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Studies on Antimutagenic and Cytotoxic Effects of Seaweeds Protein-Polysaccharides

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Abstract

Polysaccharide content in protein-polysaccharides (PPS) extracted from sea mustard, sea tangle and fusiform were 40.61, 38.42 and 52.80%, respectively. 5% of sea tangle PPS showed highest inhibitory activity on 4-nitroquinoline-1-oxide (4-NQO) against *Salmonella typhimurium* TA100 compared to the other seaweed PPS. 5% of sea mustard PPS showed highest inhibition ratio of 62% on 2-nitrofluorene (2-NF) against *Salmonella typhimurium* TA98. These PPS extracts showed cytotoxic activity against human colon cancer cell (SW-480), and showed mild cytotoxic activity on human stomach cancer cell (SNU-1) and human hepatic cancer cell (HepG 2).

Key words : seaweed, protein-polysaccharides, antimutagenicity, cytotoxicity

INTRODUCTION

Seaweed have been used for curing gout, eczema, curare, helminthiasis and gallstone as folk medicine (1). Major component having physiological activities in sea weed is known as polysaccharide. One of the seaweed polysaccharides, alginic acid have been studied with respect to inhibitory activity on aging and adult desease (2). It is reported that seaweed polysaccharides with low calorie have an eliminating effect of toxins and an inhibiting effect on heavy metal cumulation in human body (3,4). *Codium, Dictyopteris* and *Sargassum* species showed inhibition of the growth rate of transplanted carcinoma and sarcoma to mice, though they inhibited in different degrees (1). Some marine organisms have been proven to have anticancer compounds including contain polysaccharides (5-7). But the preliminary studies that can support the anticancer activity of seaweed polysaccharides have not been elucidated until now. There is a report that ether fraction of *Halocynthia roretzi* skin showed antimutagenic activity by screening unused marine resources in ames test (8). This paper reports the findings from the antimutagenicity and cytotoxicity studies of seaweed PPS against *Salmonella typhimurim* and human cancer cell lines.

MATERIALS AND METHODS

Materials

Sea tangle, sea mustard and fusiform were obtained at Haebyeon market, Pusan area.

Extraction of protein-polysaccharide fraction (PPS)

80 g of each seaweed were homogenized and boiled in 1 L distilled water for 10 hours for extracting PPS. After filtration, the residue was reextracted in 800 ml distilled water for 8 hours. The combined extracts were precipitated by adding equal volume of 95%-ethanol and being allowed to stand at 4°C for 14 hours. The precipitates were collected by centrifugation at 10,000×g for 30 min and dissolved in distilled water. After filtration, PPS were dialyzed with Spectra/Por Membrane(mwco : 12-14,000) at 4°C for 48 hours using distilled water (9,10).

Analysis of polysaccharide and monosaccharide

Polysaccharide contents were quantitatively determined by anthrone test using D-glucose as a standard (11). Monosaccharide component of PPS was analyzed by HPLC. Sample was treated according to Choi's method (12). 5 g sample was extracted three times with 80% ethanol 100 ml at waterbath of 75~80°C. After filtering (Watman No. 5A) lyophillic components, the extracted solution was removed by separating funnel with ether. This water fraction was centrifuged at 8,000 rpm for 15 min. and filtered 0.45 μm membrane filter, and then analyzed. Glucose, galactose, mannose, fructose and xylose standard were used. 25 μl sample was analyzed by HPLC system (Bio-LC DX-300, Dionex, USA), and mobile phase was 16 mM NaOH and flow rate 1.0 ml/min.

