03-Crystallography of Biological Macromolecules

PS–03.04.15 CRYSTALLISATION AND PRELIMINARY STRUCTURE ANALYSIS OF PROTEIN R2 MUTANTS OF *ESCHERICHIA COLI* RIBONUCLEOTIDE REDUCTASE

Xiao-Dong Su*, Bert-Ove Persson, Britt-Marie Sjöberg and Hans Eklund#
*Department of Cell and Molecular Biology, Karolinska Institutet, Box 60400, 104 01 Stockholm, Sweden
#Department of Molecular Biology, Swedish University of Agricultual Sciences, Biomedical Centre, Box 590, 751 24, Uppsala, Sweden
Department of Molecular Biology, University of Stockholm, 106 91 Stockholm, Sweden

Ribonucleotide reductase is a key enzyme in DNA synthesis. It catalyses the de novo production of deoxyribonucleotide precursors for all species. In E. coli it is composed of two different dimeric proteins, R1 and R2. The crystal structure of E. coli R2 has been solved and 6 conserved residues responsible for binding the two irons in the active site have been identified(1). In order to further investigate the enzymatic mechanism, we have mutated the six iron-binding residues, Asp84, Glu115, His118, Glu204, Glu238, His241. Crystals have been obtained for six of the mutants, D84H, E115A, H118A, E204H, E238A, and H241A in the absence of iron, using similar conditions as have been used for wild type and apo R2 protein(2,3). High resolution data to 2.0Å have been collected from the crystals of E115A. The E115A crystal is of space group $P2_12_12_1$, a=74.9Å, b=86.0Å, c=115.7Å which is isomorphous to wild type R2 crystals.

The preliminary structure analysis of the E115A crystal shows besides the obvious change at Glu115, a major movement of Glu238 and rearrangement of water molecules have occurred. This implies a hydrogen bonding pattern in the E115A active site which is different from that of the apo wild type R2(3).

References:
(1)Nordlund, P., Sjöberg, B.-M. and Eklund, H. (1990) Nature, 345, 593
(2)Nordlund, P., Uhlin, U., Westergren, C., Joelson, T., Sjöberg, B.-M. and Eklund, H.(1989) FEBS Lett. 258, 251
(3)Åberg, A., Nordlund, P., and Eklund, H. (1993) Nature, 361, 276

PS–03.04.16 DOMAIN INTERACTIONS AND THE TYPE II Cu ACTIVE SITE IN NITRITE REDUCTASE By Elinor T. Adman, and S. Turley, Dept. of Biological Structure, University of Washington, Seattle, WA, 98195 USA

Copper-containing nitrite reductases occur in the dissimilatory denitrification pathway in which nitrate is ultimately reduced to nitrogen gas. Nitrite reductase (NIR) converts nitrite to NO. We have shown (Godden, J. W. *et al.* (1991). Science 253, 438–442.) that the structure of NIR from *Achromobacter cycloclastes* consists of a trimer of total mw 108,000, with six copper atoms per trimer. Three of these copper atoms are type-I Cu with characteristic Cys, 2His, Met ligands and distorted tetrahedral coordination. The other three are type-II Cu, and form part of the interface between monomers, ligated by two His residues from one monomer and one His from another. The type-II site has been shown, both crystallographically (Godden, J. W. *et al.* (1991). Science 253, 438–442.) and chemically (Libby, E. & Averill, B. A. (1992). BBRC 187, 1529–1535.), to be the site at which NO_2^- is reduced.

Although bonds to the type-II Cu form the only covalent bonds between monomers holding the trimer together, the occupancy of that site appears to be quite variable. There is however a relatively large protein-protein interface (about 1700 Å^2) for one surface which includes an extended C-terminal arm consisting of residues 330–340 which contribute to holding the trimer together. We have determined the structures of a type-II Cu depleted form of the protein (material kindly provided by Y. Yang & B. A. Averill, Univ. Virginia), as well as another crystal form at pH 6.2 and pH 6.8 with the trimer in the asymmetric unit.

