

antithrombotic agents *in vitro* and *ex vivo*, and its antithrombotic effect *in vivo*. Chungpesagan-tang, Rhei Rhizoma and Scutellariae Radix potently inhibited ADP-, collagen-induced rat platelet aggregation in a dose-dependent manner *in vitro*. Chungpesagan-tang and most of its ingredients did not affect such coagulation parameters as APTT, PT and TT in human plasma. However, Rhei Rhizoma potently protected plasma clotting. Chungpesagan-tang treated group was significantly inhibited platelet aggregation in orally administered rats *ex vivo*. Puerariae Radix and Rhei Rhizoma also showed significant prolongation of tail bleeding time in conscious mice. Chungpesagan-tang showed significantly protection from death due to pulmonary thrombosis in mice.

[PC2-5] [ 10/20/2000 (Fri) 15:30 – 16:30 / [Hall B] ]

### Purification and Characterization of novel salt-inducible heparinase II from *Bacteroides stercoris* HJ-15

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*Bacteroides stercoris* HJ-15, which is human colon gram-negative rod cell, has been known to degrade heparin, acharan sulfate and chondroitin sulfate. Recently we found to produce new five heparinases: one heparinase I, three heparinase II, one heparinase III. Among three heparinase II, one is a salt-inducible enzyme. In the present study, we tried to purify the enzyme. *Bacteroides stercoris* HJ-15 was cultured in 100L of tryptic soy broth containing hepain, collected, sonicated and centrifuged at 18,000rpm for 70min at 4°C. From the supernatant, the salt-inducible heparinase II was purified to homogeneity by QAE-cellulose, DEAE cellulose, CM Sephadex C-50, hydroxyapatite and phosphocellulose. The specific activity of the purified heparinase II was 81.3 μmole/mg. Its molecular weight was 94KDa. The optimal activity was observed to be pH 7.3 containing 50mM KCl. Cu<sup>+2</sup>, Ni<sup>+2</sup>, Co<sup>+2</sup>, Fe<sup>+2</sup> and Zn<sup>+2</sup> inhibited the enzyme activity. These findings suggest that the biochemical properties of the purified enzyme should be different from the previously purified heparinase II.

[PC2-6] [ 10/20/2000 (Fri) 15:30 – 16:30 / [Hall B] ]

### Purification and Characterization of *Staphylococcus epidermidis* Urease

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The multi-subunit urease from *Staphylococcus epidermidis* ATCC12228 was purified 1,127-fold by ion exchange and gel filtration chromatography. The enzyme activity was highest when the cells were grown up to late exponential phase in LB supplemented with 50 μM nickel chloride.

The specific activity of the purified urease was 993.8 μmol urea degraded min<sup>-1</sup>. The enzyme was shown to have a M<sub>r</sub> of 409,000 and consist of three different subunits of 73.3, 22.7, and 13.8 kDa. The Michaelis constant (K<sub>m</sub>) was 8.9 mM urea. Activity gel-staining of the native urease with urea and cresol red revealed two major peptide bands.

[PC2-7] [ 10/20/2000 (Fri) 15:30 – 16:30 / [Hall B] ]

### Expression control of the *dnak* operon in *Streptococcus pneumoniae*

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Heat shock response plays a central role in cellular adaptation to stress and hostile environments. Of several heat shock proteins (hsps), DnaK (hsp70 family) and GroEL (hsp60 family) function as molecular chaperones in the folding of nascent protein chains and in the refolding of proteins after thermal damage. Expression of dnaK operon and groEL operon in *S. pneumoniae* and other gram positive organisms is negatively controlled by a repressor encoded by *hrcA*, the first gene of dnaK operon. HrcA repressor bound to CIRCE elements at the promoter site of dnaK and groEL operon. Since HrcA depends on GroEL to acquire on active conformation, GroEL seems to be specific modulator of the CIRCE regulon. Previously we demonstrated that the dnaK operon has three promoter sites and binding of HrcA to CIRCE element was stimulated by supplement of calcium. In this study, to elucidate the pathological role and expression control of dnaK operon in *S. pneumoniae*, nucleotide sequence of the dnaK operon was completely determined and effect of *hrcA* mutation on dnaK expression was examined. In the *hrcA* mutant, the basal and induced levels of DnaK protein and dnaK transcript were much higher than that of the wild type. When the effect of calcium on hsp expression was determined by Western blot analysis, both GroEL and HrcA proteins were decreased to the basal level by 1mM calcium but DnaK was not affected by calcium. These results suggest that calcium may play a differential roles in either promoter 1, 5' to the *hrcA* or promoter 3, 5' to the dnaK.

[PC2-8] [ 10/20/2000 (Fri) 15:30 - 16:30 / [Hall B] ]

**ermAM and ermK are also inducibly expressed by 16-membered-ring macrolide antibiotics.**

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The *erm* family is one of the major groups of genes responsible for inducible macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) antibiotics resistance. The 16-membered-ring macrolides have been generally considered noninducers except in the case of *Streptomyces* and the selected mutant strains. In this study, the induction specificity of *ermAM*, *ermK*, and *ermC* for MLS<sub>B</sub> resistance was studied by *lac* reporter gene assay. The unexpected MLS<sub>B</sub> resistance phenotypes were observed. *ermAM* was induced by the 16-membered-ring macrolides tylosin, kitasamycin, josamycin, and rokitamycin more strongly than the lincosamide clindamycin known as inducer of *ermAM*. *ermK* was only induced by the tylosin of the 16-membered-ring macrolides tested. However *ermC* was not induced by any 16-membered-ring macrolides.

[PC2-9] [ 10/20/2000 (Fri) 15:30 - 16:30 / [Hall B] ]

**Ulcerative colitis induced GAGs degrading enzymes of intestinal bacteria**

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Ulcerative colitis(UC) is a non-infectious chronic intestinal inflammatory disease in humans. The mechanisms underlying their pathogenesis are not well known, although it has been long discussed.

We produced acute experimental UC models in mice by several methods (5% dextran sulfate