High pressure as a effective tool to induce dissociation and aggregation of a very stable tetramer of transthyretin

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Transthyretin (TTR) is a protein present in plasma and in the cerebral spinal fluid being involved in a several amyloid diseases, which seem to be triggered by tetramer dissociation and partial monomer unfolding that subsequently aggregate into amyloid fibrils. Mice transgenic for few copies of amyloid-prone human TTR variants, including the aggressive Leu55Pro variant failed to develop deposits. Silencing the murine TTR (Mu-TTR) gene in the presence of Leu55Pro human gene resulted in aggregate deposits. In the present study we investigate the effects of HP on the tetramer of Mu-TTR by following the changes in intrinsic and extrinsic (bis-ANS binding) fluorescence. Differently of human TTR, Mu-TTR showed a strong dependence on the pH being more effectively dissociate by HP at more acid pH (pH 5.0). HP induces dissociating of Mu-TTR showed concentration dependence and a strong dependence on time suggesting it dissociation-unfolding is a slow process. TTR binds bis-ANS into the thyroxine-bind channels. At pH 7.0 and at a moderate pressure values, bis-ANS unbinds suggesting that probably these channels are being disrupted and consequently that the tetramers are being dissociate. As pressure is further increased, bis-ANS binds again to the protein, suggesting that under high pressure the apart monomers are not completely unfolded retaining part of the tertiary fold. At pH 5.0, the bis-ANS profile was different and at an elevated pressure values the apart monomers did not bind bis-ANS, suggesting they are unfolded. Interestingly, after pressure removal at pH 5.0 and 37?, Mu-TTR aggregates forming congo-red positive aggregates wich under EM imaging presented as a intertwined fibrils.