Differential mode of interaction of ThioflavinT with native structural motif in Human 1-acid glycoprotein and cross beta sheet of its amyloid: Biophysical and molecular docking approach

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Abstract:

The present study details the interaction mechanism of Thioflavin T (ThT) to Human 1-acid glycoprotein (AAG) applying various spectroscopic and molecular docking methods. Fluorescence quenching data revealed the binding constant in the order of 104 M1 and the standard Gibbs free energy change value, G = 6.78 kcal mol1 for the interaction between ThT and AAG indicating process is spontaneous. There is increase in absorbance of AAG upon the interaction of ThT that may be due to ground state complex formation between ThT and AAG. ThT impelled rise in -sheet structure in AAG as observed from far-UV CD spectra while there are minimal changes in tertiary structure of the protein. DLS results suggested the reduction in AAG molecular size, ligand entry into the central binding pocket of AAG may have persuaded the molecular compaction in AAG. Isothermal titration calorimetric (ITC) results showed the interaction process to be endothermic with the values of standard enthalpy change H0 = 4.11 kcal mol1 and entropy change TS0 = 10.82 kcal.mol 1. Moreover, docking results suggested hydrophobic interactions and hydrogen bonding played the important role in the binding process of ThT with F1S and A forms of AAG. ThT fluorescence emission at 485 nm was measured for properly folded native form and for thermally induced amyloid state of AAG. ThT fluorescence with native AAG was very low, while on the other hand with amyloid induced state of the protein AAG showed a positive emission peak at 485 nm upon the excitation at 440 nm, although it binds to native state as well. These results confirmed that ThT binding alone is not responsible for enhancement of ThT fluorescence but it also required beta stacked sheet structure found in protein amyloid to give proper signature signal for amyloid. This study gives the mechanistic insight into the differential interaction of ThT with beta structures found in native state of the proteins and amyloid forms, this study reinforce the notion that ThT is amyloid specific dye and interacts differently with the beta structures in native protein and that of the structures found in aggregated form of the same protein.

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