Glutamate dehydrogenase (GDH) reversibly catalyzes the NADP or NAD dependent oxidative deamination of L-glutamate to form 2-oxoglutarate and ammonia. Despite several studies, the structural basis of coenzyme specificity and allosteric property of the NADP dependent enzymes remain elusive. No structure of fungal enzyme is available till date. We have determined the first crystal structure of Aspergillus niger glutamate dehydrogenase (AnGDH), a unique NADP dependent fungal allosteric enzyme that gets forward inactivated by formation of mixed disulfide. The structures of the active enzyme have been determined as its apo form, complexed with NADPH and 2-oxogultarate (AnGDH-NADPH-AKG), as well as its complex with inhibitor (isophthalate) and NADPH (AnGDH-NADPH-IPT) at resolutions of 2.8, 1.8 and 1.9 Å, respectively. The high resolution (1.9 Å) structure of the forward inactivated enzyme (fAnGDH) is also solved. The hexameric structure of apo-AnGDH is unique and reveals several inter-subunit contacts may be responsible for enzyme’s cooperative behaviour. Structural analysis shows significant substrate and coenzyme induced structural changes that have not been observed before. The complexed structures also reveals for the first time, that the phosphate group of NADPH is anchored to the enzyme through polar interactions involving residues Lys122, Gin282, Ser253, Lys277, and His84. A crystal structure of AnGDH as a complex with a reaction intermediate -iminoglutarate has been determined at 1.7 Å resolution. The analysis indicates important active site structural features involved in positioning 2-oxoglutarate, NADPH and ammonium ion in the AnGDH active site and explain the reaction the catalytic mechanism for the first time.

References:

Keywords: glutamate dehydrogenase, Aspergillus niger, crystal structure