

P XVII B.16 Preferential binding of heterocyclic amines to purines in polynucleotides

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Non-covalent binding of mutagenic heterocyclic amines (HAs) to DNA, RNA, homopurine polynucleotides, and homopyrimidine polynucleotides was studied. For this purpose, a series of polynucleotide-chitosan complexes, which were insoluble in phosphate-buffered saline at neutral pH, were prepared and used for the screening. All of the nine HAs tested showed affinities to DNA and RNA. Four representative HAs, IQ, MeIQx, PhIP and Trp-P-2, were investigated most extensively. All of them showed larger affinities to denatured DNA than to native DNA. For poly (G), even stronger affinities were observed, particularly with Trp-P-2: With Trp-P-2, the order of affinity toward four homopolynucleotides was poly (G) > poly (A) >> poly (U) > poly (C). This order of affinities was observed also for IQ, MeIQx and PhIP, although the strengths of the affinities were remarkably less than those of Trp-P-2. These results suggest that the HAs have affinity to purines, particularly to guanine, in regions of polynucleotides lacking double helical structure. It appears relevant to note that for many HAs including IQ, MeIQx, PhIP and Trp-P-2, it has been reported that the major adducts formed in DNA are those of guanine.

Keyword(s): Heterocyclic amines; Affinity to guanine

P XVII B.17 Effects of benzenediols and benzenetriols on *N*-nitrosation reactions

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N-nitrosatable amino compounds are commonly present in both foodstuffs and drug, and it has been unequivocally demonstrated that carcinogenic *N*-nitroso compounds are formed from these precursors in the acidic conditions of the human stomach. Several chemically defined phenolic compounds as well as complex mixtures of plant phenolics have been shown to block nitrosation reactions, but a systematic study of the relationship between chemical structure and inhibiting activity has not yet been performed. As a first approach we have examined the influence of the number and position of hydroxyl groups on the benzene ring. Phenols tested were: phenol; 1,2-, 1,3- and 1,4-benzenediol; 1,2,3-, 1,2,4- and 1,3,5-benzenetriol. Propranolol, a β -adrenergic blocking drug, which has previously been found to react with nitrite under simulated gastric conditions with formation of the corresponding genotoxic *N*-nitrosamine, was chosen as the nitrosatable test compound. Its nitrosation was carried out under the standard conditions recommended by the WHO (10 mM propranolol hydrochloride, 40 mM sodium nitrite, pH 3.5) in the absence and in the presence of the seven phenols added to the nitrosation mixture in concentrations ranging from 2 to 40 mM. Phenol was substantially inactive. The yield of *N*-nitrosopropranolol (NOP) was reduced, with potency decreasing in the following order, by 1,2-benzenediol > 1,2,3-benzenetriol > 1,4-benzenediol > 1,2,4-benzenetriol. The inhibiting effect was dose-dependent. The maximum reduction of the yield of NOP (93%) was obtained with 40 mM 1,2-benzenediol at 60–120 min from the starting of the reaction. In sharp contrast, the yield of NOP was increased by 1,3-benzenediol and 1,3,5-benzenetriol, but the effect was inversely related to their concentration. The maximum increase of NOP formation (10.8-fold as compared to control) was obtained with 2 mM 1,3,5-benzenetriol at 15 min from the starting of the reaction. 1,2-Benzenediol and 1,3-benzenediol produced on the nitrosation of proline effects of the same type of those observed with propranolol. The results of this study indicate that depending on the positions of hydroxyl groups on the benzene ring benzenediols and benzenetriols may inhibit or hasten nitrosation reactions.

