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Antimutagenic and Cancer Cell Growth Inhibitory Effects of Seaweeds

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Abstract

The antimutagenic and cancer cell growth inhibitory effects of methanol extracts from 9 kinds of seaweed were studied in the Ames assay and cell culture systems, respectively. The methanol extracts from the seaweeds of sea lettuce, chlorella, sea tangle, sea mustard, sporophyll of sea mustard, fusiforme, seaweed papulosa, purple laver and ceylon moss showed antimutagenicities against aflatoxin B_1 (AFB_1) and N-methyl-N'-nitro-N-nitrosoguanidine(MNNG) in the *Salmonella typhimurium* TA100. These extracts revealed relatively higher antimutagenicity against AFB_1(indirect mutagen) than MNNG(direct mutagen). Sporophyll of sea mustard and seaweed papulosa exhibited strong antimutagenic activity against AFB_1, and sporophyll of sea mustard, sea tangle and ceylon moss also reduced the mutagenicity induced by MNNG. The sporophyll of sea mustard exerted the highest antimutagenic activity among the samples treated. The methanol extracts from 9 kinds of seaweed inhibited the growth of two cancer cell lines, AGS human gastric adenocarcinoma cells and HT-29 human colon carcinoma cells. Sea tangle, sea mustard and sporophyll of sea mustard inhibited the growth of cancer cells significantly. These results suggest that various seaweeds show not only antimutagenic activity but also growth inhibitory effect on some cancer cells.

Key words: seaweeds, antimutagenicity, human cancer cells

INTRODUCTION

Hypothesis for chemical carcinogenesis suggests that there are several important stages during the induction of tumor cells. Especially an initiation stage consists of genetic alterations such as damage, breakage and repair of nuclear DNA induced by mutagenic or carcinogenic compounds(1,2). In contrast, some inhibitory compounds against mutagenesis or carcinogenesis have been identified in foods of plant origin which shows divergent chemical structures(3). Diet is thought to be an important factor in cancer prevention. Cross-cultural comparison of cancer death rates shows several fascinating differences(4). Breast cancer is associated with one of the most extreme variations in the rates(5). For example, breast cancer incidence is 3 times lower for premenopausal Asian women than for American women. Seaweed consumption can be one of important factors in explaining the lower incidence of certain cancers, especially breast cancer. Epidemiological data are compatible with a role for brown seaweed in breast cancer prevention(6,7). There are many different diet habits between Asian women and American women. One of the examples is the preference of Asian population for brown seaweeds. Some seaweeds such as sea mustard and sea tangle have long been used in traditional Chinese herbal medicine for the treatment of cancer(8,9). Several studies(10-15) have shown that hot water extracts of seaweeds inhibit the growth of sarcoma-180, Meth-A, B-16 melanoma and L-1210 leukemia cells and decrease in the incidence rate of chemically induced carcinogenesis.

In Korea, seaweeds such as sea mustard, sea tangle and fusiforme have been traditionally used as food and also as an additive or seasoning with various foodstuffs. However, the details of the antimutagenic activity and anticancer activity of edible seaweeds have been poorly elucidated until now. This study was designed to determine the antimutagenic activity and cancer cell growth inhibitory effect of methanol extracts from common edible seaweeds harvested in Korea.

MATERIALS AND METHODS

Preparation of samples

Nine kinds of seaweed, such as *Ulva lactuca*(sea lettuce),

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Chlorella pyrenoidosa(chlorella), *Laminaria japonica*(sea tangle), *Undaria pinnatifida*(sea mustard), sporophyll of sea mustard, *Hizikia fusiforme*(fusiforme), *Ecklonia cava* (seaweed papulosa), *Porphyra tenera*(purple laver) and *Gelidium amansii*(ceylon moss) were purchased from the Jagalchi market in Pusan, Korea. Dried and powdered seaweeds were extracted with 20 vol(w/v) of methanol three times, by shaking 12 hrs for each. The methanol extracts were concentrated under a vacuum rotary evaporator and then dissolved in dimethyl sulfoxide(DMSO).

