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TISSUE (Na + K)-ACTIVATED ADENOSINETRIPHOSPHATASE ACTIVITIES IN FRESHWATER AND BRACKISH WATER BIVALVE MOLLUSCS[1]

LEWIS E. DEATON[2]

Department of Biological Science, Florida State University, Tallahassee, FL 32306, U.S.A.

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SUMMARY

The (Na + K)-activated ATPase activity was measured in microsomal preparations of gill, mantle, and kidney tissues from four bivalves (*Rangia cuneata, Corbicula manilensis, Polymesoda caroliniana*, and *Lampsilis claibornensis*) acclimated to fresh water (3 mOsM) and to brackish water (200 mOsM).

In freshwater acclimated *P. caroliniana, R. cuneata*, and *C. manilensis*, the mantle and kidney enzyme activity increased over that in animals acclimated to 200 mOsM. The activity of (Na + K)-activated ATPase in gill tissue was higher in osmoconforming *P. caroliniana, R. cuneata*, and *C. manilensis* (200 mOsM) than in osmoregulating individuals (3 mOsM). There were no salinity related changes in enzyme activity in *L. claibornensis* tissues. This lack of response probably reflects the long geologic history of the Paleoheterodont subclass (which includes *L. claibornensis*) in freshwater habitats.

Key words: (Na + K)-ATPase, ion regulation, freshwater bivalves, estuarine bivalves, mollusc.

INTRODUCTION

Marine bivalves are isosmotic with the environment over the entire range of their salinity tolerances, but the minimum salinity tolerated by the most euryhaline of them is 3 ‰. A few oligohaline bivalves can penetrate more dilute waters, and several successful families are fully adapted to fresh water. The hemolymph of all bivalves in habitats more dilute than 3 ‰ is hyperosmotic to the medium (Gainey and Greenberg, 1977). Moreover, these animals take up both Na and Cl ions from very dilute solutions against a large electrochemical gradient (Krogh, 1939; Chaisemartin et al., 1968; Dietz and Branton, 1975; Dietz, 1979).

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[2]*Present address:* Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, N.Y. 11794, U.S.A.

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The membrane bound enzyme, (Na + K)-activated adenosine triphosphatase, has been proposed as an important mechanism of hyperosmotic regulation in many aquatic animals (Lockwood, 1977; Kirschner, 1979). In a number of freshwater and brackish-water invertebrate species, the enzyme activity has been shown to increase in animals exposed to lowered salinity. For example, the (Na + K)-activated ATPase activity in the gills of a number of crustaceans acclimated to low salinity is higher than that in animals acclimated to high salinity (Towle et al., 1976; Spencer et al., 1979). In the oligohaline clam *Rangia cuneata* (Grey), mantle (Na + K)-activated ATPase activity likewise increases in lower ambient salinity (Saintsing and Towle, 1978).

Here I describe (Na + K)-activated ATPase activity as a function of salinity in four bivalve molluscs variously well adapted to fresh water: *Polymesoda caroliniana* (Bosc) and *R. cuneata* are both brackish water species with limited tolerance of fresh water in the laboratory (Deaton, 1981); *Corbicula manilensis* (Phillipi) is a freshwater species found occasionally in dilute brackish water; and *Lampsilis claibornensis* (Lea) is a stenohaline freshwater species.

The blood Na concentration of individuals of these four species acclimated to salinities below 3 ‰ (100 mOsM) is well above ambient, implying that Na is taken up from the medium (Deaton, 1981). The gills and mantle are likely to be sites of ion uptake in bivalves since they are exposed to the ventilatory current. The kidney of unionid bivalves absorbs Na from the filtrate during the formation of a hypotonic urine (Chaisemartin, 1968), and must contain (Na + K)-activated ATPase activity. I have assayed these three tissues from the four species listed above for (Na + K)-activated ATPase activity following acclimation of the animals to fresh water and dilute brackish water to determine the response of the enzyme activity to the changeover from osmoconformity to hyperosmotic regulation of the body fluids.

