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Thermus themophilus 由来グルタミン酸脱水素酵素における活性調節の分子機構の解析

Molecular mechanism for allosteric regulation of glutamate dehydrogenase from *Thermus thermophilus*

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Glutamate dehydrogenase (GDH) catalyzes the reversible conversion between glutamate (Glu) and 2-oxoglutarate (2-OG). GDH is a key enzyme that is responsible for amino acid biosyntheses and controls the flux of carbon/nitrogen metabolism.

Thermus thermophilus possesses two glutamate dehydrogenase genes, *gdhA* and *gdhB*, putatively forming an operon on the genome. To elucidate the functions of these genes, the gene products were purified and characterized. GdhA showed no GDH activity, while GdhB showed GDH activity for reductive amination 1.3-fold higher than that for oxidative deamination. When GdhA was co-expressed with his-tag fused GdhB, GdhA was co-purified with his-tagged GdhB. Compared to GdhB alone, co-purified GdhA/GdhB had decreased reductive amination activity and increased oxidative deamination activity, resulting in a 3.1-fold preference for oxidative deamination over reductive amination. Addition of hydrophobic amino acids affected GDH activity of the co-purified GdhA/GdhB hetero-complex. Among the amino acids, leucine had the largest effect on activity: addition of 1 mM leucine elevated GDH activity of the co-purified GdhA/GdhB by 974 % and 245 % for reductive amination and oxidative deamination, respectively, while GdhB alone did not show such marked activation by leucine. Kinetic analysis revealed that the elevation of GDH activity by leucine is attributed to the enhanced turnover number of GDH. In this hetero-oligomeric GDH system, GdhA and GdhB act as regulatory and catalytic subunits, respectively, and GdhA modulates the activity of GdhB through hetero-complex formation, depending on the availability of hydrophobic amino acids.

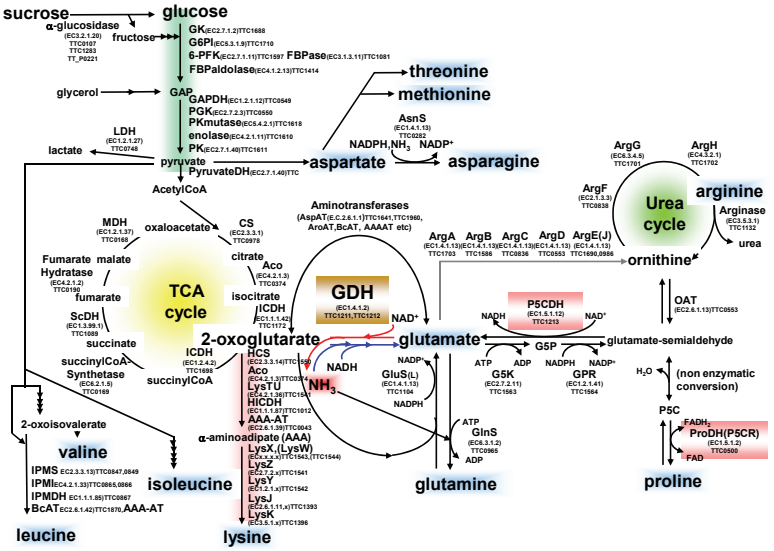

Fig. 1 Metabolic map around the GDH reaction of *T. thermophilus*

To elucidate the molecular mechanism of allosteric regulation of GDH, we determined the crystal structures of GdhA, GdhB/Glu complex, and GdhA/GdhB/Leu at 2.2, 2.1, and 2.6 Å resolution, respectively (Fig. 2). GdhA takes an unique homo-tetrameric structure which is composed of two pairs of dimer. Whereas the GdhB/Glu complex takes a homo-hexameric structure common to most GDHs of this family. The GdhA/GdhB/Leu complex is hetero-hexamer which is composed of four GdhA subunits and two GdhB subunits, forming two GdhA dimers and one GdhB dimer, respectively. GdhA subunit in this complex was superposed well with that in the structure of GdhA homo-tetramer with rmsd of 0.9 Å. In contrast, GdhB subunit in this complex was similar to that in the structure of GdhB homo-hexamer with rmsd of 0.4 Å. Six Leu molecules were bound at the interfaces of three distinct subunits. The six Leu binding sites were classified into three types according to the subunit types surrounding the Leu binding sites. The first is composed of two GdhB subunits and one GdhA subunit (Leu site 1), the second is composed of one GdhB subunit and two GdhA subunits (Leu site 2), and the third is composed of three GdhA subunits (Leu site 3). All the α-carboxyl and α-amino group of bound Leu were recognized by residues conserved in both subunits. The aliphatic side chains of Leu were recognized by hydrophobic residues for all the sites, however hydrophobicity of each site is higher in site 2 and site 3 than site 1. In the GdhB/Glu complex, Glu molecule was bound at the same site, suggesting the possible cause for 1.5-fold enhancement of the specific activity by addition of Glu. These facts suggest that ratio of GdhA dimer in the hexameric structure determines the specificity of amino acids at the effector binding site to exert complex allosteric regulation of GDH.

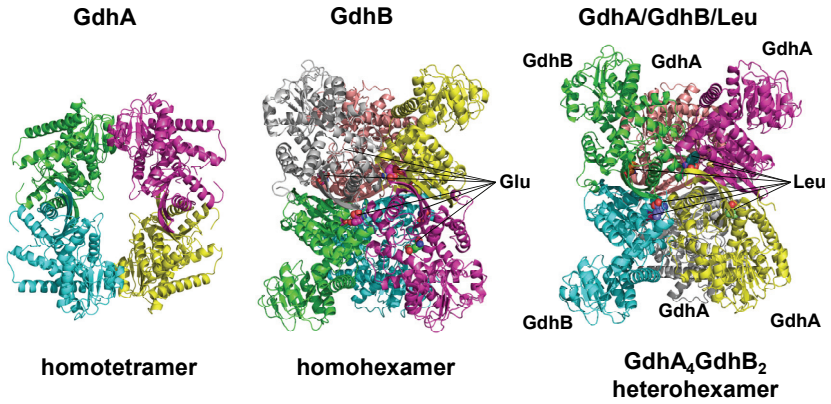

Fig. 2 Crystal structures of GdhA, GdhB/Glu, GdhA/GdhB/Leu