Ames test

Aflatoxin B_1(AFB_1) and N-methyl-N'-nitro-N- nitrosoguanidine(MNNG) were used as mutagens. AFB_1 was purchased from Sigma Chemical Co.(St. Louis, Mo, USA) and dissloved in DMSO. MNNG was obtained from Aldrich Co.(Milwaukee, WI, USA) and dissloved in distilled water. *Salmonella typhimurium* TA100 bacterial strain, histidine requiring mutant, was provided by Dr. B.N. Ames(Univ. of California, Berkerly, CA, USA), and was maintained as described by Maron and Ames(16). The genotype of the test strain was checked routinely for the histidine requirement, deep rough(*rfa*) character, UV sensitivity(*uvr* B mutation) and the presence of R factor. S9 mixture to activate the indirect mutagen, AFB_1, was also prepared by the method of Maron and Ames(16). Mutagenicity test(17,18) was carried out by a modified plate incorporation test(liquid preincubation of the organism with the test compound). In preincubation test, 0.5ml of S9 mixture (or 0.5ml phosphate buffer for direct mutagen. MNNG) was distributed into sterilized capped tubes on ice bath and then 0.1ml of test strain cultured overnight ($1\sim2\times10^9$ cells/ml) and 0.1ml of test compound (50μl of mutagen and 50μl of methanol extracts from seaweeds) were added. The tubes were vortexed gently and preincubated at 37°C for 20 min and then 2ml of the top agar kept at 45°C was added to each tube and vortexed. The resulting entire mixture was poured on the minimal agar plate. The plates were incubated at 37°C for 48 hrs and then the revertant bacterial colonies on each plate were counted.

Dose response test(16) of the mutagens on the test strain was carried out to determine the regions of revealing mutagenicity induced by the mutagens. Toxicity test for the different concentration of the methanol extracts was also carried out. The methanol extracts used for antimutagenicity test did not show any toxicity on the test strain.

Cell culture

Dulbecco's modified eagle's medium (DMEM), fecal calf serum(FCS), trypsin-EDTA and penicillin-streptomycin were purchased from GIBCO Co.(Gaithersburg, MD, USA). The AGS human gastric adenocarcinoma cell and HT-29 human colon carcinoma cell were obtained from Korea Cell Line Bank(KCLB, Seoul, Korea). The cells were cultured in DMEM supplemented with 10% FCS and 1% penicillin-streptomycin. The cultured cells were dissociated with 0.05% trypsin-0.02% EDTA and seeded 2×10^4 cells/ml for the growth inhibitory test. The cells were incubated in 5% CO_2 incubator at 37°C for 24hrs, and then the media supplemented with the methanol extracts from seaweeds were changed every two days. In control experiment, the cells were treated with DMSO. After 6 days, the cells were washed with phosphate buffered saline, treated with trypsin-EDTA and then counted by hemocytometer.

RESULTS AND DISCUSSIONS

Seaweeds can be classified into 4 groups based on the vertical distribution of their habitats, which are green algae(sea lettuce, chlorella), brown algae(sea tangle, sea mustard, fusiforme, seaweed papulosa), red algae(purple laver, ceylon moss) and blue algae. The antimutagenic activity and the growth inhibitory effect on cancer cells of 9 kinds of seaweed were studied.

The methanol extracts from the seaweeds showed antimutagenic activity against AFB_1 in *Salmonella typhimurium* TA100(Table 1). Most of methanol extracts from seaweeds exerted strong antimutagenic effect, especially sporophyll of sea mustard and seaweed papulosa exhibited the strongest activity among them. In particular, sporophyll of sea mustard showed antimutagenic effect more than 50% at the lowest concentration(0.625mg/plate) tested. Table 2 revealed the antimutagenicity of methanol extracts from seaweeds against MNNG. Most of the methanol extracts exhibited weaker antimutagenic effect against direct mutagen, MNNG, than against indirect mutagen, AFB_1. The methanol extracts from sea tangle, sporophyll of sea mustard and ceylon moss showed higher antimutagenic effect against MNNG than any other seaweed. These results suggest that common edible seaweeds have antimutagenic effect on AFB_1 and MNNG. Especially,

Table 1. Effect of methanol extracts from 9 seaweeds on the mutagenicity induced by aflatoxin B_1(AFB_1, 0.2μg/plate) in *Salmonella typhimurium* TA100

