Emulsifying Ability of Porphyran Prepared from Dried Nori, *Porphyra yezoensis*, a Red Alga

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A suspension of low-quality dried nori processed from *Porphyra yezoensis*, a red alga, was autoclaved at 120 °C for 30 min, and from the supernatant, five preparations of porphyran of differing molecular masses and chemical compositions were obtained by preprecipitation with ethanol at stepwise-increasing concentrations of 50 and 67% followed by size-exclusion chromatography. The porphyran preparations exhibited a high emulsifying activity index and high emulsion stability over a wide range of pH and temperature and also in the presence of sodium chloride. An adequately high coefficient of correlation between the median diameter of oil droplets and their 3,6-anhydrogalactose content suggests that 3,6-anhydrogalactose could take part in emulsification with porphyran.

Keywords: *Porphyran; emulsifying ability*

INTRODUCTION

Porphyra, a red alga, is abundantly cultivated in eastern Asia including Japan for food use. From *Porphyra yezoensis*, dried nori is obtained through a process that involves washing the raw laver roughly with water, cutting it coarsely, and drying it with an automatic nori-drying machine. Nori prepared in this manner is widely consumed as a familiar daily food in Japan. The dried nori usually contains ∼11−13% water, ∼29−36% proteinaceous components, ∼39−40% carbohydrates including 5−7% crude fiber, ∼0.6−0.7% lipids ∼8−11% ash, and some vitamins (for example, total ascorbic acid = 240 mg%) (Kayama et al., 1983; Mumford and Miura, 1988) and is therefore thought to be a low-energy food, rich in dietary fiber.

Porphyran originates from the cell wall and intercellular regions of the raw laver, *Porphyra*, and is known to be closely related to agarose in its basic structure, whereas it is very different in terms of having L-galactose-6-sulfate. The primary structure of porphyran shows alternating 1,4-linked 3,6-anhydro-L-galactose units and 1,3-linked β-D-galactose units, which sometimes occur as the L-galactose-6-sulfate and 6-*O*-methyl derivative, respectively (Mackie and Preston, 1974; Morrice et al., 1983). Recently, it has been reported that porphyran has some physiological functionality such as the effect of improvement of the microflora and environment of the cecum (Kawadu et al., 1995), antitumor activity (Noda et al., 1990), antihypertensive and antihyperlipidemic effects (Ren et al., 1994), and macrophage stimulation activity (Yoshizawa et al., 1993, 1995). However, because porphyran shows a relatively low viscosity and cannot form a gel due to the high amount of sulfate present as compared to agarose, which shows high viscosity and excellent gelling properties, porphyran has not received more general application.

Matsuo et al. (1993) found that saccharide-6-sulfate could be converted to 3,6-anhydrosaccharide by 6-O-desulfation with *N*,*O*-bis(trimethylsilyl)acetamide, resulting in gelation of porphyran. This gelation induced by desulfation means that the electrostatic repulsion among porphyran chains decreases with desulfation and that porphyran chains can then associate and bind to each other through noncovalent bonds such as by hydrophobic interaction or electrostatic interaction via hydroxyl groups. If porphyran chains can potentially interact through hydrophobic interaction, this may affect porphyran's amphiphilic properties, suggesting the possibility of its use as a new polysaccharide surfactant.

Kayama et al. (1983) reported that dried nori evaluated to be of low quality, which is cheap and of no practical value, tends to have a high carbohydrate content, suggesting a high porphyran content. In the present study, dried nori of low quality was thus used to prepare porphyran preparations, and their chemical features and unique emulsifying ability were investigated.

MATERIALS AND METHODS

Materials. Dried nori evaluated as being of low quality, which was obtained by processing raw laver, *Porphyra yezoensis*, a red alga cultivated in the Ariake Sea in Kyushu, Japan, in 1996, by washing it roughly with water, cutting it coarsely, and drying it with an automatic nori-drying machine, was used.

