LIGAND-MEDIATED REGULATION OF SERUM ALBUMIN FIBRIL FORMATION

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Amyloid fibrils are protein aggregates that form from monomers through a complicated mechanism which is still not fully understood. While many proteins exhibit a lag phase of fibril formation due to a slow nucleation stage, serum albumins near their denaturation temperature and acidic or neutral pH levels aggregate rapidly without a lag phase. Despite many examples of the inhibition of albumin fibrillization having been described in literature, kinetics and mechanism of inhibition have not been thoroughly studied.

In our work, we investigated the fibril formation of bovine and human serum albumins at 65 °C or higher temperatures and pH 7 in the presence of a series of organic ligands, predominantly clinical drugs. Kinetic curves of fibril formation were obtained using a Thioflavin T spectrofluorimetric assay. Additionally, albumin denaturation curves in the presence of the studied compounds were obtained using differential scanning calorimetry (DSC).

It was shown that albumin fibril formation obeys reversible first-order kinetics, and is not sensitive to seeding with preformed fibrils. The addition of substances exhibiting high albumin affinity significantly suppresses fibril growth and decreases the final yield. Using DSC data, the fraction of unfolded protein form α at the incubation temperature was determined for each ligand, concentration, and temperature. The initial aggregation rate appears to be proportional to α, and the final fibril yield is governed by the equilibrium between the protein monomer and fibrillar aggregates, which shifts towards the monomer by binding ligands. The results indicate that albumin fibrils are formed only from its unfolded form which slowly transforms into some specific, aggregation-prone conformation that quickly aggregates into fibrils. Strong native protein binders act as the best inhibitors of fibril formation, which appears to be a general rule for many different globular proteins.