inactivation of PON. Based on these results, it is proposed that the PON inactivation during LDL oxidation may be ascribed mainly to the $Cu^{2+}$-catalyzed oxidation. This work was financially supported by research grant (1-209-003-2) from the Korea Science and Engineering Foundation, Korea.

[PC1-29] [ 10/19/2001 (Fri) 09:00 - 12:00 / Hall D ]

## Protein disulfide isomerase-catalyzed multimerization of partially reduced bovine thyroglobulin

Liu XiWen°, Sok DaiEun

College of Pharmacy, Chungnam National University

Multimerization of thyroglobulin (Tg) is known to be a way to store Tg at high concentrations in the lumen of the thyroid follicles. Multimerization of bovine thyroglobulin has been intermolecular reactions through the oxidative processes, such as disulfide and dityrosine formation, as well as isopeptide formation. It is known that the disulfide formation is more responsible for Tg multimerization than the other reactions. Here, we investigated the protein disulfide isomerase (PDI) or peroxidase-induced multimerization of normal bovine Tg or partially denatured thyroglobulins, which were prepared from the treatment with thiol, urea or thiol/urea, on the basis of SDS-PAGE analyses. In addition, the enzymatic multimerization was compared with non-enzymatic multimerization, thiol-mediated or thermal. The thiol-induced multimerization of normal Tg was dependent on GSH concentration, in the presence of low GSH concentrations, the dimeric form, 660 kD, was predominant, while multimeric forms were the major in incubations with higher GSH concentration. In addition, the multimerization of Tg under thermal denaturation at 60 ℃ also required glutathione. Meanwhile, the multimerization of partially reduced Tg was accelerated by PDI in a time-dependent manner up to 30 min at 38 ℃. Similarly, the multimerization of partially reduced Tg was also mediated by peroxidase. However, PDI showed no remarkable effect on the peroxidase-mediated or nonenzymatic multimerization. Accordingly, the PDI-mediated multimerization of thyroglobulin through the disulfide linkage may require a specific molecular form of thryglobulin, optimally reduced and denatured. This work was supported by Korean Research Foundation Grant (KRF-2000-F00302).

[PC1-30] [ 10/19/2001 (Fri) 09:00 - 12:00 / Hall D ]

## BNip3 expression in hypoxia-induced neuronal apoptosis

Lee HyeJa°, Park JaeHoon, Rho YoungSoo

Department of Medicinal Chemistry and Pathology, College of Pharmacy and Medicine, KyungHee University, Seoul 130-701, Korea

Hyoxia/ischemia is one of the most common causes of neuronal injury and implicated in the pathogenesis of degenerative diseases, including dementia. It has been reported that hypoxia/ischemia induces acute neuronal necrosis or delayed cell death, depending on severity and duration of hypoxic injury. Hypoxia-induced delayed neuronal death (apoptosis) plays critical roles in the process of ischemia progression. Even though putative molecules mediating hypoxic injury, including p53 and Bax, were suspected, the precise molecular mechanisms are still unknown. Recently, it was shown that NBip3, a memember of pro-apoptotic Bcl-2 family proteins, was transcriptionally activated by hypoxic injury, suggesting certain roles in neuronal apoptosis. The object of this study is to clarify the functions of BNip3 in the apoptotic [rocess induced by hypoxia and we show evidences that BNip3 is one of the strong candidate molecules mediating hypoxic injury.

[PC1-31] [ 10/19/2001 (Fri) 09:00 - 12:00 / Hall D ]

## The release of GPI-anchored proteins from the renal proximal tubules by Bacillus

## cereus phosphoinositide-specific phospholipase C

Park EunMi°1, Yoon HyunJoong2, Park HaengSoon1

1 Department of Pharmacy, College of Pharmacy, 2 Department of Biology, College of Natural Science, Chonnam National University, Kwangju 500-757, Korea

Renal dipeptidase (RDPase, EC 3.4.13.19) and alkaline phosphatase (APase, EC 3.1.3.1) are known as glycosylphosphatidylinositol (GPI)-anchored proteins of renal proximal tubules. The bacterial PI-PLC, which was obtained from pure culture of Bacillus cereus, induced the release of RDPase and APase from porcine renal proximal tubules at 37℃ in a time- and protein concentration-dependent manners. Any effect of NO on the release of GPI-anchored RDPase and APase by the B. Cereus PI-PLC was examined. The bacterial culture was added directly to the proximal tubules in the presence and absence of sodium nitroprusside (SNP, direct NO donor) and incubated as a function of time. After incubation for 8 hours, it was observed that the RDPase release was decreased to 37.0 ±5.0% of the control in the presence of 0.1mM SNP, whereas APase release was not changed significantly. It was also confirmed with the result of partially purified bacterial PI-PLC from the bacteria cultured in the presence of SNP. RDPase and APase have slightly different structures at the lipid part of the GPI-anchor, RDPase having diacyl-glycerol whereas APase having alkylacyl-glycerol. The results suggest that there may be different isoforms of bacterial PI-PLC and NO may affect negatively the synthesis of the one responsible for RDPase release.

[PC1-32] [ 10/19/2001 (Fri) 09:00 - 12:00 / Hall D ]

## The release of renal dipeptidase from proximal tubules in the presence of insulin is a [Ca2+]i-dependent process

Yoon HyunJoong2°, Park EunMi1, Park HaengSoon1

1 Department of Pharmacy, College of Pharmacy, 2 Department of Biology, College of Natural Science, Chonnam National University, Kwangju 500-757, Korea

Renal dipeptidase (RDPase, EC 3.4.13.19), an ectoenzyme of renal proximal tubules, is covalently bound to outer leaflet of lipid bilayer via glycosylphosphatidylinositol (GPI)-anchor. *In vivo* release of RDPase was observed in urine of various animals including rat, rabbit, pig and human. Porcine and human RDPase were identified as a hydrophilic form as they were mostly partitioned into the aqueous layer by phase separation with Triton X-114. Insulin has been known to stimulate the release of several mammalian GPI-anchored cell surface proteins. *In vitro* release of RDPase was diminished by depletion of $[Ca^{2+}]i$ but restored by $Ca^{2+}$ supply. EGTA ($Ca^{2+}$ chelator), TMB-8 (inhibitor of $Ca^{2+}$ release from intracellular $Ca^{2+}$ stores) and nifedipine (L-type $Ca^{2+}$ channel blocker) decreased RDPase release but ionomycin ($Ca^{2+}$ ionophore) increased it. These $[Ca^{2+}]i$-regulating agents also synergistically controlled the releases of RDPase and other Cross-Reacting Determinant (CRD, inositol-1,2-cyclic monophosphate)-containing proteins such as porcine renal alkaline phosphatase (ALPase) and acetylcholinesterase (AChE) in the presence of insulin. These results demonstrate that the release of RDPase from its GPI-anchor in the presence or absence of insulin is a $[Ca^{2+}]i$-dependent process, different from the trypanosomal GPI-PLC which is independent of $Ca^{2+}$.

[PC1-33] [ 10/19/2001 (Fri) 09:00 - 12:00 / Hall D ]

## Modulation of Oxidative Status by Calorie Restriction in Mini rat

Lee Ji Hyeon°, Kim Aera, Kim Ji Young, Kim Chul Hong, Park Dae-Ui, Han Suk Kyu, Shimokawa Isao, Chung Hae-Young