College of Pharmacy, Kangwon National University and 1R&D center, Coreana Cosmetic Co., Korea

The flowers of Prunus persica Batsch have been used for skin disorders in East Asia from ancient time. In this investigation, the ethanol extract from this plant materials was prepared and evaluated for the protective effects on solar ultraviolet (UV)-induced skin damages including UVB-induced erythema in guinea-pigs, ear edema in ICR mice and skin carcinoma in SKH-1 mice. The P. persica extract clearly inhibited UVB-induced erythema formation dose-dependently when topically applied (IC50 = 0.5 mg/cm2). And it also inhibited UVB-induced ear edema (49% inhibition at 3.0 mg/ear). In addition, the extract greatly reduced UVB-induced skin tumor formation (42% reduction of tumor incidence and 71% reduction of total number of tumors in 28 weeks) at the initial dose of 5.0 mg/cm2/day and the maintenance dose of 2.5 mg/cm2/day). From the extract, four kaempferol glycoside derivatives were successfully isolated and their contents were measured with HPLC. Among the derivatives, the content of multiflorin B was highest and this compound inhibited UVB-induced erythema formation, indicating that multiflorin B is one of the active principles of the extract. All these results suggest that the flowers of P. persica extract may be useful for protection against UV-induced skin damage when topically applied.

[PC1-29] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Cloning of nitric oxide synthase from Staphylococcus aureus

Park JH°, Yang SY[1], Lee HY[1], Han JH, Lee HW

College of Pharmacy, SungKyunKwan University, 1Departments of Pharmacology, College of Medicine, Konyang University

Recently, we have reported the existence of bacterial nitric oxide synthase (NOS) in the prokaryotic cell, *Staphylococcus aureus* (*S. aureus*). The bacterial NOS in *S. aureus* requires the existence of cofactors (FMN, FAD, NADPH, BH_4 and calcium/calmodulin) and is induced by methanol. We compared the cofactor binding sites of murine iNOS with total genomic sequence of *S. aureus* and found seven matching sequences. Using homologous primers, we amplified 0.85kb DNA fragment from genomic DNA library of *S. aureus*. Screening of genomic DNA library produced twelve positive clones. Four DNA fragments (2, 4.5, and two 6 kb) were selected by Southern blot analysis and ligated into mammalian expression vector, pcDNA3.1.

[PC1-30] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Detection of Proteins in Sodium Dodecyl Sulfate -Polyacrylamide Gel Electrophoresis Using a Counter Ion Dye Staining Technique with Coomassie Brilliant Blue R / Neutral Red

Choi Jk°, Yoo GS, Lee JK

Chonnam National University, College of Pharmacy, Analytical Biochemistry Lab.

Coomasie Brilliant Blue R (CBBR) staining method has been used the most widely in the detection of proteins in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). However, it requires long staining time and can't be visualized in course of staining. Here we describe fast, sensitive and simple staining method that can be visualized in the course of staining employing a counter ion dye staining technique. Acidic dye, CBBR(0.005%) was mixed up with basic dye, Neutral Red (NR) (0.0005%). After electrophoretic separation in SDS-PAGE, gels were fixed with 40%MeOH/7%HAc for 30min. Then, gels were stained in 0.005%CBBR-0.0005%NR, in 30% MeOH/7%HAc staining solution for 30min-1hr. It was preferable to wash gels for 10 minutes after

staining. The procedure was completed in 1hr to 1hr 30min. In counter ion dye staining, NR inhibits the binding of CBBR to gel matrix, so enhances the staining effect of CBBR on protein bands, and also reduces background. As the result, this showed twofold increase in sensitivity comparing with CBBR staining and had very low background so stained protein bands could be visualized at the same time of staining. We consider that CBBR-NR staining method can be a recommendable alternative of CBBR for the detection of proteins in SDS-PAGE.

[PC1-31] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Modified Coomassie Brilliant Blue (CBBR) Staining with Phenosafranin in SDS - PAGE Using a Counter Ion Dye Staining Technique.

Choi JK°, Yoo GS, Lee JK, Jung DW

Chonnam National University, College of Pharmacy, Analytical Biochemistry Lab.

There are many staining methods for proteins in Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Coomassie Brilliant Blue R (CBBR) staining method among them has been used the most widely. However, it requires long staining and destaining time. In addition, a large quantity of dye is used so that protein bands can't be visualized in the course of staining. This study was set out to find the alternative staining method replacing CBBR staining. A fast, sensitive and simultaneously visualized staining method has been developed employing a counter ion dye staining technique. Acidic dye, CBBR(0.005%) was mixed up with basic dye, Phenosafranin (PS) (0.0010). After electrophoresis in SDS-PAGE, gels were fixed with 40% MeOH/7%HAc for 30min. Then, gels were stained in 0.005%CBBR-0.0010%PS in 20%MeOH/7% staining solution for 30min-1hr. It was preferable to wash gels for 10 minutes after staining. The procedure was completed in 1hr to 1hr 30min. This showed twofold increase in sensitivity comparing with CBBR staining and had very low background so stained protein bands could be visualized at the same time of staining. We consider that CBBR-PS staining method can be a recommendable alternative of CBBR for the detection of proteins in SDS-PAGE.

[PC1-32] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Purification and Identification of Human Recombinant Cathepsin P As A Novel Cysteine Proteinase

Kim MK°, Park BS, Son MH, Bae EJ, Kim SH and Kim WB; †Lee CH, Lee BY and Lee JW

Research Laboratories, Dong-A Pharmaceutical Co. Ltd., # 47-5, Sanggal-Ri, Kiheung-Up, Yongin-Si, Kyonggi-Do 449-900, and †Yuhan Research Institute, # 27-3, Tangjeong-Dong, Kunpo-Si, Kyonggi-Do 435-715, Korea

Cathepsin P is thought to be expressed ubiquitously in various types of tissues unlike cathepsin S, K, and W. Although biological and physiological functions of cathepsin P have not yet been completely established, it has been reported that cathepsin P seems to fulfill the housekeeping role like cathepsin B, L, H, and O. The mature cathepsin P is primarily a carboxypeptidase and has extremely poor endopeptidase activity. The carboxypeptidase activity of cathepsin P shares a similar profile with that of cathepsin B. The latter has been implicated in normal physiological events as well as in various pathological states such as rheumatoid arthritis, Alzheimer's disease and cancer progression.
Cathepsin P is the cysteine proteinase like cathepsin B, L, and S, which partly share the substrate and/or inhibitor with cathepsin K. But its unique properties different from cathepsin B, L, and S, have been obvious. Thus, cathepsin P has become the interesting enzyme for our study.
In order to construct the human recombinant, mature cathepsin P gene was inserted in protein expression vector. Approximately 31 kDa protein was over-expressed in *Escherichia coli* [BL21] and purified by size-exclusion and subsequent ion-exchange column chromatographic method. The purified protein was analyzed by N-terminal amino acid sequencing. The enzymatic activity