MS09-2-1 Disulfide bridge explains dimer formation for rsCherryRev1.4
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Abstract
Since the green fluorescent protein (GFP) was first discovered in the early 1960s [1], there is a vast number of novel fluorescent proteins (FPs) that have been found in nature or successfully engineered. The spectral diversity and the special photophysical behaviours of fluorescent protein have enabled their applications in many biology-related studies. However, one of the major handicaps of FPs that restricts greatly their utility as a label in studying live-cell systems is the oligomerization tendency to some extent. Extensive efforts were devoted to preventing their oligomer formations and generating monomeric FPs. Previous structural studies revealed that the oligomerization in FPs was mainly formed by electrostatic interactions and/or hydrogen bonds between monomers. Hence, the common strategy to create a monomeric form of most FPs is to mutate key residues at their interfacing positions to break down the interactions between units [2].
rsCherryRev1.4 was reported as a monomeric red reversible photo-switchable FPs that was developed from rsCherryRev by introducing six mutations: G24C, R36H, R125C, V144F, R149Q, and E160L [3]. Compared to rsCherryRev, rsCherryRev1.4 is considered as an improved version exhibiting faster off-switching speed and lower switching fatigue at a high light intensity, thus increasing the application of rsCherryRev1.4 in the RESOFLT technique. Nevertheless, rsCherryRev1.4 has some limitations such as dimerization tendency and complex photophysical properties. We now have determined the crystal structure of rsCherryRev1.4 and discovered that its dimer formation is the result of disulfide bonding.
A plate-like crystal of rsCherryRev1.4 was obtained by the sitting drop vapour diffusion method after a few weeks. Diffraction data up to 2 Å resolution were collected at the I04 beamline of Diamond Light Source at 100K using an EIGER detector. The structure was solved in space group I2 by molecular replacement using mCherry (PDB code 2h5q) [4] as the phasing model. The orientation and interface regions between two monomers in rsCherryRev1.4 are very similar to those observed in the AB interface of DsRed [5],[6]. However, structural analysis using PDBePisa reveals that only CYS24 and SER21 are involved in one disulfide bond and one hydrogen bond formed between the two monomers in rsCherryRev1.4. In DsRed, there are 13 hydrogen bonds and three salt bridges created between chain A and chain B. This comparison suggests a crucial contribution of CYS24 to the dimerization tendency of rsCherryRev1.4, and CYS24 is one of the mutations introduced in rsCherryRev that is not prone to form a dimer. Other experiments including mutagenesis, electrophoresis and chromatography were also performed. Obtained results give further evidence for the disulfide link formed by two residues CYS24 as observed in the rsCherryRev1.4 dimer.

References
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