ATP CONTENT AND RELEASE IN *GLYCERA DIBRANCHIATA* COELOMOCYTES

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Cell volume regulation is a complex process involving the transport of intracellular ions and metabolites across the plasma membrane in response to osmotic changes. Novel roles for intra- and extracellular ATP in cell volume regulation have been recently forwarded. P-glycoprotein, for example, is involved in the regulation of an osmotically activated chloride-permeable conductance (Hardy, et al., EMBO J. 14: 68-75, 1995), and has more recently been demonstrated as an electrodiffusional pathway for the release of cellular ATP which is involved in the regulation of anisoosmotic changes in cell volume (Wang, et al., Proc. Natl. Acad. Sci. USA 93: 12020-12025, 1996). This is in agreement with previous reports indicating that P-glycoprotein is partly responsible for ATP release (Abraham, et al., Proc. Natl. Acad. Sci. USA 90: 312-316, 1993; Bosch et al., Am. J. Physiol. 271: C1527-1538, 1996). It is thus relevant to further assess the regulatory mechanisms associated with the osmotic activation of these pathways.

In this context, the blood of the sea worm *Glycera dibranchiata* may provide a model for the study of osmotically associated adaptive mechanisms, as this invertebrate adapts to varying environmental osmotic conditions. In this report we have begun a study of the ATP content and release of sea worm coelomocytes (red blood cells), which may be directly affected by environmental changes in sea water osmolarity.

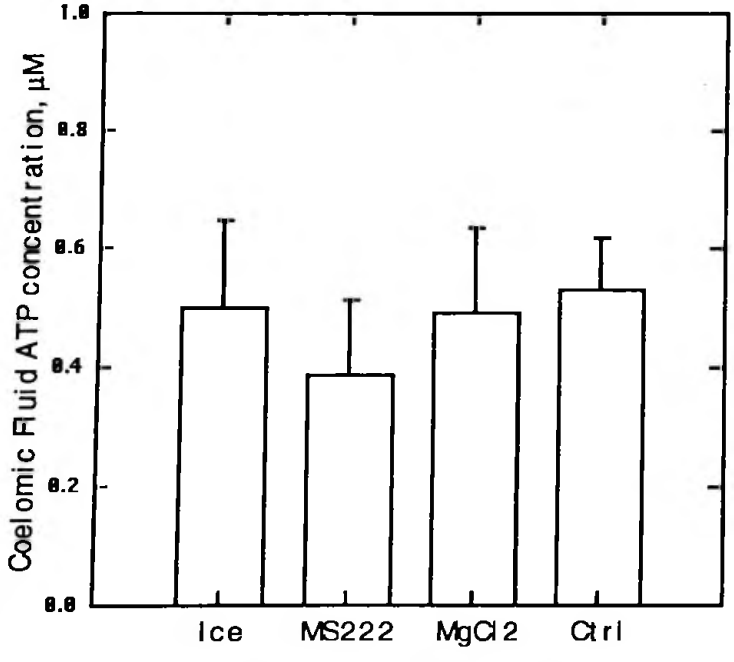
Sea worms (*Glycera dibranchiata*) were obtained from a local supplier and kept in an oxygenated sea water tank. *Glycera* "blood" was drawn from the animals to separate into the coelomic fluid (plasma), gametes and white blood cells (which were discarded), and coelomocytes by a 5 min centrifugation at 1000 rpm at temperatures ranging from 0-6°C. Blood was drawn from either control (Ctrl) animals without anesthetic, or worms anesthetized with either (1) 3.5% $MgCl_2$, (2) 3.83 mM methane sulfonate salt (MS222), or (3) ice cold artificial sea water (NaSW) kept at -20°C for 10 min.

After centrifugation ATP levels were measured in the coelomic fluid and coelomocytes with the luciferin-luciferase assay using a luminometer (Monolight 2010, Analytical Luminescence Laboratory, Ann Arbor, MI). The ATP assay mix (0.1 ml, Firelight LB, Analytical Luminescence Laboratory), was diluted in 0.5 ml of NaSW. ATP concentrations were measured from three different volumes (10, 50,

and 100 microliters) to assess any quenching effect of the coelomic fluid. ATP content and release in coelomocytes were also measured using three different concentrations of suspended cells (2, 3, and 6 microliters). To measure the total intracellular ATP in the coelomocytes, the cells were added to the assay mix and then lysed with alamethicin (10 μM, Sigma), and then sonicated for one minute.

The anesthetic conditions had varying effects on the sea worms. The worms anesthetized with MS222 were inactive after 20 min in the solution, but appeared to have a little hemolyzed. However, animals from the $MgCl_2$ and the iced NaSW conditions were not completely anesthetized and displayed certain motility. After centrifugation of the whole blood, it was noticed that the blood from animals anesthetized with $MgCl_2$ and MS222 had a thinner layer or no gametes and/or white blood cells when compared to Ctrl blood. The red blood cell counts from MS222 and $MgCl_2$ were higher than those from the iced NaSW and Ctrl conditions. It was also noted that the MS222 treatment had shrunken the cells, but the iced NaSW treated cells were larger than the controls.

To determine coelomocyte cell volume (Volume=$4/3\pi$ $(A/2)^2B/2$; A=longest diameter, B=shortest diameter), cells were photographed under a confocal microscope.



Glycera coelomic fluid ATP levels were obtained for the various methods of collection as indicated in the abcissa. Values are from 16 measurements in 4 different experiments under each condition. No statistical differences were observed for values among the various groups.

The correlation between the amount of plasma added (10, 50, and 100 μl) and the calculated amount of ATP present in the coelomic fluid indicated that there was an inversely proportional effect of the amount of coelomic fluid added to the cuvette. Values for "plasma" ATP after calculation from extrapolating the values to "zero" volume (thus no quenching) are expressed in micromoles of ATP per liter (see Fig.).

The ATP concentration of the coelomic fluid ranged from 0.384 μM (MS222) to 0.529 μM (Ctrl). This 27% difference, however, was not statistically significant. Coelomocyte intracellular ATP was also not statistically different

among the various groups, thus indicating that the stress of blood collection in the absence of anesthesia did not affect either coelomic fluid or cellular ATP. However, these findings raise the possibility that ATP being released may be rapidly metabolized in the extracellular compartment, a phenomenon which will require further investigation.

Future experiments will also be required to assess whether environmental stress such as exposure to osmotic changes may affect the content and/or the release of cellular ATP from coelomocytes as well as the levels of ATP in the coelomic fluid.

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