Biochemistry and structural biology of microbial enzymes and medical application

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Mechanism of proline specific peptidases and aminopeptidase N of microorganisms was studied by molecular biology and x-ray crystallography. Application of enzymes from microorganism for diagnostics analyses was also studied.

I, Structure and function of peptidases from pathogenic bacteria

Several proline specific peptidases were found in pathogenic bacteria by us. Among them, prolyl tripeptidyl aminopeptidase from *Porphylomonas gingivals* and prolyl aminopeptidase from *Serratia marcescens* were purified. Complex structues of those enzymes and inhibitors were clarified by x-ray crystallography. On the other hand, aminopeptidase N which has wide specificity for amino acids, distribute in pathogen. The crystal structure of the aminopeptidase N elucidated reasons of the wide substrate specificity but inert to X-Pro bond. Thus shows that proline specific peptidases and aminopeptidase N cooperatively degrade the collagen for the uptake of amino acids as nutrition at the infection of these bacteria. (*J. Biol. Chem.* **276**, 18557- 18562 (2001). *J. Bacteriol.* **188**, 1599-1606 (2006), *J. Biol. Chem.* **280**, 33664-33676 (2006), *J. Mol. Biol.* **362**, 228-240 (2006)).

II, Application of microbial enzymes for diagnostics analyses.

A series of creatinine-metabolizing enzymes was found in *Pseudomonas putida* by us. Creatininase, creatinase and sarcosine oxidase were coupled and have been developed for diagnostic analysis kit of the renal function. The structures of the native and the Mn^{2+}-activated creatininases were determined by a x-ray crystallography. Based on the structure, the activated enzyme was used for the improved assay kit. The structure of D-3-hydroxybutyrate dehydrogenase from *Pseudomonas fragi* was also clarified by crystallography. The enzyme is useful for diagnostic analysis of diabetes mellitus while monitoring the ketone bodies. (*J. Mol. Biol.* **337**, 399-416 (2004), *J. Mol. Biol.* **355**., 722-733 (2006)).