1P145**Cold adaptation of the thermophilic enzyme 3-isopropylmalate dehydrogenase from *Sulfolobus tokodaii***

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Homologous enzymes that have adapted to different temperatures show different physical properties. Enzymes isolated from thermophilic microorganisms possess higher thermal stability than do the mesophilic counterparts. Thermostable enzymes also exhibit high stability against organic solvents acidic and alkaline pH and detergents. These characteristics are of great advantage for industrial applications. One serious drawback in the application of thermophilic enzymes is the low catalytic activity at moderate or lower temperatures. We have performed random mutagenesis coupled with selection to isolate mutant enzymes with high catalytic activities at low temperature from thermophilic archaea 3-isopropylmalate dehydrogenase (IPMDH) originally isolated from *Sulfolobus tokodaii*. IPMDH encoded by *leuB* gene catalyzes the oxidative decarboxylation of 3-isopropylmalate in the presence of NAD⁺ as a cofactor and is an ideal enzyme for the present study because many mesophilic, thermophilic and psychrophilic counterparts are known to have close structural similarities. The enzyme is tetrameric protein composed of four identical subunits, each having 336 amino acid residues. Five cold-adapted mutant IPMDHs were obtained by random mutagenesis followed by screening for the activity in the *leuB* *E. coli* strain at 25 °C. All mutants showed higher activity than the wild-type at 30°C and are K_m -improved type. The isolated mutant enzymes were then analyzed to elucidate the adaptation mechanism of enzymes to lower temperatures.

1P147**2-dimensional crystallization of mutant bacterioferritin**

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The bacterioferritin (BFR) of *Escherichia coli* is an iron-storage protein consisting 24 identical subunits and contains 12 heme groups. It forms a stable complex with 4/3/2 symmetry and spherical protein shell surrounding an approximately 8 nm central cavity. This cavity can store up to 4500 iron atoms theoretically. BFR is one of suitable proteins to obtain iron nano particles by biotechnology. Moreover, it is possible to make ordered nano-particle lattice by 2-dimensional crystallization of BFR. Previously, we succeeded to find 2-dimensional crystals of recombinant BFR that containing an iron core. These crystals of BFR had a square lattice, and each BFR particle was in contact by the 4 symmetry axis. We assumed that surface charges of BFR would be important for interaction between BFRs and planed to change the surface charge to obtain larger crystals. We designed mutant BFRs that were inserted a few Glu or Lys at the 4 symmetry axis to add negative or positive charge, respectively. These BFR mutant genes were constructed by two-step PCRs and each of them was inserted an expression vector (pET17b) including T7 promoter. The resulted plasmids were used for expression by using BL21(DE3)pLysS. We are going to observe 2-dimensional crystals of those mutants using transmission electron microscopy and discuss the effects of surface charge changes for crystallization.

1P146**Attempt to understand the information encoded in the amino acid sequence of photoactive yellow protein by the simplification of sequence**

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In order to understand the information encoded in an amino acid sequence, we have attempted to simplify the amino acid sequence of photoactive yellow protein (PYP). We previously reported that the simplified PYPs, which are designed to reduce overlapping structural information, lost the chromophore binding ability with completely different structures than a native conformation, and that partial simplification was one of the successful methods to elucidate the information when the simplification of the entire sequence was impossible. However, whether the hydrophobic residues and the conserved residues can allow for the simplification or not had yet to be determined. Consequently, we examined the simplification for their residues by creating chimeric proteins composed of WT and the fully simplified PYP, which was composed of only 9 species of amino acids (S, V, D, K, F, M, G, P and C). The whole sequence of PYP was divided into 7 blocks based on the location of the secondary structural features of the protein: the 1st block ($\alpha 1$ and $\alpha 2$), the 2nd block ($\beta 1$ and $\beta 2$), the 3rd block ($\alpha 3$ and $\alpha 4$), the 4th block ($\beta 3$ and $\alpha 5$), the 5th block ($\beta 4$), the 6th block ($\beta 5$), and the 7th block ($\beta 6$). The reconstitution of the variants that simplified each block was examined by absorption spectroscopy. The chimeras that simplified the 1st and 2nd block were found to be reconstitutable. This result suggests that all residues in the 1st and 2nd blocks can tolerate the simplification. In the presentation, we will also discuss the simplification for other blocks.

1P148**Study of expression of PSD-95 in neuron-glia mixed culture cells using adenovirus**

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Because of the preferential infection of adenovirus to glia vs. neurons, it is difficult to infect into neuronal cells in neuron-glia mixed culture. Here we showed the improvement of the infection efficiency of adenovirus into neuronal cells. Neuron-glia mixed culture was prepared from hippocampus of embryonic day 19 Sprague-Dawley rats. PSD-95 cDNA was subcloned into the pENTER/D-TOPO vector and was transferred to the pAd/CMV/V5-DEST vector. The purified and linearized plasmids were then transfected into 293A cells. The viral titer was determined by calculating 50% tissue culture infectious dose ($TCID_{50}=4.4 \times 10^7$). Aliquots of the adenovirus-containing medium were added to hippocampal primary culture. Our preliminary experiments showed that infection on 1 week after plating of hippocampal cells increases the infection efficiency. To obtain the higher infection efficiency, the primary hippocampal cultures were exposed to the various concentrations of adenoviral vectors on 1 week after plating, and were fixed with 4% paraformaldehyde and immunostained on 4 and 7 days after infection. When hippocampal culture was infected with 1.3×10^7 pfu/ml of adenovirus, the highest efficiency (about 90%) was obtained. Immunostaining with anti-synaptophysin revealed that about 70% of PSD-95-clusters colocalized with synaptophysin, indicating synapse formation. Our results represented that it was possible to obtain the high infection efficiency when neuron-glia mixed culture was infected on 1 week after plating with the high concentration of virus.