Preparation of Porphyran. Dried nori (80 g) was pulverized with a home-style mixer, immersed in 1600 mL of water, and autoclaved at 120 °C for 30 min. After filtration through a Tetoron cloth followed by centrifugation at 8000 rpm for 30 min, the supernatant was prefractionated with ethanol at stepwise-increasing concentrations of 50 and 67%, and the precipitate recovered after centrifugation at 8000 rpm for 30

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min at 4 °C was dissolved in distilled water. After dialysis against distilled water and lyophilization, the crude porphyran was obtained.

The crude porphyran was fractionated by size-exclusion chromatography (SEC) as follows. The crude porphyran (0.5 g/250 mL) was applied to a Toyopearl HW-65S column (104 i.d. × 600 mm, Tosoh, Tokyo, Japan) and eluted with 0.2 M phosphate buffer (pH 7.0) as the mobile phase at a flow rate of 10 mL/min, and 10 mL fractions were collected. Saccharides and proteinaceous components in each tube were detected by measuring the absorbance at 490 nm after treatment with phenol−sulfuric acid and at 280 nm, respectively. The tubes containing the saccharides were pooled into five fractions according to the fraction number. After dialysis against distilled water, five preparations of porphyran differing in molecular mass were recovered by lyophilization.

Evaluation of the Emulsifying Ability. The emulsifying ability was evaluated according to the method of Pearce and Kinsella (1978). Porphyran was dissolved in 0.1 M citric acid−disodium hydrogenphosphate buffer (pH 3.0, 7.0, or 8.0) at a final concentration of 0.1% as saccharide. 0.5 mL of corn oil was emulsified with 2 mL of this porphyran solution at 25 °C by means of a Polytron PTA-7 (Kinematica, Switzerland) for 1 min at 24000 rpm. Fifty microliters of the emulsion was taken from the bottom of a test tube and diluted to 2.5 mL with 0.1% sodium dodecyl sulfate (SDS) solution prior to measurement of the absorbance at 500 nm. The emulsifying activity was determined as the emulsifying activity index (EAI), which was calculated by means of the following formula: $EAI = 2T/\phi c$, where $T = 2.3A/l$ [A being the absorbance at 500 nm immediately after emulsification and l (light pass) $= 10^{-2}$ m] and c is the concentration of porphyran (1000 g/m^3), with ϕ (oil phase volume) = 2. The emulsion stability (ES) was determined as the absorbance 30 min after emulsification.

Measurement of Particle Size of Oil Droplets. The emulsions prepared as described above were diluted with 0.1% SDS solution until the absorbance at 680 nm reached 0.2. The particle size distribution in the diluted emulsions was evaluated using a Shimadzu SALD-2000J (Kyoto, Japan) laser diffraction particle size analyzer.

Measurement of the Molecular Mass of the Porphyran in Each Preparation. The molecular mass of the porphyran in each preparation was measured by SEC. Samples were applied to a TSK gel G4000 PW_{XL} column (7.8 i.d. × 300 mm, Tosoh, Tokyo, Japan) and eluted with 0.2 M phosphate buffer (pH 7.0) at a flow rate of 0.6 mL/min. The refractive index was monitored.

Analytical Methods. The total saccharide content, protein content, sulfate content, and anhydrosaccharide content were measured by using the phenol−sulfuric acid method (Dubois et al., 1956) with galactose as the standard, the Lowry method (Lowry et al., 1951) with bovine serum albumin as the standard, the rhodizonate method (Terho and Hartiala, 1971) with sulfuric acid as the standard, and the resorcinol method (Aresenault, 1971) with fructose as the standard, respectively.

