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Desmutagenic Effect of Extracts Obtained from Seaweeds

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Extracts of six seaweeds obtained from solvents such as water, methanol, hexane and ethyl ether have been tested for desmutagenic activities in the *Salmonella typhimurium*/microsome systems.

Water extracts obtained from tanglé, sea-tangle, and gompi were moderately effective on the mutagenicity of Trp-P-2 and aflatoxin B_1.

Methanol extracts of sea-tangle and gompi were effective against Trp-p-2, and all samples of methanol extracts were very effective from 1.0 *mg* to 2.0 *mg* per plate on the mutagenicity of MeIQ and aflatoxin B_1.

Hexane extracts of tangle, ses-tangle, gompi and sea-straghorn were very effective on the mutagenicity of Trp-p-2, MeIQ and aflatoxin B_1 except laver and green laver.

Ethyl ether extracts of gompi and sea-straghorn were most effective from 1.0 *mg* to 2.0 *mg* per plate on the mutagenicity of Trp-P-2. Especially, ethyl ether extracts of all samples were great effective on the mutagenicity of MeIQ and aflatoxin B_1.

Introduction

A new mutagens of heterocylic amines were isolated as very potent mutagens in food processing, cooking and storage. They are 3-amino-1, 4-dimethyl-5H-pyrido(4, 3-b) indole (Trp-P-1) and 3-amino-1-methyl-5H-pyrido(4, 3-b) indole (Trp-P-2) from tryptophan pyrolysates[1,2], 2-amino-6-methyldipyrido(1, 2-a:3′, 2′-b) imidazole(Glu-P-1) and 2-aminodipyrido (1, 2-a:3′, 2′-d) imidazole(Glu-P-2) from glutamic acid pyrolysates[3,4]. Moreover, from broiled fish, two other highly mutagenic heterocylic amines, 2-amino-3-methyl-imidazo(4, 5-f) quinoline (IQ) and 2-amino-3, 4-dimethyl-imidazo (4, 5-f) quinoline(MeIQ) were isolated[5]. Another mutagenic heterocylic amine, 2-amino-3, 8-dimethylimidazo(4, 5-f) quinoxaline(MeIQx) was purified from fried beef[6], and also aflatoxin was isolated from fermented foods[7].

Since the pyrolysis products of foods may play a role in human carcinogenesis, their potential or actual health harzard sucessfully applied to estimate the mutagenic and antimutagenic properties of manmade and naturally occuring chemicals by *in vitro* test system in Ame's strain of *Salmonella typhimurium*[8]. Recently, epidemiological evidence points to an inverse relationship between the consumption of vegetables and the incidence of cancer at various sites[9,10,11].

The search for the protective components in these vegetable has focused on β-carotene and vitamin A[12,13,14,15] and ascorbic acids[16,17].

Also Morita *et al*[18],. were found that extract of cabbage, brocoli, egg plant, green pepper, apple, pineapple, ginger, mint leaf had antimutagenic factors.

Concurrent this type of research, we have examined the capacities of inactivation of the mutagenic principles of foods in six seaweeds extracts obtained by solvents such as water, methanol, hexane, and ethyl ether.

Materials and Methods

Materials

Samples of tangle(Undaria pinnatifida(Harv.) Suringar), sea tangle(Laminaria cichoriodes Miyabe), gompi(Ecklonia stolonifera Okamura), seastraghorn(Codium fragila(Sur.) Hariot), laver(Porphyra tenera Kjellman) and green laver(Enteronorpha linza(Linne) J. Agardh) were obtained from jachalchi market in Pusan.

Trp-P-2 and MeIQ were kindly gift from T, Sugimura. Japan National cancer center Research Institute, Aflatoxin B_1, NADP and glucose-6-phosphate were purchase from Sigma.

Preparation of desmutagenic factors from sample; Dried samples of each were extracted by solvents such as water. methanol, hexane and ethyl ether for 15 hrs in round flask with reflux condenser.

After extraction, the samples were centrifuged and the supernatants were concentrated for further use.