Seaweed sample \ Concentration (mg/plate)	Revertants/plate		
	0.625	1.25	2.5
AFB_1(Control)		986 ± 18^{a}	
Spontaneous		138 ± 5	
AFB_1 + Sea lettuce	734 ± 59^{c} (48)[1)]	441 ± 17^{def}(64)	428 ± 25^{cd} (66)
+ Chlorella	876 ± 50^{ab} (13)	695 ± 22^{b} (18)	689 ± 45^{b} (35)
+ Sea tangle	790 ± 37^{bc} (23)	506 ± 6^{cde} (57)	374 ± 32^{cde}(72)
+ Sea mustard	576 ± 37^{de} (48)	434 ± 17^{ef} (65)	398 ± 23^{cde}(69)
+ Sporophyll of sea mustard	515 ± 37^{e} (56)	365 ± 27^{f} (73)	268 ± 28^{e} (85)
+ Fusiforme	681 ± 9^{cd} (36)	522 ± 26^{cd} (55)	402 ± 20^{cde}(69)
+ Seaweed papulosa	681 ± 46^{cd} (36)	350 ± 4^{f} (75)	282 ± 15^{de} (83)
+ Purple laver	631 ± 19^{cd} (42)	563 ± 22^{c} (50)	472 ± 38^{c} (61)
+ Ceylon moss	808 ± 32^{bc} (22)	587 ± 33^{c} (47)	342 ± 15^{de} (76)

[1)]The values in the parantheses are inhibition rate(%).
[a~f]Means with the different letters are significantly different at the 1% level of significance as determined by Duncan's multiple range test.

Table 2. Effect of methanol extracts from 9 seaweeds on the mutagenicity induced by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 0.45μg/plate) in *Salmonella typhimurium* TA100

Seaweed Sample \ Concentration (mg/plate)	Revertants/plate		
	0.625	1.25	2.5
MNNG(Control)		1091 ± 32^{a}	
Spontaneous		109 ± 25	
MNNG + Sea lettuce	804 ± 10^{c} (29)[1)]	667 ± 39^{c} (43)	623 ± 32^{cd} (48)
+ Chlorella	649 ± 2^{e} (45)	520 ± 22^{c} (48)	590 ± 24^{de} (51)
+ Sea tangle	707 ± 9^{de}(39)	640 ± 26^{c} (46)	572 ± 33^{def} (53)
+ Sea mustard	786 ± 38^{cd}(31)	769 ± 30^{b} (33)	662 ± 36^{c} (44)
+ Sporophyll of sea mustard	632 ± 18^{e} (47)	570 ± 31^{c} (53)	517 ± 41^{f} (58)
+ Fusiforme	976 ± 27^{b} (12)	798 ± 26^{b} (30)	787 ± 28^{b} (31)
+ Seaweed papulosa	737 ± 40^{c} (36)	649 ± 4^{c} (45)	571 ± 8^{cd} (53)
+ Purple laver	822 ± 19^{cd}(27)	658 ± 44^{c} (44)	625 ± 25^{ef} (47)
+ Ceylon moss	679 ± 39^{e} (42)	571 ± 4^{c} (53)	541 ± 7^{def} (56)

[1)]The values in the parantheses are inhibition rate(%).
[a~f]Means with the different letters are significantly different at the 1% level of significance as determined by Duncan's multiple range test.

sporophyll of sea mustard showed the highest antimutagenic effect against AFB_1 and MNNG. As sea mustard grows, sporophyll of sea mustard is formed at the base of stem. Both sea mustard and sporophyll of sea mustard can be used for foods.

Ryu et al.(19) and Yasuji et al.(20,21) reported that water extracts from sea tangle and seaweed papulosa had moderate inhibitory activities on the mutagenicity of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b] indole(Trp-P-1) and AFB_1, and also ethyl ether extracts of the seaweeds showed antimutagenic activities on 2-amino-3,4-dimethyl-imidazo[4,5-f]-quinoline(MeIQ) and AFB_1.

