FLUORESCENCE AND MOLECULAR DOCKING STUDY OF THE COMPLEXES BETWEEN FIBRILLIZED INSULIN AND LYSOZYME

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During the past decades increasing attention has been given to elucidating the determinants of the toxic potential of amyloid fibrils, the deposition of which is associated with multiple human disorders, such as neurological diseases, systemic amyloidosis, type II diabetes, etc. Despite significant progress in understanding the cytotoxic action of amyloid aggregates, the role of protein-fibril interactions in the amyloid toxicity remains obscure. To fill this gap, in the present study the fluorescence and molecular docking techniques have been used to investigate the interactions between the insulin fibrils (InsF) and a globular cationic protein lysozyme (Lz), known for its antibacterial, antiviral, immunomodulatory, antitumor and anti-inflammatory activities. Insulin, a polypeptide hormone playing a pivotal role in glucose metabolism, is capable of forming amyloid fibrils in vivo, at the sites of its multiple injection in diabetic patients, and in vitro, under conditions of low pH, elevated temperature, hydrodynamic stress, etc. To explore the InsF-Lz interactions, at the first step of our study the fluorescence spectral behavior of the amyloid-sensitive phosphonium dye TDV has been examined in the InsF and its mixture with lysozyme partially unfolded at low pH. The binding of TDV to InsF was followed by a more than 30-fold increase of the fluorescence intensity at the emission maximum 610 nm. Using the log-normal asymmetric function we decomposed the TDV fluorescence spectra for the system TDV-InsF into three spectral components with maxima at ~ 572 nm, 608 nm and 649 nm. The addition of lysozyme to the TDV-InsF system led to ~ 1.4-fold enhancement of the fluorescence intensity of band at ~ 572 nm coupled with ~ 8 nm bathochromic shift of its position. This effect was accompanied with the significant decrease of the amplitude of bands at ~ 608 nm (~ 5.3 times) and 649 nm (~ 8.6 times). Moreover, the lysozyme brought about the increase of the contribution of the first spectral component in the overall spectra (from 18 % to 65 %) and decrease of that for the third component (from 31 % to 12 %), compared with the system TDV-InsF. The changes in the TDV fluorescence response in the presence of lysozyme most likely reflect the redistribution of TDV molecules between the binding sites located at InsF, Lz and protein-protein interface. To define possible modes of the lysozyme binding to the insulin amyloid fibrils, the molecular docking was conducted using the HDOCK server. The TDV structure was built in MarvinSketch and the dye geometry was further optimized in Avogadro. The PatchDock and FireDock online tools were used for TDV docking to the InsF-Lz complexes selected from the HDOCK outcomes. The selected docking poses were visualized with the UCSF Chimera software and were further analyzed with Discovery Studio Visualizer. The docking results showed that the phosphonium head of the TDV molecule preferentially interacts with the lysozyme and N-termini of the InsF, while the rest of the TDV molecule is located in the solvent exposed groove Gln15_Glu17. More specifically, our docking studies indicate that the phosphonium head of the TDV molecule tends to form π-alkyl bond with Val2 of Lz and Val2 of InsF N-terminus, while the rest of the dye binds to the groove through hydrogen bonding with Gln15, Leu16, electrostatic interaction with Glu17 and π-alkyl interaction with Leu16. In addition, the complex was found to be stabilized by hydrogen bonding with Asn37 of Lz and van der Waals forces with Glu17 of InsF. Overall, the molecular docking data lend support to the idea that TDV sensitivity to InsF-Lz interactions is largely determined by the dye ability to reside at the fibril-protein interface and to form contacts with both fibrillar insulin and lysozyme.