Determination of protein and amino acid

Protein contents were determined using bovine serum albumin (BSA, Sigma Chem. Co. USA) as a standard protein by Lowry-Folin method (13). Amino acid analysis of PPS was performed according to the hydrolysis method of Spackman et al. (14). Protein oxidation prior to hydrolysis was done for reducing the loss of cysteine, cystine and methione. Sample was oxidized with 5 ml of oxidation mixture (30% H_2O_2 0.5 ml, 88% formic acid 4.5 ml and 25 mg phenol were mixed at room temperature for 30 min. and then preserved at 0°C) at 0°C for 16 hours. 0.8 g sodium disulfite was added to oxidize sample, and then this sample (20 mg) was hydrolyzed in 5 ml of 6N-HCl at 110±5°C for 24 hours in a cap tube filled with nitrogen gas. After filtration, the hydrolysate was evaporated and dissolved in 10 ml of 0.2 N sodium cit-

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rated buffer (pH 2.2). Amino acids were analyzed with amino acid autoanalyzer (Hitachi, model 835, Japan).

Ames mutagenicity test

Mutagens/Carcinogens

4-Nitroquinoline (4-NQO) and 2-nitrofluorene (2-NF) were used as positive control. Those reagents were purchased from Sigma Chemical Co. (St. Louis, USA) and dissolved in dimethylsulfoxide (DMSO) or autoclaved distilled water.

Bacterial strains

Salmonella typhimurium TA98, TA100 strain, histidine requiring mutant, were kindly provided by Dr. B.N. Ames (Univ. of Califonia, Berkeley, CA, USA) and were maintained as described by Maron and Ames (15). The genotype of the tester strain was checked routinely for the histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and the presence of R factor.

S9 mix preparation

S9 fraction was prepared as follows according to Maron and Ames (15). Sprague-Dawley male rats were injected intraperitoneally with Aroclor 1254 dissolved in corn oil (500 mg/kg of body wt.). Five days after the injections, the rats were sacrificed, and the livers were removed and minced in 0.15 M KCl, and then homogenized, followed by centrifugation at 9000×g for 10 min. in a refrigerated centrifuge and the supernatant (S9 fraction) was stored in -80°C until used for mutagenic studies. S9 fraction was thawed immediately before being used for the preparation of S9 mix. Final concentration of S9 mix is as follows; 10% of S9 fraction, 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 0.1 M phosphate buffer.

Mutagenicity test

A modified plate incorporation test (16) in which 30 min liquid preincubation of the organism with the test compounds was employed. In the preincubation test, 0.5 ml phosphate buffer was distributed in sterile capped tubes in ice bath and then 0.1 ml of tester strain from overnight culture ($1\sim2\times10^9$ cells/ml), 0.1 ml of test compound was added. The tubes were vortexed for 3 seconds. The resulting entire mixture was overlaid on the minimal agar plate. The plate were incubated at 37°C for 48 hrs and then the revertant bacterial colonies on each plate were counted. Dose response tests on the mutagens on the tester strain were carried out to determine the regions of revealing mutagenicity induced by test compound. 0.1 ml of autoclaved distilled water was used in control plate.

The inhibition rate (%) was calculated as follows:

Inhibition rate(%)=[(a-b)/(a-c)]×100

a: revertant colony with mutagen only

b: revertant colony with mutagen and test compound

c: revertant colony without mutagen and test compound

Cytotoxicity test in cancer cell

MTT(3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide) is converted to formazan by mitochondrial dehydrogenase in living cell, and this conversion can be read on a scanning multiwell spectrophotometer (ELISA reader, Hyperion, Model ^{TM}4, Miami, FL, USA). Proper number of various cancer cells in 180 μl of 10% fetal bovine serum and RPMI media were incubated for 24 hrs at 37°C in 5% CO_2 incubator. After 20 μl seaweed PPS (25, 50, 100, 200 μg/well) were added in to the well, cells were incubated for 48 hrs. Produced formazan was dissolved in 150 μl DMSO and the absorbance at 540 nm was measured in ELISA reader.

