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.