Regulation of Protein Function: Crystal Packing Interfaces and Conformational Dimerization

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Supporting Information

Bridging Ions: Crystallisation of L14F Pc in the presence of 0.2 M MgCl_2 resulted in a monomeric arrangement with a $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$ complex bound to the protein surface. One of the water molecules from this complex simultaneously hydrogen bonds to the carbonyl oxygen of Pro25 and to a carboxylate oxygen from Asp27 [note that the Asp27 carboxylate does not directly chelate the Mg^{2+} ion as this would involve the energetically unfavourable process of desolvation (*1*)]. Despite substantial differences in crystal packing, the hydrogen bonding pattern of Asp27 is the same in all three structures. The $\text{O}^{\delta 2}$ atom is hydrogen-bonded to the amide of His24 and to a water molecule, whilst the $\text{O}^{\delta 1}$ is hydrogen-bonded to the amino terminus as well as to two water molecules. In the *Monomer* structure one of these water molecules is replaced by a water molecule coordinated to Mg^{2+} . The $[\text{Mg}(\text{H}_2\text{O})_6]^{2+}$ complex also acts as a weak bridging ion in the crystal packing interface, *via* water-mediated hydrogen bonds to the hydroxyl and carbonyl oxygen of Thr22 on a neighbouring monomer.

The role of bridging ions is greater in the *Trimer* structure where zinc directly facilitates the formation of protein-protein contacts [this is also the case for the WT structure 1baw, 2.8 Å

resolution (2)]. Each trimer interface contains a Zn^{2+} ion co-ordinated by the imidazole of His61 and the carboxylates of Asp44 (as a bidentate ligand) and Asp45 (monodentate) from the neighbouring monomer. Two water molecules complete the distorted octahedral co-ordination of the Zn^{2+} ion. An additional zinc-mediated interface, which was not observed in the WT structure, is present between chains A and C. This Zn^{2+} site is co-ordinated by the imidazole of His24 from two different protein monomers and by the carboxylate of Glu104 (as a bidentate ligand). Again, two water molecules complete the co-ordination site. There is no analogous Zn^{2+} site involving chain B and the $\text{N}^{\epsilon 2}$ of His24 is displaced by $\sim 2 \text{ \AA}$ from its position in chains A and C.

Water Molecules: As discussed above for Asp27, the first solvation layer (water molecules that make direct hydrogen bonds or van der Waal's contacts with the protein) is maintained in the different crystal forms (3, 4). Besides waters of hydration a number of buried water molecules are observed in the Pc structures. In all of the structures an internal water molecule is hydrogen-bonded to the hydroxyl of Tyr87 and to the carbonyl oxygens of Pro18 and Ala19. This water molecule is conserved in two other cyanobacterial Pc structures (5, 6) [but was observed only in chain C in the WT protein (2)]. Apparently the size and orientation of the side chain at position B8 [Gough and Chothia numbering (7)] determines whether or not a buried water is present. For example in plant Pcs, B8 is usually a buried Phe or Leu whereas in *Phormidium* Pc the smaller Val21 creates a cavity, which can be occupied by a water molecule. An additional buried water molecule was found in the vicinity of the copper site in the *Dimer* and in chains A and B of the *Trimer* structure. This water molecule is hydrogen-bonded to the carbonyl oxygens of Gly12 and Lys35, makes van der Waal's contacts with Gly8, Phe14, Leu36 and His39 (a ligand to the copper) and may play a role in redox modulation of the copper site (8).

Figure S1. Temperature (B) factors for the C^α atoms in each of the six protein chains in the three crystal forms of L14F Pc. Note the on average higher B values in the *Trimer* structure. Regions of β -strand and α -helical secondary structure are identified by the black and grey boxes, respectively. As in Figure 1, there is a general pattern of peaks and troughs corresponding to regions of flexible and rigid structure respectively.

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