Dried Green and Purple Lavers (Nori) Contain Substantial Amounts of Biologically Active Vitamin B_{12} but Less of Dietary Iodine Relative to Other Edible Seaweeds

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Vitamin B_{12} concentrations of dried green (*Enteromorpha* sp.) and purple (*Porphyra* sp.) lavers (nori) were determined by both *Lactobacillus leichmannii* ATCC 7830 microbiological and intrinsic factor chemiluminescence methods. The values determined by using the microbiological method (63.58 ± 2.90 and 32.26 ± 1.61 μg/100 g of dry weight) were identical to those found by using the chemiluminescence method (69.20 ± 2.21 and 25.07 ± 0.54 μg/100 g of dry weight) in both dried green and purple lavers, respectively. A silica gel 60 thin-layer chromatography of both laver extracts shows that non-coenzyme forms (hydroxo and cyano forms) of vitamin B_{12} predominate in both dried lavers. The dried lavers contained lesser amounts of dietary iodine (∼4−6 mg/100 g of dry weight) relative to other seaweeds, suggesting that excessive intake of the dried lavers is unlikely to result in harmful intake of dietary iodine. These results indicate that the dried lavers (nori) are the most excellent source of vitamin B_{12} among edible seaweeds, especially for strict vegetarians.

Keywords: *Vitamin B_{12}; dried edible seaweeds, green and purple lavers; nori; intrinsic factor; Lactobacillus leichmannii; dietary iodine; vegetarians*

INTRODUCTION

Various types of seaweeds (arame, carragheen, dulse, hijiki, kelp, laver, wakame, and so on) are available as food items. The seaweeds are known to be rich in vitamins and minerals as well as dietary fibers (Resources Council, 1984).

Most of the vitamin B_{12} found in seaweeds has been reported to be inactive B_{12} analogues so that they may not be bioavailable in mammals (Dagnelie et al., 1991; Herbert and Drivas, 1982; van den Berg et al., 1988; Yamada et al., 1996). Rauma et al. (1995), however, have demonstrated that some seaweeds can supply adequate amounts of bioavailable B_{12} in strict vegetarians. Dried lavers (nori) appear to be most widely eaten in the world among the seaweeds, and they have been reported to contain substantial amounts of B_{12}, which is assayed according to a *Lactobacillus leichmannii* ATCC 7830 microbiological method and/or a radioisotope dilution assay (RIDA) with hog intrinsic factor (IF) (van den Berg et al., 1988; Resources Council, 1995; Herbert, 1996). Thus, the bioavailability of the seaweed B_{12} in mammals is not well understood.

Because IF specifically recognizes the structure of the vitamin B_{12} molecule (Kolhous and Allen, 1977), it is used as the most specific B_{12}-binding protein in the fully automated B_{12} analyzer and/or RIDA kits. *L. leichmannii* ATCC 7830 used for the determination of B_{12} in foods cannot utilize cobinamide (α-ligand-free corrinoids), but the B_{12} analogues inactive for human as well as intact B_{12} and both deoxyribosides and deoxynucleotides may substitute B_{12} (Schneider, 1987). Indeed, in spirulina (*Spirulina* sp.) tablets (a health food fad) containing substantial amounts of inactive B_{12} analogues (Herbert and Drivas, 1982), the value determined by the *L. leichmannii* microbiological method has been shown to be 8-fold greater than the values obtained by the RIDA or IF chemiluminescence method (van den Berg et al., 1988; Watanabe et al., 1998). Except foods containing substantial amounts of inactive B_{12} and/or B_{12}-substitutive compounds, the observed correlation coefficient between the microbiological and IF chemiluminescence methods in foods is excellent ($r = 0.99$, $y = 1.2x - 1.1$) (Watanabe et al., 1998). Thus, in this paper, B_{12} concentrations of dried green and purple lavers (two types of nori) are determined by both *L. leichmannii* microbiological B_{12} assay and IF chemiluminescence methods. We also describe the characterization of B_{12} from the dried lavers and discuss the bioavailability of the laver B_{12} in mammals.

MATERIALS AND METHODS

Materials. Cyano-B_{12} (CN-B_{12}), hydroxo-B_{12} (OH-B_{12}), adenosyl-B_{12} (AdoB_{12}), and methyl-B_{12} (MeB_{12}) were obtained from Sigma (St. Louis, MO). Silica gel 60 thin-layer chromatography (TLC) aluminum sheets were obtained from Merck (Darmstadt, Germany). A B_{12} assay medium for *L. leichmannii* was obtained from Nissui (Tokyo, Japan). All other reagents used were of the highest purity commercially available. The dried green (*Enteromorpha* sp.) and purple (*Porphyra* sp.) lavers (nori) tested were purchased from a local market in Kochi-city, Japan.

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A Hitachi (Tokyo, Japan) spectrophotometer (U-1000) and a Shimadzu (Kyoto, Japan) UV−visible spectrophotometer (UV-1600) were used for measuring the turbidity of *L. leichmannii* test culture in the microbiological method and the absorbance of authentic B_{12} and it's analogues in the paper chromatography, respectively. A fully automated chemiluminescence B_{12} analyzer ACS 180 (Chiron Diagnostics, East Walpole, MA) was used for the B_{12} assay.

