

03-Crystallography of Biological Macromolecules

93

the site occupied by the inhibitor N atom and may thus be released from the lock-in position with OG1 atom of Thr199 as observed in the active form of the native enzyme (Merz, K.M., *J. Mol. Biol.*, 1990, 214, 799-802). This may sterically inhibit the binding of the substrate CO₂ molecule.

PS-03.05.38 CRYSTAL STRUCTURE DETERMINATION OF THE FLAVIN DOMAIN OF CORN NITRATE REDUCTASE By Guoguang Lu†, Wilbur Campbell¶, Ylva Lindqvist† and Gunter Schneider†, † Department of Molecular Biology, Swedish University of Agricultural Sciences, Box 590, BMC, 751 24 Uppsala, Sweden, ¶ Plant Biotechnology Group and Department of Biological Sciences, Michigan Technological University, Houghton, MI49931, USA

Nitrate reductase (NR, EC 1.6.6.1) of higher plants catalyzes the two-electron reduction of nitrate to nitrite using NADH as the electron donor. This reaction is the first step in the reduction from nitrate to ammonium, via which higher plants assimilate nitrogen from soil. NR is a homodimer where each subunit is composed of an approximately 100kD polypeptide and three cofactors, FAD, Fe-heme and Mo-pterin. The subunit can be divided into three functional domains, each of which contains one cofactor.

Crystals of the flavin domain of corn nitrate reductase expressed in *E. coli* have been obtained at room temperature using 0.1M TES pH=8.0 and 0.54-0.64M sodium citrate as precipitant. Crystals belong to the rhombohedral space group R3 with cell dimensions a=b=145.4Å, c=47.5Å, α=β=90°, γ=120°. All crystals are seriously twinned and a method has been

developed to reduce the intensity data from such a twin crystal to a dataset corresponding to a single crystal. Diffraction datasets of native crystals were collected on a Xentronix area detector to 3.2Å resolution with Rmerge=8%, and at the synchrotron radiation source at Daresbury by film to 2.5Å resolution. We are presently trying to solve the structure by molecular replacement using a model of Ferredoxin Reductase. Application of the rotation and translation functions gave an initial solution that is presently refined.

PS-03.05.39 Crystal structures of active site mutants of T4 endonuclease V: a pyrimidine-dimer-specific excision repair enzyme, K. Morikawa¹, M. Ariyoshi¹, K. Katayanagi¹, T. Doi¹, D. Vassilyev¹, E. Ohtsuka², ¹Protein Engineering Research Institute, 6-2-3 Furuedai, Suita, Osaka 565, and ²Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Hokkaido 060, Japan

Irradiation with ultraviolet light causes the formation of pyrimidine-dimers in DNA which is lethal and mutagenic *in vivo*. Bacteriophage T4 endonuclease V (T4 endoV) is an enzyme responsible for the first step of the excision repair in bacteriophage T4-infected *E. coli* cells. This enzyme has two distinct catalytic activities, a pyrimidine-dimer glycosylase and an apurinic/aprimidinic endonuclease, although it is a rather small protein consisting of 137 residues. The crystal structure of the wild type enzyme was refined at 1.45 Å resolution. The molecule is formed by a single compact domain which consists of three α-helices, five reverse turns and loops. In conjunction with results from site-directed mutagenesis experiments, the refined structure allows the identification of the catalytic center for the glycosylase which consists of Glu23 and surrounding basic residues, such as Arg 3, Arg 22 and Arg 26. The crystal structures of three active site mutants, E23Q, E23D, and R3Q, were also determined at atomic resolution. The results suggest that Glu23 is directly involved in the glycosylation catalysis, and that Arg3 has an important role for substrate binding.

PS-03.05.40

A COMPARISON OF THE ACTIVE SITE COPPER SUBSTITUTED LIVER ALCOHOL DEHYDROGENASE AND TWO CONFORMATIONS OF NATIVE Zn-LADH AT 1.8 AND 1.9 Å RESOLUTION. By Salam Al-Karadaghi and Eila Cedergren-Zeppezauer, Department of Structural Chemistry, Arrhenius Laboratories of Natural Sciences, Stockholm University, 10691 STOCKHOLM, Sweden.

Horse liver alcohol dehydrogenase (alcohol:NAD⁺ oxidoreductase, EC 1.1.1.1) is a zinc enzyme that catalyzes the transfer of two electrons and one proton between primary or secondary alcohols and NAD⁺. The enzyme is a dimer of two subunits, each consisting of 374 amino acids divided into two domains. The physico-chemical properties of LADH have been extensively studied and several crystal forms of the protein have been obtained. High resolution data was collected at the Hamburg synchrotron of two conformational states of the protein (Al-Karadaghi et al., *Acta Cryst. Section D*, 1993, submitted and Cedergren-Zeppezauer, Al-Karadaghi, Lamzin, Dauter and Wilson, in preparation). We present here a detailed comparison of the refined, closed structures of native Zn-LADH in complex with NADH and the inhibitor DMSO (Zn-ERD) at 1.8 Å, the similar complex of copper substituted LADH (Cu-ERD) (Al-Karadaghi et al., 1993, *PROTEINS*, submitted) and an open conformation of Zn-LADH to 1.9 Å resolution without cofactor or inhibitor bound.

The active site Zn(II) ion of LADH, essential for binding of the substrate, is coordinated to two sulfur atoms (from cysteines), one nitrogen (from histidine) and the oxygen of water in the open conformation (in which no cofactor is bound). The Zn-coordination sphere in both enzyme conformations have distorted tetrahedral geometry. Only small differences could be observed for the metal ligand parameters in spite of the fact that in the Zn-ERD complex the water molecule has been exchanged by DMSO, the nicotinamid ring of NADH is at van der Waals distance to the metal and a large domain movement has influenced secondary structure elements delivering ligands to the zinc ion.