Possibility of substrate promiscuity at cyclase enzyme of calditol carbocycle from carbohydrate in Sulfolobus acidcardarius

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The lipid of thermophilic archaea is characteristic ether-bonded isoprenoidal macrocyclic lipid and consist of a double-face monolayer membrane. Furthermore, the genus Sulfolobus, has a characteristic lipid named carditoglycerolcaldoarchaeol (GDCT). GDCT has a 5-membered carbocycle bonded with glycerol by ether linkage. Gambacorta et al and our group performed the biosynthetic studies of calditol and the occurrence of cyclization of glucose to the 5-membered carbocycle was proved. However, the key step of cyclization, the involvement of catalytic oxidoreduction, was not shown by these results directly, while the inversion of C-4 stereochemistry at glucose to calditol was needed to occur. Furthermore, some of the enzymes of the central metabolism of Sulfolobus have shown to posses a substrate promiscuity that enables to catalyze glucose and galactose, the C-4 stereoisomer of hexose, with a similar degree. So, the observation of the fate of hydrogen of glucose and galactose at C-4 within the calditol formation in Sulfolobus acidcaldarius were performed.

At first, glucose and galactose labeled at C-4 were synthesized. Then, GDCT was isolated from hydrolysate of extracted lipid and converted to its acetate and 1H NMR was observed. The C-4 hydrogen at the carbocycle of calditol was highly deuterated. The deuterium incorporation from glucose was 35 %. Interestingly, the deuterium-incorporation experiment of galactose at C-4, the deuterium incorporation was 22 and 27 %. On the other hands, the deuterium of labeled gulose, C-3 stereoisomer of glucose, was not incorporated to the 5-memberd ring of calditol. The results indicate galactose was converted to the carbocycle of calditol with a similar extent, and suggest the substrate promiscuity at C-4 of hexose was also expressed to the cyclization enzyme of calditol. Further the lower incorporation of deuterium at the C-4 of glucose compared with the incorporation of glucose labeled at the C-1 and C-6 suggest the involvement of a single enzyme of cyclization and the oxidoreduction on the C-4 at the cyclization reaction.

The glycerol-labeling experiments, the origin of glycerol portion of calditol was not clarified by the experiment because no incorporation of deuterium was observed at the glycerol portion, however, the deuterium of [1,1-2H2]glycerol was incorporated to the C-3 hydrogen of the carbocyclic portion of calditol. This result suggests the efficient conversion of glycerol to glucose through the modified Entner-Doudoroff pathway and charactaristic KDG-aldolase existed in Sulfolobus.