Photoactivation of novel fluorescent proteins from the sea salp Thalia democratica

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Photoactivatable fluorescent proteins exhibit fluorescence that can be modified by a light-induced chemical reaction. Upon photoactivation, some of these proteins become brighter, others change their fluorescence spectrum. Here we report two novel photoactivatable fluorescent proteins, named ThFP1 and ThFP2, from the salp *Thalia democratica*. Based on transcriptome analysis, two GFP-like proteins were identified. These proteins were cloned, expressed in *Esherihia coli* and purified by metal-affinity and gel-filtration chromatography. The electrophoretic mobility of the purified protein in SDS-PAGE corresponded to 26 kDa. Absorption spectra of purified proteins showed maxima at 400 and 488 nm for ThFP1, and at 337 and 510 mm for ThFP-2. Fluorescence emission spectra of ThFP-1 peaked at 512 nm, while that of ThFP-2 peaked at 527 nm. ThFP1 and ThFP2 proteins were photoactivatable upon irradiation with 365 and 445 nm light, resulting in a significant increase of long-wavelength bands in the absorption spectra of the proteins. Both proteins were capable of reversible photo-switching, showing very similar activation barriers (22.36 and 23.97 kcal/mol for ThFP1 and ThFP2, respectively). Despite the close values of activation barriers at all tested temperatures the relaxation rate of ThFP1 was 1.2-1.5 times lower than that of ThFP2. ThFP2 photoswitching was shown to be a multistep process, while the ThFP1 curve was monophasic under the same conditions. Gel-filtration chromatography indicated the oligomeric status of both proteins, with ThFP2 showing a tendency to monomerise upon photoactivation.
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