RESULTS AND DISCUSSION

Chemical Features of the Porphyran Preparations. The crude porphyran prepared by ethanol precipitation at a concentration of 67% from the autoclaved extract of dried nori was obtained in a high yield (~11% based on the weight of the dried nori), and it showed a low protein content (~21%) as compared with the yield and protein content (~1 and ~31%, respectively) in the case of the 50% ethanol-precipitated fraction. SEC of the crude porphyran obtained by the 67% ethanol precipitation was thus performed, and five distinct porphyran preparations were obtained, fractions 1−5, numbered according to the order of elution from the column. The chemical features of these porphyran preparations are shown in Table 1. The molecular mass of the main component decreased from 660 to 6 kDa in fractions 1−5, showing that suitable fractionation according to molecular mass had been achieved. In particular, the homogeneity of fraction 3 was considered to be highest because of a relatively sharp SEC pattern (data not shown). Fractions 1 and 5 each had a protein content of 8.6−10.5%, whereas fractions 2−4 did not contain any protein. The sulfate content showed a tendency to increase with decreasing molecular mass except for fraction 5. However, the 3,6-anhydrogalactose content of each porphyran preparation was independent of molecular mass, and that of fractions 3 and 4 was relatively high. The 3,6-anhydrogalactose content based on the total saccharides was ~5−14%, similar to that of dried nori as reported by Araki et al. (1977).

Table 1. Chemical Compositions of Porphyran Preparations (Fractions 1−5)

fraction	total saccharide content[a] (%)	protein content[b] (%)	sulfate content[c] (%)	3,6-anhydro-galactose content[d] (%)	molecular mass[e] (kDa)
1	30.7	8.6	8.4	1.9	657
2	57.6		8.9	4.1	48
3	82.2		9.2	11.2	40
4	79.3		9.8	10.6	7
5	28.8	10.5	1.5	1.4	6
crude	62.9	21.1	17.3	13.5	

[a] Measured by using the phenol−sulfuric acid method (Dubois et al., 1956) with glucose as the standard. [b] Measured by using the Lowry method (Lowry et al., 1951) with bovine serum albumin as the standard. [c] Measured by using the rhodizonate method (Terho and Hartiala, 1971) with sulfuric acid as the standard. [d] Measured by the resorcinol reaction (Aresenault, 1971) with fructose as the standard. [e] Evaluated by SEC as a main component.

Emulsifying Ability of Porphyran. The emulsifying ability of the five porphyran preparations (fractions 1−5) was evaluated on the basis of the absorbance at 500 nm of their O/W emulsions at pH 7.0 at a concentration of 0.1% as saccharide. The results are shown in Figure 1. Because each porphyran preparation showed much higher absorbance than the control at each time point, it was evident that porphyran had good emulsifying ability. Fractions 1 and 5 each showed a very high EAI (129 and 111, respectively) as compared with those (77−98) of fractions 2−4. Because fractions 1 and 5 contained small amounts (8.6 and 10.5%, respectively) of proteinaceous components, not only porphyran but also proteinaceous components may have some effects on the initial emulsification. However, the absorbance of the emulsions prepared with fractions 1 and 5 rapidly decreased with time. The absorbance of the control emulsion had almost reached equilibrium within 30 min, so the absorbance 30 min after emulsification was taken to be an index of the emulsion stability (ES). The ES of the control emulsion was 0.05, whereas that of each of fractions 1−5 was in the rage of 0.14−0.31. In particular, fractions 3 and 4, lacking proteinaceous components, showed high ES (0.31 and 0.29, respectively). The porphyran itself is thus considered to exhibit the high ES.

The particle size distribution of the oil droplets in the emulsions prepared with the porphyran preparations was evaluated by means of a laser diffraction size distribution analyzer just after emulsification. Because each emulsion was a polydispersed system (particle size = 0.3−200 μm), the median, mode, and mean diameters of the oil droplets were taken as representative values (Table 2). In the emulsions prepared with fractions 1

