Dietary Effects of Porphyran from Porphyra yezoensis on Growth and Lipid Metabolism of Sprague-Dawley Rats

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Effects of porphyran (POR), a sulfated galactan from an edible red alga Porphyra yezoensis (Susabinori), on growth and lipid metabolism were examined using Sprague-Dawley rats fed a cholesterol-free diet. Rats were divided into four dietary groups: those fed diets containing 5% cellulose (control), agar or two types of POR differing in sugar composition and sulfate content (low sulfate content, LS-POR and high sulfate content, HS-POR) for 3 weeks. Ingestion of the diets containing LS- and HS-POR resulted in a significant decrease in food intake and body weight gain relative to the control diet. Renal adipose tissue weight and serum cholesterol level were also significantly lower in the LS-POR group and the HS-POR group than in the other groups. In contrast, agar, which consists of the same sugar components as POR, had no effect on the above-mentioned growth and lipid parameters. Fecal excretion of neutral sterols was markedly enhanced by POR ingestion, suggesting that POR has a potent effect to interfere with the absorption of neutral sterols within the gastrointestinal tract. Fecal excretion of fatty acids and neutral sterols was significantly higher in the LS-POR group than in the HS-POR group, and it was suggested that the ability to interfere with the absorption of cholesterol and fatty acid within the gastrointestinal tract depends on the sulfation rate of porphyran.

Keywords: porphyran, Porphyra yezoensis, agar, sulfate group, non-starch polysaccharide, hypolipidemic effect, cholesterol

Non-starch polysaccharide (NSP) has been consumed for centuries and has been recognized as having nutritional benefits. Many reports have demonstrated that ingestion of NSP lowers serum cholesterol and lipoprotein levels (Marlett, 1997; Anderson et al., 1994; Buhman et al., 1998), helps to normalize serum glucose and insulin levels (Haack et al., 1998) and promotes normal laxation (Cummings, 1993). Marine algae, among various foodstuffs, have the potential to serve as a good supplier of NSP.

Porphyran, which is considered an NSP, can be extracted with hot water from Porphyra sp (Peat et al., 1961), an edible seaweed cultivated abundantly in eastern Asia. Porphyran is distributed in the intercellular matrix of the algal body and its basic structure is closely related to that of agar, although it differs by having L-galactose-6-O-sulfate (Turvey & Rees, 1961; Rees, 1961). It is well known that the primary structure of this sulfated-polysaccharide is an alternating DL-galactan comprising β-(1→4)-linked units of 3,6-anhydro-α-L-galactopyranosyl-(1→3)-D-galactopyranose that is partially substituted by galactose-6-O-sulfate of the L units and 6-O-methyl-galactose of the D units (Anderson et al., 1965; Morrice et al., 1983), respectively.

In recent years, it has been reported that porphyran also has health benefits as other NSPs: e.g., prebiotic activity (Kawadu et al., 1995), antitumor activity (Yoshizawa et al., 1993, 1995), and antihyperlipidemic activity (Ren et al., 1994). The study in rats (Ren et al., 1994) suggested that dietary porphyran prevents hypercholesterolemia that is induced by feeding diet containing 1.5% cholesterol–0.5% bile salts. However, little is known about how dietary porphyran affects growth and lipid metabolism in the feeding of a normal diet.

This investigation was performed to examine the action of dietary porphyran on these two factors when a normal diet is fed. In spite of the knowledge that cultivating manner or harvesting time alter the sulfation rate of l-galactose of porphyran (Rees et al., 1962; Araki et al., 1977), the effect of such a structural difference on physiological action have not yet investigated. Therefore, we prepared two-types of porphyran which differ in sulfate content from Porphyra yezoensis (Susabinori), and compared the effects of these algal preparations and agar, which has less sulfates, with those of cellulose.

Materials and Methods

Materials Cellulose and agar were purchased from a commercial source (Sigma-Aldrich Corp., St. Louis, MO). Semi-purified porphyran with relatively low sulfate content (LS-POR) and one with relatively high sulfate content (HS-POR) were prepared according to the method of Hama et al. (1998) from two kinds of Porphyra yezoensis that differ in their harvesting time. Small pieces of dried algal sample (2.500 g)
were suspended in 100 ml of 85% ethanol, heated at 75°C for 1 h and filtered to remove 85% ethanol-soluble substances. This step was repeated three times. The residue was dried in vacuo to obtain 2,000 g of decolorized sample.

