Photophysical properties of Green Fluorescent Protein in solution and polyvinyl alcohol film at room temperature

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Fluorescent proteins are now widely used as biological markers for in vitro and in vivo imaging using a variety of spectroscopic methods.
Green Fluorescent Protein has a β-barrel shape, composed of 11 β-bands arranged mainly in an antiparallel manner. The hydrogen bonds between adjacent β-bands allow the form a closed structure with an α-helical segment buried inside the cyllinder. Three residues in this segment (in the wild-type protein they are Ser65, Tyr66 and Gly67) in the presence of molecular oxygen, generates a p-hydroxybenzylidazolidone chromophore, responsible for the visible green fluorescence.

The chromophore is tightly shielded from the solvent molecules and held in the appropriate plane by the hydrogen bonding system inside the protein. This location in the structure ensures efficient fluorescence of the chromophore, protecting it from quenching by molecular oxygen or by radiationless relaxation due to the ring mobility.

Polyvinyl alcohol is a polar, water-soluble compound used i.a. in luminescence studies of organic compounds. As a polar compound, PVA readily forms hydrogen bonds with trapped in it molecules. It causes the stiffening of organic molecules makes it difficult to dispose of their energy in a radiation-free manner. It enables studies of the properties of trapped dyes such as fluorescence, delayed fluorescence and phosphorescence. Anisotropy of fluorescence or phosphorescence is also observed for such molecules, which is not visible in aqueous solutions. PVA-modified materials are used to stabilize biological molecules such as proteins (i.a. lipase, acetylcholinesterase, glucose, oxidase).
In this work, we compare the spectroscopic properties of EGFP entrapped in PVA and in aqueous solution. In addition, we check how such properties change for uranine, an organic molecule for which the excitation and fluorescence emission spectra fall into similar wavelength ranges as for EGFP and an increase in anisotropy and fluorescence intensity is observed after immobilisation in film.

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