Bacterial strains

Salmonella typhimurium TA 98 was kindly supplied by Prof. B. N. Ames, University of California.

Induction of rat liver microsomal enzymes

The rat liver enzymes were induced with a polychlorinated biphenyl(PCB) mixture(Aroclor 1254).

The induction procedure was similar to the method of Czygan *et al*[19]. A single i.p. injection of Aroclor 1254(dilutede in corn oil to a concentration of 200 *mg/ml*) at a dosage of 500 *mg/Kg* was given to rat five days before sacrifice.

Preparation of liver homogenate (S-9) and S-9 mix

The microsomal preparation was made according to Garner *et al*[20]. The rat liver was washed in an equal volume of 0.15*M* KCl, minced with sterile scissors in three volumes of 0.15*M* KCl and homogenized with a potter Elvehjem apparatus. The homogenate was centrifuged at 4℃ for 10 *min* at 8000×*g*. The supernatant was collected and stored at −80℃. As required, sufficient S-9 fraction was thawed(at room temperature) and kept in ice. S-9 Mix contained per *ml*; 0.3 *ml* of S-9 fraction, 8 *mM* $MgCl_2$, 33 *mM* KCl, 5 *mM* glucose-6-phophate, 4 *mM* NADP and 100 *mM* sodium phosphate, pH 7.4.

Assay of desmutagenic test

Desmutagen assays were carried out using Ame's strain of *Salmonella typhimurium* TA 98 and histidine reversion were measured by his standard method[8] with some modification. The standard method of assaying the desmutagenic factors of seaweeds on the mutagenicity of Trp-P-2, MeIQ and aflatoxin B_1 were as follow. Usually mutagens made 0.005 *ppm* of Trp-P-2, 0.00125 *ppm* of MeIQ, and 0.0125 *ppm* of aflatoxin B_1 with dimethylsulfoxide(DMSO), and were combined with 0.5 *mg*, 1.0 *mg* and 2.0 *mg* of each extracts of seaweeds and kept for 30 min at 37℃. The incubated mixture was treated with for 10 min at 100℃ in order to reduced by active factor in seaweed extracts. The mixture 0.1 *ml* of bacterial culture of TA 98 and 0.5 *ml* of S-9 mixture at 4℃. The reaction mixture were incubated at 37℃ for 20 min and added 2 *ml* of 0.6% molten top agar containing 0.5% sodium chloride, 12.4 μg of histidine, and 9.6 μg of biotin. Vortex for 30 sec at low speed and poured on selective minimal glucose agar plate. All the plate were incubated at 37℃ for 48 hrs and then the number of His^+ revertants were counted. For each reaction mixture, triplicated were used and the mean value of His^+ revertants colonies was calculated.

Results and discussion

We have recently reported[21] that legumes and edible plant saponins have desmutagenic activities on the mutagenicity of Trp-P-2, MeIQ and aflatoxin B_1.

