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Time-dependent PPO activity was determined at 4°C and 30°C. The result of activity determination, PPO extracted by phosphate buffer containing triton x-114(tPPO) was more stable than PPO by phosphate buffer(bPPO). The result of electrophoresis, at first a band was appeared at 48kd. After 1-3days a partial degrade band was appeared in bPPO and three partial degrade bands in tPPO. No activity band was appeared in PPOs at 30°C and bPPO at 4°C after 4 days. Two degrade bands (39kd and 37kd) in tPPO were remained after 30 days at 4°C. The result of activity and electrophoresis, detergent like triton x-114 was important for stability of PPO.

[PC1-6] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Proteomic analysis of nitrated and HNE-adducted proteins in the aging process**

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Peroxynitrite and 4-hydroxynonenal (4-HNE) are highly reactive molecules which are generated under oxidative stress condition and during aging. Many proteins in living organism are modified by them and consequently associated with various diseases including cardiovascular and neurodegenerative diseases. We hypothesize that peroxynitrite and 4-HNE modified serum proteins are also associated with aging process. To establish information on peroxynitrite and 4-HNE adducted proteins for aging study, we used proteomic methods, 2D-PAGE and MALDI-TOF MS, to identify modified proteins from young (7-month) and old (25-month) rat serum. As a result of immunodetection, levels of nitrotyrosine, HNE-histidine, and free HNE were increased in old rat serum. Also, we identified 16 immunopositive proteins like alpha-1-macroglobulin, apolipoprotein H, albumin, prothrombin, transferrin, T-kininogen I, and haptoglobin from young and old 2D-gels. Among of nitrated proteins, Alpha-1-inhibitor III and inter-alpha-inhibitor H4 heavy were shown in young rat serum, but T-kininogen I and alpha-1-antiproteinase were observed in old rat serum. In HNE-adducted proteins, T-kininogen I, apolipoprotein E, and haptoglobin were shown in old rat serum. Moreover, some proteins were double modified by both 4-HNE and peroxynitrite. These modified proteins are involved in homeostasis, transport, regulation of proteolysis and peptidolysis, and acute-phase responses. Our data indicate dysfunction of serum proteins through 4-HNE adduction and nitration, which may be associated with aging-related vascular diseases via endothelial cell damage and contribute to vascular aging and aging process.

[PC1-7] [ 2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function ]

### **Celecoxib Attenuates Nitric Oxide-Induced Apoptosis in PC12 Cells by Inhibiting AP-1 Activation and COX-2 Expression.**

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Recent studies suggest that inflammatory events are implicated in a variety of ailments such as cancer and neurodegenerative diseases, and certain non-steroidal anti-inflammatory drugs have beneficial effects for the treatment or prevention of these disorders. Cyclooxygenase-2 (COX-2), the rate-limiting enzyme in the prostaglandin (PG) synthesis, is induced by various pro-inflammatory stimuli including nitric oxide (NO) and has been reported to cause and/or aggravate neuronal cell death. In this study, we have investigated the possible protective effect of celecoxib, a selective COX-2 inhibitor, against inflammatory cell death induced by the NO releasing compound sodium nitroprusside (SNP) in cultured rat pheochromocytoma (PC12) cells. PC12 cells treated with SNP underwent apoptotic cell death as revealed by cleavage of poly(ADP-ribose)polymerase, decreased mitochondrial membrane potential ( $\Delta Y_m$ ), an increased Bax/Bcl-XL ratio and internucleosomal DNA