Description of zinc-binding sites on human transthyretin: implication for amyloidogenesis

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Transthyretin (TTR) is a human serum protein involved in a series of amyloidosis with no available pharmaceutical treatment. It has been shown that in humans zinc homeostasis might be involved in the modulation of expression, as well as serum concentration of TTR and recently zinc was found as the major mineral component of *ex vivo* ocular amyloid fibrils of TTR. In the present study, we determined X-ray structures at atomic resolution of TTR in pH ranging from 7.5 to 4.6 in the presence of Zn(II) to unravel if there is any putative zinc binding site. Structures of Zn(II):TTR complex revealed four classical zinc binding sites per monomer and a dissimilar structure compared to TTR in the absence of zinc, having large deviations in some regions but a topological folding nearly identical of Zn(II) free TTR. The most striking difference in Zn(II):TTR complex is observed in the a-helix region. Curiously, recent structure of TTR determined at acidic, amyloidogenic condition also showed perturbations in that region, suggesting that these changes might be related to its amyloidogenic potential. Further analysis by using TROSY – HSQC of $ZnCl_2$ titrations over TTR H^2/N^{15} elucidated the pathway of Zn(II) binding as well as the structural perturbation previously observed by X-ray and that probably one site found in crystallographic structure exist only stabilizing the crystal and not in solution. These data indicates that zinc homeostasis might regulate TTR binding of holo-retinol binding protein, once a-helix region is known to mediate interaction with this protein.

Transthyretin; zinc; amyloidosis.