MATERIALS AND METHODS

Animals

Rangia cuneata and *Polymesoda caroliniana* were collected from the broad upper reaches of Ochlockonee Bay in Wakulla County, Florida; *Lampsilis claibornensis* and *Corbicula manilensis* were gathered from the Ochlockonee River in Gadsden County. The animals were acclimated, for 4 wk, to either pure Sopchoppy River water (3 mOsm) or to a mixture (200 mOsm) of river water and sea water from the Gulf of Mexico. The osmolalities of these solutions were measured with a freezing point depression osmometer (Precision Systems Osmette).

Tissue preparation

From 1 to 15 animals, depending on their size, were used for each tissue preparation, and, in general, the methods of Towle et al. (1976) were followed. From 0.3 to 1.0 g of gill, mantle, and kidney tissue were excised, weighed, and added to chilled homogenizing medium (Hendler et al., 1972); the proportions used were 20 ml medium/g wet weight of tissue. The tissues were then homogenized with 16 strokes at 1500 rpm in a glass-Teflon Potter-Elvejem apparatus. The homogenate was centrifuged at 10,800 $\times$ *g* for 35 min, and the resulting supernatant was re-centrifuged at 105,000 $\times$ *g* for 60 min to obtain the microsomal pellet, which was resuspended in homogenizing medium and homogenized (3 strokes, 1500 rpm).

Assay of (Na + K)-activated ATPase activity

The coupled enzyme assay system of Schwartz et al. (1969) as modified by Saintsing and Towle (1978) was used to determine the enzyme activity of the microsomal preparations. The assay medium (pH 7.6) contained 5 mM $MgCl_2$, 20 mM imidazole, 100 mM NaCl, 10 mM KCl, 5 mM disodium ATP (Sigma Chemical Co., vanadium free), 0.1 mM NADH, 2.5 mM phosphoenolpyruvate, and 20 μl of a pyruvate kinase-lactate dehydrogenase suspension (Sigma). All reagents were in excess. Protein determinations were by the method of Lowry et al. (1951), with bovine serum albumin as a standard.

Statistics

Differences between the means of the data sets were tested for significance ($\alpha = 0.05$) by Student's *t*-test.

RESULTS AND DISCUSSION

Table I summarizes the (Na + K)-activated ATPase activity in gill, mantle, and kidney from the four bivalve species acclimated to river water and dilute brackish water.

In freshwater acclimated (and, therefore, osmoregulating) *Polymesoda caroliniana, Rangia cuneata,* and *Corbicula manilensis*, the (Na + K)-activated ATPase activity in mantle and kidney is higher than that in corresponding tissues from animals acclimated to 200 mOsM, a medium in which these species are osmoconformers (Deaton, 1981). The gill enzyme activity was higher in brackish water *P. caroliniana* and *C. manilensis* than in freshwater animals. There is no trend of increasing enzyme activity in *Lampsilis claibornensis* tissues from freshwater animals. Exposure of *Carunculina texasensis* to deionized water did not change gill (Na + K)-activated ATPase activity (Dietz and Findley, 1980).

TABLE I

(Na + K)-activated ATPase activities in bivalve tissues.

Species	Tissue	Acclimation medium	
		3 mOsM	200 mOsM
Polymesoda caroliniana	gill	29.0 ± 14.9 (6)	58.0 ± 8.4 (6)*
	mantle	33.6 ± 17.0 (8)*	14.4 ± 9.5 (7)
	kidney	257.0 ± 64.7 (5)*	96.6 ± 38.2 (5)
Corbicula manilensis	gill	17.5 ± 2.0 (3)	38.1 ± 10.3 (3)*
	mantle	26.9 ± 10.7 (4)*	8.3 ± 5.8 (4)
	kidney	21.2 ± 5.3 (4)*	7.4 ± 2.6 (4)
Rangia cuneata	gill	22.5 ± 11.7 (3)	27.5 ± 7.7 (3)
	mantle	32.6 ± 5.5 (4)*	13.3 ± 5.3 (4)
	kidney	73.8 ± 5.4 (4)*	51.5 ± 11.4 (5)
Lampsilis calibornensis	gill	12.4 ± 6.5 (3)	12.8 ± 5.2 (3)
	mantle	6.4 ± 3.3 (3)	7.6 ± 3.6 (3)
	kidney	19.8 ± 16.7 (5)	14.3 ± 2.3 (2)

Values are nmol P_i/min per mg protein ± SD (*n*).