Keyword(s): Nitrosation reactions; Phenols; Antimutagens

P XVII B.18 Mutagenicity of aqueous fecal extracts with umu test

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It is well known that human feces contain many mutagens. Since the majority of mutagens are also carcinogens, it is possible that fecal mutagens is a one of the etiology of colon cancer. It is also suggested that the development of the cancer is dependent of the environmental factors, especially, the dietary patterns. Considering these searches and backgrounds, we studied the fecal mutagenicity and dietary pattern of 208 healthy male and female Japanese (age: 40–70 years old). Mutagenicity of aqueous fecal extract were measured with umu test using *Salmonella typhimurium* TA1535/pSK1002 and the O-AT-overexpressing strain NM2009, with and without microsomal activation. We detected the fecal mutagenicity from 0.2 g feces (dry weight). 21.9% of the subject showed positive responses (two fold over the background level) without microsomal activation, but only 5.7% with S9 activation. Similar results were obtained using the strain NM2009. The mutagenicity of feces was higher in lower age population (under 50 years old) than higher age population (over 60 years old).

Keyword(s): aqueous fecal extract; dietary pattern; SOS response; umu test; mutagenicity; *Salmonella typhimurium*TA1535; pSK1002; *Salmonella typhimurium*NM2009

P XVII B.19 Mutagenicity of chlorinated tap water in Bangkok

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Chlorination has been shown to increase the mutagenicity of surface water in many countries. In Bangkok, the capital of Thailand, tap water is usually prepared by chlorination of Chao Phraya River water, and only in a few villages that it is prepared from ground water. In this study, we therefore measured the mutagenicity of tap water samples collected from 16 different areas of Bangkok and as well as from 2 tap water producing plants. Water samples were concentrated at pH 2 by using Amberlite® XAD-2 resin and organic compounds were eluted by ethyl acetate, dissolved in DMSO and then tested for mutagenicity towards *Salmonella typhimurium* strain TA100 and YG1024 both in the presence and absence of metabolic activation. All samples of chlorinated water including those from producing plants exhibited significant mutagenicity towards strain TA100 in the absence of S9 mix, being 2,000–4,500 revertants per litre. Addition of S9 mix markedly decreased the mutagenicity activity. The mutagenicity towards strain YG1024 was much lower, and only some samples showed significant mutagenicity when tested at 100–400 ml per plate. Interestingly, boiling of chlorinated water, a normal way for preparing drinking water in many families, decreased mutagenicity significantly and 15–20 min. boiling was enough to abolish the total mutagenicity. As expected, no mutagenicity was observed in all ground water samples. Results in the present study demonstrated that chlorinated tap water in Bangkok area which is prepared from Chao Phraya River water contain direct acting mutagens causing base pair substitution, and that boiling for at least 15 min. can destroy these mutagenic effects.

Keyword(s): Chlorinated tap water; Mutagenicity; *Salmonella typhimurium*

P XVII B.20 Effects of *Alpinia oxyphylla* (zingiberaceae) on apoptosis in human promyelocytic leukemia (HL-

60) cells and tumor promoter-induced inflammation in mice

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There have been considerable efforts to search naturally occurring substances for intervention or prevention of carcinogenesis. Many components from dietary or medicinal plants have been identified to possess potential anti-tumor properties. These include curcumin (*Curcuma longa* Linn., Zingiberaceae) and gingerol (*Zingiber officinale* Roscoe, Zingiberaceae), which have been shown to inhibit tumor promotion in animal and *in vitro* systems. Another zingiberaceous plant used in oriental herbal medicine includes *Alpinia oxyphylla* Miquel. The methanolic extract of *Alpinia oxyphylla* contains phenolic compounds which have structures similar to that of curcumin. In the present study, we investigated whether the extract of *Alpinia oxyphylla* possesses potential cancer chemopreventive activity using cultured human promyelocytic leukemia (HL-60) cells. Treatment of HL-60 cells with the methanolic extract of *Alpinia oxyphylla* significantly reduced the viability of the treated cells by 27, 54, 74 and 82% compared with the solvent-treated control after 5 hr incubation with 10, 20, 33 and 67 µg/ml of the extract, respectively. Microscopic examination of the treated cells showed characteristic morphology of apoptosis. In addition, incubation of HL-60 cells with the extract induced DNA fragmentation in a dose and a time-dependent manners. Nuclear DNA fragmentation was observed after incubation of the cells with 20–67 µg/ml of the extract for 5 hr followed by agarose gel electrophoresis. In another study, topical application of *Alpinia oxyphylla* extract onto mouse ear ameliorated inflammation induced by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The anti-tumor promotional effect of *Alpinia oxyphylla* is under investigation using the 7,12-dimethylbenz(a)anthracene-initiated and TPA-promoted two stage mouse skin carcinogenesis model (supported in part by a grant from the RCNDD-KOSEF and a grant of the '97 Good Health R&D Project, Ministry of Health and Welfare, Republic of Korea).

