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Structure–Function Relationship in the Tocopherol Transfer Protein

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ABSTRACT: The role of specific amino acid residues in mediating the biochemical functions of tocopherol transfer protein (TTP) was investigated using site-directed mutagenesis and functional assays. These findings further current understanding of TTP mechanism of action and its role in human health.

KEYWORDS: tocopherol transfer protein; vitamin E

Tocopherol transfer protein (α-TTP) is soluble 32-kDa protein expressed in liver that selectively binds alpha tocopherol. That TTP plays a critical role in vitamin E transport is evident from the physiological impact of mutations in the TTP gene. Thus, humans harboring heritable mutations in the TTP gene display low plasma vitamin E levels and a neurodegenerative syndrome termed ataxia with vitamin E deficiency (AVED). Essentially identical symptoms arise in mouse models in which TTP expression is specifically disrupted. In cultured hepatocyte cell lines, overexpression of TTP leads to enhanced secretion of cellular tocopherol to the media. From these observations a model for TTP function was developed in which the protein somehow regulates a novel, alpha-tocopherol–specific secretion pathway. However, the molecular mechanisms underlying this activity are, at present, unknown. *In vitro* characterization of TTP relies on the protein's "signature" activity, namely, the facilitation of tocopherol transfer between lipid bilayers. Neither the molecular mechanism underlying tocopherol transfer, nor its relevance to TTP function *in vivo* are well understood. To arrive at a better-defined picture of TTP function, we used site-directed mutagenesis to generate a series of substitution mutations in the protein. In our choices of target residues for mutagenesis, we were guided by the naturally ocurring mutations in human AVED patients, and by the recently solved three-dimensional structure of TTP. To test the functionality of the TTP mutants, we purified them from overexpressing bacteria, and measured their tocopherol transfer activity *in vitro*. We have also utilized urea-induced denaturation experiments to assess

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overall folding and stability of the mutated proteins in comparson to the wild-type TTP.

We find that all AVED TTP mutations retain significant tocopherol transfer activity. Mutations associated with the severe, early-onset form of the disease (namely, the R59W, E141K and R221W substitutions) exhibit a 2–3-fold kinetic impairment in the ability to catalyze tocopherol transfer). TTP variants possessing the mild, later-onset AVED substitutions (R192H, H101Q, and A120T) or a mutation in the protein's basic patch (R68A) exhibited tocopherol transfer activities that were virtually indistinguishable from the wild-type protein. These results show that TTP's activity in the tocopherol transfer assay *in vitro* does not fully capture its biochemical activities *in vivo*. Thus, we propose that for proper maintainance of tocopherol status *in vivo*, TTP is likely to have additional, as-yet unknown functions, such as interactions with other cellular components.

We also find that occupancy of the ligand-binding pocket significantly protects TTP against denaturant-induced unfolding. Possibly TTP's amphipathic helical "lid," which changes conformation upon tocopherol binding, contributes significantly to the overall stability of the protein.