some decapods excess sodium is secreted *via* the digestive tract and not the gills. Green et al.⁵⁾ also suggested that the digestive tract may function in sodium secretion in *U. pugilator*.

The results of the present study showed that *E. japonicus* is a hyper- and hyposmoregulator. Change of the gill Na⁺-K⁺-ATPase activity suggested that the posterior gills may play a role in sodium absorption in freshwater and hyposmotic conditions while the anterior gills participate in sodium excretion in hyperosmotic conditions.

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