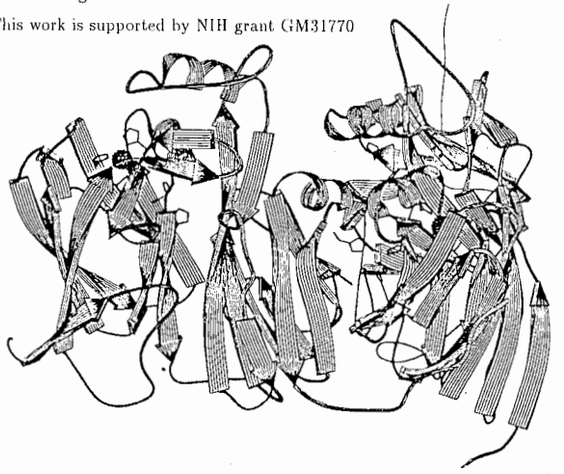
Crystals of the type-two-depleted NIR (T2D) were obtained in the same manner as the native material, yielding the same cubic space group, $P2_13$, cell edge 97.9 Å. Data to 1.6 Å were collected at the Molecular Structure Corporation facility on an R-Axis II image plate. 121,969 observations of 35,654 unique reflections to 1.6 Å (92% complete) gave an R_{merge} of 0.07 overall, 0.19 in the highest resolution shell. The $P2_12_12_1$ form has cell edges 99.5, 115.3, and 115.9 Å. Data to 2.8 and 2.5 Å were collected on a Siemens area detector for the pH 6.8 and pH 6.2 forms respectively.

A difference map at 2.3 Å between native data and type-II depleted data shows a peak at the type-II Cu of ~8.5σ confirming the loss of the copper at that site. However, a difference map of the T2D data and the model also shows a peak at that site indicating that either there is some residual copper at that site, or most likely, a water replaces the Cu and aids in maintaining the geometry of the site. Refinement (using X-PLOR) of the model with a water in that site, and about 75 other ordered solvent molecules gives an R-factor of 0.193 at this stage.

Solution of the orthorhombic form was by the Patterson correlation method in X-PLOR, and the current models have R-factors of 0.216 and 0.187 (no solvent) for the two forms. The N and C terminal residues of one of the three subunits are altered by packing interactions in this form. Difference maps between the pH 6.2 and 6.8 forms indicate differences in occupancy of solvent bound at the type-II Cu in the interface (Adman, E. T. & Turley, S. (1993). Bioinorganic Chemistry of Copper, edited by Karlin, K. D. & Tyeklar, Z. pp. 397–405. ed. New York: Chapman Hall).

The changes at the interface appear to be small, but may be significant, since the mechanism of NO_2^- reduction at the type-II Cu is likely to involve solvent reorganization.

This work is supported by NIH grant GM31770



PS–03.04.17 STRUCTURE OF BUFFALO LACTOTRANSFERRIN By A. Raman and T.P. Singh, Department of Biophysics, All India Institute of Medical Sciences, New Delhi - 110 029, K.L. Bhatia, National Dairy Research Institute, Karnal, India

Lactotransferrin is an iron-binding protein. It consists of a single polypeptide chain. The molecular weight is around 80000 Daltons. There are two glycans attached to the protein chain through N-glycosidic linkages. It is able to bind tightly, but reversibly, two ferric ions per protein molecule but only with the synergistic binding of two bicarbonate or carbonate anions. It has been isolated and

purified from buffalo colostrum. The purified lactotransferrin has beam crystallized from a 10% ethanol solution. The crystals are orthorhombic and the space group is $P2_12_12_1$ with unit cell dimensions a = 111.40A, b = 150.80A, c = 158.10A. The asymmetric unit contains three molecules of the protein with a solvent content of about 59%. The crystals were stable in the X-ray beam and diffracted beyond 3.0A resolution. The intensity data upto 3.00A resolution on the native crystals have been collected. The molecular replacement method has been used to determine the structure of the protein using the models of human lactoferrin and rabbit serun transferrin protein. The protein possesses a bilobal structure. Further refinement of the structure is in progress.

PS-03.04.18 CRYSTAL STRUCTURE OF CYTOCHROME c_3 FROM *Desulfovibrio desulfuricans* ATCC 27774.

J. Morais*, C. Frazão, P. M. Matias and M. A. Carrondo

C.T.Q.B., Apartado 127, 2780 Oeiras, Portugal

The three-dimensional X-ray structure of cytochrome c_3 from sulphate-reducing bacteria *Desulfovibrio desulfuricans* ATCC 27774 (Dd 27774) (M.W. 13kDa, 107 residues, 4 heme groups) has been determined by the method of molecular replacement, using as model the refined structure of cytochrome c_3 from *Desulfovibrio vulgaris* Miyazaki F (DvM) (Higuchi *et al.*, J. Mol. Biol., 1984, 172, 109-139), since the two proteins share a 45% sequence homology. The c_3 DvM coordinates were retrieved from the Protein Data Bank.

