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.

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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

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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

was measured using the specific cathepsin P substrate [Abz-FRF(4NO_2)-OH] and nonspecific substrate [Z-FR-AMC]. As the results, cathepsin P showed the significant preference for Abz-FRF(4NO_2)-OH. This purified human cathepsin P will be used in the screening of selectivity of proteinase inhibitors.

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[PC1-33] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

Purification and Characterization of Polyphenol Oxidase from Perillae Folium

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Polyphenol oxidase (PPO) was purified from an triton X-114 extract of Perillae Folium by ammonium sulfate fractionation and chromatography on DEAE-cellulose. From chromatography on DEAE-cellulose two fractions with PPO activities were separated. Their fractions were examined by sodium dodesyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The result of electrophoresis molecular weight was 48Kd from first fraction and 37Kd from second fraction. PPO activity according to pH showed the similar value through broad pH range(4.0-9.0) at first fraction. While at second fraction optimum pH of PPO is 6.0. Substrate specificities of PPO showed the same result at the first and second fraction.

[PC1-34] [10/20/2000 (Fri) 15:30 - 16:30 / [Hall B]]

The Novel 40 kDa Cytosolic Phospholipase A2 Is Implicated in Ca2+-dependent Arachidonic acid Release from Mammalian Red Blood Cells

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Many recent lines of evidence show that red blood cells (RBC) can modify platelet pathophysiology through the release of arachidonic acid (AA) and eicosanoids formation including thromboxane A_2 and thus influence thrombosis and hematosis. The release of AA is known to be a rate-limiting step for the production of eicosanoids and platelet-activating factor and occur by activation of phospholipase A_2 (PLA_2) as a major pathway. Recently we purified and characterized a novel 40 kDa form of cytosolic PLA_2, termed rPLA_2, from bovine RBC, which was further identified as an unknown protein in MALDI-TOF mass spectrometric analysis. To examine whether this rPLA_2 cause the Ca^{2+}-dependent AA release from human and bovine RBCs, we developed a derivative of naphthaquinone, EA4, which inhibited rPLA_2 in a competitive pattern, and found this inhibitor also significantly decreased the Ca^{2+}-dependent AA release from human and bovine RBCs metabolically labeled with [^{3}H]AA. In contrast, methyl mercury and CNU-2 as cPLA_2 inhibitors and ETYA as sPLA_2 inhibitor did not change the Ca^{2+}-dependent AA release. These results suggest that this rPLA_2 may be implicated in the Ca^{2+}-dependent release of AA from mammalian RBCs.