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-