RESULTS AND DISCUSSION

Composition of PPS fraction

Polysaccharide contents of PPS extracted from sea mustard, sea tangle and fusiform were 40.61, 38.42 and 52.80%, respectively (Table 1). High performance liquid chromatography of the water soluble revealed the presence of six mono saccharides, glucose, galactose, mannose, fructose, xylose, and fucose. Three seaweed contained galactose most with the highest content of 64.00% in sea tangle. These results are similar to that of another seaweed, *Sargassum thunbergii* (17), which also showed anticancer activity. Protein contents and amino acid composition of PPS were shown in Table

Table 1. Polysaccharide content and monosoccharide composition in PPS of seaweeds

	Sea mustand	Sea-tangle	Fusiform
Polysaccharide (%)	40.61	38.42	52.80
Monosaccharide (%)			
Glucose	21.78	11.74	11.33
Galactose	59.98	64.00	40.81
Mannose	1.01	1.46	13.22
Fructose	11.02	17.22	9.22
Xylose	4.92	3.92	23.90
Fucose	1.29	1.66	1.52

Table 2. Protein contents and amino acid composition in PPS of seaweeds

	Sea mustand	Sea-tangle	Fusiform
Protein (%)	2.96	2.94	6.02
Amino acid (mg%)			
ASP	14.38	12.11	14.00
THR	3.60	4.33	10.40
SER	6.75	4.46	8.90
GIU	16.87	15.00	13.00
PRO	1.82	-	-
GIY	13.02	8.71	7.29
AIA	7.21	10.78	8.20
CYS	6.13	11.04	12.01
VAL	9.13	11.74	8.47
MET	-	-	-
ILE	6.37	6.55	4.92
LEU	5.10	7.41	4.83
TYR	-	-	5.05
PHE	3.26	3.0	1.02
HIS	1.04	-	-
LYS	3.64	4.86	0.96
ARG	1.68	-	-

2. Fusiform showed highest protein content, 6.02%. These proteins of three seaweed consisted of about 15 kinds of amino acid. Sea mustard contained 16.87 mg% glutamic acid, 14.38 mg% aspartic acid, 13.02 mg% glycine. Sea tangle consisted of glutamic acid, aspartic acid, valine, cystine, alanine with 15.00, 12.11, 11.74, 11.04 and 10.78 mg%, respectively and fusiform also consisted of aspartic acid, glutamic acid, cystine, threonine with 14.00, 13.00, 12.01 and 10.40 mg%, respectively (Table 2). These results are almost similar to the amino acid composition of *Codium pugniformis* (18). From these results, it is suggested that PPS of sea mustard, sea tangle and fusiform was composed of six monosaccharides and fifteen amino acids at least.

Antimutagenicity of seaweed PPS

Before examining the antimutagenicity of seaweed PPS, we tested the mutagenicity of seaweed PPS. We tested the mutagenicity of seaweed PPS itself in S-9 mixed plate and plate without S-9 at the contcentration below 7.5 μg of test material. Mutagenicity of seaweed PPS itself was not observed (data not shown). 5% of sea tangle PPS showed highest inhibition ratio of 61.4% on 4-NQO against *Salmonella typhimurium* TA100. 7.5% of sea tangle PPS and fusiform PPS showed 60.6%, 54.7% respectively in this system (Fig. 1). In another system, 5% of sea mustard PPS showed highest inhibition ratio 62.8% on 2-NF against *Salmonella typhimurium* TA98 (Fig. 2). Another two seaweed PPS also showed highest inhibition ratio at 5% PPS, and the ratio was 60.6% for seatangle PPS and 59.6% for fusiform PPS. From these results, it is suggested that three seaweed PPS showed highest inhibition ratio at 5% concentration in TA98 system, and the inhibition ratio tended to decrease as the concentration used get higher than 5%.

