Development of Immunocomplex Reagent for One-step Fluorescence Polarization Immunoassay of DDT

Hong Ji Youn°, Kim Jong-Hyun†, Choi Myung Ja*

*Bioanalysis & Biotransformation Research Center,
Korea Institute of Science & Technology, Seoul 136-791, Korea
†Department of Chemistry, Seoul Women's University, Seoul, 139-774, Korea

DDT and its related metabolites (DDA, DDE, DDD) were investigated using a homogeneous fluorescence polarization immunoassay (FPIA). Fluorescence polarization immunoassay (FPIA) for DDT was developed using a fluorescence polarization analyzer in photo check mode. FPIA is based on the increase in fluorescence polarization of a small fluorescent-labeled tracer when it was bound by specific antibody. If the sample contains DDT, tracer will compete with DDT for the antibody binding and the polarization signal will decrease.
Nine fluorescence-labeled DDT tracers were synthesized and characterized by the combination of three DDT derivatives, DDA, DDHP and DDT7, and three fluorescence labels, fluoresceinamine isomer I (AF1) and II (AF2), and ethylenediamine fluoresceinthiocarbamyl (EDF). The bindings of tracers with specific DDT antibody produced from DDT7-KLH immunogen were investigated to select optimal pair of tracer and antibody. Significant differences were found in titer level, sensitivity, and assay kinetics with pairs of various combination. Among them, a pair of DDT7 and AF2 tracer (Rf=0.3 in $CHCl_3$:MeOH, 4:1) showed best response.
To simplify the FPIA procedure, the immunocomplex reagent, that is a pre-equilibrated mixture of antibody and tracer, was prepared. This immunocomplex could be used as one direct single reagent for the measurement of displacement of tracer from immunocomplex after sample addition. Thus, we could measure a fluorescence polarization of DDT analyte with only one-step addition of sample without incubation. The detection limits of DDT, DDE and DDD by FPIA in optimal immunoreagents and condition is approximately 10 ng/ml for DDT derivatives using 50 ul samples within 7 minutes. DDA is 100 times less sensitive. The immunocomplex reagent has proven to be significantly stable comparing with respective solution of antibody and tracer.

[PC1-48] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

AgingPath : Database programmed to investigate aging process

Park Dae Ui°, Lee Ji Hyeon, Kim Heun Jin, Kim Chul Hong, Hong Seong Eui, Chang Gregory Y., Yu Byung Pal, Jung Hae Young

Pusan Bioinforamtics and Biocomplexity Research Center, Pusan Nat. Univ., College of Pharmacy, Pusan Nat. Univ., Dept. of Neurology, Univ. of Southern California, Los Angels, Dept. of Physiology, Univ. of Texas Health Center at San Antonio, Texas

Aging is an inevitable biological process that affects all living organisms. The process is time-dependent and inevitably leads to a functional decline. Underlying pathophysiologic process may best be explained by considering several biologic processes. Programmed genetic processing (e.g. apoptosis), oxidative stress, concomitant disease process, and factors not yet identified may all work to determine the rate and rapidity of aging. We programmed an aging regulatory pathway database (AgingPath) based on known biomolecules that have a role in aging, in order to better understand the process. In addition, beneficial effect of how caloric restriction (CR) may work to slow this process is also investigated. AgingPath is divided into two main sections, A) list of biomolecules that vary with aging, and B) list of various biomolecules which are modulated by CR. Currently, AgingPath is further divided into five different categories, under each category, search function is available. Many diagrams or graphic figures contain hot-links, which when activated, result in more detailed information. Pre-defined users (data entry person) are able to submit a new biomolecule or edit an existing biomolecule to reflect a latest development. AgingPath, with latest updated information, can help find a new biomarker. Similarly, the mechanism of CR on slowing of aging process may better be defined. AgingPath is freely available at http://pro.bio.pusan.ac.kr.

[PC1-49] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

BandW : The 2-DE image analysis software using MATLAB

Park Dae Ui°, Kim Chul Hong, Hong Seong Eui, Jung An Seong, Chang Gregory Y., Jung Hae Young

Pusan Bioinformatics and Biocomplexity Research Center, Pusan National University, Pusan, Korea, College of Pharmacy, National University, Pusan, Korea, Dept. of Neurology, University of Southern California, Los Angels, USA

Since genomics represents only the first step in understanding celluar physiology, it needs to be complemented by systematic analysis of the proteins, termed proteomics. The primary means studying protemics has been with use of 2-demer sional electrophoresis (2DE) since 1975. 2DE initially separate each protein according to its electric charge content by using isoelectric focusing and further separation of similar charged proteins are accomplished by using soduim dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE). But Analyzing two-dimensional gel electrophoresis(2DE) is complicated and time-consuming. In order to facilitate the process, we compiled "BandW" which is an image analysis tool made using MATLAB(Matrix oriented computing engine). BandW analyzes the input image using M by N matrix which has a value between 0 to 255. Each value represent a pixel intensity. Image output is processed to TIF(Tagged Indexed Fileformat) format. BandW has simplified layout which utilizes image processing toolbox and graphic user interface engine embedded in MATLAB 5.3. During analysis, spot volume is calculated using contour algorithm. Volume calculation starts with combining outer boundary of same pixel intensity in the adjacent matrix until the circular loop is completed. The area within the loop actually represent a three dimentional volume. We tested BandW by analyzing 2DE in liver mitochondria of young (LCY) or liver mitochondria of old (LCO) rats. Our results, using BandW, were identical to values obtained from available commercial softwares. In addition, BandW is 3D capable as only one other highly priced software.

[PC1-50] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Activation of PI3K is not Sufficient but Required for H-ras-induced Invasive Phenotype of MCF10A Cells

Shin Il-Chung°, Moon Aree

College of Pharmacy, Duksung Women's University, Seoul, Korea

We have previously shown that H-ras, but N-ras, induces an invasiveness and cell motility in human breast epithelial cells (MCF10A), while both H-ras and N-ras induce transformed phenotype. It has been recently shown that phosphatidylinositol 3-kinase (PI3K) plays an important role on cell migration. In the present study, we wished to investigate the functional role of PI3K in H-ras-induced invasive phenotype in MCF10A cells. The activation of PI3K was examined by detecting phosphorylation of Akt, a downstream molecule of PI3K, by Western blot analysis. We show that phosphorylated Akt level was upregulated both in H-ras MCF10A cells and N-ras MCF10A cells comparing to the parental MCF10A cells while the amount of Akt was equal in the parental, H-ras and N-ras MCF10A cells. The data indicate that activation of PI3K is not sufficient for invasiveness and motility since PI3K is also activated in the non-invasive and non-motile N-ras MCF10A cells. We investigated the functional significance of PI3K activation in invasion and motility by using PI3K inhibitors, LY294002 and wortmannin. Treatment of LY294002 and wortmannin significantly reduced invasive phenotype and motility of H-ras MCF10A cells, suggesting the requirement of PI3K activation for H-ras-induced invasion and motility. We then examined the effect of the PI3K inhibitors on matrix metalloproteinase(MMP) expression. Treatment of LY294002 inhibited secretion of MMP-2 and MMP-9 in a dose-dependent manner while wortmannin did not affect MMP levels in H-ras MCF10A cells. The possible role of Rac1 in H-ras-induced invasive phenotype in MCF10A cells are currently under investigation.

[PC1-51] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Enhancement of Proliferation and Migration of Glioma Cells by Glial Cell-derived Neurotrophic Factor (GDNF) for the Development of an Artificial Nerve Tubing

Song Hyun°, Dong June Chung[1], Pill-Hoon Choung[2], Aree Moon