*Values marked by an asterisk are significantly higher ($\alpha = 0.05$) than the corresponding value in the other acclimation medium.

This result may reflect the early (Devonian) establishment of the paleoheterodont bivalves in freshwater habitats (Haas, 1969). There are no brackish-water or marine species in this subclass. The unionids are paleoheterodonts, while the other species in this study are in the subclass Heterodonta, in which freshwater species are a much more recent development (Keen and Casey, 1969). Hence, the response of tissue (Na + K)-activated ATPase in *C. manilensis* to lower salinity is more similar to the brackish-water species than to *L. claibornensis*. There are also differences between *C. malinensis* and unionids in other aspects of ion regulation (Dietz, 1979).

Saintsing and Towle (1978) also found the mantle (Na + K)-activated ATPase activity in low salinity *R. cuneata* to be higher than that of animals acclimated to salinities greater than 3‰. Hedgepeth and Lynch (1974) reported increased enzyme activity with increasing salinity in the gills of the oyster *Crassostrea virginica* (Gmelin) and the clam *Mercenaria mercenaria* (L.). Neither of these species is capable of hyperosmotic regulation, and the function of the increase in gill (Na + K)-activated ATPase in these animals as well as *P. caroliniana* and *C. manilensis* (Table I) may not be connected with osmotic regulation. Dietz and Findley (1980) suggest that the enzyme may be involved primarily in cellular ionic balance.

The values reported here for (Na + K)-activated ATPase activity in *R. cuneata* tissues are higher than those obtained by Saintsing and Towle (1978), but their use of tissue homogenates instead of microsomal preparations probably accounts for the differences. My values for activity in *L. claibornensis* gill tissue are similar to those reported for microsomal preparations of gill tissue from other unionid mussels (Lagerspetz and Senius, 1979; Dietz and Findley, 1980).

The (Na + K)-activated ATPase activity in bivalve gill and mantle tissue is about an order of magnitude lower than that of comparable preparations from freshwater fish gills. Kamiya and Utida (1969) found values ranging from 127 to 230 nmol P_i/min per mg protein in gill homogenates from a number of freshwater and euryhaline teleosts acclimated to fresh water. Microsomal preparations from gills of the killifish *Fundulus heteroclitus* (L.) acclimated to fresh water have (Na + K)-activated ATPase activity of 1.1 μmol P_i/min per mg protein (Towle et al., 1977). The blood Na concentrations of freshwater fish are much higher than those of freshwater bivalves, but the magnitude of the net Na influx in bivalves is about the same as that of freshwater fish (Dietz and Branton, 1975).

The concentration of Na in the urine collected by Chaisemartin (1968) from the unionid *Margaritana margaritifera* (L.) is higher than that found in the waters in which the animals live, suggesting that some extra-renal Na uptake is necessary to maintain the hyperionic Na concentration in the body fluids. The response of the mantle (Na + K)-activated ATPase activity to dilution suggests that the mantle is involved in ion uptake from dilute media. Whether the increases in enzyme activity following dilution occur due to activation of in situ, but inactive enzyme (Towle et al., 1977), or because of synthesis of new enzyme protein, is still open to question (Neufeld et al., 1980).

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REFERENCES

Chaisemartin, C., 1968. Place de la fonction rénale dans la régulation de l'eau des sels chez *Margaritana margarantifera* (Unionides). C.R. Soc. Biol. 162, 1193–1195.

Chaisemartin, C., Martin, P.N. and Bernard, M., 1968. Homeoionémie chez *Margaritana margarantifera* L. (Unionides), étudiée à l'aide des radioéléments ^{24}Na et ^{35}Cl. C.R. Soc. Biol. 169, 523–526.

Deaton, L.E., 1981. Ion regulation in freshwater and brackish water bivalve mollusks. Physiol. Zool. 54, 109–121.

