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THE MECHANISM OF ACCUMULATION OF METALS, ESPECIALLY HIGH LEVELS OF VANADIUM, BY ASCIDIANS

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High levels of vanadium were found by mere chance in the blood cells of an ascidian, known as sea squirt, by the German chemist M. Henze in 1911. Subsequently, many investigators including analytical chemists, physiologists, bioinorganic chemists and chemists of natural product, joined in attempts to characterize the metal in living organisms partly because of the initial interest in its very presence in such organisms and partly because of the primary interest in its involvement in carrying oxygen as the third metal following iron and copper. However, studies on the mechanism of accumulation and physiological significance of vanadium have made little progress, since the problem needed to interdisciplinary investigations, to be elucidated.

First, we reexamined the vanadium content of several tissues from 20 ascidian species, employing the extremely sensitive method of neutron-activation analysis for the quantification of this metal. Consequently, the highest concentration of 350 mM vanadium was found in the blood cells of *Ascidia gemmata* belonging to the suborder Phlebobranchia, which concentration corresponds 10^7 times higher than that in sea water. Ascidian blood cells have been classified morphologically as being about 10 types. Morula cells, one type of vacuolated cell, have been thought to be the so-called vanadocytes that might be involved in the accumulation of vanadium. Our combination techniques of cell fractionation and neutron-activation analysis revealed that signet ring cells, another type of vacuolated cell, were the true vanadocytes rather than the morula cells.

Furthermore, the oxidation state of vanadium in the blood cells has been a subject of controversy. Our ESR (electron spin resonance) measurements of the oxidation state in the fractionated blood cells revealed that the vanadocytes contained vanadium, 97.6% of which was in the +3 oxidation state with the rest being in the +4 oxidation state. After Henze's initial discovery of 1 N acidity in ascidian blood cells, low pH values have been reported by several investigators and the mechanism of accumulation and oxidation state of vanadium have been suggested to be associated with a very low pH within the cells. However, K. Kustin's group recently claimed that the intracellular pH was neutral on the basis of measurements made by their new technique. We considered that one of the reasons for this extreme controversy was that the measurements of pH were made using the entire population of blood cells, that is to say, without the separation by fractionation of the subpopulations of blood cells. Applying ESR spectrometry to the measurement of pH values of fractionated subpopulations, we could confirm that the vanadocytes in *A. gemmata* had a low pH value of 2.4 while these cells contained the highest level of vanadium.

These facts suggest the possibility that proton concentrated by a H^+-ATPase are energetically linked to the accumulation of vanadium. We made, therefore, immunological search for H^+-ATPase in the vacuolar membrane of signet ring cells as the first step. It was consequently revealed that antibody raised against the vacuolar-type H^+-ATPase reacted with the vacuolar membranes of signet ring cells and addition of bafilomycin A_1, a specific inhibitor of vacuolar-type H^+-ATPase, actually inhibited the uptake of protons by the vacuoles of signet ring cells, with resultant neutralization of the contents of the vacuoles.

Hereupon, there have been certain complications involved in the identification of signet ring cells, the so-called vanadocytes, partly because of difficulties associated with morphological discrimination between several types of blood cells and partly because of our inadequate knowledge as to the cell-lineages of blood cell. We postulated that monoclonal antibodies would be powerful tools for resolving these issues. The antibody that we generated reacted with the signet ring cells not only of *A. sydneiensis samea* used as antigen but also with those of the other species and recognized a protein of about 45 kDa.

This antibody was also a useful tool to determine the time at which the accumulation of vanadium commenced during embryogenesis. Using neutron-activation analysis and an immunofluorescence method, we found that amounts of vanadium per individual increased dramatically two weeks after fertilization, and by two months the amount accumulated in larvae was about 600,000 times greater than that in the unfertilized eggs of *A. gemmata*. A vanadocyte-specific antigen, recognized by a monoclonal antibody specific to the signet ring cells, first appeared in the body wall coinciding with the significant accumulation of vanadium.

Characterization of vanadium-binding proteins extracted from the blood cells of vanadium-rich ascidians is in progress,

which must have a key how ascidians selectively and highly accumulate vanadium from sea water.

The unusual phenomenon whereby some ascidians accumulate vanadium to levels more than ten million times higher than that in sea water has attracted investigators in various fields. Ascidians with this unusual physiological characteristic may help up to clarify not only how ascidians but also how other living organisms accumulate transition metal, as well as the physiological roles that these metals play in living organisms.