Cytotoxic effect of seaweeds against cancer cell

Cytotoxicity of seaweed PPS on human stomach cancer cell (SNU-1), human colon cancer cell (SW-480) and human hepatic cancer cell (HepG 2) were shown in Fig. 3, 4 and 5. Inhibitory effects of seaweed PPS on SNU-1 increased with the concentration up to 200 ug/well. 200 ug of sea mustard, sea tangle and fusiform PPS each showed inhibition ratio of 57.6%, 39.6% and 33.0% respectively. Higher concentration than 200 ug/well showed decreased inhibition ratio (Fig. 3). It is considered that the highest inhibition ratio of sea mustard 57.6% was less than that of ethanol extract of *Artemisia iwayomogi* on gastric cancer cell (19). Inhibitory effects of seaweed PPS on SW-480 also increased according to the concentration up to 200 ug/well. 200 ug of sea mustard, sea tangle and fusiform PPS showed inhibition ratio of 60.0%, 52.24% and 39.27%, respectively (Fig. 4).

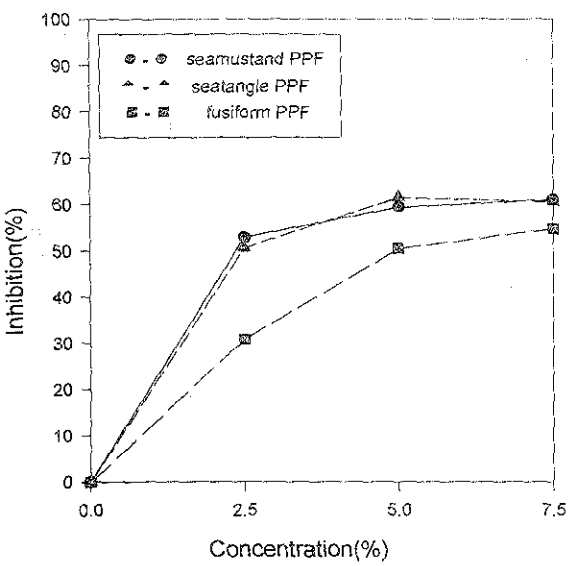

Fig. 1. Antimutagenic effects of seaweed PPS on 4-nitroquinoline (0.5 μg/plate) against *Salmonella typhimurium* TA100.

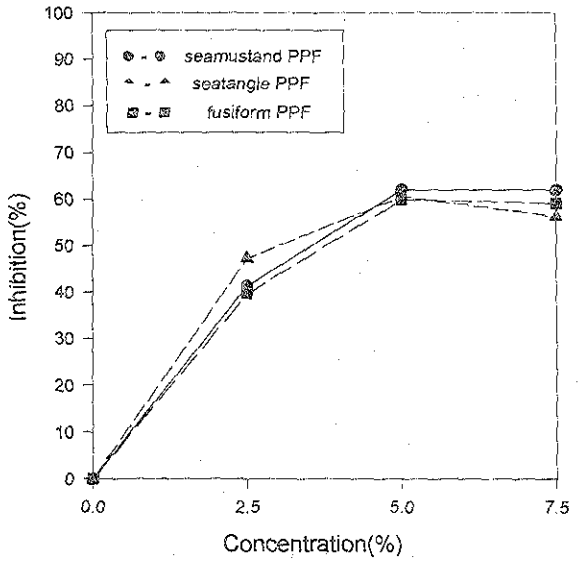

Fig. 2. Antimutagenic effects of seaweed PPS on 2-nitrofluorene (4 μg/plate) against *Salmonella typhimurium* TA98.