Two kg of decolorized sample was heated in 100 ml of water at 95°C with constant stirring for 1.5 h and the slurry was put through a nylon mesh to remove the residue. The hot-water extract was filtered to complete the removal of debris and concentrated to 10 ml using an ultrafiltration apparatus. Sodium acetate was added to the concentrate to a concentration of 0.3 M, and then ethanol was added to a concentration of 35% (v/v). After 1 h, the ethanolic suspension was filtered to remove the precipitate. Ethanol was added to the filtrate to a concentration of 60% (v/v) and the formed precipitate was collected by filtration, then washed with 75% ethanol to remove the excess salts, and dried in vacuo to obtain 350 g of semi-purified porphyran preparation.

Sugar and sulfate analyses Component sugars of agar and porphyran were quantified according to the method of Hama et al. (1999), briefly, polysaccharides were anhydrolyzed mercaptoylized, trimethylsilylated and analyzed by capillary GLC (Model 5890 Hewlett Packard, Avondale, PA) equipped with a capillary column (column, DB-1, 0.32 mm i.d. x 30 m, J&W Scientific; column oven, 180–240°C at 2°C/min; carrier gas, He). Two hundred fifty µg of polysaccharide was dissolved in 0.5 ml of 1 M HCl, hydrolyzed (100°C, 4 h) to liberate the ester sulfate, which was quantified using a capillary electrophoresis instrument (Model C1A, Waters Corp., Milford, MA).

Animals and diets Four weeks old male Sprague-Dawley (SD) rats (Seac Yoshitomi, Yoshitomi, Japan) were housed individually in stainless steel mesh cages with a controlled temperature of 20–23°C and light from 08:00 to 20:00, and given access to a commercial rat diet and deionized water for 7 days to allow adaptation to the environment. All experimental procedures were conducted in accordance with the Guidelines for Animal Experiments in the Faculty of Agriculture and Graduate Course of Kyushu University, and according to Law No. 105 and Notification No. 6 of the Japanese government.

Rats were fed a AIN-93G diet that contained 36.7% cornstarch, 20% casein, 13.2% α-cornstarch, 10% sucrose, 10% safflower oil, 3.5% mineral mixture, 1% vitamin mixture, 0.3% L-cystine, 0.25% choline bitartrate, 0.0014% tert-butylhydroquinone and 5% test material as a dietary fiber. The test materials were agar, LS-POR, HS-POR or cellulose (as a control diet). The rats were divided at random into four treatment groups of approximately equal weight (n=5) and fed experimental diets for 3 weeks and weighed every two days. All feces were collected during the experimental period, lyophilized and weighed. At the end of that period, the rats were killed by withdrawing blood from the abdominal aorta under diethyl ether anesthesia. Liver and other visceral organs of each rat were immediately excised and weighed.

Determination of serum lipid levels The levels of serum cholesterol, HDL cholesterol, triglyceride and phospholipid were enzymatically determined with commercial kits: Cholesterol Test, HDL-Cholesterol Test, TG-G Test and PL-B Test (all from Wako Pure Chemicals, Osaka, Japan).

Determination of fecal fatty acids and neutral sterols Lyophilized fecal samples over a 4-day period during day 10–13 were used to measure fatty acids and neutral sterols in feces. Pentadecanoic acid (50 µg) and 5β-cholanic acid (50 µg) was added to 100 mg of fecal sample (exactly weighed) as internal standard. Fatty acids and neutral sterols were repeatedly extracted with heptane–diethyl ether–95% ethanol by the method of Jeejeebhoy et al. (1970) and the combined solution was evaporated under N2, dissolved in 5 ml solvent. The extracted fatty acids and neutral sterols were simultaneously quantified by capillary GLC as their n-buty1 ester-trimethylsilyl ether derivatives according to the method of Batta et al. (2002); column, DB-1, 0.32 mm i.d. x 30 m, J&W Scientific; column oven, 150–310°C at 7°C/min; carrier gas, He.

Statistics The data were analyzed by Duncan’s new multiple-range test (1955) to determine the exact nature of the differences among the groups.

Results Porphyran preparation and sugar analyses As shown in Table 1, two kinds of porphyran preparations differing in sugar composition and sulfate content were obtained. The agar used in this study was similar to porphyran in the component sugars but different in its composition and with few sulfates.

Effect of algal galactan on the growth of rats Table 2 shows body weight gain, food intake, food efficiency and fecal dry weights of the animals from the four dietary groups during the experimental period. Body weight gain was significantly lower in the LS-POR group and the HS-POR group than in the cellulose (control) group or the agar group. Food intake was significantly higher in the agar group and significantly lower in both the LS- and the HS-POR groups than in the control group. Food efficiency was significantly lower in the LS-POR group than in the other groups. Also, fecal dry weights were significantly higher only in the LS-POR group, while the HS-POR group did not differ from the agar and the control groups.