In order to screen systematically desmutagenic

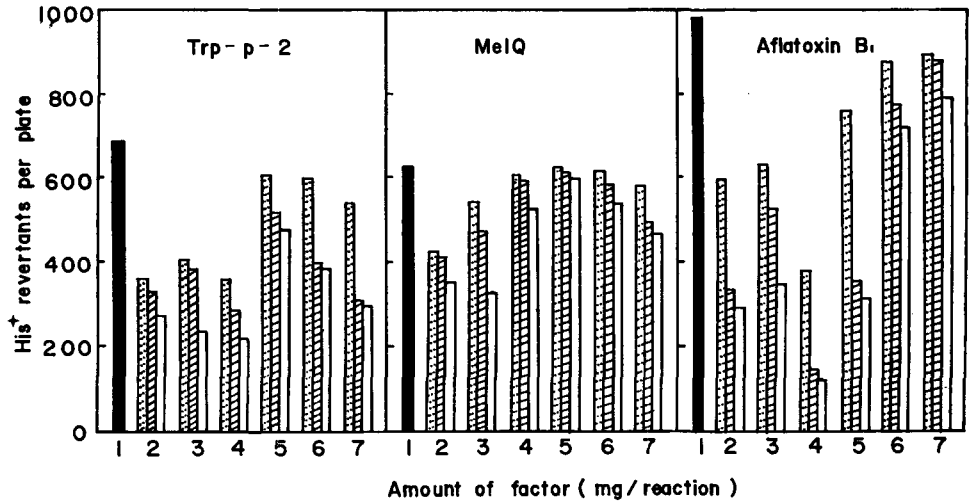

Fig. 1. Desmutagenic effect on the mutagenicity of Trp-p-2, MeIQ and aflatoxin B_1 by water extract.
1. Indicator ; 0.005 *ppm* of Trp-p-2, 0,00125 *ppm* of MeIQ and 0.0125 *ppm* of aflatoxin B_1
2. tangle; 3. sea-tangle; 4. gompi; 5. sea-straghorn; 6. laver; 7. green-laver
▨ : 0.5 *mg* per plate ▨ : 1.0 *mg* per plate □ : 2.0 *mg* per plate

factors in seaweeds, we collected six samples and prepared extracted from them. Their desmutagenic activities were carried out at several doses giving His^+ revertants per plate of each mutagenic/saponin reaction mixture using *Salmonella typhimurium* TA 98 as indicator. Their mutagenic activities were measured by *Salmonella syphimurium* TA 98 with reactivation by the rat liver homogenate(S-9). The desmutagenic frequency on plates were compared with plate containing mutagens at 0.005 *ppm* of Trp-P-2, 0.00125 *ppm* of MeIQ and 0.0125 *ppm* of aflatoxin B_1 per plates.

Desmutagenic effects of seaweeds by water extracts effects of treatment with water extracts on the mutagenicity of Trp-P-2, MeIQ and aflatoxin B_1 are shown in Fig. 1. Desmutagenic factors were obtained from tangle, sea tangle, gompi, sea straghorn, laver and green laver. Concentration of each samples with water extracts were contained at 0.5*mg*, 1.0 *mg* and 2.0 *mg* per plate. Most samples have no effects or very moderately activities in reducing the number of His^+ revertants. Tangle, sea-tangle and gompi were considerably effective on the mutagenicity of Trp-P-2 and aflatoxin B_1. On the contrary, sea-straghorn, laver and green laver were no effective or very low effective on the mutagenicity of MeIQ.

Desmutagenic effect of seaweeds extracted from tangle, sea-tangle, gompi, sea-straghorn, laver and green laver were tested for their capacity to develope the desmutagenic activities resulting from methanol extracts. Fig. 2 are showed the effects of methanol extracts obtained from seaweeds on the mutagenicity of Trp-P-2, MeIQ and aflatoxin B_1. Most samples have moderately activities in reducing the number of His^+ revertants containing at 0.5 *mg* and 1.0 *mg* per plate except laver. Sea tangle and gompi were great effective against Trp-P-2. Laver and green laver were low effective in reducing the number of His^+ revertants containing at 0.5 *mg* per plate, but tangle, sea-tangle, gompi, sea-straghorn, green laver were very effective at ranging from 1.0 *mg* to 2.0 *mg* of methanol extracts per plate. Samples of methanol extracts were very effective at ranging from 1.0 *mg* to 2.0 *mg* per plate on the mutagenicity of aflatoxin B_1.

Desmutagenic effect of seaweeds extracted by hexane (Fig. 3) are showed the desmutagenic effect of hexane extracts on the mutagenicity of Trp-P-2,