Keyword(s): chemoprevention; *Alpinia oxyphylla*; apoptosis

P XVII B.21 DNA strand scission and cell death induced by salsolinol through redox cycling

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Oxidative DNA damage has been implicated in pathogenesis of certain neurodegenerative disorders, including Parkinson's disease. Autoxidation of biogenic catecholamines has been proposed to generate reactive oxygen species, such as hydroxyl radicals, that can mediate oxidative damage to DNA. Certain transition metals catalyze the redox cycling between catechols and their quinoid forms. The naturally occurring neurotoxin, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol; SAL), has been speculated to contribute to Parkinson's disease and neuropathology of chronic alcoholism. Metabolic formation of (R)-SAL is considered to be mediated through irreversible condensation of dopamine with acetaldehyde. In contrast, (S)-SAL is a predominant enantiomer in certain beverages and food stuffs, including soy sauce, beer and bananas. In the present study, we found the capability of SAL to cause DNA cleavage in the presence of Cu (II). Incubation of SAL and CuCl₂ with calf thymus DNA caused strand breaks. Likewise, SAL in combination with Cu (II) mediated the strand scission in αX174RFI or pBR322 supercoiled DNA in a time-related manner. Neither Cu (II) nor the catechol alone induced any appreciable DNA cleavage. Catalase protected against SAL- and copper-dependent DNA strand scission. The reaction of SAL with Cu (II) was accompanied by the reduction of Cu (II) to Cu (I). Furthermore, SAL induced cell death in cultured PC 12 cells, which was exacerbated by Cu (II). From these data, it seems likely that SAL undergoes redox cycling catalyzed by Cu (II) to generate reactive

species which may be responsible for the neurotoxic and possible genotoxic action of this catechol isoquinoline.

Keyword(s): Salsolinol; Redox cycling; DNA strand scission

P XVII B.22 DNA strand breaking activity and mutagenicity of a Maillard reaction product, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one

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We have previously identified one of active compounds with DNA strand breaking activity in ethyl acetate extract of Maillard reaction mixture of glucose and glycine as 4-hydroxy-2-hydroxymethyl-5-methyl-3(2H)-furanone (HHMF). In this study, we identified a new DNA-breaking compound and elucidated the mechanisms for DNA breaking and mutagenicity. When the ethyl acetate extract of the Maillard mixture was subjected to reversed phase HPLC, several UV-absorbing peaks were observed and two of the peaks were active to break DNA. The first active peak was HHMF. We purified the second active peak and identified it as 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) known as a Maillard product. DDMP was mutagenic to *Salmonella typhimurium* TA 100 without metabolic activation. DDMP induced DNA single strand breaks against supercoiled pBR 322 DNA at pH 7.4 and 37 °C. The DNA breaking activity of DDMP increased with dose of DDMP and the incubation time. It was active at pH 7.4 and 9.4, while it was inactive at pH 4.4. The DNA breaking by DDMP was inhibited by active oxygen scavengers, spin trapping agents and metal chelators, and enhanced by Fe (III) ion. ESR-spin trapping using DMPO revealed the generation of hydroxyl radical from DDMP. DDMP reduced Fe (III) ion into Fe (II) ion dose dependently. Hence, DDMP generated superoxide, hydrogen peroxide and hydroxyl radical by means of trace amount of metal ions to induce DNA strand breaks. The oxidative DNA damage by DDMP through active oxygen species may participate in the mutagenesis.

Keyword(s): 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one; DNA damage; Active oxygen species