Purification and Characterization of Two Novel Chondroitinases from Bacteroides stercoris HJ-15

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Bacteroides stercoris HJ-15, which is human colon Gram-negative rod cell, had been known to degrade acharan sulfate and heparin. Recently it was found to produce chondroitinases and We tried to purify the chondroitinase. *B. stercoris* HJ-15 was cultured in 10 L of tryptic soy broth containing chondroitin sulfate A, collected and sonicated, followed by centrifugation at 18,000 rpm for 60 min. at 4℃. The supernatant was applied into QAE-cellulose, CM-Sephadex C-5, hydroxyapatite, phosphocelluose, and Sephacryl S-300 coumn chromatography. Two kinds of chondroitinases, chondroitinase ABC and chondroitinase AC were purified. They were consisted of monomer and dimer subunit, respectively. The specific activities, molecular weight and optimal pH of chondroitinase ABC were 45.7 μmole/min/mg, 114 KDa and pH 7.0. The specific activities, molecular weight and optimal pH of chondroitinase AC were 57.0 μmole/min/mg, 84 KDa and pH 5.8. They were inhibited by Ni^{+2} , Mg^{+2}, Zn^{+2}, Cu^{+2} and Co^{+2}, and Cu^{+2}, Pb^{+2}, Zn^{+2}, Ni^{+2} and Co^{+2}, respectively. These findings suggest that the biochemical properties of the purified enzymes were different from the previously purified enzymes.

[PC2-5] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Biochemical characterization of HrcA in Sreptococcus pneumoniae.

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Heat shock response plays a central role in cellular adaptation to stresses and hostile environments. Of several heat shock preteins(hsps), DnaK and GroEL play a key role in the folding of nascent protein chains and in the refolding of proteins after thermal damage. HrcA acts as a repressor of groEL and dnaK operon in Streptococcus pneumoniae or other gram positive organisms.
To elucidate the biochemical nature of the HrcA, the HrcA was overproduced in E.coli and purified. we determined stability of HrcA in various conditions of PH and temperatures. Also biophysical nature of HrcA in native condition was determined by nondenaturing PAGE analysis. HrcA was found to form dimer in the absence of CIRCE and PI value and molecular weight were 5.05 and 39.3 kDa, respectively. To identify the binding of HrcA to CIRCE element, the band shift assay was performed. To determine the effect of metal ion on binding of HrcA to CIRCE, several metal ions were supplemented to band shift assay buffer.
HrcA bound to CIRCE element, especially hairpin-loop structure, which is the promoter site of groESL and dnaK operon. The binding between HrcA and CIRCE element was stimulated by supplementation of calcium and zinc. Also supplementation of calcium affected synthesis of GroEL after heat shock.

[PC2-6] [04/21/2000 (Fri) 14:50 - 15:50 / [1st Fl, Bldg 3]]

Molecular Characterization of HSP104 in Steptococcus pneumoniae

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Several stress conditions induced the synthesis of heat shock protein(HSP)s in S. pneumoniae. One

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

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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

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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