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# Osmotic and Ionic Regulation and the Gill $Na^+ + K^+$-ATPase Activity in the Japanese Shore Crab *Hemigrapsus sanguineus*

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Osmotic, sodium and chloride regulations of hemolymph in *Hemigrapsus sanguineus* were investigated. The crabs exhibited strong hyperosmoregulation in the range of 50–75% SW. Sodium and chloride ions were also well regulated in the same salinity range. The specific activity of gill $Na^+ + K^+$-ATPase was higher in the posterior gills than in the anterior gills. In the posterior gills, the $Na^+ + K^+$-ATPase activity showed an increase of 1.5 times with acclimation from 100% SW to 50% SW. Absorption of sodium ion by *H. sanguineus* in low salinity may be attributed to the function of the posterior gills which have a high level of $Na^+ + K^+$-ATPase activity.

The Japanese shore crab *Hemigrapsus sanguineus* inhabits the intertidal or littoral zone where the environmental salinity largely fluctuates. It has been reported that crabs inhabiting such an environment generally exhibit hyper-, or hyper- and hypo-osmoregulation[1] and their gills function as an osmoregulatory organ.[1-8] Sodium and potassium activated adenosine triphosphatase ($Na^+ + K^+$-ATPase) has been known to be a mediator of active sodium transport.[4] In the crab gills, also, evidence is available that this enzyme is involved in the osmoregulatory mechanism.[5-8]

This paper describes the pattern of osmotic and ionic regulation of hemolymph and changes of the gill $Na^+ + K^+$-ATPase activity in *H. sanguineus* in response to alternation of the environmental salinity.

## Materials and Methods

Japanese shore crabs *Hemigrapsus sanguineus* were collected from the shore of southern Hokkaido, and maintained in natural seawater (100% SW) at 15°C. Four to eight intermolt crabs of 26-39 mm in carapace width were used for each experiment.

Animals were exposed to various concentrations of artificial seawater (50-175% SW) for 1 to 15 days. The serum was obtained by centrifugation of hemolymph at 3000×g for 10 min and used for determinations of the osmotic and ionic concentrations. The osmotic concentrations of the serum and the experimental media were determined by freezing point osmometry using an osmometer (Precision System, Model 2007). The sodium and chloride concentrations were determined by flame photometry and coulometric titrations respectively, using an atomic absorption spectrophotometer (Hitachi, Model 518) and a chloridometer (Jokoo, Model C-50).

The gill $Na^+ + K^+$-ATPase was assayed with crabs maintained in 100% SW and those acclimated in 50% SW for 10–14 days. The gills were excised separately in each of the three anterior and posterior pairs. The vestigial pairs from the maxillipeds were excluded for the enzyme assay. The gills were homogenized in 20 vol. (V/W) of a solution containing 0.3 M sucrose, 5 mM EDTA (disodium salt), and 100 mM imidazole (pH 7.2). The procedure for the enzyme assay was the same as previously described.[8] The $Na^+ + K^+$-ATPase activity was expressed in μmoles Pi released per mg protein per hour.

## Results

Changes of the osmotic, sodium and chloride concentrations of the hemolymph after transfer of crabs from 100% SW to 50–175% SW are shown in Figs. 1–3. Each of the three factors reached a stable level shortly after transfer. The hemolymph osmotic concentration in 50% SW crabs showed a considerably hyperosmotic level compared to that of the medium (Fig. 1). In 75–175% SW crabs, the hemolymph was isosmotic or hyperosmotic compared to each medium (Fig. 1). The hemolymph sodium and chloride concentrations showed similar changes to the osmotic concentration (Figs. 2 and 3); considerably hyper-

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ionic to the medium in crabs transferred to 50% SW and iso- or hyperionic in those exposed to 75–175% SW.

