Purification of Ginsenoside Rb1-Metabolizing β-Glucosidase from Fusobacterium K-60, a Human Intestinal Anaerobic Bacterium

Park, S.Y.[O,1], Bae, E.-A.[1], Kim, D.-H.[1]

[1]College of Pharmacy, Kyung Hee University

Fusobacterium K-60, a ginsenoside R_{b1}-metabolizing bacterium, was isolated from human intestinal feces. From this *Fusobacterium* K-60, a ginsenoside R_{b1}-metabolizing enzyme, β-glucosidase, has been purified. The enzyme was purified to apparent homogeneity by a combination of butyl-toyopearl, hydroxyapatite ultrogel, Q-sepharose, and sephacryl S-300 HR column chromatographies with a final specific activity of 1.52 mmol/min/mg. It had optimal activity at pH 7.0 and 40°C. The molecular mass of this purified enzyme was 320 kDa, with 4 identical subunits (80 kDa). The purified enzyme activity was inhibited by Ba^{++}, Fe^{++}, and some agents that modify cysteine residues. This enzyme strongly hydrolyzed sophorose, followed by p-nitrophenyl β-D-glucopyranoside, esculin, and ginsenoside R_{b1}. However, this enzyme did not change 20-O-β-D-glucopyranosyl-20(S)-protopanaxadiol (IH-901) to 20(S)-protopanaxadiol, while it weakly changed ginsenoside R_{b1} to IH-901. These findings suggest that the Fusobacterial β-glucosidase is a novel enzyme transforming ginsenoside R_{b1}.

[PC2-8] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Molecular cloning and characterization of groESL operon in Streptococcus pneumoniae

Seung-Whan Kim, Suhk-Neung Pyo, and Dong-Kwon Rhee

College of Pharmacy, Sungkyunkwan University

GroEL is a major target of the immune defense in infection and seems to be negatively regulated by HrcA in gram-positive organisms. But HrcA's mechanism has not been elucidated. To elucidate the role of groEL in Streptococcus pneumoniae, the groESL operon was cloned in Escherichia coli. The promoter region of the pneumococcal groESL operon contained sA type promoter and an inverted repeat (CIRCE). Northern blot analysis of the groESL operon demonstrated that the groESL operon is transcribed as a bicistronic mRNA, and reached maximum expression 7.5 to 10 min after heat shock. Primer extension analysis showed a potential transcription start point, 155 bp upstream of the translation start site, preceding the groES gene. The putative negative regulator of groEL gene, hrcA, of S. pneumoniae was recovered by PCR-based chromosomal walking from grpE locus and sequence analysis showed an sA type promoter flanked by 2 CIRCE elements. His-tagged HrcA was overexpressed in soluble form in E. coli and bound to CIRCE regions in the promoter of both groESL and dnaK operons in vitro. Additionally, a helix-loop helix motif, a putative DNA binding domain, was found at the C-terminal of HrcA. These results will provide to determine the nature of HrcA in groESL repression.

[PC2-9] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Characterization of antibiotic resistance and stress protein in Staphylococcus aureus and Streptococcus pneumoniae

Jeong HY, Jang SJ, Kong HS, Lee SD, Kim EU, Chang JY, Choi SO, Lee KH, Shin HS, Kim KW, Joe BJ, Rhee DK

Korea Food and Drug Administration, Division of Antibiotics; College of Pharmacy, Sungkyunkwan University

Since bacterial resistance has been a major problem in Korea, we monitored antibiotic resistance of Staphylococcus aureus and Streptococcus pneumoniae strains isolated from hospital patients in Korea and studied resistance mechanisms of them in relation to stress proteins.
From minimum inhibitory concentrations (MICs) of 107 S. aureus strains isolated from hospital patients in the year 2000, the resistance rates were as follows; penicillin resistant, 99%; oxacillin resistant (MRSA), 80%; vancomycin resistant (VRSA), 0%. In the presence of Triton X-100, bacterial lysis of ATCC25923 (methicillin-susceptible S. aureus) and STA007 (methicillin-resistant S. aureus) were suppressed after heat shock (culture temperature was shifted from 30 ℃ to 40 ℃ for 10 minutes) and the suppression of lysis by heat shock was greater in the STA007 than in the ATCC25923.
When lysis of the wild type SKP3026 and it's clpL mutant of S. pneumoniae strains by tetracycline were compared, lysis of the clpL mutant was faster than that of the wild type.
Heat shock suppressed bacterial autolysis in S. aureus and 84-kDa stress protein (ClpL) of S. pneumoniae suppressed autolysis by tetracycline. Therefore stress proteins do not seem to be the major mechanism of antibiotic resistance, but contribute to increase viability in resistant strains of S. aureus and S. pneumoniae.

[PC2-10] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Bacterial Arylsulfate Sulfotransferase as a Reporter System

Yun HJ°, Kwon AR, Lim JA, Kang JW, Kim SY, Min YH, Choi EC

College of Pharmacy, Seoul National University

In order to investigate whether the arylsulfate sulfotransferase (ASST) is suitable as a sensitive reporter system for Gram-positive bacteria, a reporter vector carrying the fragments of the *astA* structural region was constructed and designated as pSY815. To test the utility of the ASST reporter system in *Bacillus subtilis*, the regulatory regions of *ermC* and *ermAMR* were inserted upstream of the coding region of the reporter gene, to generate the vectors pSY815-EC and pSY815-ER, respectively. In the absence of an inserted regulatory region, the plasmid displayed very low background activity. The ASST activity under the control of the *ermC* regulatory region was increased 4.42-fold when induced by 0.1 ㎍/ml of erythromycin. Under the *ermAMR* regulatory control, the activity was increased 1.66-fold when induced by 0.2 ㎍/ml of tylosin. These results were consistent with a *lacZ* reporter gene assay of the *ermC* and *ermAMR* regulatory regions. This indicates that this reporter system is very sensitive.
The lack of endogenous activity, the simple detection of enzyme activity in the living cell, the commercially available non-toxic substrates, and the high sensitivity make ASST a useful genetic reporter system for monitoring gene expression and understanding gene regulation in Gram-positive bacteria.

[PC2-11] [04/19/2001 (Thr) 15:30 - 16:30 / Hall 4]

Genetic Characterization of Vancomycin-Resistant Enterococci from Raw Milk

Lee JW1, Nam HJ2, Choi SS3, Kim KJ1, Kim BS2 and Ha NJ1

1Department of Pharmacy Sahmyook University, 2 Lab. of Nosocomial Pathogen National Institute of Health Korea, 3 Department of Food Science Sahmyook College