Acta Cryst. (2002). A**58** (Supplement), C294

DOMAIN MOTION IN THE MECHANISM OF GUANINE NUCLEOTIDE EXCHANGE FACTORS

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Activation of small GTP-binding proteins is mediated by guanine nucleotide exchange factors (GEFs) that catalyze the dissociation of the tightly bound GDP nucleotide, allowing its replacement by cellular GTP. The reaction is initiated by the formation of a low-affinity G protein/GDP/GEF complex, which isomerizes to form a high affinity nucleotide-free complex that is eventually dissociated by the entry of GTP. Structural studies have shown that the small G proteins undergo large conformational changes in the course of the exchange reaction. We have investigated how GEFs recognize and accompany the structural changes of their substrates by a crystallographic and modelling analysis of GEF domains for G proteins of the Arf and Rho/Rac/cdc42 families. These studies suggest that domain closure of the GEF is an integral component of the exchange reaction. We propose that the reaction is initiated by anchoring of the GDP-bound G protein onto a docking subdomain, subsequently allowing a catalytic subdomain to close and catalyze the dissociation of GDP. This separates the exchange reaction into a recognition and a catalytic step, which may be critical to establish specific recognition at the low-affinity G protein/GDP/GEF stage.

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Keywords: GUANINE NUCLEOTIDE EXCHANGE FACTORS SMALL GTP BINDING PROTEINS TRANSPORT

Acta Cryst. (2002). A**58** (Supplement), C294

PHASING BY MAXIMAL-MINIMAL NON-ISOMORPHIC SUB-SUPER-GROUPS RELATIONSHIP IN FOUR-HELIX BUNDLE DESIGNED PROTEIN

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Artificial metalloproteins mimicking natural systems are important for development of novel materials, catalysts, and biosensors. A very simple model protein, DF1, has been recently designed and the crystal structure of the zinc derivative of DF1 determined.1 DF1 is a homodimer of helix-loop-helix hairpins assembled to form a 4-helix bundle motif with a site similar to the binuclear iron site of bacterioferritin. 1 The core of DF1 is extremely well packed, and Leu 13 and Leu 13 occupy the space proximal to the di-metal site, preventing access of substrate molecules. Substitution of these residue with smaller side chain amino acids, like Ala and Gly, leads to form an hole near the di-metallic site, which should allow the access of substrates.2 The di-Mn(II) form of DF1, DF1-L13A and DF1-L13G variants were crystallised and structurally characterised by X-ray diffraction experiments at the Elettra Synchrotron. Four crystalline forms were isolated for these manganese derivatives. Phasing problem were solved by molecular replacement and by maximal-(minimal) non-isomorphic sub-(super)-groups relationship. Exogenous ligands coordinated to the metal ions have been found in the hole formed by the substitution of the Leu13.

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Keywords: ARTIFICIAL-METALLOPROTEIN, PHASING PROBLEM, MAXIMAL-MINIMAL NON-ISOMORPHIC SUBGROUPS

Acta Cryst. (2002). A**58** (Supplement), C294

RAMACHANDRAN PLOTS OF ALL 20 AMINO ACIDS

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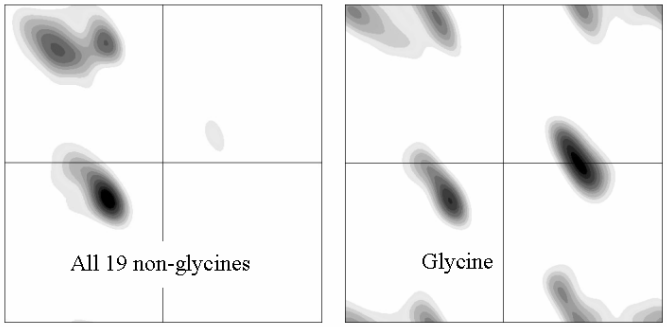
Ramachandran plots were calculated for each of the 20 amino acids, separately for HELIX, SHEET and Random coil. 237 384 amino acids from 1042 protein subunits with at least 2.0 Å resolution from the PDB were used. All areas in the plot follow the diagonal Φ- Ψ, rather than being parallel to any of the axes as in the original Ramachandran plot. Thus the two torsion angles are coupled. Gly has 5 allowed regions, 3 of which are not in the areas predicted by Ramachandran. The B-sheet region is split into two regions. Those are due to amino acids in B-strands and in Random coil. Amino acids in parallel and anti-parallel B-strands have nearly the same conformations. Each amino acid has its characteristic pattern. Asp and Asn have the most complicated plots.

Hovmöller, Zhou & Ohlson Acta Cryst D (2002) in press.



Keywords: RAMACHANDRAN PLOTS AMINO ACID CONFORMATIONS SECONDARY STRUCTURE

Acta Cryst. (2002). A**58** (Supplement), C294

A NOVEL MULTI-HEME STRUCTURE OF HIGH MOLECULAR-WEIGHT CYTOCHROME C FROM *D. VULGARIS HILDENBOROUGH*

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Desulfovibrio vulgaris Hildenborough is a sulfate reducing bacterium that contains various c-type cytochromes: cytochrome c-553, cytochrome c3 and high-molecular weight cytochrome c (HMC) are well known. HMC consists of one polypeptide chain of 545 amino acids and 16 heme groups that are bound through the CXXCH motif. We wish to report a quite novel three-dimensional structure of HMC. Crystals of HMC were obtained within one week from a solution containing polyethylene glycol. They were red hexagonal and grew up to 0.1x0.1x0.05mm^3. Data collection was performed at the SPring-8 BL44B2 beamline. The crystal diffracted up to 2.5Å resolution. It belongs to hexagonal space group $P6_2$ with unit cell dimensions a=107.5 Å , c=101.5 Å and γ=120°. Structure determination was carried out by the moleculer replacement method using the structure of HMC from *Desulfovibrio vulgaris* Miyazaki F as a starting model unpublished result. All heme groups but one have two histidine ligands as 5th and 6th coordination site. One heme located in the domain IV and 15th heme from the N-terminus, lacks 6th ligand. This is the first observation that a c-type cytochrome containing c3-like component has a penta-coordinated heme. Remarkably both propionate groups of the heme are protruded from the surface of the molecule, meaning that an electron might be transferred from redox partners via one of these solvent-accessible propionate groups.

Keywords: HIGH-MOLECULAR WEIGHT CYTOCHROME C HEXADECAHEME PROTEIN ELECTRON TRANSFER PROTEIN