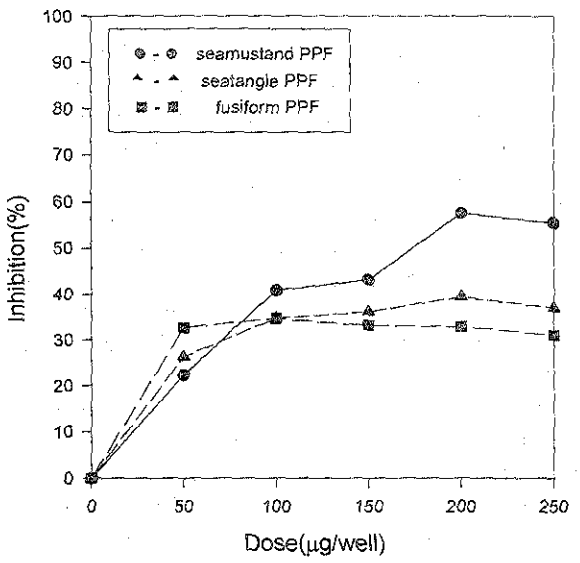

Fig. 3. Inhibitory effect of seaweed PPF during the growth of human stomac cancer cell (SNU-1).

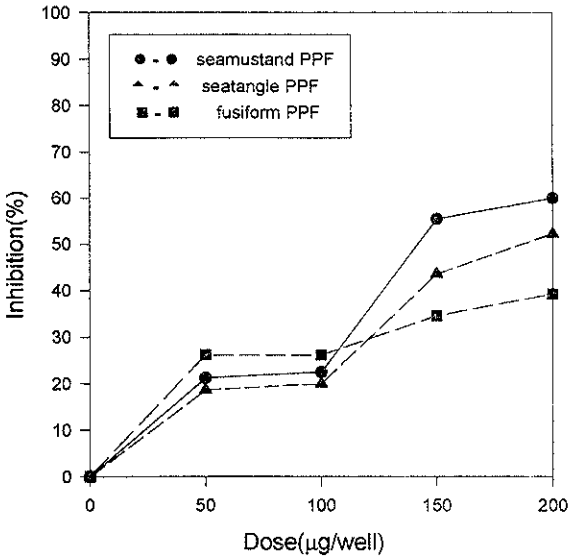

Fig. 4. Inhibitory effect of seaweed PPF during the growth of human colon cancer cell line (SW-480).

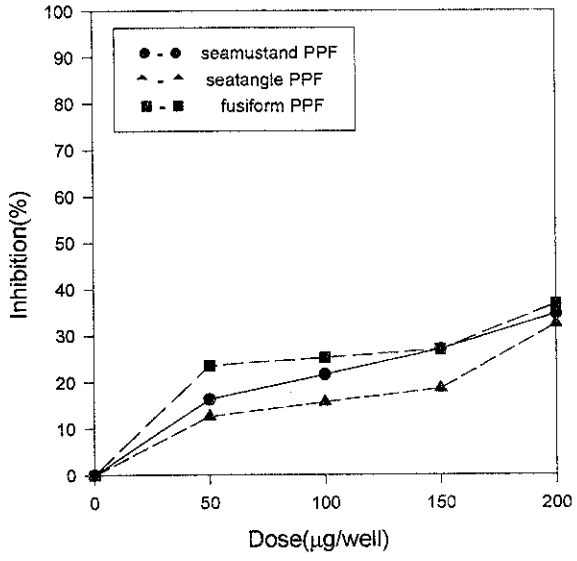

Fig. 5. Inhibitory effect of seaweed PPF during the growth of human hepatic cancer cell line (Hepg 2).

Inhibitory effecf of seaweed PPS on HepG2 also increased according to the concentration up to 200 ug/well. 200 ug of sea mustard, sea tangle and fusiform PPS showed inhibition ratio 36.7%, 34.6% and 32.42% respectively (Fig. 5). The inhibitory activity of the PPS on HepG2 were very week compared to their effect on SNU-1 and SW-480 cancer cell. Sea mustard showed highest inhibition ratio with 60.0% in colon cancer cell and the other two seaweed PPS also showed enhanced inhibition on colon cancer cell compared to the other cancer cells. From these results, it is suggested that seamustard PPS may be used as an inhibitor of human colon cancer cell growth. This inhibitory effect of sea mustard on colon cancer is not so strong as that of chloroform fraction of garlic which inhibited HT-29 colon carcinoma cells about 80% (20). Further study is needed by using more fractionated chromatography.

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