

Crystal Structure of Human α -Tocopherol Transfer Protein Bound to its Ligand: Implications for Ataxia with Vitamin E Deficiency

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Human α -tocopherol transfer protein (ATTP) plays a central role in vitamin E homeostasis, preventing the degradation of α -tocopherol (α -T), a lipophilic molecule, by routing it for secretion by hepatocytes (liver cells). Mutations in the gene that encodes ATTP have been shown to cause a severe deficiency in α -T, which results in a progressive neurodegenerative spinocerebellar ataxia, a disorder of neurons primarily in the brainstem and spinal cord, known as ataxia with vitamin E deficiency (AVED). We have determined the high-resolution crystal structure of human ATTP with (2R, 4'R, 8'R)- α -T in the binding pocket. One mutation associated with human disease is located directly in the binding pocket, likely disrupting binding to α -T.

Ataxia with Vitamin E Deficiency (AVED) is a recessively inherited condition in which afflicted individuals develop problems with balance, typically starting in the late teen years. As the disease progresses, many individuals are limited to a wheelchair, lose sensation in their hands and feet, and have difficulties with slurred speech. Some develop retinitis pigmentosa, a blinding condition caused by the degeneration of the retina. Mutations in α -tocopherol transfer protein (ATTP) have been shown to cause AVED.

ATTP exists in the fluid portion of a cell's cytoplasm and is expressed mainly in the liver. The heterologous expression of ATTP in cell culture confers its ability to secrete α -T by a non-Golgi pathway, preventing the degradation of α -T. Patients with AVED absorb α -T from their diets normally, but have nearly immeasurable steady-state plasma levels due to the defective incorporation of α -T for transport with very low density lipoprotein. A transgenic mouse in which the ATTP gene was deleted replicated the phenotype of the human disease.

There are eight forms of vitamin E (including α - δ tocopherol and α - δ tocotrienol), which differ in two structural features: the degree of methylation (decreasing from α -T to δ -T) of the chroman ring and saturation of the phytyl tail. All forms are potent lipophilic antioxidants, but α -T is the most biologically potent due to the action of ATTP. Of the eight possible stereoisomers, i.e. variations, of α -T, only (2R, 4'R, 8'R)- α -T occurs naturally. Synthetic vitamin E supplements are racemic mixtures, but ATTP is sensitive to stereochemistry and particularly so at the C2 stereocenter.



The high-resolution 1.5Å structure of ATTP in a complex with (2R, 4'R, 8'R)- α -T was determined from multiwavelength anomalous diffraction (MAD) data measured at beamline X4A using a selenomethionyl recom-

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binant protein crystal. The structure of ATTP is composed of two domains: an N-terminal all-helical domain and a C-terminal domain, which at its core is composed of a $\beta\alpha\beta\alpha\beta$ fold and contains the binding pocket for α -T (**Figure 1**). The most striking feature of the structure is the buried, solvent-inaccessible binding pocket, which would require significant conformational changes to release α -T. Most of the interactions between ATTP and α -T are through van der Waals contacts, although there are three well-ordered water molecules that appear to form a hydrogen bonding network with the hydroxyl group of α -T (**Figure 2A**).

In ATTP, a number of missense mutations – which occur when one DNA base pair is substituted for another – have been associated with AVED. One mutation, L183P – a change from one amino acid, leucine, to another, proline – was found to map directly to the binding pocket (**Figure 2A**). The sidechain of L183 has extensive van der Waals contacts with α -T, and a change to proline at this position would not only be expected to disrupt these interactions, but would also affect the residues in the nearby α -helix that also form contacts with α -T. When expressed in *E. coli*, the L183P mutant was insoluble and could not be further characterized.

Three other missense mutations, R59W (arginine-to-tryptophan), R192H (arginine-to-histidine), and R221W, involve a change in positively charged residues, which help to form a prominent positively charged surface on ATTP (**Figure 2B**). Although the function of this region is not understood, it seems unlikely to affect binding to α -T directly. We have proposed that it may represent a site of protein-protein or protein-lipid interaction that regulates the release of α -T from the binding pocket of ATTP. Interestingly, a similar mutation (R233W) in a related protein, cellular retinaldehyde binding protein, causes a hereditary retinopathy, suggesting that this region of the protein may have a conserved function across multiple members of this family of lipophilic transfer proteins.

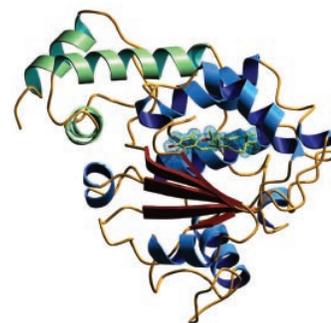


Figure 1. Ribbon diagram of ATTP viewed down the β -sheet. α -T is colored in yellow. The N-terminal domain helices are indicated in green, and the C-terminal domain's helices are in blue and its strands in red. The electron density that covers α -T is drawn as a blue mesh at 1σ .

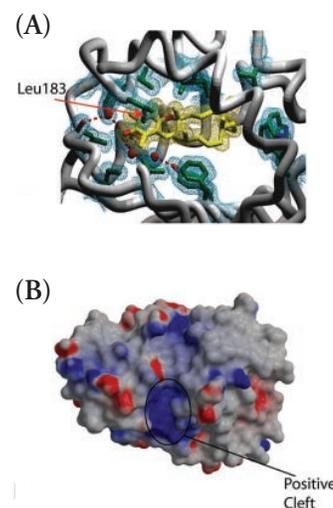


Figure 2. (A) Close-up view of the ligand binding pocket. Side chains (in green) that form van der Waals contacts with α -T (in yellow) or participate in hydrogen-bonding networks in the ligand-binding pocket are shown. Three well-ordered water molecules located in the binding pocket are shown as red spheres. The protein backbone is shown as a worm model, excluding residues 216-220 and 249-275 for clarity. Electron densities that cover the residues of interest, α -T, and three water molecules are drawn as mesh at 1σ . Dashed red lines indicate hydrogen bonds. (B) Electrostatic potential surface map of ATTP. A positively charged cleft indicates where a number of disease-causing mutations are located.