Thesis Title Partial Characterization of Mutagen(s) in Shallot (Allium ascalonicum Linn.) and the Effect of Some Chemicals on Its Mutagenic Activity

Author Mr. Prayad Pantasri

M.Sc. Biochemistry

Examining Committee :

Assist.Prof. Dr.Vic ai Wongchai.....Chairman

Assist.Prof. Dr.Usanee Vinitketkumnuen..Member

Assoc.Prof. Dr.Maitree Suttajit.....Member

Assist.Prof. Dr.Umnat Mevatee.....Member

ABSTRACT

Shallot mutagenicity and its modulation were studied by Salmonella mutation, preincubation technique. The methanol extract of shallot has stronger mutagenicity to S. typhimurium strain TA98 than to TA100, both with and without metabolic activation. The direct mutagenic substances of shallot reported could undergo metabolic activation to become higher by enzymes in rat-liver S9 fraction.

Modification of shallot mutagenesis in Salmonella mutation system by phase II reaction was studied by including glutathione (GSH) or uridine-5'-diphosphoglucuronic acid (UDPGA) in the system. The decrease mutagenicity of the mutagenic substances in shallot occurred through conjugation reaction both with either GSH or UDPGA. In addition, GSH could

react directly with the mutagenic substances in shallot. The chemical reaction might occur via -SH group of GSH. Another thiol-containing compound, dithiothreitol (DTT) also suppressed the mutagenicity of shallot without S9 mix.

Retinoic acid and ascorbic acid could not modify the shallot mutagenesis in Salmonella mutation system.

After nitrite treatment, the mutagenicity of shallot was demonstrated toward TA100, with and without metabolic activation. The nitrosation products might be probably formed from precursor in shallot.

Partial purification of active mutagenic substances in shallot by SEP-PAK (uBonda Pak) column was done. The mutagenic substances were eluted with 50 % and 100 % methanol. Further purification by Sephadex LH-20 column chromatography of 50 % methanol eluate gave 4 mutagenic peaks. The major mutagenic peak was further re-chromatographed. This peak had the same retention time as standard quercetin on Sephadex LH-20 column chromatography. By Silica gel 60 G thin-layer chromatography, the R_f value of this peak is closed to that of quercetin in a solvent system (chloroform : ethanol : butanone : acetyl acetone; 16:10:5:1) but different in the other system (chloroform : methanol : water ; 65:45:12).

Its UV absorption spectra showed a pattern similar to that of standard quercetin and quercetrin (quercetin-3-rhamnoside). Its co-chromatography of the compound under this peak with authentic quercetin did not show any enlargement of the peak but still showed some shoulder of the obtained peak. Therefore, the mutagenic compound in this peak might possibly be quercetin glycoside.

The 100 % methanol eluate still showed to contain more than one mutagenic peaks by sephadex LH-20 chromatography. However the major mutagenic peak was not sufficient for further purification.