MS06 P01

Reductive Alkylation of *Geobacillus* sp. Strain T1 Thermostable Lipase Mahiran Basri[a], Cheong Kok Whye[b], Raja Noor Zaliha Abd. Rahman[c], Mohd Basyaruddin Abdul Rahman[a] and Abu Bakar Salleh[c], [a]Faculty of Science, [b]Institute of Bioscience, [c]Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
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Keywords: alkylation, lipase, molten globule

The functional and physical properties of proteins can be tailored to the process of interest by changing the structure of the proteins. Small changes made to the protein structure could give an insight into the relationships between the structure and function. One way is by using a simple method such as by attaching chemical modifiers to specific amino acid residues of the protein molecules. Chemical modification was carried out on T1 thermostable lipase via reductive alkylation. Alkylation was carried out using propionaldehyde with different degree of modification to represent different level of hydrophobicity. The targeted alkylation sites were lysine, in which T1 lipase possessed 11 residues. Four residues (Lys84, 102, 138 and 251) were found to be exposed, four residues (Lys185, 329, 344 and 345) were moderately exposed and three were buried residues (Lys28, 207 and 229). The structural features of both native and modified state enzyme were studied using circular dichroism, MALDI-TOF MS and fluorescence spectroscopy. Comparison of the far-UV circular dichroism spectra between native and alkylated enzyme suggested formation of molten globule (MG)-like structure. This was further supported by 8-anilino-1-naphthalenesulfonic acid (ANS) probed fluorescence which indicated higher exposure of hydrophobic residues, consequential of chemical modification. Based on MALDI-TOF MS analysis, a number of lysine residues were found to be alkylated.

MS06 P02

New Protein Crystallization Device by Counter-Diffusion Method and *In Situ* Structure Determination Tomokazu Hasegawa[a], Kensaku Hamada[a], Masaru Sato[b], Moritoshi Motohara[b], Satoshi Sano [b], Tomoyuki Kobayashi[b], Tetsuo Tanaka[b], Yukiteru Katsube[a], [a]*PharmAxess,Inc., Osaka, Japan.* [b]*Japan Aerospace Exploration Agency "JAXA", Tsukuba, Japan.* E-mail: tomokazu@pharmaxess.com

Keywords: Protein crystallization device, Counter-diffusion method

We have developed a new protein crystallization device, "Micro Chip", using PDMS (polydimethylsiloxane). The "Micro Chip" is used for scanning protein crystallization conditions widely by using counter-diffusion method. The device requires few amounts, only 2 ul, of a protein solution, and the directions and pre-preparation is also easy. Although diffraction experiments can be carried out directly with an obtained crystal without taking out from "Micro Chip", taking out the crystal is also quite easy.
Since "Micro Chip" is portable, it is going to use it to a space experiment in the JAXA-New-GCF (JAXA-NGCF) project.
We will show some experimental data of "Micro Chip".

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MS06 P03

DLS & chromatography for rapid protein evaluation in crystallographic trials. Kohei Shiba[a], Koji Inaka [b], Ulf Nobbmann [c], Katsuhiko Jindo [a], and Atsushi Nakagawa[d] [a]*Symex Corporation, Japan.* [b]*Maruwa Foods and Biosciences, Inc.,Japan.* [c]*Malvern Instruments, UK.* [d]*Institute for Protein Research, Osaka University, Japan.* E-mail: Shiba.Kouhei@sysmex.co.jp

Keywords: dynamic light scattering, protein purification characterization, crystallography biological

Recent progress in structure determination of proteins allows us to reveal the interactions of protein molecules at atomic level. NMR and X-ray crystallography are widely used for determination of the three-dimensional structure of the proteins in detail. In both techniques, preparation of good samples is now the most difficult and important step. In addition, crystallization of the sample is required for X-ray crystallography. However, a serious and frequently encountered problem is that crystals cannot easily be obtained, even after an enormous number of crystallization trials have been attempted. Gel-electrophoresis, such as SDS-PAGE and native-PAGE, gel-filtration and dynamic light scattering (DLS) measurement are often used for evaluation of samples quality, and these measurements are often used to evaluate the possibility of crystallization.
DLS provides us the dispersity of the protein molecules in solution, and some results show strong correlation between quality of the crystals and dispersity of the protein solution. However, it takes a couple of minutes for each samples to measure the polydispersity of molecules, and also, the measurements are performed in batch mode. Therefore, a real-time DLS system, which can be used on line during the preparation, is required for crystallography. A real-time DLS system and some ideal trials from the preparation for crystallization will be presented.

