БІОФІЗИКА КЛІТИНИ

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SPECTRAL BEHAVIOR OF AMYLOID-SPECIFIC DYES IN PROTEIN-LIPID SYSTEMS. II. CONGO RED INTERACTIONS WITH HEMOGLOBIN

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A number of so-called conformational diseases including neurological disorders (Parkinson's, Alzheimer's and Huntington's diseases), type II diabetes, spongiform encephalopathies, systemic amyloidosis, *etc*., are associated with the deposition in tissue of highly ordered aggregates of specific proteins. Amyloid fibrils are usually detected by several techniques including Thioflavin T fluorescence, Congo Red (CR) birefringence of spectrophotometric assay, electron microscopy, *etc*. However, application of amyloid-specific agents such as CR to amyloid detection may be hampered by the dye ability to associate not only with fibrillar structures but also with monomeric protein species. In view of this reasoning the present study was directed toward the examination of the interactions between CR and hemoglobin (Hb), the protein with well-characterized structure and physicochemical properties. The binding of CR to native and denaturated Hb was studied using absorption spectroscopy technique. Differential absorption spectrum of CR associated with denaturated protein was found to exhibit maximum close to that characteristic of fibrillar structures (545 nm), thereby providing arguments in favor of Hb fibrillization. Formation of CR complexes with native Hb was followed by the long-wavelength shift (~10 nm) of absorption maxima being indicative of the probe transfer to the environment of lower polarity. Based on analysis of Hb crystal structure the tentative location of CR in the protein molecule has been identified. The most probable dye binding site was assumed to involve the hydrophobic cavity between Lys16 and Lys60 serving as anchors for two negatively charged CR sulfonic groups. Quantitative parameters of CR complexation with native Hb – association constant (K_b) and number of binding sites (n) – were determined by analyzing the dependencies of dye absorbance changes upon varying protein concentration. Approximation of experimental dependencies by Langmuir binding model yielded the values of K_b and n *ca.* 2.6×10^5 M^{-1} and 1.4, respectively. For thermally denaturated Hb, the shape of CR binding curve was revealed to change from Langmuir-like to sigmoidal. Simulation results showed that such a behavior of binding curve is characteristic of preferential dye association with aggregated protein species.

KEY WORDS: Congo Red, hemoglobin, protein-dye complexes