

Evolutionary conservation of structure and function in the plant aldehyde dehydrogenase 12 family

The aldehyde dehydrogenase (ALDH) superfamily of enzymes catalyzes a broad range of biologically important reactions. Although several ALDH subfamilies have been thoroughly characterized structurally, biochemically, and genetically, other ALDH superfamily members remain uncharacterized. One such subgroup is ALDH12 from plants. ALDH12 has been annotated as possessing the function of L-glutamate- γ -semialdehyde dehydrogenase (GSALDH, also called ALDH4A1). GSALDH proteins function in the second step of proline catabolism, which is a two step process in which proline is converted to glutamate. Interestingly, ALDH12 family members from plants bear very low sequence identity to the annotated GSALDH isozymes (<30% identity). Herein, we describe the structural and biophysical characterization of *Zea mays* ALDH12. Further, we document a structure determination pipeline for solving the phase problem for proteins that share very low sequence identity to proteins currently in the Protein Data Bank. ZmALDH12 is a novel member of the GSALDH family of enzymes. Although the active site bears strong resemblance to ALDH4A1 isozymes from other organisms, the overall quaternary structure is novel for GSALDHs. SAXS and analytical ultracentrifugation analysis reveal ZmALDH12 is tetrameric in solution. Shape reconstruction from experimental SAXS data reveals the in solution tetramer is consistent with the *in crystallo* tetramer. Overall, our analysis provides both a method for difficult structural determination by molecular replacement and structural analysis of a novel plant enzyme that broadens the complexity of the ALDH superfamily.

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