**Fluorescence and molecular dynamic study of lysozyme amyloid fibrils**

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**Флуоресцентне та молекулярно-динамічне дослідження амілоїдних фібрил лізоциму**

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The accumulation of the highly ordered protein aggregates, amyloid fibrils, in various tissues and organs is associated with a variety of human diseases, including Alzheimer’s, Parkinson’s disease, systemic amyloidosis, type II diabetes, etc. These diseases are caused by protein misfolding and occur because the partially folded intermediates leave pathway of native folding and self-assemble in β-stranded amyloid fibrils. One of the most powerful approaches to the detection of amyloid fibrils involves the use of fluorescent dyes. Extensive research efforts are currently focused on looking for the effective amyloid-specific flurophores. The goal of the present study was twofold: 1) to assess the amyloid-sensing propensity of the two newly synthesized near-infrared cyanine heptamethine dyes, AK7-5 and AK7-6 through comparing the fluorescence responses of these dyes in the presence of the native and fibrillar lysozyme; 2) to gain insight into the role of mutations in the amyloidogenesis of lysozyme using the molecular dynamics simulation. Lysozyme is a multifunctional protein with bactericidal, antitumor and immunomodulatory activities, whose amyloid formation is related to hereditary systemic nonneuropathic amyloidosis.

In an organic solvent and in the sodium-phosphate buffer (pH 7.4) the examined dyes have the emission maxima at 835/839 nm for AK7-5/AK7-6, respectively (monomeric band). In the presence of the native lysozyme we observed the appearance of hypsochromic band at 700 nm, whose intensity increased with the protein concentration. In the presence of lysozyme fibrils, both heptamethine dyes demonstrated significant rise of the monomeric bands (by a factor of 4.5/3 for AK7-5/AK7-6, respectively). These results suggest that the native lysozyme has more binding sites for the dye aggregates than fibrillar lysozyme, while the fibril grooves represent specific binding site for the dyes monomers.

To understand the conformational behavior of partially folded lysozyme, we have performed 100 ns molecular dynamic unfolding simulations of seven lysozyme mutants (I56T, F57I, W64R, D67H, F57I/T70N, EAEA, EAEA-I56T) at high temperature (500 K) using the GROMACS suite of programs and Charmm36 force field. MD simulation of WT lysozyme at 300 K showed that protein structure remains stable, the RMSD did not exceed 0.15 nm, indicating that the force field is reasonable in terms of the protein stability. The 100 ns simulations at 500 K performed for WT protein and mutants revealed the destruction of α-helices and β-sheets to coils and turns. The main findings can be summarised as follows: The destruction time of A-D-helices increases in the rows:

A-helix (residues 5-15): D67H<EAEA<**WT**<EAEA-I56T<F57I<I56T<F57I/T70N< W64R;

B-helix (residues 25-36): F57I/T70N <EAEA<W64R<**WT**< EAEA-I56T< F57I< D67H< I56T;

C-helix (residues 89-100): F57I/T70N< D67H<EAEA< EAEA-I56T<**WT**< I56T< F57I< W64R;

D- helix (residues 110-116): F57I/T70N< D67H, F57I<EAEA< I56T< **WT**< EAEA-I56T< W64R.

The stability of the main β-structures (B1-B3) follows the order:

B1 (residues 43-46): F57I/T70N<EAEA<**WT**< D67H< W64R < EAEA-I56T, F57I< I56T;

B2 (residues 51-54): F57I/T70N< EAEA<**WT<** W64R < EAEA-I56T< I56T< F57I< D67H;

B3 (residues 59-60): F57I/T70N<< EAEA<**WT<** EAEA-I56T< I56T, W64R< F57I< D67H;

In summary, it can be concluded that:

1. The revealed spectral behavior of the cyanine dyes, viz. significant distinctions in the fluorescence spectra in the presence of monomeric and fibrillar forms of lysozyme, point to the possibility of using these molecules as fluorescent amyloid markers along with the classical amyloid marker Thioflavin T.
2. The β-sheets of the examined lysozyme mutants show higher stability than those of WT protein, except F57I/T70N and EAEA variants;
3. The extent of β-sheet formation during the simulation was similar for WT and mutated proteins, suggesting that stability provides a basis for hypothesis that stability of original β-structures of lysozyme plays an important role in its transition into amyloidogenic state.