

Glutathione S-Transferase Having Vanadium-Binding Activity Isolated from a Vanadium-Accumulating Ascidian, *Ascidia sydneiensis samea*

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Several species of ascidians accumulate vanadium in their vanadocytes, vanadium containing blood cells, at high concentration and with high selectivity. Through the accumulation process, almost vanadium ions in the +5 oxidation state are reduced to the +3 oxidation state via the +4 oxidation state and stored in the vacuole of vanadocytes.

For hunting new factors involved in this unique phenomenon, we have tried to isolate novel vanadium-binding proteins from tissue extracts of a vanadium-rich ascidian, *Ascidia sydneiensis samea*, by using a vanadium-chelating column, and consequently several vanadium-associated proteins have been isolated. We aimed at one of the proteins expressed highly in the digestive organ, and its N-terminal amino acid sequence was determined. To screen the cDNA coding the protein, a degenerate primer corresponding to the amino acid sequence and cDNA library of vanadocytes were used for polymerase chain reaction. As a result, a single DNA fragment was amplified. Analysis of the DNA sequence revealed that the estimated amino acid sequence of the protein shows a striking homology with glutathione S-transferase (GST). Therefore, the protein was named as AsGST.

Through analysis of the recombinant protein, we confirmed that AsGST certainly has vanadium-binding activity, forms dimer as same as other GSTs and has GST activity with 1-chloro-2,4-dinitrobenzene (CDNB), one of general substances of GSTs.

One of the most important functions of GSTs in organisms is protection against oxidative stress induced by heavy metals. Additionally glutathione (GSH), the cofactor of GSTs, is known to modulate mobilization and toxicity of metals such as cadmium and copper, and behaves as a reducing agent for metals including vanadium. Therefore, we supposed that AsGST and GSH might play important roles during the process of vanadium accumulation in ascidians.

The correlation between vanadium-binding property and GST activity and detailed localization of AsGST are under investigation.