of the HSP, the 104 kDa HSP was induced by ethanol shock, and amount of the 104 kDa HSP (p104) reached up to 18% of total cellular protein. But unlike other bacteria, GroEL, DnaK and Clp protein was not induced by ethanol shock.
The aims of this study was to determine and characterize physiological role of HSP104 in S. pneumoniae. S. pneumoniae defective in p104 was produced by insertion deletion mutagenesis by tripartite PCR followed by transformation. To elucidate mechanism of the p104 in thermotolerance, viability of the cells was determined after heat shock. In basal thermotolerance test, the p104 mutant showed no significant difference with that of the wild type. However, the mutant cells were 10 times more sensitive than the wild type in induced thermotolerance test. These result suggested that the p104 has chaperone fuction. Immunological crossreactivity of anti-pneumococcal p104 with other organisms(B.subtilis, S.aureus, S.pyogen, E.coli, S.typi) cell lysates was measured by Western blot. But anti-pneumococcal p104 antibody did not crossreact with other organism's cell lysates protein.

[PC2-7] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Inhibition of acyl CoA synthetase by phenazine-1-carboxylic acid

Kim KJ°, Song MS

Dept. of Life Science, Soonchunhyang University, 336-745 Asan, Korea

Incubation of phenazine-1-carboxylic acid (PCA) with acy CoA synthetase from Pseudomonas leads to enzyme-catalyzed depletion of PCA, to transient inhibition, and to irreversible inactivation of the enzyme. Both the transiently inhibited and irreversibly inactivated species show a marked increase in the absorbance at 360 nm that is proportional to the decrease in enzyme activity. Hydroxylamine treatment of irreversibly inactivated enzyme restores about one-third of the catalytic activity, with a concomitant decrease in absorbance at 360 nm. Polyacrylamide isoelectric focusing of the irreversibly inactivated enzyme shows three bands of approximately equal intensity, different native enzyme. Upon hydroxylamine treatment, one of the three bands disappear and now focuses identically with native enzyme. It is evident that the irreversible inactivation of enzyme by an excess of PCA generates three products, one of which can be reactivated by hydroxylamine.

[PC2-8] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Characterization of the Salmonella typhimurium ATCC-13311 astJ Gene, Encoding an Arylsulfate Sulfotransferase

Kang JW°, Kwon AR, Choi EC

Department of Pharmacy, Seoul National University

Sulfoconjugation is an important pathway in the detoxification of compounds which have phenolic functional group. It is reported that many intestinal florae use arylsulfate sulfotransferase (ASST) whose donor substrates are phenyl sulfate esters, for conjugation.
The gene encoding ASST from the Salmonella typhimurium ATCC-13311 was cloned and expressed in Escherichia. coli TH2. On the basis of restriction enzyme map, insert DNA was subcloned and sequenced by Sanger dideoxy termination method. The substrate specificity of recombinant ASST was same with that of parent ASST. Using p-nitrophenyl sulfate as a donor substrate, phenol is the best acceptor substrate, followed by 1-naphthol, resorcinol, tyramine, acetaminophen, and tyrosine

[PC3-1] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

EFFECTS OF CURCUMIN AND RELATED DIARYLHEPTANOIDS ON INDUCIBLE

CYCLOOXYGENASE (COX-2) EXPRESSION AND NF-κB ACTIVATION IN HUMAN BREAST EPITHELIAL (MCF10A) CELLS

Kim Jung- Hwan[o1], Lee Min Won[2], Surh Young-Joon[1]

[1]College of Pharmacy, Seoul National University and College of Pharmacy, [2]Chung Ang University, Seoul, Korea

Up-regulation of the inducible form of cyclooxygenase (COX-2) has been often observed in various types of cancerous and transformed cells. Recently, targeted inhibition of COX-2 is recognized as one of the promising strategies for the treatment of cancer as well as inflammation. As part of a program to evaluate the cancer chemopreventive potential of anti-inflammatory phytochemicals, we initially measured the COX-2 inhibitory activity of some naturally occurring diarylheptanoids structurally related to curcumin. Treatment of human breast epithelial (MCF10A) cells with the tumor promoter, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) induced *cox-2* mRNA and COX-2 protein expression in time- and concentration-related manners. Hirsutanone from *Alnus hirsuta* var.sibirica and curcumin present in turmeric (*Curcuma longa* L.) inhibited TPA-induced COX-2 expression at both transcriptional and post-transcriptional levels. There is some evidence that expression of COX-2 is regulated by the eukaryotic transcription factor NF-kappa B. In support of this notion, we found that the NF-kappa B inhibitor, pyrrolidine dithiocarbamate strongly suppressed the expression of COX-2 induced by TPA in MCF10A cells. Hirsutanone as well as curcumin attenuated the TPA-stimulated NF-kappa B activation, which was associated with inhibition of degradation of the inhibitory unit I-kappa B and subsequent translocation of the functionally active NF-kappa B subunit, p65. The luciferase reporter assay revealed that inactivation of NF-kappa B by hirsutanone led to blockade of its transcriptional activity. TPA treatment transiently induced the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERK1 and ERK2) which are known to a play important role in inflammation. TPA-induced activation of p38 and ERK1/2 was substantially suppressed by curcumin treatment.

[PC3-2] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Apoptotic Death in MDA-MB-231 Human Breast Cancer Cell Induced by Trichostatin A

Kim SH[o], Kang HK, Chung HY, YH Choi[1], Kim ND

Department of Pharmacy, Pusan National University, Pusan 609-735, Korea, [1]Department of Biochemistry, College of Oriental Medicine, DONG-EUI University Pusan, 614-054, Korea

Trhichostatin A (TSA) is a Streptomyces product, which inhibits the enzyme activity of histone deacetylase. It is also known as an inducer of apoptosis on several human cancer cell lines. In this study, we investigated the mechanism of apoptosis process by TSA especially on MDA-MB-231 human breast carcinoma cells. The cytotoxicity of TSA on MDA-MB-231 cells was assessed by MTT assay. The cell viability was decreased dose-dependently and the IC 50 value was about 100 ng/ml after 24 h treatment with TSA. Morphological change and DNA ladder formation, the biochemical hallmark of apoptotic cell death, were observed after treatment of TSA in a concentration- dependent manner, which was accompanied with poly (ADP- ribose) polymerase cleavage and caspase-3 activation. TSA treatment up-regulated the expression of a cyclin-dependent kinase inhibitor p21(Waf1/Cip1) protein, a key regulatory protein of the cell cycle. We also observed down-regulation of Bcl-2 protein by TSA without alteration of Bax expression. These results demonstrated that TSA might inhibit cell growth and induce apoptosis on human breast carcinoma MDA-MB-231 cells.