学位論文内容の要旨

博士の専攻分野名称：博士（農学）　　氏　名：Nongluck Jaito

学位論文題名

Function, structure, and application of microbial enzymes related to CE-MGP pathway

（微生物由来CE-MGP経路関連酵素の機能，構造ならびに応用に関する研究）

β-Mannan, composed mainly by β-(1→4)-linked D-mannosyl residues, is a polysaccharide included in hemicellulose. It is known to be utilized as a carbon source of bacteria, and generally considered to be degraded by hydrolysis. But recently its new degradation pathway (CE-MGP pathway), involving an epimerase and phosphorylases, has been found in obligate anaerobes, *Bacteroides fragilis* and *Ruminococcus albus*. In this pathway, Man_2, a hydrolytic product of β-mannan, is epimerized to 4-*O*-β-D-mannosyl-D-glucose (Man-Glc) by cellobiose 2-epimerase (CE), and Man-Glc is phosphorolyzed to α-D-mannosyl phosphate (Man1*P*) and D-glucose by 4-*O*-β-D-mannosyl-D-glucose phosphorylase (MGP). Although known MGPs have been found only in anaerobes, the putative MGP genes are also found in aerobes. An aerobe, *Rhodothermus marinas*, has a putative MGP gene, *Rmar_2440*, upstream *CE* (*Rmar_2439*, RmCE). There are some studies about RmCE including structure analysis. RmCE, having an $(\alpha/\alpha)_6$ barrel fold catalytic domain, forms *N*-acetyl-D-glucosamine 2-epimerase (AGE) superfamily together with AGE, D-mannose isomerase (MI), and aldose–ketose isomerase. Only CE acts on oligosaccharides unlike the other members, but the structural basis for its disaccharide specificity has not been fully understood. Furthermore, this superfamily includes many putative function-unknown proteins with low sequence identity to any function-known proteins. In this study, characteristics of putative *R. marinus* MGP and a function unknown AGE superfamily protein, *Marinomonas mediterranea* Marme_2490, and important amino acid residues for the disaccharide specificity of RmCE were analyzed. As an application of CE and MGP, the colorimetric quantification methods for Man-Glc and Man_2 were established.

1. Biochemical characterization of Rmar_2440

Recombinant Rmar_2440 was produced in *E. coli* and characterized. Rmar_2440 catalyzed the phosphorolysis of Man-Glc to Man1*P* and D-glucose. In the reverse

phosphorolysis, Rmar_2440 had high acceptor preferences for D-glucose, 6-deoxy-D-glucose, and D-xylose. Methyl β-D-glucoside and 1,5-anhydro-D-glucitol served as acceptors unlike *R. albus* MGP. This finding of MGP activity suggests that the CE-MGP pathway is not limited in anaerobes but distributed even in aerobes.

2. Molecular basis for high disaccharide specificity of RmCE

The protein structure of RmCE suggested that D188 and W385 are involved in the binding of disaccharide substrates on the non-reducing side through hydrogen-bonding and stacking interactions. The D188A and W385A mutants significantly lost the epimerization activity particularly on disaccharide substrates. The results suggest that D188 and W385 are important for high specificity for disaccharides.

3. Biochemical characterization of Marme_2490

Recombinant Marme_2490 clearly showed MI activity. It catalyzed the interconversion between D-mannose and D-fructose with the higher k_{cat}/K_m for D-mannose compared with other known MIs. In addition, Marme_2490 has epimerization activity to D-talose and β-(1→4)-linked disaccharides, Man_2, Glc-Man, and epilactose, which is a distinctive property in the AGE superfamily.

4. Colorimetric quantifications of Man-Glc and Man_2

A quantification method for Man_2 and Man-Glc was established. MGP phosphorolyzed only Man-Glc with its high specificity to produce the equal molar of D-glucose, which can be quantified with a conventional colorimetric method. In the presence of CE, Man_2 can be also quantified. The method is simple and easy, and saves time in quantifying the mannooligosaccharides.