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Development of the Protein Microcrystal Handling Technique Using a Laser Tweezers. Takaaki Hikima[a], Tetsuya Shimizu[a], Masaki Yamamoto[a].
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In synchrotron facilities, X-ray microbeam will be utilized for protein crystallography. It will be possible to collect diffraction data from a protein microcrystal with the size in the range from 1 μm to 10 μm. Until now a protein crystal is picked up manually from a crystallization droplet using a cryoloop and it mounted on a goniometer head. However it seems to be impossible to manipulate the protein microcrystals are very small and fragile against a shock. So we are developing an automatic microcrystal pick-up system. In the system, we applied laser tweezers to manipulate the fragile protein microcrystal. It was reported that the laser tweezers at the near-infrared region traps and manipulates a cell without critical photodamage. We applied a laser tweezers with two single-mode lensed fiber probes. The system could manipulate the protein microcrystal with lower emission power than the laser tweezers based on conventional condensing lens. The lensed fiber probe was small, which had an advantage to manipulate a microcrystal in the crystallization droplets at various crystallization plates. The laser tweezers succeeded in trapping protein crystals smaller than 25 μm and levitating it onto the cryoloop. X-ray measurement of the manipulated protein microcrystals at SPring-8 BL41XU indicated that laser trap with 1064 nm wavelength hardly affected the result of X-ray structural analysis.

Keywords: crystallography of protein small molecules; laser technology; microcrystals

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Crystal Structures of Metal Ion Containing Adenylate Kinase from Desulfovibrio Gigas. A. Mukhopaadhyay[a], A.V. Kladova[a], J. Trincão[a], S.A. Bursakov[a,b], I. Moura[a], J.J.G. Moura[a], M.J. Romão[a].
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Adenylate kinases (AK) from Gram-negative bacteria are usually devoid of metal ions in their LID domain.. Crystal structures of substrate free AK from *Desulfovibrio gigas* (AK_{gig}) with three different metal ions (Zn^{2+}, Co^{2+} and Fe^{2+}) bound in its LID domain have been determined by X-ray crystallography to resolutions 2.1, 2.0 and 3.0 **Å**, respectively. The zinc and iron forms of the enzyme crystallized in the space group *I222*, whereas the cobalt form crystals were *C2*. The presence of the metals was confirmed by calculation of anomalous difference maps and by X-ray fluorescence scans. The work presented herein is the first report of a structure of a metal containing Gram-negative. Native Co/Zn enzyme was crystallized, but only zinc was detected in its LID domain. Co- and Fe- AK forms were obtained by overexpressing the protein in *E. coli* grown in minimal medium supplemented with the appropriate MCl_2 (where M is Co^{2+}, Zn^{2+}, Fe^{2+}). .Zn- and Fe- forms of AK crystallized as monomers in the asymmetric unit, whereas the Co-AK crystallized as a back to back dimer However, all three crystal structures are very similar to each other with the same LID domain topology with the only change being the presence of different metal atoms in it. Absence of fully occupied metal sites in all three structures may indicate the weak binding of the metal atom to the enzyme. In the absence of any substrate, the LID domain of all holo forms of AK was present in a fully open conformation state. The normal mode analysis was performed and fluctuation of the LID domain along the catalytic pathway was predicted.

[1] Gavel O.Yu., Bursakov S.A., Rocco G. D., Trincão J., Pickering I. J., George G. N., Calvete J. J., Shnyrov V. L., Brondino C. D., Pereira A. S., Lampreia J., Tavares P., Moura J. J.G., Moura I., **2008**, *Journal of Inorganic Biochemistry*, 102, 1380–1395.

Keywords: metalloenzyme X-ray crystallography; MAD phasing; gram negative bacteria

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Proton Transfer Mechanisms in Multi-copper Oxidases: Studies in CotA-laccase. Catarina S. Silva[a], Zhenjia Chen[a], Paulo Durão[a], Peter F. Lindley[a], Lígia O. Martins[a], Cláudio M. Soares[a], Isabel Bento[a].
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Multi-copper oxidases (MCOs) constitute a family of enzymes responsible for coupling substrate oxidation with the reduction of dioxygen to water. Widely distributed in nature, MCOs include ascorbate oxidase, ceruloplasmin and laccases, the latter ones being the simplest representative members of this family of enzymes. As observed amongst its members, these enzymes contain at least two different copper centres as their minimal functional unit: a mononuclear type 1 blue copper centre (T1) and a trinuclear cluster comprising two type 3 and one type 2 copper atoms (T2/T3 site). The substrate oxidation occurs at the mononuclear centre, shuttling electrons to the trinuclear centre where dioxygen binding and reduction occurs along with the production of water molecules.
Using CotA-laccase as a model system, we have proposed a putative mechanism of oxygen reduction for this type of enzyme [1]. However, many questions relating to its catalytic mechanism remain to be addressed.
In nearly all multi-copper oxidases it is observable the existence of a carboxylated group conserved in the neighbouring of the trinuclear cluster, suggested to be involved in protonation events [2], [3]. Our CE/MC