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Mechanism of allosteric regulation of glutamate dehydrogenase from *Thermus thermophilus*

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Introduction

Glutamate dehydrogenase (GDH) catalyzes reversible conversion between glutamate and 2-oxoglutarate using NAD(P)(H) as coenzymes. Although mammalian GDH is regulated by GTP through the antenna domain, little is known about the allosteric activation mechanism by leucine. As for the bacterial GDH lacking such a domain, the structural basis of the regulatory mechanisms has not yet been elucidated. *Thermus thermophilus* possesses GDH with a unique subunit configuration composed of two different subunits, GdhA (regulatory subunit) and GdhB (catalytic subunit)[1]. *T. thermophilus* GDH is unique in that the enzyme is subject to allosteric activation by leucine. To elucidate the structural basis for leucine-induced allosteric activation of GDH, we determined the crystal structures of the GdhB/Glu complex and GdhA/GdhB/Leu complex at 2.1 and 2.6 Å resolution, respectively.

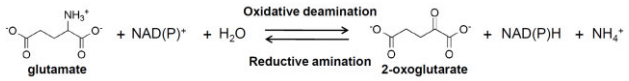

Fig. 1. Reaction of GDH.

Materials and Methods

Preparation of crystals – Crystallization of GdhB/Glu and GdhA/GdhB/Leu were performed by the hanging drop vapor diffusion method. For GdhB/Glu complex, the reserviour solution containing 0.1 M HEPES-NaOH, pH 7.0, and 0.6 M ammonium phosphate was used. For GdhA/GdhB/Leu complex, the reserviour solution containing 0.1 M sodium citrate tribasic pH 5.6, and 0.2 M ammonium phosphate monobasic was used.

Results and Discussion

Homo-hexameric structure of GdhB/Glu complex – GdhB takes a homo-hexameric structure similar to other bacterial and mammalian GDH50s (Fig. 2A). A hexamer binds twelve glutamate molecules; six molecules are bound at the active sites, while other six molecules are bound at the subunit interfaces. Glutamate molecules bound to the active sites are stabilized by specific interaction (Fig. 2B). We assume that this active sites may represents a low-activity state not activated by leucine. Six other glutamate molecules are bound at the interfaces of three subunits (Fig. 2C).

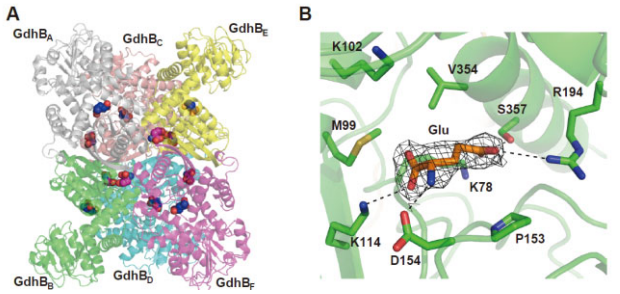

Fig. 2. Structure of GdhB/Glu complex. (A) Structure of GdhB/Glu complex. (B) Glutamate-binding site at the active site of GdhB/Glu complex.

Hetero-hexameric structure of GdhB/Glu complex – GdhA/GdhB forms hetero-hexameric structure, which is composed of four GdhA and two GdhB subunits (Fig. 3A). Although no substrate is found in the active site of GdhA/GdhB/Leu complex, six leucine molecules are found at the interfaces of three subunits (Fig. 3A and B). The six leucine binding sites can be classified into three types (sites 1, 2, and 3) based on the subunit organization. Here, we describe about the site 1, which is formed by three GdhA subunits. At site 1, leucine is recognized tight hydrophobic interaction and hydrogen bonds. The enzymes with mutations around leucine binding site in the crystal structure, exhibited markedly decreased sensitivity to leucine. These results demonstrated that these residues play major roles in binding leucine for allosteric activation.

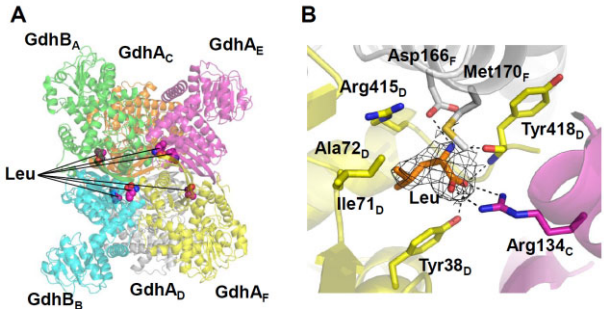

Fig. 3. Structure of GdhA/GdhB/Leu complex. (A) Structure of GdhA/GdhB/Leu complex. (B) Leu site 1 in GdhA/GdhB/Leu complex.

Reference

1) T. Tomita *et al. Microbiology*. **156**, 3801-13 (2010).

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