CRYSTALLOGRAPHY OF BIOLOGICAL MACROMOLECULES

PS04.02.45 CRYSTAL STRUCTURE OF THE GOWER II HUMAN EMBRYONIC HEMOGLOBIN (α2ε2). A.J. Sutherland-Smith, H.M. Baker, and E.N. Baker, Department of Biochemistry, Massey University, Palmerston North, New Zealand and R.M. Mould, O.M Hofmann and T. Brittain, School of Biological Sciences, University of Auckland, New Zealand

Three hemoglobin molecules (Gower I, Gower II and Portland) are synthesised by the human embryo between two weeks and twelve of gestation. These hemoglobins appear to function as scavengers of O2 from the mother’s interstitial fluid before the placenta has developed. Functional Gower II hemoglobin is a tetramer comprising the adult ε chain and the embryonic ε, which has 79% sequence identity to the adult β. Binding studies have indicated that Gower II hemoglobin binds O2 cooperatively, with a higher affinity than the adult molecule, and displays similar allosteric behaviour towards H+, Cl- and 2,3 DPG.

Gower II hemoglobin with carbon monoxide bound was crystallised. The crystals proved to be tetragonal (spacegroup P43212) with one α2ε2 tetramer in the asymmetric unit. The 3D structure has been solved at 2.8 Å resolution by molecular replacement and refined to a crystallographic R-factor of 0.204 (Rfree of 0.279) with good geometry.

The quaternary structure is very similar to that of the adult molecule. Within the ε subunit the main difference from the adult β is a small shift of the N terminal helix over the central cavity of the tetramer. The environment of the heme pocket is like that of the adult with the major variation being a closer packing of Ser 138 (Ser in β). Close density is visible for the bound CO ligand.

PS04.02.46 CRYSTAL STRUCTURE OF A BACTERIAL COPPER-CONTAINING AMINE OXIDASE FROM ARTHROBACTER GLOBOFONNIS AT 2.8Å RESOLUTION. Matthew C.J. Wilce, Hans C. Freeman, J. Mitchell Guss, Vinay Kumar (University of Sydney, NSW 2006, Australia) and William S. McIntire, (Department of Veterans Affairs Medical Center, San Francisco, CA 94143, USA)

We present the crystal structure and structural analysis of a bacterial copper-containing amine oxidase (AO). AOs are homodimeric proteins with molecular weights of between 70-90 kDa per subunit. Their function is the oxidative metabolism of amines in the presence of molecular dioxygen. They are involved in many fundamental cellular processes including: tissue differentiation, tissue development, wound healing, cancer and possibly programmed cell death. AOs are of particular importance in gram-positive methyloptrophs, including Arthrobacter sp., as these organisms are able to utilise methyamine as their sole carbon and energy source.

A. globofonnis AO crystallises in a number of forms with and without the presence of ammonium salts that are known to inactive the enzyme. The structure of the crystal form known as type II is reported here. The crystals were grown from LiSO4. The space group is C2 (a=157.6, b=64.3, c=92.6 Å, β=112.6°) with one subunit per asymmetric unit. Molecular replacement was used to determine an initial phase set. Both the pea seedling and E. coli AO [Parsons, M.R. et al. (1985). Structure, 3, 1171-1184] structures were successfully used as search models. The structure has been refined at 2.8Å resolution. The structure of A. globofonnis AO is compared with both the E. coli AO and pea seedling AO structures with particular reference to the active site.