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

S. Geremia1,2, L. Di Costanzo1,2, L. Randaccio1,2, A. Lombardi2,3, V. Pavone2,3, M. Campagnolo1,3, K.R. Acharya1,2, M.C. Hares1, D.D. Leonidas,4, S. Geremia1

1University of Trieste / Department of Chemical Sciences Centre of Excellence In Biocrystallography Via L. Giorgieri 1 TRIESTE 34127 ITALY
2Center for Biochemical and Biophysical Sciences and Excellance In Biocrystallography Via L. Giorgieri 1 TRIESTE 34127 ITALY
3University of Napoli Federico II, Department of Chemistry
4Institute of Biological Research and Medicine and Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115, U.S.A.

Acta Cryst. (2002). A58 (Supplement), C277

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 C2 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

ENGINEERING GREEN FLUORESCENT PROTEIN TO A REDOX SWITCH

A. Henriksen1, H. Oestergaard1,2, F. Hansen1,3, J.R. Winther1
1Carlsberg Laboratory Department of Chemistry Gamle Carlsberg Vej 10 VALBY 2500 DENMARK 2Technical University of Denmark

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-π-terpene (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-dependent spectral changes in the absorption and emission spectra of the protein.

References

Keywords: PROTEIN ENGINEERING REDOX SWITCH GREEN FLUORESCENT PROTEIN