The correlations of the osmotic, sodium and chloride concentrations between the media and hemolymph 10 days after transfer are illustrated in Figs. 4–6. It is clear that *H. sanguineus* is a hyperosmoregulator. The hemolymph osmotic

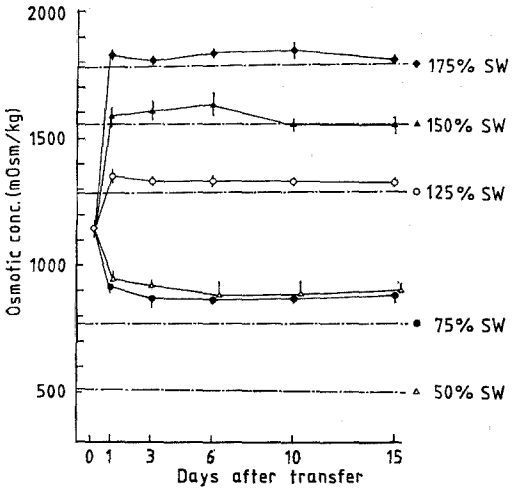


**Fig. 1.** Changes of the hemolymph osmotic concentration of *H. sanguineus* after transfer to various concentrations of artificial seawater. Bars represent standard deviations for 4-8 samples. Solid and dashed lines are the respective levels of hemolymph and experimental medium. ◇, 100% SW; △, 50% SW; ●, 75% SW; ○, 125% SW; ▲, 150% SW; ◆, 175% SW.

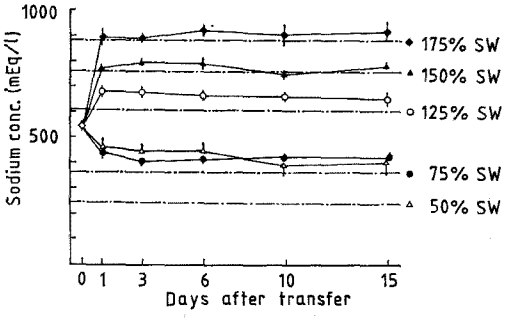


**Fig. 2.** Changes of the hemolymph sodium concentration of *H. sanguineus* after transfer to various concentrations of artificial seawater. Bars represent standard deviations for 4-8 samples. Solid and dashed lines are the respective levels of hemolymph and experimental medium. ◇, 100% SW; △, 50% SW; ●, 75% SW; ○, 125% SW; ▲, 150% SW; ◆, 175% SW.

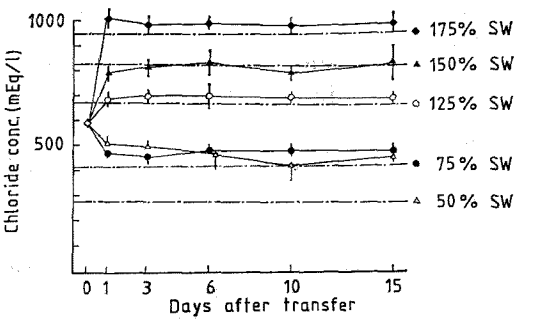


**Fig. 3.** Changes of the hemolymph chloride concentration of *H. sanguineus* after transfer to various concentrations of artificial seawater. Bars represent standard deviations for 3-8 samples. Solid and dashed lines are the respective levels of hemolymph and experimental medium. ◇, 100% SW; △, 50% SW; ●, 75% SW; ○, 125% SW; ▲, 150% SW; ◆, 175% SW.

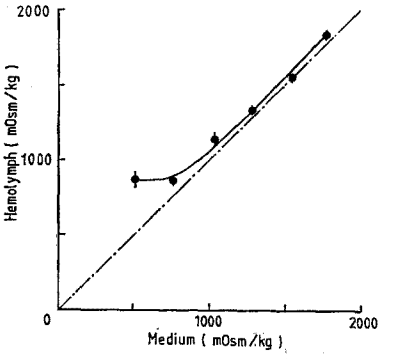


**Fig. 4.** Correlation between the osmotic concentrations of the hemolymph and the medium. Bars represent standard deviations. Dashed line represents the isosmotic line.