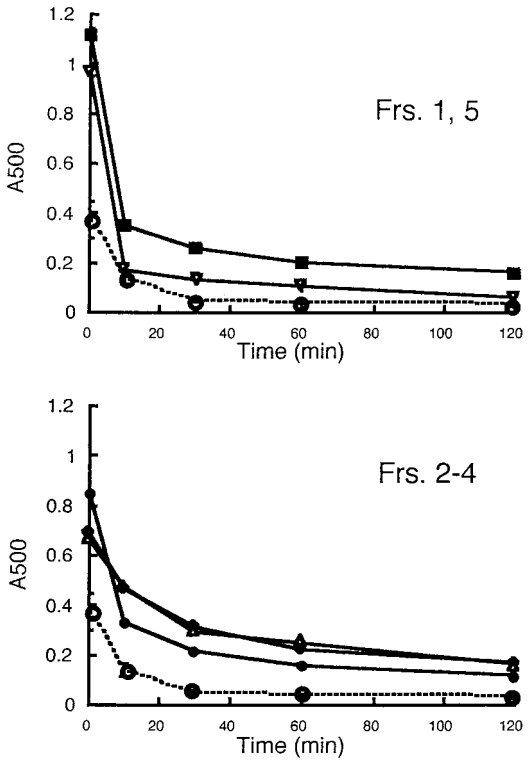

Figure 1. Emulsifying ability of porphyran preparations (fractions 1−5). O/W emulsions [corn oil/ 0.1% porphyran solution, 20:80 (v/v)] were prepared at pH 7.0 and held at 25 °C: ■, fraction 1; ●, fraction 2; ◆, fraction 3; △, fraction 4; ▽, fraction 5; ○, control (without porphyran). The emulsions were 50-fold-diluted with 0.1% SDS solution, and the emulsifying ability was evaluated by measuring the absorbance at 500 nm.

Table 2. Particle Size Distribution of Emulsions Prepared with Porphyran Preparations (Fractions 1−5)[a]

fraction	median diameter[b] (μm)	mode diameter[c] (μm)	mean diameter (μm)
1	33.25	37.88	27.60
2	23.64	46.32	16.63
3	11.28	56.63	11.44
4	11.54	46.32	11.22
5	29.48	30.97	27.57
control	52.43	56.64	39.66

[a] The particle size distribution of emulsions prepared with porphyran preparations was measured by means of a laser diffraction particle size analyzer after dilution with 0.1% SDS. [b] At 50% frequency. [c] At maximum frequency.

and 5, oil droplets with relatively large particle size predominated, whereas oil droplets with relatively small particle size predominated in those prepared with fractions 3 and 4. This suggests that an emulsion with small particle size could be prepared with a porphyran preparation having a suitable chain length or chemical composition such as fractions 3 and 4. Therefore, the correlation between the median diameter of the oil droplets in the emulsions and the chemical structure of the porphyran preparations was analyzed. Coefficients of correlation between the median diameter (y) and the molecular mass (x), sulfate content (x), and 3,6-anhydrogalactose content (x) of the porphyran preparations were 0.622 ($y = 0.022x + 18.44$), −0.539 ($y = -1.59x + 33.87$), and −0.979 ($y = -2.09x + 34.06$), respectively. In addition, high correlation coefficients [0.852 ($y = 1.75x + 33.42$) and −0.933 ($y = -1.62x + 28.36$), respectively] were obtained for the relationship between 3,6-anhydrogalactose contents (x) and the mode (y) or mean (y) diameter. The adequate correlation between the particle size of oil droplets and the 3,6-anhydrogalactose content suggests that 3,6-anhydrogalactose may contribute substantially to the emulsification with porphyran. Because 3,6-anhydrogalactose is considered to have high hydrophobicity as compared with galactose, galactose-6-sulfate, and 6-*O*-methyl galactose, 3,6-anhydrogalactose may interact with the surface of the oil droplets, resulting in orientation of the porphyran chain. However, this explanation should be proved in a separate study.

Table 3. Effect of pH on the EAI of Porphyran Preparations (Fractions 2−4)[a]

pH	fraction 2	fraction 3	fraction 4
3.0	61.5 ± 8.5	39.3 ± 2.0	30.3 ± 0.3
7.0	98.0 ± 0.6	79.5 ± 5.6	76.7 ± 1.0
8.0	140.7 ± 17.5	80.0 ± 3.0	84.5 ± 0.3

[a] O/W emulsions [corn oil/0.1% porphyran (fractions 2−4) soln, 20:80 (v/v)] were prepared at pH 3.0−8.0 and 25 °C. EAI was calculated from the absorbance at 500 nm of the emulsions after 50-fold dilution with 0.1% SDS. Data are shown as mean ± SD ($n = 3$).

