Poster Presentation

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Enzymatic hydrolysis of the β−β amide linkage in poly-β-aspartate

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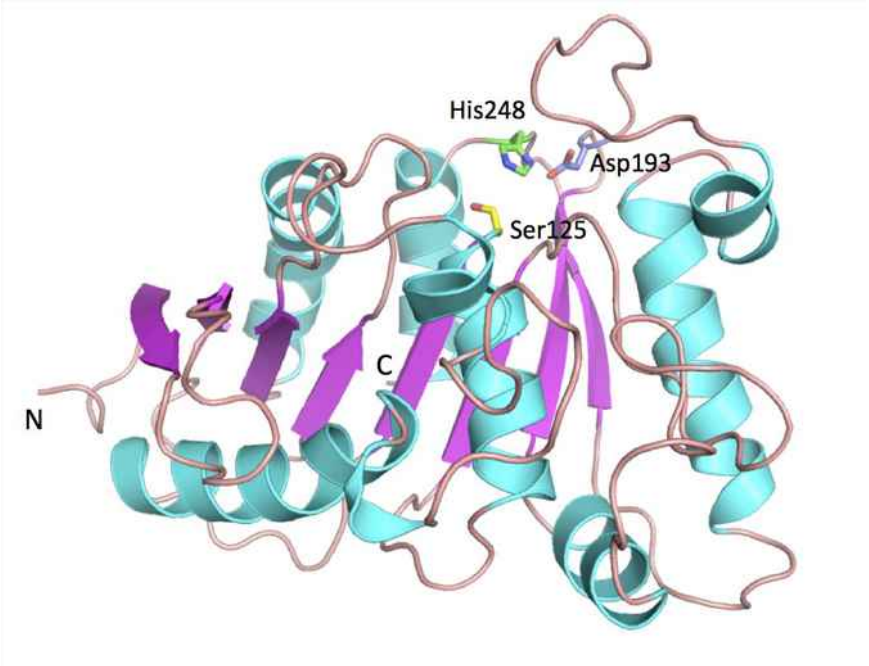
Polyaspartate is a biodegradable and water-soluble polymer, and an important material as an alternative to polyacrylate. Polyacrylate is widely used as a builder of detergents, pigment dispersant, cosmetic moisturizer, water treatment agents (scale preventive, metal corrosion preventive), water absorptive polymer of disposable diaper, and so on, and are likely accumulated in the environment. Replacement of the polymer by biodegradable polyaspartate should reduce the accumulation and therefore environmental pollution. Thermally synthesized polyaspartate contains both α- and β-amide linkages. Recently, three bacterial enzymes that degrade polyaspartate have been isolated and characterized. An enzyme from Pedobacter sp. KP-2 specifically catalyzes the hydrolysis of the β−β-amide linkage in the polymer. The enzyme is a monomeric periplasmic protein, and the mature form contains 265 amino acid residues with a molecular weight of about 30,000. To understand the mechanisms underlying the interactions with the polymer substrate and hydrolysis of the β−β amide linkage, we set out the crystallographic study of the enzyme. We succeeded in crystallization of the enzyme by vapor-diffusion using polyethylene glycol 4,000. Crystals belonged to orthorhombic space group C222. Addition of cobalt salt in the mother liquor altered the space group of crystals to P6422 and greatly improved the diffraction quality. Data sets for native and mutant enzymes were collected to a resolution of 1.9 Å at SPring-8, Japan. Phases were calculated by cobalt-SAD. The obtained structure represented an α/β hydrolase fold which is often observed for a large number of serine esterases such as lipase and PHB depolymerase. Residues Ser125, Asp193, and His248 were within hydrogen bonding interactions, forming a catalytic triad. Structures of subsites 1 and 2 for binding of monomer units of the polymer were determined for the mutant enzyme complexed with an oligomer substrate. The monomer unit bound to subsite 1 was in extensive hydrogen bond interactions with residues including arginine. Though overall structural similar between the enzyme and PHB depolymerase from Penicillium funiculosum, residues that may interact with polymer substrates were totally different.



Keywords: biodegradable polymer, α/β hydrolase fold, enzyme-substrate complex