PS-03.04.15 CRYSTALLISATION AND PRELIMINARY STRUCTURE ANALYSIS OF PROTEIN R2 MUTANTS OF \textit{ESCHERICHIA COI} 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 inorganic activators have been identified (1). In order to further investigate the enzymatic mechanism, we have mutated the six iron-binding residues, Asp84, Glu115, His116, 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 apo R2 protein (2), (3). High resolution data to 2.0 Å have been collected from the crystals of E115A. The E115A classic is of space group P2_1_2_1, a = 74.9 Å, b = 86.0 Å, c = 115.7 Å, which is isomorphous to wild type R2 crystals.

The preliminary structure analysis of the E115A crystal shows besides the obvious charge change at Glu115, a major movement of Cu238 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).

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PS-02.04.16 DOMAIN INTERACTIONS AND THE TYPE II Cu ACTIVE SITE IN NITRITE REDUCTASE

By Elmer T. Adman, and S. Turley, Dept. of Biological Structure, University of Washington, Seattle, WA 98195 USA.

Copper-containing nitrate reductase occur in the denitrification denitrification pathway in which nitrate is ultimately reduced to nitrogen gas. Nitrite reductase (NIR) converts nitrite to NO. We have shown (Cochran, J. W. et al. (1991) Science 253, 438-444) that the structure of NIR from 

\textit{Azobacter vinelandii} contains a trimer of total mass 108,000, with six copper atoms per trimer. Three of these copper atoms are type II 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 (Cochran, J. W. et al. (1991) Science 253, 438-444) and chemically (Libby, B. & Avellin, B. A. (1992) BBRC 187, 1529-1533), to be the site at which NO is reduced. Although bonds to the type II Cu from the only conserved 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 Å²) for one surface which includes an extended C-terminal arm consisting of residues 310-346 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. Avellin, Univ. Virginia), as well as another crystal form at pH 2.0 and pH 6.8 with the trimer in the asymmetric unit.

Crystals of the type II-depleted NIR (TID) were obtained in the same manner as the native material, yielding the same cubic space group, P3_1_2_1, cell edge 37.9 Å. Data to 1.6 Å were collected from the Molecular Structure Corporation facility on an R-AxisII image plate. 41,964 observations were reconciled to 1.6 Å (1726 complexes) gave a completeness of 0.97 overall, 0.99 in the highest resolution shell. The TID form has cell edges 99.4, 115.3, and 115.0 Å. Data to 2.8 Å were collected on a Siemens area detector for both pH 2.0 and pH 6.2 form respectively. A difference map at 2.8 Å between native data and type II-depleted data shows a peak at the type II Cu of 3.8 e/Å^3 confirming the loss of the copper at that site. However, a difference map of the TID and the model also shows a peak at that site indicating that there is some residual copper at that site, or now, likely, a water replaces the Cu and aids in maintaining the geometry of the site. Replacing (using X-PLOR) of the model with a water in that site, and about 75 other ordered solvent molecules gives an R-factor of 0.13 at this stage.

Solutions 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 (two 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. Differences maps between the pH 2.0 and 6.8 forms indicate differences in occupancy of solvent bound at the type II Cu at the interface (Adman, E. T. & Turley, S. (1993), Bioorganic Chemistry of Copper, ed. Karlin, R. D. & Tyszkiewicz, 26, 397-406, ed. New York: Chapman Hall).

The charge at the interface appear to be small, but may be significant, since the mechanism of NO reduction at the type II Cu is likely to involve solvent reorganization.

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PS-03.04.17 STRUCTURE OF BUFFALO LACTOTRANSFERRIN

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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 a glycosidic linkage. 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