Kinetic Stabilization of Transthyretin and its Role as an Inhibitor of Aβ Amyloid Formation

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i Lilla Hörsalen, KB.E3.01, KBC-huset, fredagen den 17 mars, kl. 10:00. Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Professor Gunilla Westermark, Department of Medical Cell Biology, Uppsala university, Sweden.
Abstract
Amyloid formation occurs when normally soluble proteins and peptides misfold and aggregate into intractable threadlike structures called fibrils. There are currently more than 30 proteins associated with this aberrant structure, including the Aβ peptide in Alzheimer’s disease (AD) and transthyretin (TTR) in TTR amyloidosis. TTR is a homotetrameric transporter protein present in both cerebrospinal fluid and plasma. Dissociation of its tetrameric structure is required for the formation of amyloid fibrils. Small molecule ligands able to bind and stabilize the tetrameric structure of TTR thus represent a potential therapeutic intervention. Interestingly, apart from TTR’s role as a toxic agent in TTR amyloidosis, it also has a role as an inhibitor of the Aβ toxicity associated with AD. The work presented in this thesis focused on small molecules that have the potential ability to prevent TTR amyloidosis. We also sought to gain a greater understanding of the interaction between TTR and the Aβ peptide with respect to Aβ fibril formation.

The ability of a drug to stabilize TTR is directly correlated to its binding affinity. However, since TTR is a plasma protein, it is of great importance that the drug binds selectively to TTR. In paper I, we used a newly developed urea denaturation assay, in combination with isothermal titration calorimetry, to show that, in a complex environment such as plasma, the enthalpy of binding correlates better with a drug’s ability to stabilize TTR than the binding affinity. In paper II, we modified the highly selective but rapidly degraded TTR ligand luteolin in order to increase its resistance against biotransformation. Using a liver-based microsome assay, in combination with HPLC, we show how the luteolin analogues have gained increased stability. However, using the urea assay, we also show that the analogues have lost much of luteolin’s selectivity. In paper III, we show that tetrabromobisphenol A is a highly selective binder of TTR in plasma and is able to rescue cells from TTR-induced toxicity. In paper IV, we studied the interaction of TTR with Aβ and its effect on Aβ fibril formation. We used a ThT fluorescence-based assay and dot blotting to show that TTR inhibits Aβ amyloid formation and promotes the formation of high molecular weight assemblies with an open N-terminus. Using surface plasmon resonance, we further show how TTR is unable to inhibit fibril elongation and instead targets the nucleation processes, both primary and fibril-catalyzed secondary nucleation. To conclude, we present new molecules with the ability to selectively stabilize TTR that can serve as scaffolds in drug design. We also elucidate TTR’s inhibiting effects on toxic Aβ amyloid formation.

Keywords
TTR, Aβ, kinetic stabilizer, luteolin, amyloid, fibrillation, urea denaturation assay, selectivity, ThT assay, liver microsomes, ITC