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Title:	Elucidating Contributions from Multiple Species during Photoconversion of Enhanced Green Fluorescent Protein (EGFP) under Ultraviolet Illumination
Authors:	Dhamija, Shaina (/jspui/browse?type=author&value=Dhamija%2C+Shaina) De, Arijit K. (/jspui/browse?type=author&value=De%2C+Arijit+K.)
Keywords:	Enhanced Green Fluorescent Protein (EGFP) Photoconversion
Issue Date:	2021
Publisher:	Wiley
Citation:	Photochemistry and Photobiology, 97(5), 980–990.
Abstract:	Photocycle in wild-type green fluorescent protein (wt-GFP) involves generation of a bright fluorescent deprotonated chromophore from feebly fluorescent protonated form via excited-state proton transfer. In addition to this usual photocycle, wt-GFP is also known to exhibit irreversible photoconversion upon illumination with ultraviolet and visible radiation. However, a detailed understanding of photoconversion in enhanced GFP (EGFP: S65T/F64L mutant of wt-GFP), which predominantly exists in deprotonated form, is yet to be explored. Using 254 nm irradiation, we study how photoconversion proceeds in EGFP. The key findings are observation of spreading out of an isosbestic point and existence of an initial lag phase in spectral kinetics of absorbance, indicative of sequential photoconversion through an intermediate. Fluorescence kinetics of EGFP and its photoproduct are estimated by assigning two unique fluorescence lifetimes which is further complicated by the fact that their fluorescence are spectrally inseparable, as evident from global analysis of fluorescence lifetime. Time-resolved fluorescence anisotropy studies further suggest minimal structural changes in protein scaffold upon photoconversion. Based on these findings, an analytic model is developed to account for the overall decay in fluorescence (as photoconversion proceeds) that inherently incorporates the initial lag phase and a summary of energetics and processes involved is provided.
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