The methanol extracts from the tested seaweeds inhibited the growth of AGS human gastric adenocarcinoma cells (Fig. 1). Those from sea tangle, sea mustard, sporophyll of sea mustard and ceylon moss($p<0.01$) showed higer inhibitory effect on the growth of AGS cells than the other seaweeds. As the concentration was increased, the growth of AGS cells was more retarded as concentration dependent manner. Especially, sporophyll of sea mustard inhibited significantly the growth of AGS cells at the low concentration (0.05 mg/ml). At the 0.2mg/ml concentration, sea mustard and sporophyll of sea mustard inhibited the growth of AGS cells more than 70%. Fig. 2 showed the inhibitory effect of the seaweeds on the growth of HT-29 human colon carcinoma cells. Sporophyll of sea mustard and fusiforme exerted higher growth inhibition rates($p<0.01$) of the HT-29 cells among the

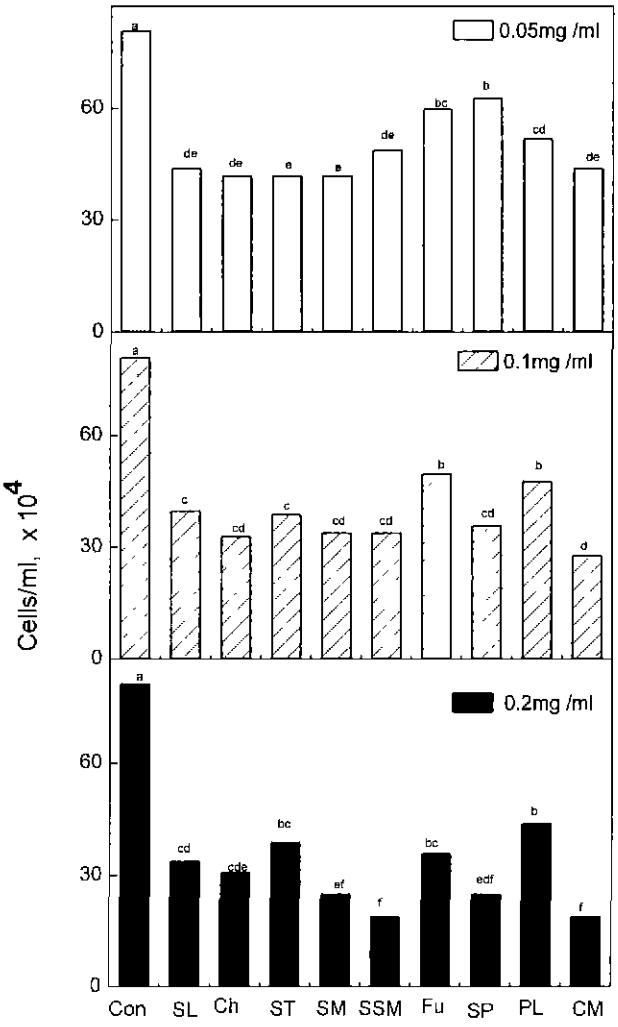

Fig. 1. The inhibitory effect of methanol extracts from 9 seaweeds on the growth of AGS human gastric adenocarcinoma cells after 6 days of incubation at 37°C.

Con.(control), SL(sea lettuce), Ch(chlorella), ST (sea tangle), SM(sea mustard), SSM(sporophyll of sea mustard), Fu(Fusiforme), SP(seaweed papulosa), PL(purple laver), CM(ceylon moss)

$^{a\sim f}$ Means with the different letters are significantly different at the 1% level of significance as determined by Duncan's multiple range test.

