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STRUCTURAL TRANSITIONS OF *PHOTOBACTERIUM LEIOGNATHI* LUCIFERASE DETERMINED BY VARIOUS OPTICAL TECHNIQUES UNDER UREA-INDUCED EQUILIBRIUM DENATURATION

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The study was aimed to identification of conformational transitions of *Photobacterium leiognathi* luciferase during equilibrium denaturation with urea using several optical techniques, including circular dichroism, stationary and time-resolved fluorescence. Gravity center and intensity ratio I_{325}/I_{390} for the fluorescence spectra, molar ellipticity at 222 nm and fluorescence lifetimes of the protein were analyzed. Investigated parameters revealed two possible transitions for *P. leiognathi* luciferase with the midpoints at 0.5—1.1 and 3.5—4.2 M of urea. Changes in the values of two lifetime components, characterizing the luciferase fluorescence reflect both transitions, while steady-state fluorescence parameters (gravity center of spectrum and I_{325}/I_{390} ratio) reveal only the second one. Far-UV circular dichroism spectra displayed transitions at 4.2 M of urea for *P. leiognathi* luciferase. Conformational transitions characteristics of *P. leiognathi* luciferase and previously studied *Vibrio harveyi* luciferase (Inlow et al., 2002) were compared. Since, according to the published data for *V. harveyi*, midpoint of the second conformational transition is at about 2.5 M of urea, the results indicate more stable secondary structure for the *P. leiognathi* luciferase under study. The possible reasons for observed differences in fluorescent characteristics of two types of luciferases during denaturation can be connected to the microenvironment variation of the tryptophan residues in their tertiary structure, namely in position 131 and 277 in α-subunit.

K e y w o r d s: bacterial luciferase, denaturation, protein intermediate states, protein fluorescence lifetime, circular dichroism