Photocycle of the photoswitchable fluorescent protein asCP

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Fluorescent proteins (FPs) are widely used in biology as tags to trace other proteins in living cells. Photoswitchable fluorescent proteins constitute a unique subclass as their photophysical properties may reversibly be changed upon laser illumination. Purified from the sea anemone *Anemonia sulcata,* asCP is one of the most studied FP of this type. Its ability to be reversibly switched between a nonfluorescent "off"-state (λ_{abs} = 565 nm) and a fluorescent "on"-state (λ_{abs} = 576 nm, λ_{em} = 610 nm) upon intense green light irradiation has been attributed to photochemical E → Z isomerization of its chromophore. A reverse isomerization proceeds either thermally or photochemically by irradiation at 445 nm ("quenching"). The details of this interconversion remain poorly characterized. Here, by using high-level quantum chemistry methods we explore the asCP photocycle at atomic level.

Structures, spectra and properties of asCP have been modelled by using *ab initio* based QM/MM and molecular cluster approaches. The results of these simulations favour a mechanism describing a majority of photoinduced asCP transformations solely relying on protein structures with the anionic form of the chromophore. The structures with the zwitterionic chromophore are energetically unfavourable in both ground S_0 and excited S_1 electronic states. The computed $S_0 \rightarrow S_1$ vertical excitation (561 nm) and $S_1 \rightarrow S_0$ vertical emission (605 nm) energies for the model system with the anionic chromophore, as well as calculated vibronic structure of the absorption band correlate well with the available experimental data. In particular, we show that a shoulder at 530 nm in the absorption band corresponds to a vibronic transition in the anionic chromophore and does not originate from contributions of other protonation states. This spectral shoulder primarily arises from two most active Franck-Condon modes, which are traced to in-plane stretching vibrations with frequencies of 830 and 1285 cm^{-1}. Internal conversion is shown to proceed through competing radiationless channels in the off-state of the protein. Two distinct types of the twisted S_0/S_1 conical intersections are found. Both conical intersections are related to internal rotation of the chromophore in the central bridge moiety. One of them corresponds to the photoinduced E → Z isomerization, and the other leads to relaxation back to the "off"-state. Small quantum yield of the asCP photoactivation originates from different topographies of S_1 along the two branches in internal conversion.

Photochemical quenching of the "on"-state of asCP is traced to the excited-state Z → E isomerization of the neutral form of the chromophore. The estimated value of the absorption maximum (437 nm) is in close agreement with the experimental maximum of quenching efficiency (445 nm). Furthermore, the neutral chromophore concentration increases upon "off"-"on" photoswitching as it has been shown by the QM/MM-based molecular dynamics study, whereas the ground state Z → E isomerization proceeds exclusively through the anionic form of the asCP chromophore.

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