Poster

Biochemical and structural insights on pH-dependent cooperativity of glutamate dehydrogenase in *Saccharomyces cerevisiae*

Arpan Banerjee[1], Barsa K. J. Godsora[1], Parijat Das[1], Prasenjit Bhaumik[1*]

[1]*Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai-400076, India*

**Corresponding author: Tel 022-25767748,*

pbhaumik@iitb.ac.in

Glutamate dehydrogenase (GDH) catalyses the reversible oxidative deamination of L-glutamate to produce α-ketoglutarate in all living organisms. *Saccharomyces cerevisiae* expresses two NADP-specific GDHs: ScGDH1 and ScGDH3. Although these enzymes share 89% sequence identity, ScGDH3 shows pH-dependent cooperativity towards the substrate α-ketoglutarate, whereas the isoenzyme ScGDH1 exhibits canonical Michaelian kinetics [1]. The exact reason(s) behind the substrate- dependent alteration in kinetic behaviours among ScGDHs is/are unknown. We have determined the crystal and cryo-EM structures of ScGDH1 and ScGDH3 (Fig. 1A-D). Based on the sequence and structural analysis of ScGDH1 and ScGDH3, we hypothesized one substituted positively charged residue that is involved in mediating salt bridge interactions, might be crucial for inter-subunit allosteric communication. Hence, we made a point mutation in ScGDH3 (SDM-1) with its homologous residue present in ScGDH1. Contrary to the kinetic properties of the native enzyme, the ScGDH3 mutant completely lost cooperative behaviour irrespective of changes in the pH. Further, the point mutation was reversed to generate a ScGDH1 mutant (SDM-2). Interestingly, the point mutation has introduced cooperativity in the natively Michaelian ScGDH1 . These observations clearly indicate the critical role of the targeted residue in the kinetic regulation of ScGDH3. Further, the high- resolution crystal structure of ScGDH3 mutant (SDM-1) confirmed the alteration in surface charge distribution that contributes toward modulating the allosteric behaviour of ScGDH3 at different pHs. The results of our study would aid in understanding the allosteric regulations in other GDHs.

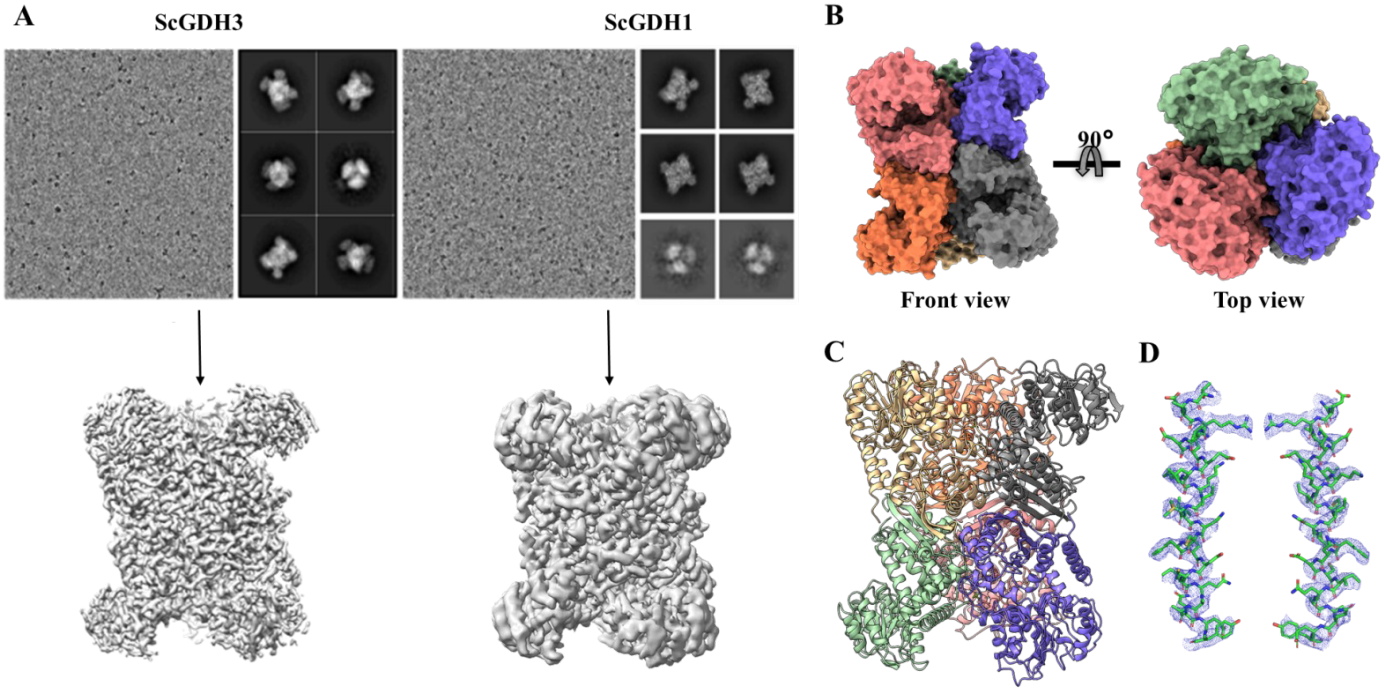

Figure 1. **(A)** Determination of the single-particle cryo-EM structures of apo-ScGDH3 and apo-ScGDH1. **(B)** Surface representation of hexameric apo-ScGDH3. **(C)** The cartoon representation shows overall structural fold of apo-ScGDH3. **(D)** Cryo-EM density map of two helices: showing density corresponds to the main chain and side chains of some amino acids.

[1] DeLuna, A., Avendano, A., Riego, L., & Gonzalez, A. (2001). *J. Biol. Chem.* **276**, 43775.