

STRUCTURAL AND FUNCTIONAL STUDIES OF PROLINE CATABOLIC ENZYMES

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ABSTRACT

Catabolism is the oxidation of organic nutrients into simple end molecules to extract energy. For proline, the catabolic pathway involves two enzymes, L-proline dehydrogenase (PRODH, EC 1.5.99.8) and L- Δ^1 -pyrroline-5-carboxylate dehydrogenase (P5CDH, EC 1.5.1.12). Via the action of PRODH and P5CDH, proline can be utilized both as a carbon and nitrogen source. The conventional view of proline catabolism was that PRODH and P5CDH appear as separate enzymes in eukaryotes and as fused bifunctional enzymes (PutA) in bacteria. Analysis of genome sequence data, however, revealed a more complex situation for bacteria. The updated view is that PutAs are indeed restricted to bacteria, but monofunctional PRODHs and P5CDHs appear in both eukaryotes and bacteria. One of these newly discovered bacterial monofunctional PRODHs was chosen and characterized. This work resulted in kinetic and structural analysis of PRODH from *Thermus thermophilus*. *T. thermophilus* PRODH was also used for studying mechanism-based inactivation by *N*-propargylglycine. This work has also resulted in the first structure of a covalently modified PRODH as well as characterization of inactivation kinetics. Physical and functional interactions between monofunctional *T. thermophilus* PRODH and P5CDH utilizing coexpression have also been studied and preliminary results reported. Finally,

the structure determination of *Bradyrhizobium japonicum* PutA from pseudomerohedrally twinned crystal is reported.