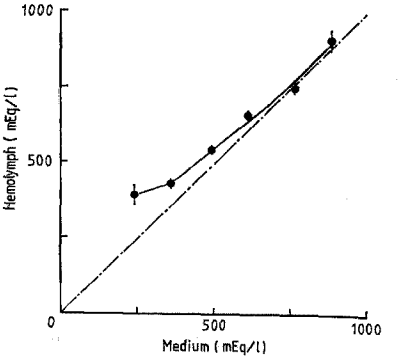


**Fig. 5.** Correlation between the sodium concentrations of the hemolymph and the medium. Bars represent standard deviations. Dashed line represents the isoionic line.

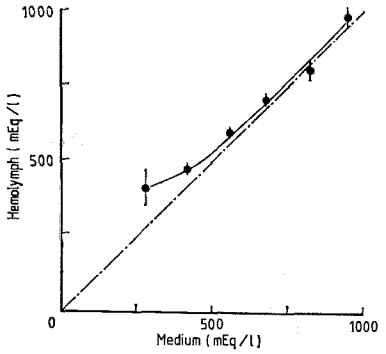


**Fig. 6.** Correlation between the chloride concentrations of the hemolymph and the medium. Bars represent standard deviations. Dashed line represents the isoionic line.

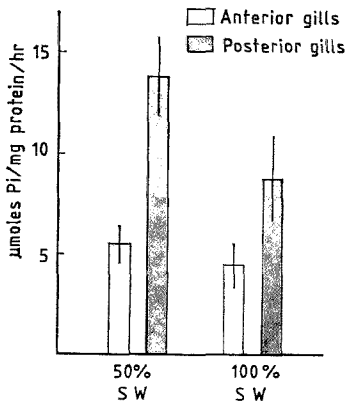


**Fig. 7.** $Na^+ + K^+$-ATPase activities of the anterior and posterior gills of *H. sanguineus* acllimated to 50 and 100% SW. Bars represent standard deviations for 7 or 8 samples.

concentration was well regulated between 50 and 75% SW, but in the range exceeding 75% SW, it conformed to the environmental changes parallel with the isosmotic line (Fig. 4). The correlation curves of sodium and chloride concentrations were similar to that of the osmotic concentration (Figs. 5 and 6). A strong hyperionic regulation of these ions was observed between 50 and 75% SW, but not in the range of 75–175% SW.

The specific activity of gill $Na^+ + K^+$-ATPase in crabs acclimated to 50 and 100% SW is shown in Fig. 7. The enzyme activity was much higher in the posterior gills than in the anterior ones in both 50 and 100% SW. In comparison of the posterior gills, the activity was 1.5 times higher in 50% SW crabs (13.34 μmoles Pi/mg protein/hr) than in 100% SW crabs (8.86 μmoles Pi/mg protein/hr). The activity of the anterior gills appeared to be slightly higher in 50% SW crabs (5.54 μmoles Pi/mg protein/hr) than in 100% SW crabs (4.42 μmoles Pi/mg protein/hr), but this difference was not statistically significant (Student's t test, $P > 0.05$).

## Discussion

The hemolymph osmotic, sodium and chloride concentrations of *H. sanguineus* showed rapid acclimation to media of various salinities between 50 and 175% SW. The period necessary for acclimation in intertidal or coastal crabs is short in general. In *Pachygrapsus crassipes*,[9] *Callinectes sapidus*[10] and *Carcinus maenas*,[11] the acclimation periods are about 1–2 days or less. In contrast to these, a catadromous crab *Eriocheir japonicus* requires 20 days for the acclimation period.[8] The immediate acclimation in *H. sanguineus* and other intertidal or coastal crabs may reflect their necessity to cope with an abrupt change in environmental salinity of their habitats.

