Crystallographic analyses of apo and reduced azurins

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Abstract

Crystals of the blue copper protein azurin, from *Alc. denitrificans*, have been prepared in the apo (metal-free) and reduced (Cu(I)) forms by soaking oxidised (Cu(II)) azurin crystals in solutions containing cyanide and ascorbic acid, respectively. The apo and reduced azurin crystals are isomorphous with the oxidised crystals, containing two protein molecules in the asymmetric unit. A CAD4 diffractometer was used to collect X-ray diffraction data from these crystals, to 2.2Å resolution for apo-azurin and 1.9Å for reduced azurin. Both crystal structures have been refined using restrained least-squares methods, treating the two protein molecules of the asymmetric unit independently, to give final R-factors of 0.158 for apo-azurin (1956 protein atoms and 275 solvent atoms) and 0.166 for reduced azurin (1967 protein atoms and 269 solvent atoms).

Analysis and comparison of these structures with that of the oxidised form show that the protein structure is essentially identical between all three forms. However, very small changes, which are consistent between the two independent molecules of the asymmetric unit, are apparent at and near the copper binding site. Upon reduction of the oxidised form, all copper distances (left unrestrained during refinement) increase by 0.05-0.10Å, while the geometry remains unchanged. Thus the copper in reduced azurin is bound strongly by one thiolate sulphur (Cys-112) and two imidazole nitrogens (His-46 and His-117) in a trigonal arrangement, while a thioether sulphur (Met-121) and a carbonyl oxygen (Gly-45) approach the copper from axial directions to complete a distorted trigonal bipyramidal geometry. The Cu...S_δ 121 distance is 3.2Å, and does not agree with the 2.7Å distance predicted by EXAFS studies despite attempts to restrain this distance during refinement. Two S...HN hydrogen bonds, between the cysteine sulphur and peptide NH groups, are shortened by ≈0.2Å upon reduction of Cu(II) to Cu(I).

The configuration of the copper ligands and the surrounding structure are maintained when the copper is removed. This shows that the folding of the polypeptide chain determines the conformation of the metal ligands, enforcing a distorted geometry on the copper centre in both oxidised and reduced forms. The structure of the copper binding site in the apo form is more similar to the oxidised form than to the reduced form.

The observations made in the analysis of these three forms of azurin have implications for the biological function of the protein, which is thought to be electron transfer. The distorted geometry of the copper, which is enforced upon the metal by the protein, supports the concept of the entatic state, whereby the copper is held in a geometry approximating the transition state between the coordination preferences of Cu(II) and

Cu(I). Since minimal changes at the active site occur upon reduction, the protein accomplishes its function of fast electron transfer by minimising the activation energy of the process.

Erratum

p. 45 Reference number 395 should be 210.

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