sphaeroides

Seung-Hyun Cho*, Sang-Hee Yun and Sa-Ouk Kang
Laboratory of Biophysics, School of Biological Sciences; Institute of Microbiology, Seoul National University, Seoul 151-742

PpsR from the facultative photoheterotroph *Rhodobacter sphaeroides* is involved in repression of photosynthesis gene expression under aerobic condition. PpsR was heterologously overexpressed and purified to homogeneity. Gel mobility shift assays showed that the purified PpsR is biologically active. The activity of PpsR in wild type was higher in aerobic condition than in photosynthetic condition. The activity was not detected in null mutant and was amplified in the exconjugant with *ppsR* carrying plasmid. Both cysteins in PpsR exist in their reduced form under the oxidation-reduction potential that is estimated in living cell. The amount of PpsR in aerobic and photosynthetic conditions in wild type maintained almost constant level by Western analyses. PpsR was not detected in null mutant and increased in the exconjugant with *ppsR* carrying plasmid by Western analyses.

E318

Characterization of Repressor Regulating the Expression of Pyruvate Dehydorgenase Gene in *Streptomyces seoulensis*

Jong-Il Yu*, Hwan Yun and Sa-Ouk Kang
Laboratory of Biophysics, School of Biological Sciences; Institute of Microbiology, Seoul National University, Seoul 151-742

Pyruvate dehydrogense repressor gene (pdhR) is located within PDH (pyruvate dehydrogenase complex) operon and shows homology with gluconate repressor (GntR) family, which is well known as DNA binding protein family. PdhR was overexpressed using pIJ702 vector in *Streptomyces seoulensis* and the growth of the overexpressed cells was retarded compared with wild type. PdhR was partially purified by heparin-sepharose and ion exchange chromatography from *S. seoulensis*. The native enzyme had a molecular mass of 45 kDa and SDS-PAGE revealed that the enzyme consists of two subunits , each with a molecular mass of 23 kDa. Electrophoretic mobility shift assay showed that PdhR binds to both promoters, Ppdh and Pace. This result indicates that PdhR acts as a repressor inhibiting transcription of PDH complex by binding to Ppdh as well as to Pace.

E319

Calcium-Induced Conformational Changes of the Recombinant CBP3 Protein and its Domains in *Dictyostelium discodeum*

Tsogbadrakh Mishig-Ochir* and Sa-Ouk Kang
Laboratory of Biophysics, School of Biological Sciences; Instituite of Microbiology, Seoul National University, Seoul 151-742

In order to characterize calcium affinity features of the CBP3 protein (Y.-H. Han and S.-O. Kang (1998) FEBS Lett. 441, 302-306) which has four EF-hand motifs in *Dictyostelium discodeum*, the protein was overexpressed in *Escherichia coli* and purified by Nickel-column using His 쌍Bind Buffer Kit (Novagen) under denaturing condition. After refolding and removal of histidine tag, this recombinant protein was further characterized by fluorescence, cysteine titration with 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB), and circular dichroism (CD) spectroscopy in the presence or absence of Ca^{2+}. The fluorescent intensity for CBP3 increased with 3-5 nm maximal wavelength

change, as the concentration of calcium ion in the solution increased. In the apo- and Ca^{2+}-binding states of the protein, the number of cysteine residues titrated with DTNB was different. The far-UV CD spectrum was sensitive to calcium binding. These results pointed out that the calcium binding induces certain conformational changes. Also we prepared the N- and C-domain of CBP3 protein. Conformational changes induced by calcium were also showed in the N- and C-domain. It seems that the CBP3 has calcium sensor character and calcium affinity of the N-terminal domain is higher than C-terminal domain. CBP3 protein and its domains have low solubility because of aggregation depending on the concentrations of protein and calcium, reducing agent, pH, etc.

E320

Functional Expression of Alternative Oxidase from *Candida albicans* in *Escherichia coli*

Young-Sug Lee*, Won-Ki Huh and Sa-Ouk Kang

Laboratory of Biophysics, School of Biological Sciences; Institute of Microbiology, Seoul National University, Seoul 151-742

In addition to the cytochrome-involved respiratory pathway, *Candida albicans* is known to possess an alternative, cyanide-resistant respiratory pathway that is mediated by alternative oxidase (AOX). But, only little information concerning the molecular structure and enzymatic features of AOX have been available. In this study, the *AOX1* and *AOX2* genes were cloned into the expression vector pGEX-4T-1 and pET 15b/ pET3a, respectively, and were expressed in *Escherichia coli*. The growth of the transformant carrying pET15b-AOX2 was cyanide-resistant. · Polypeptides with molecular masses of 69 kDa and 44 kDa were found in the cytoplasmic membrane of *E. coli* carrying pGEX-4T-1-AOX1 and pET 15b-AOX2, respectively, and were recognized by antibody against plant-type AOX from *Sauromatum guttatum*. The ubiquinol oxidase activity found in the membrane of the transformant was insensitive to cyanide, while that of the control strain, which contained vector alone, was inhibited. These results clearly showed the functional expression of *C. albicans* AOX in *E. coli*. When *C. albicans AOX* genes (*AOX1/AOX2*) were expressed in alternative oxidase-deficient *Saccharomyces cerevisiae*, it could also confer cyanide-resistant respiration on *S. cerevisiae*.

E321

Identification of a Protein that Interacts with Calcium-Binding Protein 3 (CBP3) in *Dictyostelium discoideum*

Sun-Young Jung*, Chang-Hun Lee and Sa-Ouk Kang

Laboratory of Biophysics, School of Biological Sciences; Institute of Microbiology, Seoul National University, Seoul 151-742

Dictyostelium discoideum, at least eight small, four-EF hand calcium-binding proteins respectively are expressed at specific stages during development. One of these proteins, calcium-binding protein 3 (CBP3), first appears just prior to cell aggregation and then maintains relatively constant levels throughout development. To determine the role of CBP3 during development, the protein was used as a bait in a yeast two-hybrid screen to reveal putative CBP3-interacting proteins. Of 7.0×10^6 independent transformants, one positive transformant carrying Actin8, which is the major component of the cellular microfilament system or actin cytoskeleton, was identified. Thus CBP3 might function to