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Biosynthetic pathway of L-gulose, a rare sugar existed in the main polar lipid of a thermophilic archaea

Yusuke Nakayama[1], Noriaki Yamauchi[2*]

[1]Grad School of Sci., Kyushu Univ., [2]Fac. of Sci., Kyushu Univ.

Thermophilic archaea live under harsh conditions such as high temperatures (60 to 100 C) and low pH (2 to 3). The resemblance of their living environment to primitive Earth conditions has been pointed out. The lipid of this species is characteristic ether-bonded isoprenoidal macrocyclic, which consists of a double-face monolayer membrane. Among them, *Themoplasma* has main polar lipid (MPL), which has L-gulose a characteristic rare sugar as a major component of polar head group of MPL. Themoplasma had no cell wall, the outer sphere of the body directly contact with the harsh environment. So, the biosynthesis of the characteristic component of MPL is intriguing at the point of the adaptation of organisms to the environment like a primitive Earth conditions.

L-Gulose was existed at the biosynthetic intermediate of L-ascorbic acid (vitamin C) in plants, and sugar component of bleomycin, a clinically important antitumor antibiotic produced by actinomyces. Biosynthetic pathways of L-gulose are existed all three domain of the category of living system, eubacteria, archaea and eucarya. This pathway might be shown an indicator of the correlation of three domains.

This study was aimed for elucidation of a biosynthesis pathway of L-gulose in Thermoplasma for the clue to the relationships of metabolic evolution of thermophilic archaea and the other two domain of living organisms. At first, classical chase experiment of labeled compound in the biosynthesis of MPL in *Thermoplasma acidophilum* was conducted.

At first, the deuterium-labeling compounds, D-[3-^{2}H] and [3,4-$^2H_2$]-glucose were synthesized. Then, incubation experiments with the labeled compounds into low-glucose-containing Themoplasma medium (7 days, 60 C, pH2.5) were performed. The lipid was extracted from the collected organism, and hydrolyzed to give a monosaccharide-containing fraction that was bonded to the MPL. The mixture was derivatized and the penta-O-TMS-monosaccharide mixture was analyzed by GC-MS. The mass spectra of the chromatographic peaks corresponding to L-glucose were further analyzed in detail, and the position and the fragments determined content of deuterium.

For the experiment with D-[3-^{2}H]-glucose, the C-3 hydrogen containing fragment (*m/z* 305 for non-labeled material) shows the highest fragment peaks at *m/z* 306, which means 1 deuterium was incorporated from the D-[3-^{2}H]-glucose to L-gulose attached at C-3. The degree of incorporation was about 40 %, extensively high for this kind of incorporation experiment. Next, for the experiment with [3,4-$^2H_2$]-glucose, almost the same result-high degree of incorporation of deuterium at C-3 was observed. This means the C-4 hydrogen was lost during the conversion of D-gluose to L-gulose.

A preliminary experiments revealed the L-gulose was biosynthesized with the step-by-step epimerization of C-2 and C-5 of D-glucose. And the possibility of most chemically simplest pathway (D-glucose can converted to L-gulose with 2 steps, reduction at C-1 and oxidation of C-6) was denied. Further, these experiments strongly indicate that the C-4 oxidation along with the enolization of C-4 and C-5 was occurred at the mechanism of stereochemical inversion of C-5. The mechanism was seen in the stereochemical inversion of hydroxyl group in the monosaccharide, and the close resemblance of this case and the biosynthesis of vitamin C were observed. In other words, L-gulose biosynthesized with a similar process existed in eukaryote (plant). And it suggests that the relation of the metabolism evolution of archaea and eucaryote compared with eubacteria at the point of metabolic evolution of monosaccharide. That is consistent with a molecular phylogenic tree made by 16S ribosomal RNA, and the distribution of two isoprenoidal biosynthetic pathways (mevalonate pathway vs MEP pathway), which also synthesize characteristic isoprenoidal lipid in archaea.

Keywords: thermophilic archaea, isoprenoidal lipid, rare sugar, biosynthesis, metabolic evolution