（学位第３号様式）

学 位 論 文 要 旨

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題　目	Functional and structural analysis of inorganic pyrophosphatase from Antarctic psychrotroph *Shewanella* sp. AS-11 （南極産好冷細菌 *Shewanella* sp. AS-11 由来無機ピロホスファターゼの機能及び構造解析）

Inorganic pyrophosphatase (PPase) is an essential enzyme in all living organisms, as it hydrolyzes inorganic pyrophosphate to phosphate. There are 2 soluble PPase families, families I and II, and the PPases in these 2 families have completely different primary structures. Both families of PPases are only active in the presence of metal ion cofactors. *Shewanella* sp. AS-11 is a bacterium isolated from the shellfish *Neobuccinum eatoni* and lives in the ice-covered seas of Antarctica, where the temperature is close to and often below 0°C. In this study, inorganic pyrophosphatase from *Shewanella* sp. AS-11 (*Sh*-PPase) was successfully cloned, expressed in *E. coli* and purified from the cell extracts by a combination of ammonium sulphate fractionation and anion-exchange chromatography.

Sh-PPase was found to be a family II PPase homodimer with a subunit molecular mass of 34 kDa and preferentially utilized Mn^{2+} and Co^{2+} over Mg^{2+} for activity. Zn^{2+} also significantly activated the enzyme. The optimal temperature for activity of *Sh*-PPase activated by Mn^{2+} was surprisingly low (5°C), while those of Zn, Co and Mg–activated enzymes were 20, 30 and 40°C, respectively. The specific activities of *Sh*-PPases activated by Co^{2+}, Mn^{2+} and Zn^{2+} were 100-, 45- and 12-fold higher than that of Mg–activated *Sh*-PPase at 5°C, respectively. *Sh*-PPase activated by Co^{2+} or Mn^{2+} was less stable than non–activated enzyme at 50°C, whereas the enzyme activated by Zn^{2+} was more stable than non-activated enzme. Activation of *Sh*-PPase with divalent cations enhanced k_{cat}, but did not significantly affect K_m. Thus divalent cations markedly influenced the catalytic efficiency, temperature dependency, and thermostability of *Sh*-PPase. Furthermore, Mn^{2+} or Co^{2+} was shown to be required to gain cold-adapted characteristics.

To investigate the possibility that the functional characteristics of *Sh*-PPases are related to conformational changes of the enzyme upon activation with divalent metal ions, I performed the fluorescence and circular dichroism (CD) spectroscopic analyses. The results of fluorescence anisotropy measurement for intrinsic tryptophan and CD spectroscopy suggest that the binding with divalent metal ions affects neither the conformational fluctuation of tryptophan residues nor the secondary structure of the enzyme. However, the observed changes in fluorescence spectra of the tryptophan residues and the fluorescence probe 1-anilino-8-naphthalene sulfonate (ANS) proved that the environments of tryptophan side chains and ANS–binding site(s) of *Sh*-PPase became less hydrophobic on activation with divalent cations. Furthermore, the activation with divalent metal ions decreased the quenching effect of acrylamide on tryptophan fluorescence. From these results together with the homology-modelled structure of *Sh*-PPase, it can be concluded that binding of divalent cation to the active site causes some conformational changes in the whole enzyme molecule, which may influence the temperature dependency and thermostability of activated enzymes.

To further understand the mechanism of cold adaptation of *Sh*-PPase, I have been trying to grow the crystals of *Sh*-PPases for X-ray crystallography. The crystals of non–activated and Mn–activated *Sh*-PPases were grown at neutral or acidic pH with polyethylene glycol as precipitant, but the quality of X-ray diffraction data of these crystals was not good enough to analyze the three dimensional structure.