the present structure is in the apo form and differs significantly in conformation with the holo form. In comparison with the *A. vinelandii* holoenzyme structure, which displayed a closed conformation which is inaccessible to isocitrate, the present structure shows the enzyme in an open conformation that allows the substrate access to the active site.

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Keywords: isocitrate dehydrogenase, citric acid cycle, coenzyme specificity

MS93.P22

Acta Cryst. (2011) A**67,** C776

TrpS – evolutionary paths to catalytic efficiency

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The efficiency of metabolic pathways in the cell largely relies on enzyme association processes that lead to the *compartmentation* of biochemical reactions. This allows to overcome barriers in metabolite production derived from the high intracellular concentrations of compounds (*cellular crowding*) that affect diffusion, equilibria, reactions rates and side reactions. It also prevents cross-talk between competing pathways and relieves cellular stress by maintaining high local concentrations of compounds but low total cellular levels. In forming metabolic compartments, multifunctional enzymes (permanent or transient) are formed that couple sequential reactions. Often, enzymes within a pathway not only evolve mutual recognition, but also complex modes of communication that allows them to share small compounds and substrates (*channelling*) as well as to mutually control their activities (*allostery*).

Tryptophane synthase (TrpS) is a well established model system for the study of substrate channelling and allostery in enzymes. TrpS consists of a central beta$_2$-dimer flanked by two alpha-subunits in a linear a-b-b-a arrangement. The alpha-subunit cleaves indole-glycerol-phosphate into glyceraldehyde-phosphate and indole, while subunit beta condenses indole with L-serine to yield L-tryptophan using pyridoxal phosphate (PLP) as cofactor. Catalytic efficiency is achieved by the channelling of indole from the active site of subunit alpha to that of beta through a hydrophobic, 25 Å long interconnecting tunnel. The reactions of the active sites are kept in phase by switching both subunits between open and close conformations to prevent indole loss. Further, the subunits mutually activate each other through the transmission of allosteric effects. Thus, the TrpS catalytic cycle requires a delicate orchestration of structural changes in the enzyme machinery [1]. Recently, subunits alpha and beta have been found as isolated proteins –TrpA and TrpB– in archea genomes. These variants, thought to be evolutionary remnants, display crippled association properties and distinct catalytic efficiencies [2]. Aiming to retrace the evolution of the sophisticated TrpS machinery from rudimentary TrpA and TrpB modules, we have elucidated the crystal structures of these TrpA and TrpB variants as well as their transient complex. The models serve as templates to analyse the role of indels (insertion and deletion sequence motifs) as dominant mechanistic factors in the evolution of catalytic features. We conclude that inter-subunit communication in TrpS is the result of a "gain-of-function" in its component subunits caused by motif deletions. Further, we uncover an alternative enzymatic strategy by which the simpler TrpA and TrpB enzymes achieve catalytic efficiency as high as that of TrpS, but in the absence of intricate subunit interrelations. This work sheds light, from a structural perspective, on the diversification of enzymatic strategies to catalytic proficiency.

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Keywords: tryptophan synthase, enzyme evolution, indels

MS93.P23

Acta Cryst. (2011) A**67,** C776

Distant residues of maize glutamine synthetase affecting substrate affinity

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Glutamine synthetase (GS, EC 6.3.1.2) is an enzyme that catalyzes the incorporation of ammonia into glutamate to form glutamine with concomitant hydrolysis of ATP. Plant glutamine synthetase plays a crucial role in the assimilation and re-assimilation of ammonia derived from a wide variety of metabolic processes during plant growth and development.

Glutamine synthetase from eucaryotes has an oligomeric structure consisting of 10 identical subunits to form two pentamer rings with 52 symmetry. The ten catalytic centers are located between two adjacent subunits in each ring, and the two rings are held together by residues around Phe150, which are distant from the catalytic centers. Kinetic study of P146G, F150G and delF150 mutant enzymes with crystallographic study of F150V and F150G revealed that the ring-ring contact sites are crucial for high affinity for the substrate glutamate and the glutamate may be incorporated into the catalytic centers through the regions near the two ring interfaces.

Alanine-scanning mutagenesis around Phe150 and kinetic study revealed that residues responsible for increasing K_m for glutamate are located at the regions spanning the ring-ring interfaces and the entry sites of glutamate into the catalytic centers. These residues are distant from the catalytic centers and hence are likely to contribute to the affinity of glutamate to the enzyme in addition to the affinity of the catalytic centers themselves for glutamate. To verify this working hypothesis crystals of two mutants G241A and W243A in these regions distant from the catalytic centers were prepared and analyzed at 2.55Å and 2.80 Å resolutions, respectively. The refined crystal structures of them indicated that the structures except for the mutated residues are similar to the wild type enzyme, without significantly changing the structure of the catalytic centers. This study supports the structural elements separate from the catalytic centers contribute to increasing the affinity for substrate glutamate.

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Keywords: enzyme, crystal, plant