MS06 P04

Prediction of Improvement of Protein Crystal Quality Grown in Microgravity Hiroaki Tanaka,[a] Masaru Sato,[b] Koji Inaka,[c] Bin Yan,[a] Sachiko Takahashi,[a] Mari Yamanaka,[a] Naoki Furubayashi,[c] Satoshi Sano,[b] Tomoyuki Kobayashi,[b] Atsushi Nakagawa[d], Tetsuo Tanaka[b], [a]*Cofocal Science Inc.,Japan.* [b]*JAXA, Japan.* [c]*Maruwa Foods and Biosciences Inc., ,Japan.* [d]*Osaka University, Japan.*
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Keywords: microgravity crystal growth, diffusion coefficient, kinetic coefficient

It is said that the microgravity environment has a positive effect on protein crystallization due to minimized convection fluid motion and sedimentation. However, the microgravity experiment was thought to have a limited potentiation to structural biology. Japan Aerospace Exploration Agency (JAXA) has conducted crystallization experiments in microgravity (JAXA–GCF project) since 2003, and has obtained know-how for obtaining high-

quality crystals, followed by the development of estimation method of the microgravity effect on crystallization.
In the project, high viscosity of the precipitant solution had positive effects on the quality of the protein crystal grown in microgravity. We have obtained high-quality crystals of alpha-amylase diffracted to 0.89 Å and of lysozyme diffracted to 0.88 Å both at SPring-8 beamline BL12B2 in the project. Both precipitant solutions contained polyethylene glycol either as a precipitant or an additive. It might be because protein and impurity depletion zones are positively formed in high viscous solutions especially in the microgravity environment.
We developed the method for estimating the diffusion coefficient (D) and kinetic coefficient (β) by a simple experiment. The value 'D/β' indicates that protein and impurity depletion zone around the crystal is formed in microgravity if D/β is low enough. Since we can predict the effects of microgravity on the protein crystal growth before performing microgravity experiment, it is possible to select samples and crystallization conditions which have high possibility to improve the crystal quality. Moreover, if we modify the crystallization condition to lower D/β, the improvement of the crystal quality can be expected in microgravity experiment.

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MS06 P05

JAXA-GCF Project --- The Past, Present and Future

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Keywords: microgravity crystal growth, atomic resolution crystallography, high-resolution X-ray diffraction

Japan Aerospace Exploration Agency (JAXA) finished JAXA-GCF project in 2006. Totally six protein crystallization experimental opportunities in space were provided to crystallographers, more than 250 protein samples were launched, using Russian flight opportunities, twice a year, from 2003. In the project, the success rate of crystallization, that is mostly the improvement of the maximum resolution, has been significantly increased to about 70% of protein that was highly purified and succeeded in the optimization of the crystallization condition. Moreover, the maximum resolution was even improved if the crystal showed already an excellent resolution around 1 Å in the ground-based experiment. We have obtained several know-how to grow high-quality crystals in space.
Based on them, JAXA has started JAXA-New-GCF (JAXA-NGCF) experiment in 2007. Three flight opportunities are scheduled, once in every six months. The first flight has already launched on Jan. 18, 2007 and will be landed in April. The purposes of JAXA-NGCF are to obtain atomic-resolution crystals for precise structural analysis, to cooperate with national project, and to transfer technology to private companies for commercial use.
We thank ESA and the Belgium government for Odissea mission and the usage of Granada Crystallization Facility (GCF), the Federal Space Agency and RSC Energia for the usage of the Russian Service Module, and NASA for the usage of the incubator in the US module. We are grateful to Professor Garcia-Ruiz and the members of his laboratory in CSIC-University of Granada for their helpful advices. We thank Protein 3000 Project (Riken and eight universities), NIAS, PCProt, and other users for providing protein samples.

MS06 P06

Cu co-crystallization and metal-ions cross-influence as a new optimization tools

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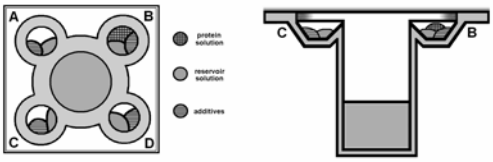
Keywords: Crystal morphology, Cupric compounds, Cross-crystallization

The effect of several metal cations (Cu^{2+}, Cd^{2+}, Co^{2+}, Ba^{2+}) was tested in attempts to improve crystallization and verify a newly discovered cross-crystallization method with two selected proteins; di-heme cytochrome c_4 from anaerobic purple sulphur bacterium *Thiocapsa roseopersicina* and sweet-tasting protein thaumatin from the African berry *Thaumatococcus daniellii*. Cu^{2+} ions promoted the most dramatic improvement in crystal morphology, internal packing and diffraction quality. This investigation qualitatively established the influence of cupric cations on the crystal growth by using the cross-crystallization procedure.



(Fig.: Schematic side and top view of Emerald BioStructures CombiClover Crystallization Plate used for sitting drop cross-crystallization experiments). It was found that influence of Cu^{2+} ions produced evidently different outer morphology and internal packing of thaumatin crystals (hexagonal prism). Usually their shape is presented as a tetragonal bipyramids. In the case of cytochrome, the good diffractable crystals were obtained only by using cross-crystallization method with metal-ion salts. Newly grown crystals (hexagonal prisms) of thaumatin and cytochrome displayed as the same primitive tetragonal system and diffracted up to 1.7 Å. Crystals were suitable for high-resolution structure analysis. (Table: Crystal morphology and internal packing influenced by metal-ion salts).