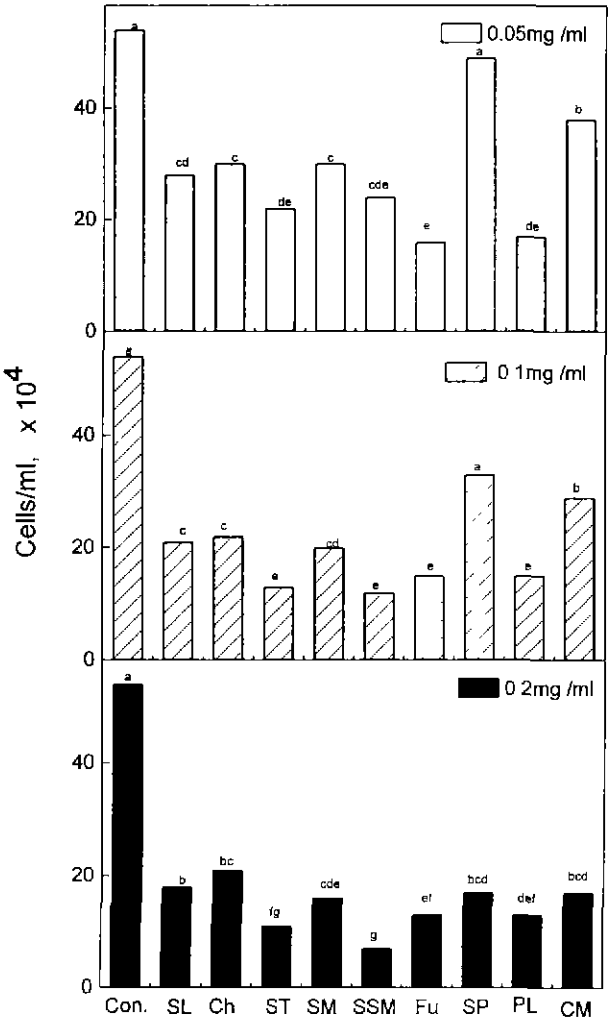

Fig. 2. The inhibitory effect of methanol extracts from 9 seaweeds on the growth of HT-29 human colon carcinoma cells after 6 days of incubation at 37°C.

Con.(control), SL(sea lettuce), Ch(chlorella), ST (sea tangle), SM(sea mustard), SSM(sporophyll of sea mustard), Fu(Fusiforme), SP(seaweed papulosa), PL(purple laver), CM(ceylon moss)

$^{a\sim g}$ Means with the different letters are significantly different at the 1% level of significance as determined by Duncan's multiple range test.

seaweeds. These results suggest that the methanol extracts of seaweeds inhibit growth of the AGS and HT-29 cancer cells. Interestingly, sporophyll of sea mustard, the base of stem of sea mustard, showed higher antimutagenic activity and cancer cell growth inhibitory effect than sea mustard itself.

Several reports(20-24) have been published on the antitumor activity of marine algae. The non-dialyzable fraction of hot-water extracts from seaweeds showed marked growth inhibition of sarcoma-180 cells subcutaneously implanted into mice, and these fraction was suggested to consist primarily of polysaccharide. Yamamoto et al.(22-24) also demostrated that partially purified polysaccharide fraction from seaweeds, the active component of which was suggested to be a sulfated glycuronoglycan, was effective against sarcoma-180 cells and that the aqueous seaweeds fraction decreased the growth of Meth-A, B-16 melanoma and L-1210 leukemia cells. Other studies(9, 10, 13, 23) have shown that daily injection with seaweed extract after tumor cell inoculation resulted

in tumor growth inhibition and increased life span of the mice. In these studies, the sulfated polysaccharide in the extract was postulated to be the active component. It seems likely that seaweeds modulate the development of tumors, affecting the promotion of tumor growth.

In addition to these results, Cho(25) demonstrated that alginic acid extracted from seaweeds inhibited growth of human cancer cells, and also showed antimutagenic effect. Alginate is a viscous dietary fiber in algae-containing foods and the main constituents of alginate are uronic acids(manuronic and guluronic acid), which give the pectin-like characterstics. It is thought that the antimutagenic and anticarcinogenic activities of alginate depend on the composition of the homopolymer 1→4 linked β-D-manuronic acid(MM-block) and α-L-guluronic acid(GG-block) in alginate.

The possible reasons for the antitumorigenic effects of dietary seaweed have been discussed elsewhere(3,4), including the abundent presence of nondigestible fiber, minerals and vitamins, antibacterial activity against fecal flora, stimulation of the immune response and cytotoxic activities of alginate and laminarin. Perhaps another important active compound is the cell wall polysaccharide sulfate ester, fucoidan, and its component fucose.

The results of this experiment suggest that seaweeds show the antimutagenic activity and the growth inhibitory effect on cancer cells. Further study is needed to identify the active compound(s) and mechanism(s) of the antimutagenicity and anticancer effect of the seaweed, especially sporophyll of sea mustard.

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