

Biochemical and crystallographic analyses of carboxypeptidase 1 from *Thermus thermophilus*

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Carboxypeptidase 1 from *Thermus thermophilus* (TthCP1, 58 kDa) is a metallopeptidase which hydrolyzes a peptide bond from the C-terminus of peptides and proteins and requires a divalent metal ion such as Zn²⁺ or Co²⁺ 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 TthCP1 to reveal the structural basis of its catalytic mechanism and heat stability, and also characterized its substrate specificity. TthCP1 was crystallized using PEG8000 as the precipitant by sitting drop vapor diffusion method. A native dataset was obtained to a resolution of 0.26 nm with an R-Axis VII detector equipped with an FR-E X-ray generator (Rigaku). The crystal structure was determined by molecular replacement using the atomic coordinates of carboxypeptidase 1 from *Pyrococcus furiosus* (Arndt, J.W. *et al.*, 2002, PDB code: 1KA2). To determine the substrate specificity of TthCP1, we incubated a few kinds of peptides and a series of N-terminally protected dipeptides with TthCP1 and analyzed the reaction products by UV/VIS spectroscopy and MALDI-TOF mass spectrometry. We are going to discuss the structural basis of substrate specificity and thermostability of TthCP1.