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