Table 1. Sugar compositions and sulfate content of the algal polysaccharides.

<table>
<thead>
<tr>
<th>Component sugars (%) (w/v)</th>
<th>Sulfate content (%) (w/v)</th>
<th>Total (%) (w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal 6-O-M-Gal 3,6-AG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar 37.9</td>
<td>17.8</td>
<td>41.0</td>
</tr>
<tr>
<td>LS-POR 56.7</td>
<td>2.7</td>
<td>19.7</td>
</tr>
<tr>
<td>HS-POR 65.1</td>
<td>1.5</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Component sugars of algal polysaccharides were determined by GLC equipped with a fused-silica capillary column (column, DB-1, 0.32 mm i.d. x 30 m; column oven, 180–240°C at 2°C/min; carrier gas, He). Sulfate content was determined using a capillary electrophoresis instrument, Gal, galactose; 6-O-M-Gal, 6-O-methyl-galactose; 3,6-AG, 3,6-anhydrogalactose; LS-POR, low-sulfated porphyran; HS-POR, high-sulfated porphyran.

Table 2. Effects of algal polysaccharides on the growth parameters of rats.

<table>
<thead>
<tr>
<th>Weight gain (g)</th>
<th>Food intake (g/day)</th>
<th>Food efficiency (g gain/g intake)</th>
<th>Fecal dry weight (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cesullose 169±5</td>
<td>20.5±0.5</td>
<td>0.39±0.01</td>
<td>1.09±0.08</td>
</tr>
<tr>
<td>Agar 175±4</td>
<td>21.7±0.1*</td>
<td>0.38±0.01</td>
<td>0.99±0.06</td>
</tr>
<tr>
<td>LS-POR 141±2</td>
<td>19.2±0.2</td>
<td>0.35±0.01</td>
<td>1.29±0.1*</td>
</tr>
<tr>
<td>HS-POR 151±4</td>
<td>19.2±0.3</td>
<td>0.37±0.01</td>
<td>0.98±0.10</td>
</tr>
</tbody>
</table>

Each data value is mean±SE (n=5). Values without a common letter are significantly different (p<0.05).
diarrhea was observed during the experimental period among the four groups.

Table 3 shows relative weights (g/100 g body weight) of visceral organs and adipose tissues. Relatively lower weights of liver, kidney and spleen were observed in both POR groups than in the control groups, though the differences were not significant. Heart and lung weights were similar among the four groups. Renal adipose tissue weight was significantly lower in both POR groups than in the control group, but, in contrast, was similar in the agar group to the control group.

Effect of algal galactan on the lipid metabolism As shown in Table 4, the level of serum total cholesterol was significantly lower in both POR groups than in the control group and the agar group. The level of serum HDL cholesterol was also significantly lower in both POR groups than in the control group. In contrast, agar feeding did not have a significant effect on serum cholesterol levels compared with control feeding. The level of triglyceride tended to be lower in the LS-POR and the HS-POR feeding groups than in the control group, though the differences were not significant. The level of phospholipids was significantly lower in the LS-POR than in the control group. Agar feeding did not induce decreases of triglyceride and phospholipid levels, nor of cholesterol level.

**Fecal fatty acids and sterols excretion** Fecal excretion of saturated fatty acids, which include palmitic, stearic, eicosanoic and docosanoic acid, was highest in the LS-POR groups, followed by the HS-POR, the cellulose, and lowest in the agar groups (Table 5). The differences were significant between each dietary group. Fecal excretion of unsaturated fatty acids (oleic, lenoleic and lenolenic acid) was significantly higher in rats fed LS-POR than in those fed HS-POR, cellulose or agar. Fecal excretion of all the fatty acids quantified were significantly higher in LS-POR–fed rats, and significantly lower in agar-fed rats, than in control or HS-POR–fed rats. Fecal excretion of neutral sterols, which include cholesterol and coprostanol, was significantly higher in LS-POR and HS-POR-fed rats than in cellulose or agar-fed rats, although only a trace of coprostanol was detected in the feces of rats fed both these diets.