Crystals of c_3 Dd 27774 were obtained in space group $P6_122$, with cell dimensions a=b=61.8Å and c=109.8Å, Z=12. An X-ray diffraction data set was measured with synchrotron radiation at SRS Daresbury, England, using an Enraf-Nonius FAST area detector diffractometer. The intensity data measurements were carried out with program MADNES. Using programs from CCP4 suite,19328 reflections were merged into 11179 unique (84.8% completeness) in the resolution range 1.75<d<20.0 Å, with $R_{merg}(I) = 5.5\%$.

Cross-rotation and translation functions were performed with ALMN and TFSGEN programs (CCP4 suite), respectively. The packing of the molecules in the unit cell was checked on a CYBER 910/470 graphics workstation with TOM/FRODO.

Rigid body refinement of the model and subsequent refinement using molecular dynamics were performed with XPLOR, achieving an R factor of 25.9%, for data up to 2.3 Å. RESTRAIN least-squares refinement is in progress, the current R factor being 22.5%, for data up to 1.9Å.

PS-03.04.19

CRYSTAL STRUCTURE STUDY OF BAR-HEADED GOOSE OXYHEMOGLOBIN. By Zhang Jian, Hua Ziqian, Lu Guangying and Gu Xiaocheng, Biology Department, Peking University, Beijing 100871, China.

Bar-headed goose (Anser indicus) is a migratory bird which inhabitates around west china's Qinhai lake. On its migratory flight over Mount Everest it is exposed to ambient pO_2 of about 50mmHg which accounts to 20% of pO_2 at sea level. The determination of its hemoglobin crystal structure may partly elucidate the high altitude respiration mechanism where the air is extremely sparse and also the effect of evolutionary pressure to the protein molecular structure.

Oxyhemoglobin crystals suitable for X- ray analysis were grown from polyethylene glycol(PEG) with average MW.6000, at 4° C, pH6.8. The crystals belong to space group $P4_22_12$. Its unit cell parameters are a=b=81.6A, c=107.3A. There is one ab dimer per asymmetric unit. The intensity data were collected from a single crystal on screenless Weissenberg camera using synchrotron X-ray(Sakabe, N.,J. Appl. Cryst. 1983, 20, 404-407). Total independent reflections were 24807 with upper resolution of 1.8A. Intensity R factor was 4.7.

The hemoglobin of Bar-headed goose has about 70% sequence homology to that of human. So the molecular replacement method was used in structure determination. 2.1A resolution human oxyhemoglobin coordinates was taken as model molecule(Shaanan, B. et al., J. Mol. Bio., 1983, 171, 31- 59). The orientation and position of the molecule in the unit cell were determined with AUTOMR program (Matsuura, Y., J. Appl. Cryst., 1991, 24, 1063-1066). The initial model was rotated about (30, 50, 40) degree (polar angle system). Cross rotation function was calculated using data between 10-4A resolution and pattern cutoff radius 30A. The highest peak appeared at (60.38, 175.73, 260.75) of polar angle system and its translation parameters were (40.8A, 0.0A, 32. 2A) calculated from 12-10A resolution data. Rigid body refinement was carried out with CORELS program, allowing only rotation and translation of molecular dimer. This brought the R-factor to 0.447 between 10-4A resolution. The model after CORELS program was then refined by the simulated annealing method with X-PLOR program(Brunger, A.T.,Science, 1987,235,458-460), the R-factor dropped to 28.9% against data between 8-2A resolution without intervention. The root-mean-square deviation of bond length and bond angle were 0.026A and 4.443 degree respectively for all atoms. The model was checked on the graphics with FRODO program. The hame group area fitted well with the 2Fo- Fc electron density map, while the N-terminal area of the peptide chain fitted relatively poor. Further refinement is under way.

PS-03.04.20 CRYSTAL STRUCTURE ANALYSIS OF HEMOGLOBINS WITH MG-SUBSTITUTED α-HEMES AND EITHER UNLIGANDED OR CO β-HEMES. By S-Y. Park*[1] A. Nakagawa[2], H. Morimoto[1], [1]Department of Biophysical Engineering, Faculty of Engineering Science, Osaka University, Osaka 560, Japan., [2]Photon Factory, KEK, Oho, Tsukuba, Ibaraki 305, Japan.

We have been studying metal substituted hybrid hemoglobins represented as $\alpha_2(M)\beta_2(Fe)$ and $\alpha_2(Fe)\beta_2(M)$, where M denotes metal ions substituted for Fe^{2+}. Depending on the kind of metal ion substituted Fe^{2+}, the subunits change their O_2 affinity from the lowest to the highest of the human hemoglobins (Morimoto et al., *Tanpaku Kakusan Koso*, 1987, 32, 557-565). Thus we can study the interaction between the state of the central ion of heme and the state of the globin moiety by comparing their properties.