03-Crystallography of Biological Macromolecules

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

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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.

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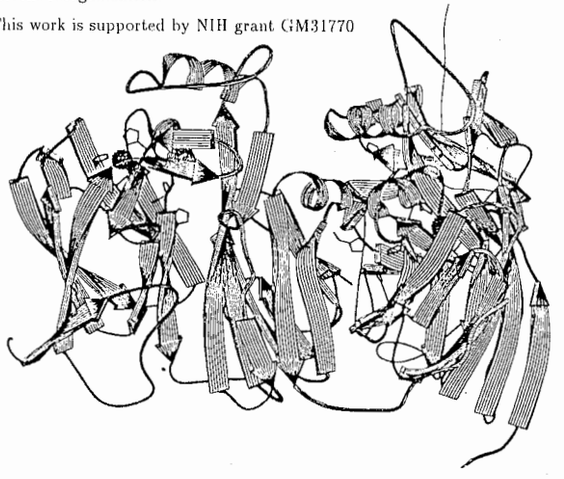

PS–03.04.17 STRUCTURE OF BUFFALO LACTOTRANSFERRIN By A. Raman and T.P. Singh, Department of Biophysics, All India Institute of Medical Sciences, New Delhi - 110 029, K.L. Bhatia, National Dairy Research Institute, Karnal, India

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

