Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences

This journal is (c) The Royal Society of Chemistry and Owner Societies 2010

Supplementary material Möglich & Moffat

Experimental

Purification and spectroscopic characterization of YtvA mutants

The coding region for full-length YtvA (residues 1-261) was amplified by PCR from *Bacillus subtilis* genomic DNA and cloned into the pET28c expression vector (Novagen, Madison, WI, USA) using NdeI and SacI restriction enzymes. Protein expression and purification was carried out as described for the isolated LOV domain of YtvA[1]. Protein concentration was determined by absorption measurements using an extinction coefficient of 12500 M^{-1} cm^{-1} at 450 nm^2. Point mutants of YtvA were generated by site-directed mutagenesis (QuickChange, Agilent, Wilmington, DE, USA) and purified as wild-type YtvA.

UV/vis absorption data were recorded on a Shimadzu UV-1650 PC spectrophotometer at (22 ± 1) ºC and at protein concentrations between 20 and 30 μM. Protein samples were photobleached by illumination for 60 s with white light from a fiber optic illuminator (model 9745-00, Cole-Parmer Instrument Co., Chicago, IL) and recovery was followed spectrophotometrically. Absorption data were fitted to exponential functions using ProFit (QuantumSoft, Uetikon, Switzerland).

Structural model of YHF

Homology models for the histidine kinase domain of *Bradyrhizobium japonicum* FixL were calculated with MODELLER[3] using structures of the cytoplasmic portion of *Thermotoga maritima* HK853 (PDB code 2C2A[4]) and of its complex with the response regulator RR468 (3DGE[5]) as templates. Structures of the PAS sensor domains were derived from the structures of

This journal is (c) The Royal Society of Chemistry and Owner Societies 2010

the isolated sensor domains (PDB codes 2PR5[1] and 1XJ3[6]). Structures of individual domains were manually assembled using MOLMOL[7] and LSQKAB[8] where linkers are assumed to adopt α-helical conformation. Molecule graphics were drawn with MOLSCRIPT[9].

References

1 A. Möglich and K. Moffat, Structural Basis for Light-dependent Signaling in the Dimeric LOV Domain of the Photosensor YtvA, *J Mol Biol*, 2007, **373**, 112-126.
2 A. Losi, E. Ghiraldelli, S. Jansen, and W. Gärtner, Mutational effects on protein structural changes and interdomain interactions in the blue-light sensing LOV protein YtvA, *Photochem Photobiol*, 2005, **81**, 1145-1152.
3 A. Sali and T. L. Blundell, Comparative protein modelling by satisfaction of spatial restraints, *J Mol Biol*, 1993, **234**, 779-815.
4 A. Marina, C. D. Waldburger, and W. A. Hendrickson, Structure of the entire cytoplasmic portion of a sensor histidine-kinase protein, *Embo J*, 2005, **24**, 4247-4259.
5 P. Casino, V. Rubio, and A. Marina, Structural insight into partner specificity and phosphoryl transfer in two-component signal transduction, *Cell*, 2009, **139**, 325-336.
6 J. Key and K. Moffat, Crystal structures of deoxy and CO-bound bjFixLH reveal details of ligand recognition and signaling, *Biochemistry*, 2005, **44**, 4627-4635.
7 R. Koradi, M. Billeter, and K. Wüthrich, MOLMOL: a program for display and analysis of macromolecular structures, *J Mol Graph*, 1996, **14**, 51-5, 29-32.
8 Collaborative Computational Project Number 4, The CCP4 suite: programs for protein crystallography, *Acta Crystallogr D Biol Crystallogr*, 1994, **50**, 760-763.
9 P. J. Kraulis, MOLSCRIPT: A Program to Produce Both Detailed and Schematic Plots of Protein Structures., *J Appl Cryst*, 1991, **24**, 946-950.