THE MECHANISM OF CYANINE DYE BINDING TO LYSOZYME AMYLOID FIBRILS

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Near-infrared cyanine dyes have found numerical applications in biomedical research. For example, these probes have been employed as noncovalent labels for detection of proteins, nuclear acids, lipids due to their high extinction coefficients and long-wavelength absorption maxima. Notably, strong van der Waals, H-bonding, electrostatic, steric, hydrophobic and stacking intermolecular interactions give rise to the formation of the dye self-associates in solution. Specific type of protein aggregates – amyloid fibrils, have been considered as one of the predominant pharmaceutical targets for early detection and treatment of a number of human pathologies, including Alzheimer's disease, systemic amyloidosis, type II diabetes, etc. The present study was aimed at developing of the new approach for bioimaging applications, based on the shift of the monomer–aggregate equilibria of heptamethine dyes AK7-5 and AK7-6 upon their binding to lysozyme amyloid fibrils, as well as the investigating the molecular mechanism of the dye-protein complexation. Indeed, disruption of the H-aggregates was observed upon the dye complexation with fibrillar lysozyme, resulting in the substantial reduction of the H-aggregate absorption band and the enhancement of the monomer absorption band. Analysis of the absorbance changes induced by the dye titration with fibrillar lysozyme, taking into account the dye monomer–aggregate equilibria in buffer, revealed high values of association constants for the dye binding to the protein aggregates (*ca.* ~ 6 μM^{-1}), suggesting their potential to be amyloid markers, and associate with specific fibril sites – grooves, running parallel to the fibril axis. It was also suggested that only the dye monomers could insert into the surface grooves because of steric constraints. To study the mechanism of AK7-5, AK7-6 binding to protein aggregates the PatchDock algorithm was employed. The online-available program calculated 10 optimal structures of protein-ligand complexes, corresponding to the maximized surface shape complementarity and minimized number of steric clashes. It appeared that the AK7-5 was bound to the Q75–N59 groove, while AK7-6 was incorporated into the S60–W62,G54–L56 groove of the lysozyme fibril core. Despite similar lipophilicity of AK7-5 and AK7-6, the latter inserts into the more hydrophobic fibril groove, probably due to the strong π-stacking interactions between tryptophan residues and phenyl rings of AK7-6. Indeed, aromatic side chains of the Y-ladders were particularly favorable binding sites for classical amyloid marker – Thioflavin T, as revealed by fluorescence measurements. In turn, molecular dynamics simulations suggested high Thioflavin T affinity for the fibril grooves formed by Y and L residues. Furthermore, hydrophobic, van der Waals and aromatic interactions between Congo Red and prion protein Sup35 disrupted stacking of the protein β-sheets, resulting in inhibition of amyloid fibril formation. As follows from the above, novel dyes could be involved in hydrophobic, π-stacking and H-bonding interactions with amyloid fibrils. Interestingly, docking studies revealed that AK7-5 and AK7-6 dimer species occupy the non-specific sites, lying perpendicular to the amyloid fibril axis due to steric constraints. Similarly, stacking of only Thiazole Orange monomers with DNA bases was revealed. The observed tendency of AK7-5 and AK7-6 to form J-aggregates in the presence of native lysozyme could be associated with their binding to the deep cleft of lysozyme molecule, allowing hydrophobic and electrostatic dye-protein interactions. Indeed, our docking studies showed that there is enough space for a dimer insertion into lysozyme cleft. In conclusion, the novel potential amyloid markers AK7-5 and AK7-6 were revealed and mechanism of their binding to lysozyme amyloid fibrils was suggested.