Crystal Structure of Carboxypeptidase 1 from *Thermus* thermophilus

Koji Nagata^{a,b}, Shiho Tsutsui^a, Woo Cheol Lee^a, Kosuke Ito^a, Masayuki Kamo^a, Yumiko Inoue^b, Masaru Tanokura^{a,b}, ^a*Graduate* School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan. ^bRIKEN Harima Iinstitute at Spring-8, Hyogo, Japan. E-mail: unagata@mail.ecc.u-tokyo.ac.jp

Carboxypepidase 1 from *Thermus thermophilus* (*Tth*CP1) is a metallopeptidase which hydrolyzes a peptide bond from the C-terminus of peptides and proteins and requires a divalent metal ion such as Zn^{2+} or Co^{2+} for its activity. The metal ion binding motif of TthCP1 differs from those of classical metalloproteases and a distinctive catalytic mechanism has been proposed. In this research, we have solved the crystal structure of *Tth*CP1 to analyze the structural basis of its catalytic mechanism and heat stability, and also characterized its substrate specificity.

*Tth*CP1 was crystallized using PEG8000 as the precipitant by sitting drop vapor diffusion method. A native dataset was obtained to a resolution of 2.6 Å Diffraction data were collected using an ADSC Quantum 210 detector system at beamline PF-AR NW12 at Photon Factory (Tsukuba, Japan) [1]. The crystal structure was determined by molecular replacement using the atomic coordinates of carboxypeptidase from *Pyrococcus furiosus* (*Pfu*CP, PDB code: 1KA2 [2]). The structure, substrate specificity and thermostability of *Tth*CP1 will be presented and compared with those of *Pfu*CP [2, 3].

[1] NagataK. et al., Acta Cryst., 2004, **D60**, 1445. [2] Arndt J.W. et al., Structure, 2002, **10**, 215. [3] ChengT. C. et al., Prot. Sci., 1999, **8**, 2474.

Keywords: carboxypeptidase, structure-function protease, thermostable enzyme