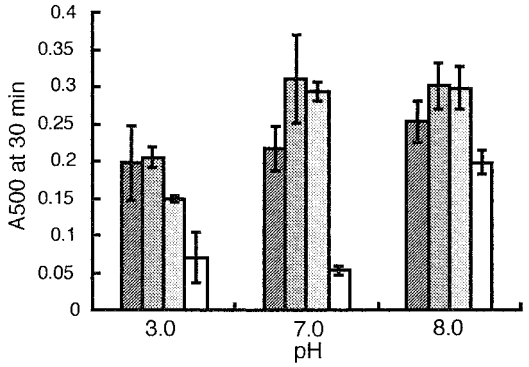

Figure 2. Effect of pH on the ES of porphyran preparations (fractions 2−4): slashed bar, fraction 2; heavily dotted bar, fraction 3; lightly dotted bar, fraction 4; white bar, control (without porphyran). O/W emulsions [corn oil/0.1% porphyran (fractions 2−4) soln, 20:80 (v/v)] were prepared at pH 3.0−8.0 and held at 25 °C. The ES was evaluated by measuring the absorbance at 500 nm 30 min after emulsification. Data are shown as mean ± SD ($n = 3$).

Effect of Environmental Factors on the Emulsifying Ability of Porphyran. The effects of several environmental factors on the emulsifying ability of porphyran were studied. The effect of pH on the emulsifying ability of porphyran was evaluated by measuring the absorbance of the emulsions prepared at pH 3.0, 7.0, and 8.0 with fractions 2−4 lacking proteinaceous components. The EAI and ES values obtained are shown in Table 3 and Figure 2, respectively. The emulsions prepared with fractions 2−4 exhibited high EAI and ES values at every pH and at every time point, indicating that the porphyran has good emulsifying ability over a wide pH range. However, because the EAI and ES values decreased by ∼40−60 and ∼10−50%, respectively, at pH 3.0 as compared with those at pH 7.0, the emulsifying ability of the porphyran is considered to decrease to some extent in the acidic region. At pH 8.0, the EAI and ES values showed a tendency to increase. The control emulsions have a similar tendency; therefore, it appears that the emulsifying ability of porphyran does not essentially change in the alkaline region.

The effect of temperature on the emulsifying ability of porphyran was evaluated by measuring the absor-

Table 4. Effect of Temperature on the EAI of Porphyran Preparations (Fractions 2−4)[a]

temp (°C)	fraction 2	fraction 3	fraction 4
4	73.8 ± 3.1	60.2 ± 2.2	64.1 ± 1.6
25	98.0 ± 0.6	79.5 ± 5.6	76.7 ± 1.0
60	76.7 ± 1.8	104.4 ± 9.4	60.9 ± 7.6

[a] O/W emulsions [corn oil/0.1% porphyran (fractions 2−4) soln, 20:80 (v/v)] were prepared at pH 7.0 at temperatures of 4−60 °C. EAI was calculated from the absorbance at 500 nm of the emulsions after 50-fold dilution with 0.1% SDS. Data are shown as mean ± SD (n = 3).

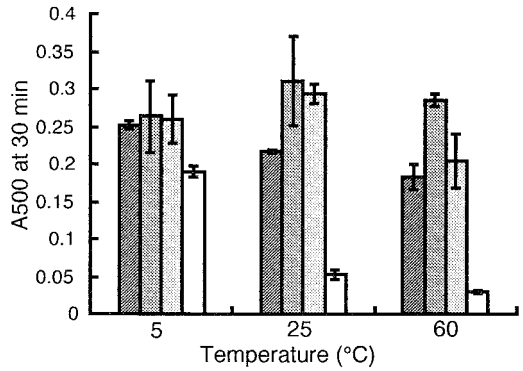

Figure 3. Effect of temperature on the ES of porphyran preparations (fractions 2−4): slashed bar, fraction 2; heavily dotted bar, fraction 3; lightly dotted bar, fraction 4; white bar, control (without porphyran). O/W emulsions [corn oil/0.1% porphyran (fractions 2−4) soln, 20:80 (v/v)] were prepared at pH 7.0 after porphyran solutions had been heated at 4, 25, or 60 °C for 10 min. The ES was evaluated by measuring the absorbance at 500 nm 30 min after emulsification. Data are shown as mean ± SD (n = 3).

