mg/kg PTZ showed progressive increase in seizure severity, and in particular, in the animals showing severe seizure responses and sudden loss of body weight, the serious brain damages were revealed by histological analysis such as Nissl staining. We found moribund cells in discrete brain regions, such as piriform, entorhinal, and parietal cortex, hippocampus, and substantia nigra (SN) using acid fuchsin and TdT-mediated dUTP nick end labeling (TUNEL) staining, respectively. Apoptotic bodies were observed in dying cells after TUNEL staining. These data are the first demonstration showing that the chronic, systemic PTZ administration induces cell death in discrete brain regions. These results suggest that the long-lasting blockage of inhibitory synaptic activity may lead to the neuronal cell death.

E121 Downregulation on Neuronal Calcium Signaling by ER stress in PC12 cells

Ji Ae Park[1*] and Hyockman Kwon[2]
[1]Department of Molecular Biology, Dankook University; [2]Department of Bioscience and Biotechnology, Hankuk University of Foreign Studies

Neuronal activity facilitates neuronal survival and can lead to increases in synaptic strength. This phenomenon is mediated by the intracellular influx of calcium during membrane depolarization. The increase in calcium activates the transcription factor CREB that results in induction of multiple gene expressions including BDNF exon III. Since ER stress perturbs intracellular calcium signaling, we investigated the effects of ER stress on depolarization-induced CREB activation and BDNF exon III expression. Tunicamycin (TM) and -mercaptoethanol (ME), that induce ER stress by suppression on protein glycosylation or formation of disulfide bonds respectively, attenuated the depolarization-induced CREB activation, but not completely. ME also reduced the expression of BDNF exon III with a similar extent as that on the activation of CREB. On the other hand, TM completely abolished the expression of CREB in spite of a significant activation of CREB. These results suggest that ER stress can attenuate activity-dependent neuronal survival in part. In addition, TM appears to suppress the expression of BDNF exon III through multiple pathways.

E122 Insect Immunity - Purification and Some Properties of Immune Protein (Hemolin) from Hemolymph of Protaetia brevitarsis

Ock-Mi Choi* and Hak-Ryul Kim
The Graduate School of Biotechnology, Korea University, Seoul 136-701

Hemolin is the 48 kDa protein induced in response to bactreial infection and binds to Lipopolysaccharide(LPS) identified in the hemolymph of two lepidopteran insect species, *Hyalophora cecripia* and *Manduca sexta*. Based on these informations, we found the protein was bacteria-induced and bound to LPS, and the molecular weight is about 48 kDa on the SDS-PAGE in the last larval hemolyph of *Protaetia brevitarsis* after bacteria injection. Hemolin-like protein has been partially purified by gel permeation chromatgraphy (Superdex) and resource Q (ion-exchange chromatography) using fast performance liquid chromatography (FPLC) system.

E123 Purification and Molecular Properties of the Ferritin from the Larval Hemlymph of Protaetia brevitarsis

Eun-young Kim* and Hak-Ryul Kim
The Graduate School of Biotechnology, Korea University, Seoul 136-701

Ferritin, an iron storage protein, has been purified in the last larval hemolymph of *Protaetia brevitarsis* (coleopteran) by KBr

density gradient ultracentrifugation and two steps of resource Q (ion-exchange chromatography) using fast performance liquid chromatography (FPLC) system. Ferritin of *P. brevitarsis* is shown to have molecular mass of 600 kDa on a Native PAGE and its subunits consist of two major polypeptide with 27 kDa and 30 kDa presented on a SDS-PAGE. Ferritin was detected by Ferene-S stain and the confirmation of ferritin was also performed by western blotting with polyclonal antibody against Wax moth ferritin. The 27 kDa of *P. brevitarsis* was shown to react intensively with that of Wax moth ferritin whereas the 30 kDa weakly reacted. Other characteristics such as amino acid composition, N-terminal amino acid sequence, and isoelectric point were investigated.

E124 Purification of Transferrin from the Larval Hemolymph of Galleria mellonella

Seong-muk Kim* and Hak-Ryul Kim
The Graduate School of Biotechnology, Korea University, Seoul 136-701

An iron-binding protein has been purified from the last larval hemolymph of *Galleria mellonella* by using rapid purification method. Following the density gradient ultracenrifugation, we purified transferrin-like protein from the hemolymph subphase by immobilized metal ion affinity chromatography (IMAC) using fast protein liquid chromatography (FPLC). This protein was detected by potassium ferricyanide staining method in native polyacrylamide gel and molecular mass was about 80 kDa in SDS-PAGE.

E125 Purification and Characterization of the Ferritin for the Larvae of Bombyx mori.

Kyoung-Hee Lee* and Hak-Ryul Kim.
The Graduate School of Biotechnology, Korea University, Seoul 136-701

Ferritin, an iron-storage protein, was partially purified from the hemolymph of *Bombyx mori* by 3 steps, KBr density gradient ultracentrifugation, gel permeation chromatography (Superdex) and reversed phase chromatography (Resourse RPC) using fast performance liquid chromatography (FPLC) system. The detection of Fe was performed by Ferene S stain. Native molecular mass of ferritin was estimated as 660 kDa by Native PAGE. The partially purified hemolymph ferritin is composed of 3 subunits and molecular masses of each subunits were determined as about 24 kDa, 26 kDa, and 28 kDa, respectively using SDS-PAGE.

E126 Pak Kinase Activity Regulates Abi Stability

Susun Chi*, Soohee Chang and Dongeun Park
School of Biological Sciences, Seoul National University, Seoul, Korea

Abi (Abl interactor) proteins were originally identified as binding partners of the Abl nonreceptor tyrosine kinase. Abi contains serine/threonine-rich regions, proline-rich regions, SH3 domain and PEST motifs involved in protein destabilization. We found that serum starvation resulted in rapid loss of Abi protein. Degradation of Abi is reduced by calpain inhibitors. Overexpression of active Pak mutant, $Pak^{H83,86L}$, inhibits the degradation of Abi upon serum starvation. Activation of Pak by overexpression of active Rac, Rac^{G12V}, also inhibits the degradation of Abi upon serum starvation. We also show that multiple bands for overexpressed Abi in Western blots disappear by overexpression of PID (Pak inhibitory domain). Treatment of calpain inhibitor or lactacystin inhibits the degradation of overexpressed Abi. Our results demonstrate that Pak activation stabilizes Abi proteins and suggest a role for Abi in Rac/Pak signaling pathway.