squalene synthases gene derived from panax ginseng

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Transgenic Eleutherococcus senticosus plants were prepared by introducing the genes for squalene synthase (SQS), hygromycin phosphotransferase (HPT) and green fluorescent Protein (GFP) through Agrobacterium–mediated transformation. The enzyme, SQS, represents a putative branch point in the isoprenoid pathway capable of diverting carbon flow specifically to the biosynthesis of phytosterol and oleanolic acid. The full SQS gene was isolated from P. ginseng roots. Early globular embryo clusters developed from embryogenic callus were used as the explant source. Following infection, selection was achieved on hormone-free MS medium containing 300-mg/l cefotaxime and 25-mg/l hygromycin at 2-week intervals. Somatic embryos were germinated and converted into plantlets after the cotyledonary embryos were pretreated with 14.4 mM GA_3. Establishment of transgenic somatic embryos was confirmed by presence of SQS and HPT genes, green fluorescence of GFP, and increased SQS enzymatic activity. The SQS enzyme activity of transgenic plant was 3 times higher than the wild type. In addition, a gas chromatographic analysis revealed that phytosterol (b-sitosterol and stigmasterol) levels in transgenic E. senticosus were increased remarkably. These results suggest that the SQS gene may play a regulatory role for phytosterol synthesis, and the produced transgenic E. senticosus plantlet can be used as the sources of medicinal raw materials.

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

Antioxidant Activity of major protein from Panax Ginseng C.A. Meyer.

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A major protein was isolated from ginseng root (Panax ginseng C.A. Meyer) using a combination of ammonium sulfate fractionation, gel filtration chromatography, ion-exchange FPLC. Electrophoretic and gel permeation chromatographic studies revealed that the major protein, GMP, is composed of two subunits of approximately 28 kDa. In this study, investigated the ability of GMP to inhibit the oxidation of low-density lipoprotein (LDL). GMP inhibited Cu^{2+} (5 μM)-promoted oxidation of LDL (125 μg protein/mL) in a dose-dependent mamer (0~5 μM), with a maximal inhibitor at GMP/copper ratio of 1:10 and an IC_{50} value of 0.2 μM, as determined by measurement TBARS. Meanwhile, bovine serum albumin (BSA) and histidine showed IC_{50} value of 2.4 μM and 8.0 μM, respectively. In related experiment, GMP was more effective than histidine in preventry against Cu^{2+}-induced formation of carbonyl in BSA, but the reverse in preventry Cu^{2+}-induced oxidation of ascorbic acid. However 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH)(1mM)-induced oxidation of LDL was not inhibited by ether GMP or histidine. These data suggest that the antioxidant action of GMP against Cu^{2+}-promoted LDL oxidation might the mainly due to the selective interaction with Cu^{2+} associated with LDL.

[PC1-14] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Inhibitory effect of benzoxathiol LYR-71 compound on inflammatory enzymes and cytokines

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The benzoxathiol LYR-71 compound was discovered as an inhibitor of NF-kB transcriptional activity with an IC50 value of 5.4 uM. Furthermore, benzoxathiol LYR-71 compound inhibited the NF-kB binding activity to DNA in a dose-dependent manner, which was identified by EMSA with oligonucleotide corresponding to NF-kB consensus sequence. It is well known that NF-kB is an important transcription factor to regulate the expression of inflammatory enzymes (iNOS and COX-2) and cytokines (TNF, IL-1 and IL-6). The benzoxathiol LYR-71 compound suppressed all expressions of iNOS, COX-2, TNF, IL-1 and IL-6 transcripts in LPS-stimulated murine

macrophages Raw 264.7. The compound inhibited not only LPS-induced NO production with an IC50 value of 6.5 uM but also TNF and IL-1 productions with IC50 values of 17.2 uM and 10.0 uM, respectively. Benzoxathiol LYR-71 compound inhibited the IL-6 signaling with an IC50 value of 4.3 uM but did not inhibit TNF or IL-1 signaling at all. Therefore, benzoxathiol LYR-71 compound would be benifical for treatment of inflammatory and immune diseases.

[PC1-15] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Anti-inflammatory and Antinociceptive Effects of Methanol Extract from the Fomes fomentarius

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As an attempt to search for bioactive natural products exerting antiinflammatory activity, we have evaluated the anti-inflammatory and antinociceptive activities of the methanol extract from the Fomes fomentarius (MEFF). MEFF (50, 100 mg/kg/day, p.o.) significantly reduced an acute paw edema induced by carrageenan in rats. When analgesic activity was measured by acetic acid-induced writhing test and hot plate test, MEFF showed a dose-dependent inhibition in animal models. In addition, MEFF potently inhibited the LPS-induced production of NO, PGE_2 and TNF-αproduction of macrophages. Consistent with these observations, the expression level of iNOS and COX-2 enzyme was decreased by MEFF in a concentration-dependent manner. These results suggest that methanol extract from the Fomes fomentarius exert anti-inflammatory effects by inhibiting NO and PGE_2 induction.

[PC1-16] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Fast visible dye staining of proteins in one- and two-dimensional sodium dodecyl sulfate-polyacrylamide gels compatible with matrix-assisted laser desorption/ionization-mass spectrometry

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A fast and matrix-assisted laser desorption/ionization-mass spectrometry compatible protein staining method in one- and two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis is described. It is based on the counterion dye staining method that employs oppositely charged two dyes, Zincon and Ethyl Violet to form an ion-pair complex. It is safe to use since the methanol used previously in staining solution was replaced with ethanol, which is not toxic. The protocol including fixing, staining and quick washing steps can be completed in 1 to 1.5 h depending upon gel thickness. It has the sensitivity comparable with the colloidal Coomassie Brilliant Blue G (CBBG) stain using phosphoric acid as a component of staining solution (4-8 ng). The counterion dye stain does not induce protein modifications like in CBBG stain using trichloroacetic acid and methanol as components of staining solution, which complicates interpretation of peptide mapping data from mass spectrometry. Considering the safety, sensitivity, speed and compatibility with mass spectrometry, the counterion dye stain may be more practical than any other dye-based protein stains for routine proteomic researches.

[PC1-17] [2003-10-10 09:00 - 13:00 / Grand Ballroom Pre-function]

Curcumin Inhibits Phorbol Ester-induced Expression of Cyclooxygenase-2 in Mouse Skin through Suppression of Extracellular Signal-Regulated Protein Kinase Activity and NF-κB Activation

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Recently, there have been considerable efforts to search for naturally occurring substances for the intervention of carcinogenesis. Curcumin, a yellow coloring ingredient of turmeric (Curcuma longa L., Zingiberaceae), has been