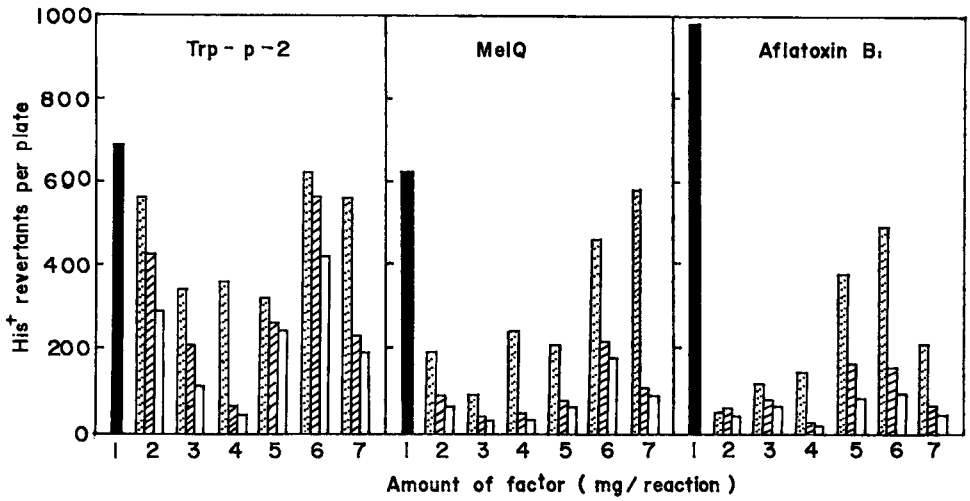

Fig. 2. Desmutagenic effect on the mutagenicity of Trp-p-2, MeIQ and aflatoxin B_1 by methanol extract.
Refer to Fig. 1.

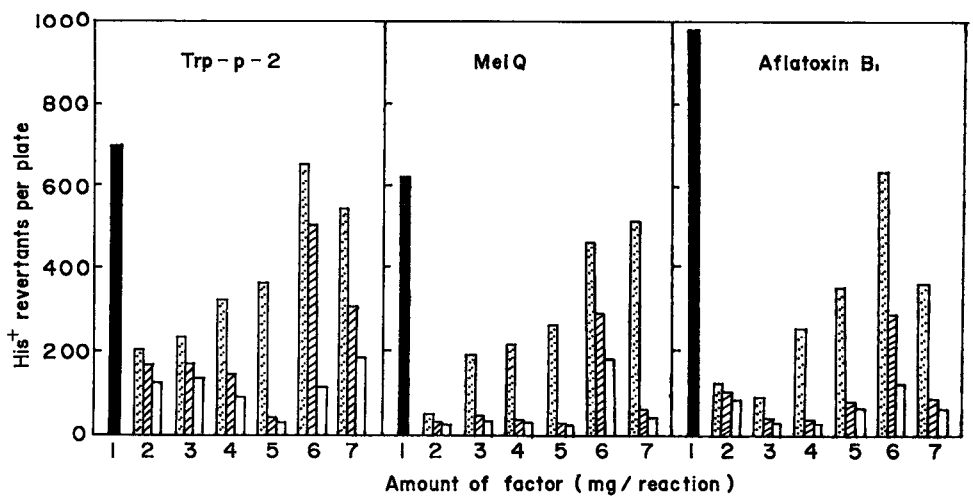

Fig. 3. Desmutagenic effect on the mutagenicity of Trp-p-2, MeIQ and aflatoxin B_1 by hexane extract.
Refer to Fig. 1.

MeIQ and aflatoxin B_1. We found that hexane extracts from tangle, sea-tangle, gompi, sea-straghorn, and green laver were very effective at ranging from 1.0 *mg* to 2.0 *mg* per plate on the mutagenicity of Trp-P-2.

All samples of seaweeds were most effective on the mutagenicity of MeIQ. Also most samples except laver were most effective on the mutagenicity of aflatoxin B_1.

Desmutagenic effect of seaweeds extracted by ethylether. All samples of ethyl ether extracts were examined as to their capacity of inactivation on the mutagenicity of Trp-P-2, MeIQ and aflatoxin B_1 (Fig. 4). Ethyl ether extracts obtained from all of samples were great effective on the MeIQ and aflatoxin B_1.