**Discussion** Although many studies showed that NSPs have nutritional benefits, little has been known about how porphyran affects growth and lipid metabolism when a normal diet is fed. We examined the effects of porphyran on these features using rats fed a cholesterol-free diet containing 5% of test materials. We also chose two-types of porphyran preparations that differ in sulfation rate and agar that had fewer sulfates, because the relation between sulfation rate and physiological actions had hardly been investigated despite the wide range of sulfation rate occurring naturally in porphyran (Rees et al., 1962; Araki et al., 1977). In this experiment, the diet containing 5% of porphyran was found to lower food intake and body weight gain whether

<table>
<thead>
<tr>
<th>Table 4. Effects of algal polysaccharides on the tissue weight of rats.</th>
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<tbody>
<tr>
<td></td>
<td>Liver (g/100 g weight)</td>
<td>Kidney (g/100 g weight)</td>
<td>Spleen (g/100 g weight)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>4.16±0.24a</td>
<td>0.34±0.01</td>
<td>0.88±0.04a</td>
</tr>
<tr>
<td>Agar</td>
<td>4.72±0.31a</td>
<td>0.35±0.00</td>
<td>0.96±0.05a</td>
</tr>
<tr>
<td>LS-POR</td>
<td>3.68±0.08a</td>
<td>0.36±0.01</td>
<td>0.80±0.02</td>
</tr>
<tr>
<td>HS-POR</td>
<td>4.03±0.35a</td>
<td>0.36±0.02</td>
<td>0.85±0.05a</td>
</tr>
</tbody>
</table>

*Each data value is mean±SE (n=5). Values without a common letter are significantly different (p<0.05).
it was highly sulfated or not. It was also found that dietary porphyran lowered serum cholesterol level and renal adipose weight, and tended to lower serum triglyceride and phospholipid levels. In contrast, agar had no effect on either growth parameters or serum lipid levels.

Historically, the hypocholesterolemic effect of NSP has been attributed to its ability to inhibit intestinal re-absorption of secreted neutral steroids or bile acids, resulting in greater fecal steroids excretion (Buhman et al., 1998; Trautwein et al., 1999; Gallaher et al., 2002). In this experiment, we evaluated the ability of algal galactans to inhibit cholesterol re-absorption by determining two major fecal neutral steroids, cholesterol and its intestinal bacterial derivative, coprostanol. The data that total fecal neutral steroid (cholesterol+coprostanol) excretion was enhanced by porphyran ingestion supported that porphyran has the ability to inhibit intestinal re-absorption of secreted steroids, at least neutral sterols, as well as other NSPs, psyllium or glucomannan. Also, the result of increased fecal fatty acid excretion by low-sulfated porphyran ingestion raised the possibility that the absorption of overall lipids within the gastrointestinal tract was interfered with coexistent porphyran. It is of interest that low-sulfated porphyran was found to accelerate the excretion of lipids more strongly than high-sulfated, suggesting that lower food efficiency observed in the ingestion of low-sulfated porphyran was partly attributable to the interference of lipid absorption.

Several investigators (Gallaher et al., 1993; Schneeman & Richter, 1993; Furda, 1990) hypothesized that viscous aqueous NSP causes an increase in the solution viscosity of the intestinal contents, which, in turn, serves to decrease the translational diffusion at the unstirred water boundary layer, or to interfere with the micelle formation in the intestine, and results in a decrease of overall lipid absorption. Both porphyran preparations form highly viscous hydrocolloids at a wide range of pH and ionic strength in vitro, therefore, it was expected that porphyran contributes to increasing the viscosity of intestinal content; however, the reason there is a significant difference in hypolipidemic behavior between the two porphyran preparations remains unclear.

Fecal coprostanol, which is the reductive derivative of cholesterol, is believed to be produced by cholesterol-reducing bacteria (Mott et al., 1980; Ren et al., 1996). In the case of porphyran ingestion, coprostanol was only found in only a trace amount, whether it was highly sulfated or not, suggesting that porphyran in the intestine strongly suppressed its formation by interfering with the cholesterol metabolism in intestinal bacteria or intestinal overall microflora. A similar tendency was observed previously in chitosan feeding (Fukada et al., 1991), however, to our knowledge, this is the first finding that showed porphyran affecting cholesterol metabolism within the gastrointestinal tract.

In conclusion, porphyran extracted from “Susabini” was shown to lower the renal adipose tissue weight and serum cholesterol levels, and to enhance the excretion of fecal cholesterol, while agar consisting of similar sugar components as porphyran had no effect. It was suggested that the ability to interfere with the absorption of cholesterol and fatty acid within the gastrointestinal tract depends on the sulfation rate of the polysaccharide. Porphyran may prove to have significant health benefits and to provide therapeutic effects.

References

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