Structure and Characterization of Proteins and Enzymes Involved in Nucleotide Metabolism and Iron-Sulfur Proteins - DTU Orbit (17/11/2019)

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This thesis presents work on two projects: the all cysteinyl coordinated D14C variant of the hyperthermostable Pyrococcus furiosus (Pf) ferredoxin (Fd) as well as wild-type (WT) and two variants of the bifunctional dCTP deaminase-dUTPase (DCD-DUT) from Mycobacterium tuberculosis (Mj). Furthermore, a program named MyCrystals has been developed to keep track of crystallization trials and results. The program combines pictures with crystallization conditions and is able to sort the pictures based on selected conditions. MyCrystals was used extensively throughout this work and allows for an overview of the crystallization results through the use of databases.

Changing the cluster coordinating aspartate to cysteine in Pf Fd proved to impair the ease with which the [Fe₄S₄]₄ cluster converted to the [Fe₂S₃]₃ cluster. A disulfide bonded dimer was observed at pH 8.0, whereas only the monomer was present at pH 5.8. The crystal structure of D14C [Fe₂S₃]₃ is the first structure with a [Fe₂S₃]₃ cluster, in which a cysteine from a full cysteine binding motif is unprotected and facing away from the cluster. The structure is in close resemblance with the WT [Fe₂S₃]₃ structure. Crystal packing in both D14C and WT [Fe₂S₃]₃ Fd shows extended β-sheet dimers. These dimers were not observed in solution and were likely a result of the high protein concentration in the crystals.

WT, A115V and A115G Mt DCD-DUT were successfully purified, and the crystal structure of the A115V variant with dTTP bound was solved. The variants were created to investigate the importance of steric hindrance on a water molecule suggested to play a key role in dephosphorylation. However, this water molecule was present in the structure of A115V:dTTP and the variant did not dephosphorylate dTTP. The dTTP pyrimidine moiety in the WT and A115V structures is rotated compared with the pyrimidine moiety of dUMPNNP in the structure of Mehtanocaldococcus jannaschii (Mj) DCD-DUT. This causes changes in the hydrogen bonding pattern of conserved residues in the active site and may give rise to less stabilization of the negative charge formed on the oxygen bridging the α-β-phosphorous of the nucleotide in the course of the dephosphorylation reaction. The flexible region consisting of residues 110-118 in the structure of A115V:dTTP is in close resemblance with the active conformation seen in Mj DCD-DUT:dUMPNNP.

A115V Mt DCD-DUT was unable to bind dTTP in the inactive conformation due to steric hindrance caused by the introduced valine side chain. In contrast, the A115V variant was inhibited by dTTP at both pH 6.8 and 8.0, albeit with a stronger inhibition at pH 8.0. The protonation state of the conserved His112 in the flexible loop is likely to play an important role herein. His112 is completely deprotonated at pH 8.0, where it is stabilized in the active conformation. The active conformation for the WT enzyme is likely to be the same as seen in Mj DCD-DUT:dUMPNNP, and this conformation does not allow dTTP binding because of steric hindrance. Hyperbolic dCTP and dUTP saturation curves support that the WT enzyme was present solely in the active conformation at pH 8.0.

The A115V variant was inhibited by dTTP at both pH 6.8 and 8.0, albeit with a stronger inhibition at pH 8.0. The flexible 110-118 loop in the A115V:dTTP structure is more loose compared with Mj DCD-DUT:dUMPNNP. This wider loop could allow dTTP binding even with protonated His112. dTTP binds more easily at pH 8.0 because completely deprotonated His112 takes up less space near the nucleotide binding site.

The A115G variant showed an opposite pH effect of dTTP inhibition compared with the WT enzyme. A115G was very sensitive to dTTP at pH 8.0, while no substantial inhibition was observed at pH 6.8. Changing alanine to glycine, which has additional allowed backbone conformations, is likely to introduce additional flexibility to the already flexible 110-118 loop. Hence, dTTP binding at pH 8.0 could simply be explained by the less restrained structure. In contrast, the lack of inhibition at pH 6.8 for the A115G variant could be caused by the protonated histidine inducing stability by binding to Gly115 O and thereby structurally arranging the very flexible loop. At pH 8.0, the A115G variant shows sigmoidal dCTP saturation and hyperbolic dUTP saturation. This distinction between dTTP and dUTP as substrates could be related to Ala115 being involved in dCTP deamination in the WT enzyme. The flexible Gly115 backbone of the A115G variant may require adjustment for the deamination to take place, whereas the conformation of this residue is indifferent for dephosphorylation.

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