

Foundational Study on Selective Extraction of the Rare Metal from Seawater, Based on the Mechanism of Accumulation of High Levels of Vanadium by Ascidians

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Summary

Ascidians that were marine invertebrate treated phylogenetically as a subphylum of the Chordate were found to contain high levels of vanadium ions in their blood cells (Henze, 1911). As the result of our redetermination by means of a neutron activation analysis, the highest level of vanadium was estimated to reach 350mM in the blood cells of *Ascidia gemmata*, corresponding to 10^7 times higher than that in seawater.

In the present experiments, we have succeeded to extract a vanadium-binding protein from the blood cells of *Ascidia sydneiensis samea*, a vanadium-rich ascidian belonging to the family of Ascidiidae. The blood collected by cardiac puncture was centrifuged at 400 xg to separate the blood cells from the serum. The obtained blood cells except giant cells were homogenized and submitted to SDS-PAGE. In order to determine vanadium-binding proteins, the each gel fraction cut at 5 mm length from the top to the bottom was analyzed its content of vanadium by a flameless atomic absorption spectrometry. Consequently, it was revealed that about 100 kDa, 40 kDa and 15 kDa proteins were vanadium-binding proteins.

Furthermore, anion-exchange chromatography, DEAE Sephacel, was used to extract a membrane-bound protein from the blood cells. The blood cells freezed at $-80\text{ }^{\circ}\text{C}$ and thawed were centrifuged at 100,000 xg for 60 min and the supernatant obtained was applied to the column and fractioned. Vanadium content in each fraction obtained was also determined. As the result, a 15 kDa protein was revealed to be a vanadium-binding protein bound with membranes of the blood cells.

To prepare the antibody against the 15 kDa protein, 1 ml solution containing 100 μg of the protein was injected to rabbit intraperitoneally three times and the antibody was obtained. Immunoblot analysis revealed that the antibody obtained specifically recognized the antigen.