

The Structural Changes of Green Fluorescent Protein Induced by Small Guanidine Hydrochloride Concentrations

Olesya V. Stepanenko¹, I.M. Kuznetsova¹, V.V. Verkhusha², V.N. Uversky³, K.K. Turoverov¹

¹ Institute of Cytology of the Russian Academy of Sciences, Tikhoretsky av. 4, 194064, Saint-Petersburg, Russia,

² Albert Einstein College of Medicine, 1300 Morris Park Avenue, 10461, New York, USA, ³ Indiana University, 10th Street 410 W, 46202, Indianapolis, USA.

Green fluorescent protein (EGFP) belongs to a large family of the so-called “fluorescent proteins” or “GFP-like proteins” characterized by the presence of chromophore which able to absorb and in the most cases to emit visible light. A great interest to fluorescent proteins (FPs) is caused by their wide use as biological markers for studying of gene expression and protein localization and tracking in living cells and tissues. In spite of low amino acid homology, all fluorescent proteins have a unique β -can fold which represents an 11 β -stranded cylinder threaded by an α -helix containing the chromophore formed from residues in the position 65-67 through an autocatalytic cyclization. The structure of FPs has been extensively investigated. It has been shown that FPs possess high conformational stability under a variety of denaturing conditions as well as that denaturation process is much slowly as compared to unfolding of proteins having structure of the other topology. This means that native and unfolded states of fluorescent proteins are separated by high energy barrier. In the recent works it is pointed out that the unfolding of GFP is not one-stage process but is accompanied by accumulation of several intermediate states. Existence of intermediate states is thought to be connected with two features of GFP structure. First, the chromophore is slightly twisted in the native protein while it is planar in the unfolded protein. Second, the central helix shows some deviations from optimal geometry. These peculiar properties are supposed to result in hysteresis of unfolding and refolding curves for sfGFP (superfolder).

In this work the effect of small guanidine hydrochloride (GdnHCl) concentrations on the structure of EGFP have been studied. The addition of such denaturant concentrations has been shown to induce no noticeable changes of secondary structure and chromophore microenvironment as can be seen from almost indistinguishable far UV and visible CD spectra; at the same time these GdnHCl concentrations induce just a small tertiary structure distortion recorded by changes in near UV CD spectra and by data of tryptophan fluorescence quenching induced by acryl amide. For all that, the addition of 0.1-0.2 M GdnHCl to EGFP results in approximately 20% increase of “green” fluorescence intensity. Furthermore, experiments on kinetics of EGFP denaturation by GdnHCl have revealed that denaturant addition leads to the sharp increase of chromophore fluorescence intensity which just after GdnHCl addition essentially exceeds the level of native protein intensity.

All experimental data allow us to conclude that both effects have the same nature and caused by the stabilizing action of denaturant. This implies that GdnHCl in small concentrations does not act as denaturant but can stabilize the protein structure through the hydrogen bonds with carbonyl group on the protein surface and through ionic interactions between the denaturant ions and the charged sites on the protein. In the presence of small GdnHCl concentrations the structure of green fluorescent protein becomes less strained. In these conditions, the chromophore being inaccessible to quenching action of water molecules gets less twisted, more planar, configuration. This leads to the increase of the conjugation of a π -electron system that in its turn increases the fluorescence quantum yield of EGFP.

Acknowledgment: This work was supported by Program “Leading Scientific School of Russia” (grant 1961.2008.4) and Russian Science Support Foundation (Stepanenko O.V., 2008).