In addition gompi and sea-straghorn were great effective in reducing the number of His^+ rever-

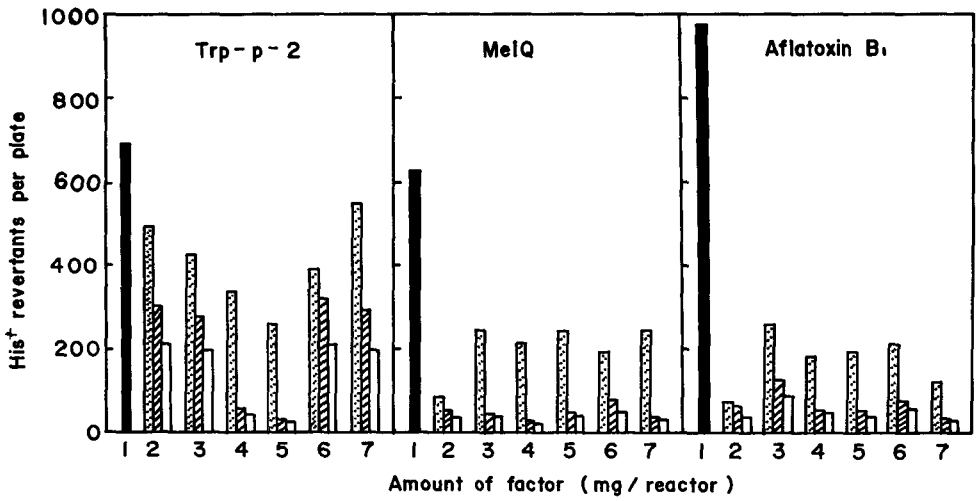

Fig. 4. Desmutagenic effect on the mutagenicity of Trp-p-2, MeIQ and aflatoxin B_1 by ethyl ether extract.
Refer to Fig. 1.

tants, containing at 1.0 *mg* and 2.0 *mg* per plate, but tangle, sea-tangle, laver and green laver were low effective on the mutagenicity of Trp-P-2.

The results are showed that ethyl ether extracts from all of samples have wide spectra desmutagenic activities than extracts obtained from water, methanol and hexane.

Some vegetables have desmutagenic factor on the tryptophan pyrolysates[23], but it was not clear yet mechanism involved inactivation of mutagenicity principles.

Many *in vitro* test systems have been sucessfully applied to estimate the mutagenic or antimutagenic activity of compounds.

Seaweeds extracts lead to effects of desmutagen which can be readily by the *Salmonella Typhymurium* mutagenicity assay.

This well-defined system was carried out examine by four different solvents such as water, methanol, hexane, and ethyl ether.

The examined methanol, hexane and ethyl ether extracts were most activity on the mutagenicity. Seaweed extracts obtained from ethyl ether was great effective in the mutagenicity. These results could be explained by supposing either that a single factor can desmutanize the mutagens of different chemical structure or that complex factors are involved for inactivation of on the some mutagenicity of Trp-P-2, MeIQ and aflatoxin B_1. The present study showed that the extracts of organic solvents were more inactivated at least on the Trp-P-2, MeIQ and aflatoxin B_1.

These studies may be also useful to show whether taking seaweeds in combination with cooked meat and fish might be helpful to decrease or neutralized the toxicity.

We don't know yet mechanisms involved in inactivation of the mutagenicity principles. It is possible to understand of mechanism by using purified desmutagenic factor from the seaweeds.

These studies are now under way to these phenomena in more detail.

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해조류의 항돌연변이 효과

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해조류중 미역, 다시마, 곤피, 청각, 파래 및 김을 물, 메타놀, 핵산 및 에칠에테르로 추출한 엑스분의 항돌연변이 효과를 *Salmonella typhimurium*/microsome 系로 실험하였다. 물로 추출한 엑스분은 Trp-P-2와 aflatoxin B_1에 다소 효과가 있었고, 메타놀의 엑스분중 다시마, 곰피는 Trp-P-2에 효과가 있었으며, 메타놀 엑스분을 plate 당 1.0 *mg*, 2.0 *mg* 함유하는 모든 시료는 MeIQ와 aflatoxin B_1에 효과가 있었다. 에칠 에테르의 엑스분은 곰피와 청각이 매우 효과가 있었고, 특히 6종류의 해조류중 에칠 에테르의 엑스분은 MeIQ와 aflatoxin B_1에 대하여 우수한 효과가 있었다.