*H. sanguineus* showed a strong ability of hyperosmoregulation in the range of 50–75% SW. This is consistent with the fact that hyperosmoregulatory crabs are commonly seen in intertidal or coastal zone.[1]

The hyperosmoregulation of crabs in dilute media is accomplished mainly by the uptake of sodium ion through their gills from the ambient water.[2,8,12] By participating in active transport of sodium, $Na^+ + K^+$-ATPase seems to play the major role in hyperosmoregulatory animals.[18] The higher activity in the posterior gills compared to the anterior gills observed in *H. sanguineus* suggests the localization of a sodium uptake site in the posterior gills. An increase of the enzyme activity in the posterior gills with acclimation to a low salinity supports this interpretation. Similar results have been reported in *E. japonicus*,[5] *C. sapidus*,[5,7] and some other hyperosmoregulatory crabs.[6,14,15] On the other hand, osmoconformers such as *Cancer irroratus*,[16] *Calappa hepatica*[6] do not show changes of the $Na^+ + K^+$-ATPase activity either in the posterior or the anterior gills regardless of a difference in the environmental salinity.

Morphological evidence supports the localization of the salt absorptive sites in the posterior gills of hyperosmoregulatory crabs.[17,18] In *H. sanguineus*, cells exhibiting a special morphology and assumed to be involved in salt absorption

were found in the epithelium of the posterior gills (WATANABE, in preparation). The hyperosmoregulation of *H. sanguineus* in media of low salinities may, therefore, be attributable to the function of $Na^+$-$K^+$-ATPase localized in the posterior gills.

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## References

1) A. P. M. LOCKWOOD: *Biol. Rev.*, **37**, 275–305 (1962).
2) E. SCHOFFENIELS and R. GILLES: in "Chemical Zoology" (ed. by M. FLORKIN and B. SCHEER), Vol. 5, Academic Press, New York and London, 1970, pp. 255–286.
3) R. GILLES and A. PÉQUEX: *J. exp. Zool.*, **215**, 351–362 (1981).
4) J. C. SKOW: *Physiol. Rev.*, **45**, 596–617 (1965).
5) D. W. TOWLE, G. E. PALMER, and J. L. HARRIS: *J. exp. Zool.*, **196**, 315–322 (1976).
6) A. M. SPENCER, A. H. FIELDING, and F. I KAMEMOTO: *Physiol. Zool.*, **52**, 1–10 (1979).
7) G. J. NEUFELD, C. W. HOLLIDAY, and J. B PRITCHARD: *J. exp. Zool.*, **211**, 215–224 (1980).
8) K. WATANABE and J. YAMADA: *Bull. Fac. Fish Hokkaido Univ.*, **31**, 283–289 (1980).
9) W. J. GROSS: *Biol. Bull.*, **112**, 43–62 (1957).
10) B. S. BALLARD and W. ABBOTT: *Comp. Biochem Physiol.*, **29**, 671–687 (1968).
11) J. SHAW: *J. exp. Biol.*, **38**, 135–152 (1961).
12) H. J. KOCH., J. EVANS, and E. SCHICHS: *Meded Vlaamch Acad. Kl. Wet.*, **16**, 3–16 (1954).
13) D. W. TOWLE: *Mar. Biol. Lett.*, **2**, 107–12 (1981).
14) A. PÉQUEX and R. GILLES: *Arch. int. Physiol Biochem.*, **85**, 425–428 (1976).
15) L. H. MANTEL and J. LANDESMAN: *Biol. Bull.* **153**, 437–438 (1977).
16) G. J. NEUFELD and J. B. PRITCHARD: *Comp. Biochem. Physiol.*, **62C**, 165–172 (1979).
17) D. E. COPELAND: *Am. Zool.*, **8**, 417–432 (1968).
18) D. E. COPELAND and A. T. FITZJARRELL: *Z. Zellforsch.*, **92**, 1–22 (1968).