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STRUCTURE OF A MINIATURISED HEMOPROTEIN BY USING THE MAD TECHNIQUE ON THE COBALT-EDGE

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One of the most challenging topics in hemoprotein chemistry is to identify and to rationalise the structural requirements for specific defined functions (dioxygen activation/transport/storage, electron transfer etc.). Recently, mimochrome-IV a synthetic based-peptide model of hemoproteins was designed [Chem.Rev. (2001) 101, 3165]. The molecule is composed by two identical nine-peptide chains covalently linked to the propionic groups of a deuteroporphyrin-IX through the epsilon-amino function of Lys. Each peptide sequence bears a His residue in the central position that may act as axial ligand in metal coordination. With the aim to study the metal environment of this system we have undertaken the X-ray diffraction experiments of the mimochrome-IV cobalt(III) derivative. After several unsuccessful attempts to solve the phase problem by molecular replacement and the direct methods, the presence of a cobalt atom in the structure suggested to perform an MAD experiment. Four MAD data sets, collected at Elettra, were used to solve the XRD structure of the lambda isomer of Co(III)-mimochrome-IV. The structure consists of two antiparallel peptide chains in α-helix conformation, sandwiching the porphyrin plane with a pseudo *C*2 symmetry. This is the first crystal structure of an artificial heme protein of the mimochrome series and represents the starting point to address the future design directions to reproduce in artificial molecules the subtle mechanisms that control the heme functions, thus ensuring the selectivity of the natural systems. Furthermore, at best of our knowledge this result represents the first structure solved using MAD technique on the cobalt absorption edge.

Keywords: MINIATURIZED PROTEIN HEMOPROTEIN MAD

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GUEST-HOST CROSSTALK IN AN ANGIOGENIN/RNase A CHIMERIC PROTEIN

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Chimeric proteins are powerful tools for tackling numerous biological problems. These include the investigation of functional differences between related proteins, the generation of proteins with novel activities, and the recreation of critical events in protein evolution. Human angiogenin and bovine pancreatic ribonuclease (RNase A) share 33% sequence identity but have distinct functions. Angiogenin is a potent inducer of angiogenesis that is only weakly ribonucleolytic, whereas RNase A is a robust RNase that is not angiogenic. A chimera (ARH-I), in which angiogenin residues 58-70 are replaced by residues 59-73 of RNase A, has intermediate ribonucleolytic potency and no angiogenic activity. We present a 2.1 Å resolution crystal structure of ARH-I that reveals the molecular basis for these characteristics. The RNase A-derived (guest) segment adopts a structure largely similar to that in RNase A, and successfully converts this region from a cell-binding site to a purine-binding site. At the same time, its presence causes complex changes in the angiogenin-derived (host) portion that account for much of the increased RNase activity of ARH-I. Guest-host interactions of this type probably occur more generally in protein chimeras, emphasizing the importance of direct structural information for understanding the functional behavior of such molecules.

Keywords: ANGIOGENIN, RIBONUCLEASE A, CHIMERIC PROTEIN

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ENGINEERING GREEN FLUORESCENT PROTEIN TO A REDOX SWITCH

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To track the potential for disulfide bond formation in living cells a pair of cysteines was introduced in the green fluorescent protein variant, YFP, [1-3]. The formation of a disulfide bond between the cysteines resulted in a more than 2-fold decrease in the intrinsic fluorescence of the protein. With this change in fluorescence it was possible to probe the redox changes that occur in *E. coli* upon disruption of the thioredoxin reductive pathway [4]. GFP is a single chain protein consisting of 238 amino acid residues in an 11-stranded β -barrel with a central irregular α -helix including an auto-oxidized fluorescent tri-peptide (Ser-Tyr-Gly). Several variations in the amino acids adjacent to the chromophore have demonstrated that a number of non-covalent interactions play a role for spectral properties of the protein. In this environment a number of cysteine pairs were inserted in positions that, from a visual inspection of the GFP structure, seemed to be suitable for disulfide bond formation. It was a criterion that the formed disulfide bond would be solvent exposed. X-ray crystallographic characterization of the most successful of the engineered gene products to 1.5 Å shows the structural mechanism responsible for the redox-dependant spectral changes in the absorption and emission spectra of the protein.

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STRUCTURE OF THE TATA-BOX BINDING PROTEIN FROM A THERMOPHILIC ARCHAEON, *SULFOLOBUS ACIDOCALDARIUS*

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The TATA-box binding protein (TBP) from a thermophilic archaeon, *Sulfolobus acidocaldarius* (optimum growth temperature, OGT, of 75°C), has been crystallized. Its structure was determined by the molecular replacement method using phases obtained from another structure [1PCZ]. The R-factor of the model is 20.4% at the resolution of 2.0 Å. With this structure and structures of two other groups of TBPs, that from Pyrococcus (OGT of 100ºC) and those of mesophilic eukaryotes, it has become possible to compare a series of protein structures which share the same secondary structure combination but modified in order to produce different thermal stabilities. On the basis of the three groups of TBP structures, amino acid positions were classified into those exposed to the solvent and those shielded inside. With this information, the amino acid sequences of a number of TBPs originated in various archaeal species have been compared. It has been concluded that with increasing OGT, the average hydrophobicity of the residues shielded increases, and, in contrast, of the residues exposed the average hydrophilicity increases. In some sense, the sum of the two values reflects the free energy of denaturation of each protein. Only three types of amino acid residues, Val, Leu, and Ile, exceed the average hydrophobicity inside the Pyrocuccs TBP. Inside the crystal structures of TBPs, a hydrophobic network between the three types of residues Val, Leu and Ile develops with increasing the thermo-stability, and this was most clearly identified with the N-domain. Only a limited number of positions are left unoccupied by the tree types of residues inside the Pyrococcus TBP, and these are the target for replacing to the three types in order to further increase the stability.

Keywords: THERMAL STABILITY ARCHAEA HYDROPHOBICITY