Engineered disulfide in the orange carotenoid protein enables control of its structural dynamics associated with photoprotection function and carotenoid transfer

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The Orange Carotenoid Protein (OCP) plays a key role in the cyanobacterial photoprotection via the reversible photoactivation from the orange to the red form, binding to the light-harvesting complexes (phycobilisomes, PBs), and heat dissipation of the excess energy. Photoactivation entails significant conformational rearrangements of a compact dark-adapted state including the detachment of the N-terminal extension (NTE) from the C-terminal domain (CTD) and domain separation. The PBs binding and quenching ability of OCP is inactivated by the Fluorescence Recovery Protein (FRP) and, in principle, can be modulated by the natural C-terminal domain homolog (CTDH) through the light-controlled carotenoid arrest. OCP functioning seems to depend on domain separation and exposure of the CTD surface covered by NTE. Here, we devise an OCP variant with the NTE trapped on the CTD via an engineered disulfide (OCPcc). NTE trapping preserves OCP photoactivity but weakens functional interaction of OCP with FRP and PBs during photoactivation, which is completely restored upon reduction of the introduced disulfide. Interestingly, NTE trapping did not fully abolish but reduced the apparent efficiency of carotenoid transfer to CTDH. Non-interacting with the dark-adapted oxidized OCPcc, FRP binds dark-adapted reduced OCPcc nearly as efficiently as OCP devoid of the NTE, suggesting that the low-affinity FRP binding to dark-adapted OCP is realized via NTE displacement. The unexpected PBs fluorescence lifetime (~600 ps) in the presence of photoactivated oxidized OCPcc indicates that it binds to PBs in an orientation suboptimal for quenching PBs fluorescence. This approach shows the effective redox controlled uncoupling of the OCP spectral and functional photoactivation. Partially supported by RFBR grant no. 20-54-12018. Previously published in: Slonimskiy YB et al. (2020) BBA - Bioenerg. 148174.