

Exploring the binding of α -tocopherol, with human transferrin using computational and spectroscopic approaches: Implication of plant-derived antioxidant in neurodegenerative diseases

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α -Tocopherol-Transferrin Binding, Robust Computational and Experimental Validation, Preservation of Transferrin Stability with Functional Relevance, A Foundation for Iron-Targeted Neuroprotective Drug Development

INTRODUCTION	METHODS	RESULTS	CONCLUSIONS	REFERENCES
Aberrant iron homeostasis is increasingly recognized as a central contributor to oxidative stress, protein aggregation, and progressive neuronal dysfunction in Alzheimer's disease. As the principal iron-transport glycoprotein, human transferrin (Htf) plays a pivotal role in regulating cerebral iron distribution, making it an attractive molecular target for therapeutic intervention. α-Tocopherol (vitamin E), a potent lipophilic antioxidant, has demonstrated neuroprotective potential; however, the molecular basis underlying its interaction with Htf remains unresolved. To address this knowledge gap, we employed an integrated computational and biophysical workflow comprising molecular docking, molecular dynamics simulations, fluorescence spectroscopy, and isothermal titration calorimetry to characterize the binding mechanism, stability, and thermodynamic profile of the α-tocopherol–Htf complex at atomic resolution.	A. IN SILICO APPROACH 1 Protein & Ligand Preparation Human Transferrin (Htf) α-Tocopherol - Htf structure retrieved from PDB (ID: 3QYT) - Protein cleaned, optimized and prepared - Ligand structure obtained from PubChem and energy minimized 2 Molecular Docking Blind docking using InstaDock and QuickVina-W - Best binding pose selected based on binding affinity - Interaction analysis using PLIP 3 Molecular Dynamics Simulations 200 ns MD simulation using GROMACS - Force field: CHARMM36 - Water model: SPC/L - System neutralized with ions 4 Trajectory Analysis RMSD, RMSF, Radius of gyration (Rg) and SASA - Structural stability and flexibility assessment 5 Advanced Analyses Hydrogen Bond Analysis Principal Component Analysis (PCA) Free Energy Landscape (FEL) - Intramolecular & intermolecular H-bonds - Conformational sampling (PCA) - Free energy landscape mapping 6 Binding Insight Identification of key interacting residues and stable binding mode of α-tocopherol in Htf B. IN VITRO EXPERIMENTAL VALIDATION 1 Fluorescence Binding Assay Intrinsic fluorescence quenching of Htf upon addition of α-tocopherol (0–4 μM) - Modified Stern–Volmer analysis to determine binding constant (K) - Indicates formation of a stable Htf–α-tocopherol complex 2 Isothermal Titration Calorimetry (ITC) Measurement of heat change upon titration of α-tocopherol into Htf - Determines thermodynamic parameters: K_d, ΔH, ΔS and stoichiometry (n) - Confirms spontaneous, endothermic and high-affinity binding 3 Data Integration & Validation - Complementary computational and experimental approaches corroborate the stable binding of α-tocopherol with Htf	This study integrates molecular modeling with complementary biophysical approaches to systematically characterize the interaction between α-tocopherol and human transferrin at the molecular level. The agreement between molecular docking, molecular dynamics simulations, fluorescence spectroscopy, and isothermal titration calorimetry provides consistent evidence for stable, spontaneous, and high-affinity complex formation with minimal perturbation of transferrin structural integrity. Importantly, the identified interaction near functionally relevant iron-coordinating residues offers a plausible mechanistic basis for further investigating the influence of α-tocopherol on transferrin-mediated iron homeostasis. Although the functional consequences of this interaction require validation in cellular and in vivo systems, these findings establish a robust molecular framework for future studies exploring antioxidant-mediated regulation of iron metabolism in neurodegenerative diseases.	Loeffler, D. A., et al. "Transferrin and iron in normal, Alzheimer's disease, and Parkinson's disease brain regions." <i>Journal of neurochemistry</i> 65.2 (1995): 710-716. Shamsi, Anas, et al. "Exploring the binding of cinchonine with human transferrin: combined experimental and computational approaches." <i>Scientific Reports</i> 15.1 (2025): 40660.	
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