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Keywords: proteases, crystallization, X-ray structure

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Do the Strong Magnetic Fields Modify the 3D Structure of Proteins?
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In this work we explore the feasibility of a new method to increase the crystal quality of biological macromolecules for X-ray crystallography. The method consist of growing protein crystals in capillary tubes containing a gelled protein/precipitant solution under the presence of a strong magnetic field of 10 Tesla. The strong magnetic field applied to the crystal growth cell was the conventional NMR magnet normally used in Chemistry laboratories.

From our preliminary results by solving the 3D structure of these analyzed protein crystals and controls, it was observed that crystals grown under the presence of a strong magnetic field in gel/capillary tubes improved substantially their electron density maps where electron density was not observed in all controls.

This promising methodology will help most of biocrystallographers to increase the crystal quality, a typical problem in most of the research laboratories for structural biology. Because of the existence of two different gels (double protective-chamber), this methodology seems to be also an available way to transport safely crystals to the synchrotron facilities without using the classical heavy laboratory Dewars for data collection.
Keywords: biomacromolecule X-ray, crystal growth from solution, crystal perfection

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Crystal Structure of the NADP-dependent 3-hydroxyisobutyrate Dehydrogenase
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3-Hydroxyisobutyrate, a central metabolite in the valine catabolic pathway, is reversibly oxidized to methylmalonate semialdehyde by a specific NAD/NADP-dependent dehydrogenase. To gain insight into the function of this enzyme at atomic level, we have determined the first crystal structures of 3-hydroxyisobutyrate dehydrogenase from *Thermus thermophilus* HB8: holo enzyme, 3-hydroxyisobutyrate complex, and sulfate ion complex. The crystal structures reveal a unique tetramer consisting of four identical protomers. The protomer folds into two distinct domains with open/closed interdomain conformations. The cofactor NADP(H) and the substrate 3-hydroxyisobutyrate are bound at the cleft between the two domains of the closed protomer. The observed tetramer structure might be important for the catalytic function through forming the active site involving two adjacent subunits. A kinetics study confirms that this enzyme has strict substrate specificity for 3-hydroxyisobutyrate and serine, but it cannot distinguish the chirality of the substrates. This enzyme prefers the physiological cofactor NADP rather than NAD. We propose a reaction mechanism based on the structures of cofactor/substrate bound at the cleft; Lys^{165} is the probable catalytic residue of the enzyme.

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Keywords: 3-hydroxyisobutyrate, 3-hydroxyisobutyrate dehydrogenase, MAD

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Visualization of the Forward and Reverse Reactions Catalyzed by Nitrite Reductase
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The process of denitrification involves the sequential reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to dinitrogen (N_2). The nitrite reductase from *A. faecalis* (NiR) is a green 110 kDa homotrimer with each monomer containing one type I and one type II copper (Cu) sites. The type I is the site of electron transfer from pseudoazurin. Electrons are then donated internally to the type II Cu site, where NO_2^- is reduced to NO. Crystals of NiR are orthorhombic with a trimer in the asymmetric unit.

To visualize the product bound at the active site, ascorbate *reduced* crystals of NiR were exposed to a NO saturated solution and frozen in liquid N_2 in the absence of oxygen. Data were collected at SSRL to 1.3 Å resolution. After refinement at full occupancy, the average B factor for NO is 29 $Å^2$, similar to that observed for water bound to the resting state of the enzyme. The N and O atoms of NO are equidistant from the Cu, thus the Cu-nitrosyl of NiR is characterized with side-on coordination of a diatomic molecule [1].

To examine the ability of the enzyme to catalyze the reverse reaction, *oxidized* crystals of NiR were exposed to a saturated NO solution. Refinement of the structure to 1.4 Å revealed nitrite bound to the copper via its oxygens, indicating completion of the reverse reaction in crystal. Spectroscopic studies [2] further support the conclusion that Cu-NiR can catalyze the reverse reaction.

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Keywords: structures of metalloproteins, copper complexes, nitric oxide

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The Structural Basis for Ser/Thr Protein Phosphatase Inhibition
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Serine/Threonine Protein Phosphatases are important in many cellular processes including glycogen metabolism and immunosuppression. Many marine prokaryotic organisms produce structurally diverse phosphatase inhibitors that can be toxic. The surface of Ser/Thr Phosphatases contain an inhibitor-binding loop which is important in inhibitor activity. How this loop determines inhibitor-specificity is unknown. We have solved the structures of Protein Phosphatase-1 (PP1) bound to four marine natural product inhibitors: okadaic acid, motuporin, clavosine and microcystin-LA(2H)[1]. These inhibitors bind in a similar manner to the phosphatase, exhibiting analogous interactions and showing no structural rearrangement of the inhibitor-binding loop. A structure solved using a mutant PP1, where the inhibitor-binding loop from calcineurin has been substituted for the native loop, reveals repositioning of only specific amino acid side-chains. These results indicate that inhibitor specificity in Ser/Thr Phosphatases is most likely due to specific interactions within the inhibitor-binding loop and not structural rearrangements. There are observable differences in the binding of inhibitors to PP1 and the PP1-calcineurin hybrid, information that may be utilized in the design of new immunosuppressant calcineurin inhibitors.