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

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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}.

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Molecular cloning and characterization of groESL operon in Streptococcus pneumoniae

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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.

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

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