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**First Structure of a Monofunctional Proline Dehydrogenase Involved in Reactive Oxygen Species Generation.**

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Nature recycles proline by converting it to glutamate. This 4-electron oxidation process is catalyzed by the sequential actions of two enzymes, the flavoprotein proline dehydrogenase (PRODH) and the NAD-dependent enzyme  $\Delta^1$ -pyrroline-5-carboxylate dehydrogenase. Human PRODH is part of the p53 signaling pathway and up-regulation of PRODH in lung, renal, and colon carcinoma cells has been shown to generate reactive oxygen species (ROS) and induce cell death by apoptosis. Also, mutations in PRODH have been linked to increased schizophrenia susceptibility. The molecular mechanism of ROS generation by PRODH and the molecular consequences of schizophrenia-linked mutations have been poorly understood due to a lack of biochemical and structural information for human PRODH, which is a mitochondrial inner membrane protein. We used bioinformatics analysis to show that PRODH homologs exist in Gram-positive bacteria and then targeted PRODH from *Thermus thermophilus* (TtPRODH) for structure determination and biochemical study. TtPRODH was crystallized in the presence of MPD and the detergent n-octyl  $\beta$ -D-glucopyranoside. The structure was solved to 2.0 Å resolution using SAD phasing from a selenomethionyl derivative combined with two-fold NCS averaging. The structure reveals a unique  $\beta_8\alpha_8$  barrel with the FAD bound at the carboxyl terminal end of the strands of the barrel. Unexpectedly, the FAD isoalloxazine is highly solvent exposed, which contrasts the highly buried FAD bound to PRODH domain of bifunctional Proline Utilization A (PutA). Biochemical studies showed that TtPRODH, like human PRODH, generates proline-dependent ROS. Our structure suggests that the solvent exposed active site of TtPRODH underlies the observed ROS production by this enzyme.