Table 5. Effect of NaCl on the EAI of Porphyran Preparations (Fractions 2−4)[a]

NaCl (%)	fraction 2	fraction 3	fraction 4
0	98.0 ± 0.6	79.5 ± 5.6	76.7 ± 1.0
0.5	73.8 ± 3.1	80.0 ± 3.0	84.0 ± 0.5
1.0	76.7 ± 1.8	63.5 ± 0.2	92.7 ± 0.5

[a] O/W emulsions [corn oil/0.1% porphyran (fractions 2−4) soln, 20:80 (v/v)] were prepared at pH 7.0 and 25 °C including NaCl. EAI was calculated from the absorbance at 500 nm of the emulsions after 50-fold dilution with 0.1% SDS. Data are shown as mean ± SD (n = 3).

bance of emulsions prepared with fractions 2−4 in a similar manner after the solutions were heated at 4, 25, or 60 °C for 10 min. The results are shown in Table 4 and Figure 3. Each of the porphyran preparations showed no essential change in the EAI and ES values in the temperature range of 4−60 °C. In particular, emulsion prepared with fraction 3 showed high EAI and ES values even at 60 °C. Thus, the emulsifying ability of porphyran is considered to be independent of temperature.

The effect of sodium chloride on the emulsifying ability of porphyran was investigated for emulsions containing 0.5 and 1.0% NaCl at pH 7.0. As shown in Table 5 and Figure 4, high EAI and ES values were maintained even in the presence of 1.0% NaCl. A proteinaceous emulsification modifier such as β-lactoglobulin loses almost all emulsifying ability in the presence of 1% NaCl, whereas β-lactoglobulin conjugated with carboxymethyl dextran retains emulsifying ability, possibly due to the ion-exchange capacity of carboxyl groups on the saccharide chain (Hattori et al., 1994; Nagasawa et al., 1996a,b). Because porphyran is a polyanionic saccharide with sulfate groups, the observed salt tolerance is considered to be due to its ion-exchange capacity as described above.

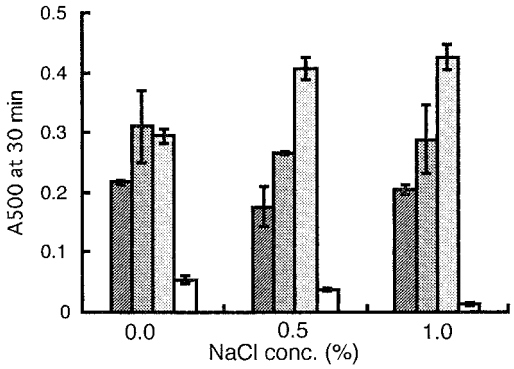

Figure 4. Effect of NaCl on the ES of porphyran preparations (fractions 2−4): slashed bar, fraction 2; heavily dotted bar, fraction 3; lightly dotted bar, fraction 4; white bar, control (without porphyran). O/W emulsions [corn oil/0.1% porphyran (fractions 2−4) soln, 20:80 (v/v)] were prepared at pH 7.0 including NaCl and held at 25 °C. The ES was evaluated by measuring the absorbance at 500 nm 30 min after emulsification. Data are shown as mean ± SD (n = 3).

Concluding Remarks. In this study, we prepared porphyran from low-quality dried nori, *P. yezoensis*, a red alga, by autoclaving the suspension, preprecipitation with ethanol, and fractionation by SEC. The porphyran-obtained preparations exhibited a high EAI and high ES over a wide range of pH and temperature and also in the presence of sodium chloride. This study will possibly contribute to the development of new polysaccharides useful as valuable emulsifying modifiers.

ABBREVIATIONS USED

EAI, emulsifying activity index; ES, emulsion stability; SEC, size exclusion chromatography; SDS, sodium dodecyl sulfate; O/W, oil in water.

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