Form 2

Dissertation Abstract

Report no.	(Course-based) No.1002	Name	Tanim Jabid Hossain
Dissertation title	Analysis of the Catabolic Pathways of Glycoconjugates (複合糖質の代謝機構の解析)		

Abstract

Glycoconjugates are the molecules in which glycan units are covalently linked to noncarbohydrate entities by a process called glycosylation. Here we studied the catabolic pathways of two glycoconjugates, namely $Man_1GlcNAc_2$ and *C*-mannosyl tryptophan (CMW) which are related to two different types glycosylation called *N*-glycosylation and *C*-mannosylation respectively.

While the biosynthetic pathway of *N*-glycosylation has been well clarified in *S. cerevisiae*, many issues remain to be clarified in their degradation processes. It has been revealed that in the cytosol of this yeast, most of the free oligosaccharides structurally related to the *N*-glycans (free N-glycans or FNGs) are generated from the misfolded glycoproteins by the action of the cytoplasmic peptide:N-glycanase (Png1). A cytosol/vacuole α-mannosidase, Ams1, then trims the free FNGs to eventually form a trisaccharide composed of Manβ1,4GlcNAc β1,4GlcNAc (Man1GlcNAc2). Whether or not the resulting $Man_1GlcNAc_2$ is enzymatically degraded further, however, is currently unknown. The objective of this study was to unveil the fate of $Man_1GlcNAc_2$ in *S. cerevisiae*. Quantitative analyses of the FNGs revealed a steady increase in the amount of $Man_1GlcNAc_2$ produced in the post-diauxic and stationary phases, suggesting that this trisaccharide is not catabolized during this period. Inoculation of the stationary phase cells into fresh medium resulted in a reduction in the levels of $Man_1GlcNAc_2$. However, this reduction was caused by its dilution due to cell division in the fresh medium. Our results thus indicate that $Man_1GlcNAc_2$ is not enzymatically catabolized in *S. cerevisiae*.

C-mannosylation, an unusual co- or post-translational modification of proteins, involves the attachment of one mannose residue to specific Trp molecule of proteins producing *C*-mannosyl proteins. Since *C*-mannosyl tryptophan (CMW) structure was detected in human urine, it is believed that the *C*-mannosyl proteins are degraded inside our body to produce CMW which is then excreted in urine into the environment. Nevertheless, CMW is not normally found in the environment, suggesting that at least microbes can catabolise this compound. In this study, we aim to find the bacteria which can degrade CMW and to determine the mechanism of the degradation by the bacteria, and we could successfully isolate the bacteria which grow in media containing CMW as the only carbon source. Surprisingly, the single colonies of bacteria, which initially grew on CMW, when inoculated in rich media, e.g., lysogeny broth, didn't grow on CMW anymore. A possible explanation is that each of the single colonies on CMW-media, in fact, has several different bacterial species and when sub-cultured through rich media one or more species, essential for CMW degradation, are lost. 16S rRNA gene analysis of few of the single colonies indicated that indeed different bacterial species inhabit each single colony. To determine which of the bacteria are actively involved in CMW catabolism we then performed next generation sequencing which revealed that abundance of only one bacterial strain from the Sphingomonadaceae family increased in the same manner as the amount of CMW decreased in the media, indicating that this strain might be the one degrading CMW.