Dietz, T.H., 1979. Uptake of sodium and chloride by freshwater mussels. Can. J. Zool. 57, 56–160.

Dietz, T.H. and Branton, W.D., 1975. Ionic regulation in the freshwater mussel, *Ligumia subrostrata* (Say). J. Comp. Physiol. 104, 19–26.

Dietz, T.H. and Findley, A.M., 1980. Ion-stimulated ATPase activity and NaCl uptake in the gills of freshwater mussels. Can. J. Zool. 58, 917–923.

Gainey, L.F. and Greenberg, M.J., 1977. Physiological basis of the species abundance-salinity curve in molluscs: a speculation. Mar. Biol. 40, 41–49.

Haas, F., 1969. Family Unionidae. In: Treatise on Invertebrate Paleontology, pt N, pp. N411–N463. Editor: R.C. Moore. Geological Society of America, Boulder, Colo.

Hedgepeth, M.E. and Lynch, M.P., 1974. Salinity related changes in ATPase levels in the Virginia oyster, *Crassostrea virginica* and the hard clam, *Mercenaria mercenaria*. Va. J. Sci., 25, 64.

Hendler, E.D., Toretti, J., Kupor, L. and Epstein, F.H., 1972. Effects of adrenalectomy and hormone replacement on Na^+-K^+-ATPase in renal tissue. Am. J. Physiol. 222, 754–760.

Kamiya, M. and Utida, S., 1969. Sodium-potassium activated adenosine triphosphatase activity in gills of freshwater, marine, and euryhaline teleosts. Comp. Biochem. Physiol. 31, 671–674.

Keen, M. and Casey, R., 1969. Family Corbiculidae. In: Treatise on Invertebrate Paleontology, pt N, pp. N665–N669. Editor: R.C. Moore. Geological Society of America, Boulder, Colo.

Kirschner, L.B., 1979. Control mechanisms in crustaceans and fishes. In: Mechanisms of Osmoregulation in Animals, pp. 157–222. Editor: R. Gilles. John Wiley and Sons, New York.

Krogh, A., 1939. Osmotic regulation in aquatic animals. Cambridge University Press, London, 242 pp.

Lagerspetz, K.Y.H. and Senius, K.E.O., 1979. ATPase stimulated by Na^+ or K^+ in gills of the freswater mussel Anodonta. Comp. Biochem. Physiol. 62B, 291–293.

Lockwood, A.P.M., 1977. Transport and osmoregulation in crustacea. In: Transport of Ions and Water in Animals. Editors: B.L. Gupta, R.B. Moreton, J.L. Oschman and B.J. Well, pp. 673–707. Academic Press, New York.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Neufeld, G.J., Holliday, C.W. and Pritchard, J.B., 1980. Salinity adaption of gill Na, K-ATPase in the blue crab, *Callinectes sapidus*. J. Exp. Zool. 211, 215–224.

Saintsing, D.G. and Towle, D.W., 1978. Na^+ and K^+ ATPase in the osmoregulating clam *Rangia cuneata*. J. Exp. Zool. 206, 435–442.

Schwartz, A., Allen, J.C. and Harigaya, S., 1969. Possible involvement of cardiac Na^+, K^+-adenosine triphosphatase in the mechanism of action of cardiac glycosides. J. Pharmacol. Exp. Ther. 168, 31–41.

Spencer, A.M., Fielding, A.H., and Kamemoto, F.I., 1979. The relationship between gill Na-K ATPase activity and osmoregulatory capacity in various crabs. Physiol. Zool. 52, 1–10.

Towle, D.W., Palmer, G.E. and Harris, J.L., III, 1976. Role of gill $Na^+ + K^+$-dependent ATPase in acclimation of blue crabs (*Callinectes sapidus*) to low salinity. J. Exp. Zool. 196, 315–321.

Towle, D.W., Gilman, M.E. and Hempel, J.D., 1977. Rapid modulation of gill $Na^+ + K^+$-dependent ATPase activity during acclimation of the killifish *Fundulus heteroclitus* to salinity change. J. Exp. Zool. 202, 179–186.