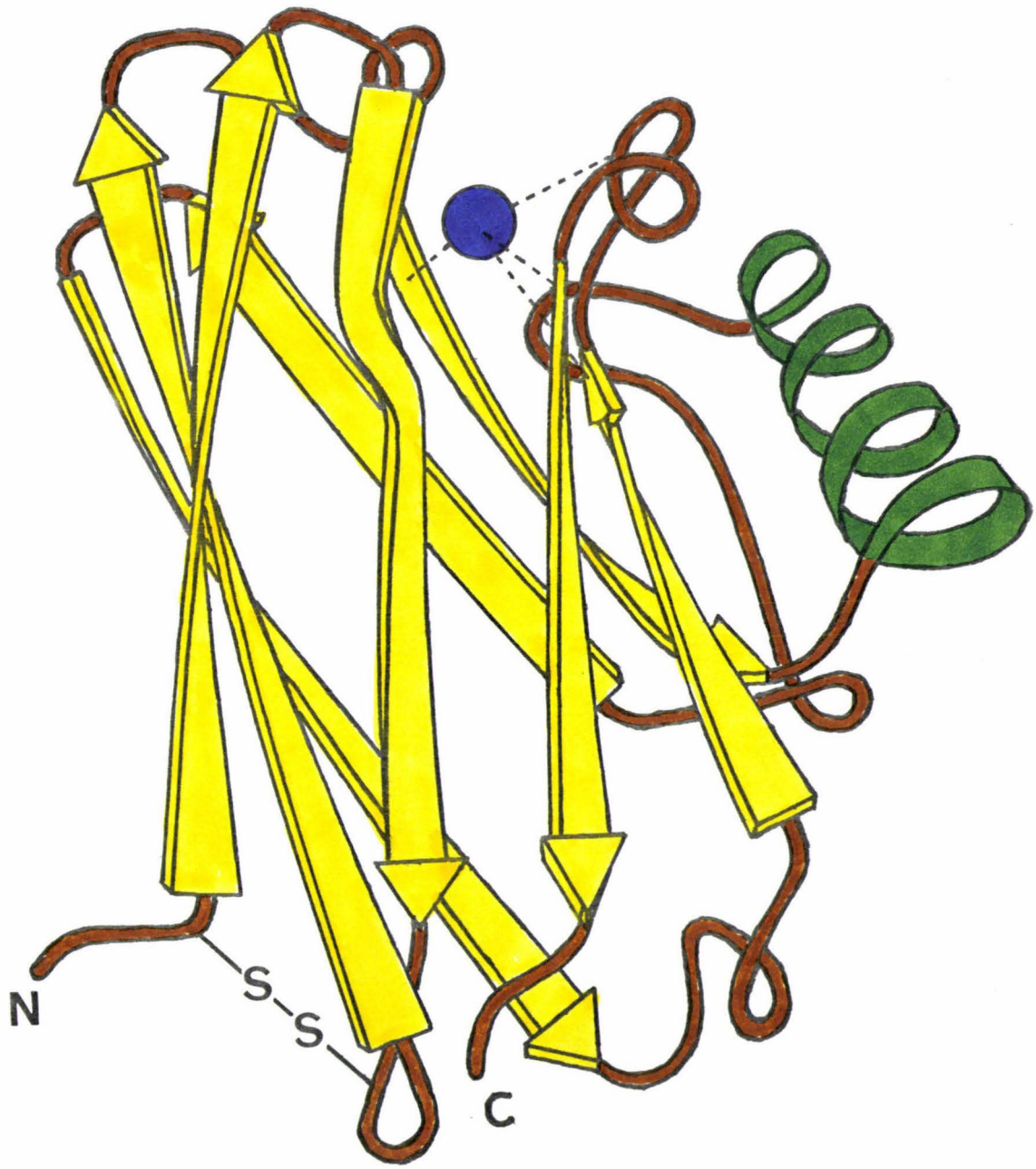
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THE THREE DIMENSIONAL STRUCTURE OF AZURIN, A BLUE COPPER PROTEIN, AT 3Å RESOLUTION

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry at Massey University.

GILLIAN E. NORRIS

1982



AZURIN

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ABSTRACT

Purifications and crystallizations of two electron transfer proteins, azurin and cytochrome c', from Alcaligenes denitrificans and Alcaligenes sp. NClB 11015 have been carried out. The azurin crystals from Alcaligenes denitrificans were found suitable for high resolution X-ray structure analysis. They are orthorhombic, space group $C222_1$ (with marked tetragonal pseudosymmetry), cell dimensions a = 75.0Å, b = 74.1Å, c = 99.5Å, with two molecules per asymmetric unit. A 3Å resolution electron density map of azurin was calculated. Four isomorphous heavy atom derivatives, prepared with $KAu(CN)_2$, uranyl acetate, $Hg(NH_3)_2Cl_2$ and ($KAu(CN)_2$ + uranyl acetate) (a double derivative) were used to calculate phases by the method of isomorphous replacement, giving an overall figure of merit of 0.614. The polypeptide chain could be followed unambiguously in both protein molecules in the asymmetric unit,with the aromatic sidechains, in particular, readily identifiable because of their distinctive appearance.

Kendrew skeletal models were built for both molecules, the polypeptide chain(consisting of 129 amino acids) being found to be folded into an eight-strand β-barrel, with an additional flap containing a short helix. There is one disulphide bridge within the barrel. The topology of the molecule was found to be the same as that of plastocyanin, and a comparison of the three dimensional structures of azurin and plastocyanin allowed the sequences to be aligned on structural rather than purely statistical grounds. It also established the probability that the two proteins have evolved from a common ancestor.

The copper atom has a highly-distorted tetrahedral co-ordination geometry, forming three shorter bonds (length approximately 2Å), with a cysteine thiolate sulphur (Cys 112) and two histidine imidazole

nitrogens (His 46 and 117), as well as a longer bond (approximately 3Å) with a methionine thioether sulphur (Met 121). A surprising result was the closeness of a peptide carbonyl oxygen, that of Gly 45, to the copper atom. At this stage of the structure analysis it is not clear whether it should be regarded as a ligand, or not.

Reduction of the protein crystals with chromous ions was attempted, and the results are discussed in terms of the possible electron transfer mechanism of the protein.

The cytochrome c' crystals from both species of bacteria are hexagonal, space group P $6_1$22 (or P $6_5$22), cell dimensions $a = b = 54.7$Å, $c \sim 185$Å $\gamma = 120^{\circ}$, with one subunit (molecular weight 14,000) in the asymmetric unit. No structural work has been carried out on these.

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