Extraction of Vitamin B_{12} in Dried Green and Purple Lavers. All procedures are done in the dark. Each (1.0 g) of the dried green and purple lavers was powdered by the use of a food mill (MX-X51, National, Osaka, Japan), added to 50 mL of 0.5 mol/L acetate buffer, pH 4.8, and homogenized with a dispersor (Polytron model PCU 11, Kinematica, Switzerland).

Total vitamin B_{12} was extracted from the homogenates by the method of boiling with KCN at acidic pH (Frenkel et al., 1980); specifically, 20 mg of KCN was added to the homogenates, which were boiled for 30 min at 98 °C in the dark. The extraction procedures were done in a Dalton (Tokyo, Japan) draft chamber. These homogenates were centrifuged at 10000*g* for 10 min. The supernatant was filtered through a filter paper (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and then used for the B_{12} assay.

Vitamin B_{12} analogues were extracted from both dried lavers by the 80% (v/v) ethanol method (Watanabe et al., 1991). Each laver powder (1.0 g), after addition of 50 mL of 80% (v/v) ethanol, was vigorously shaken, heated at 98 °C for 30 min, and then cooled in an ice bath. The solution was centrifuged at 10000*g* for 10 min. The supernatant was allowed to evaporate to dryness and dissolved in a small amount of distilled water. The solution was used as a sample for the TLC analysis.

Silica Gel 60 TLC. All procedures were done in the dark. The 80% (v/v) ethanol−B_{12} extracts of the dried green and purple lavers were spotted quantitatively (each 2000 pg) on the silica gel 60 TLC sheet and developed with 1-butanol/2-propanol/water (10:7:10) as a solvent in the dark at a room temperature. The TLC sheet was dried and cut into small pieces (5 × 10 mm) with scissors. B_{12} was extracted from the pieces with 1-butanol/2-propanol/water (10:7:10) containing 20 mg/L KCN several times, evaporated to dryness, dissolved in 2.0 mL of distilled water, and used as samples for the B_{12} assay. Authentic B_{12} analogues (OH-B_{12}, AdoB$_{12}$, CN-B_{12}, and MeB$_{12}$) extracted from the silica gel under the same conditions were determined with a Shimadzu UV−visible spectrophotometer (UV-1600) by measuring absorbance at 551 nm. The R_f values of authentic OH-B_{12}, AdoB$_{12}$, CN-B_{12}, and MeB$_{12}$ on this TLC system were 0.03, 0.20, 0.22, and 0.36, respectively.

Assay of Vitamin B_{12}. B_{12} was assayed according to the microbiological method with *L. leichmannii* ATCC 7830 and a B_{12} assay medium (Nissui, Tokyo, Japan) and by the fully automated chemiluminescence B_{12} analyzer ACS 180 (Chiron Diagnostics, East Walpole, MA) according to the manufacturer's instructions. The above B_{12} extracts were directly applied to the chemiluminescence B_{12} analyzer. They were diluted with distilled water up to a B_{12} concentration range of 0.01−0.2 μg/L and used as samples for the microbiological method. The turbidity (100 − %*T*) of the *L. leichmannii* test culture was measured at 600 nm with a Hitachi spectrophotometer (U-1000).

Assay of Dietary Iodine. The concentrations of dietary iodine were spectrophotometrically determined in the dried green and purple lavers according to the method of Houston (1950).

Statistics. Statistical analysis was performed using GraphPad PRISM 2.0 (GraphPad Software, San Diego, CA). Statistical significance was determined using Student's *t* test; $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Vitamin B_{12} concentrations were determined in the dried green and purple lavers by both *L. leichmannii* microbiological and IF chemiluminescence methods (Table 1). The values determined by using the microbiological method are identical to the values determined with the chemiluminescence method in the dried green (∼63.6−69.2 μg/100 g of dry weight) and purple (∼25.1−32.3 μg/100 g of dry weight) lavers. The result indicates that both dried lavers contain biologically active B_{12}, but not inactive B_{12} analogues and/or B_{12}-substituted compounds.

Table 1. Vitamin B_{12} Concentration of Dried Green and Purple Lavers

laver	vitamin B_{12} concn[a] (μg/100 g of dry wt)	
	chemiluminescence assay	microbiological assay
green	69.20 ± 2.21	63.58 ± 2.90
purple	25.07 ± 0.54	32.26 ± 1.61

[a] All values obtained represent mean ± SEM (n = 4). The detailed procedures were described in the text.

van den Berg et al. (1988) have reported that nori (*Porphyra* sp.) contains 12.0−68.8 μg/100 g of dry weight of vitamin B_{12}, which is determined by the RIDA. By the use of the *Lactobacillus* microbiological B_{12} assay method, the B_{12} contents of the dried green (31.8 μg/100 g of dry weight) and purple (83.6 μg/100 g of dry weight) lavers have been reported to be remarkably higher than those of other dried seaweeds (kelp, 0.1; hijiki, 0 or trace; wakame, 0.6 μg/100 g of dry weight) (Resources Council, 1995). These data and the results in Table 1 indicate that the dried green and purple lavers (nori) contain substantial amounts of biologically active B_{12}, like cattle liver (52.8 μg/100 g of edible portion) (Resources Council, 1995).

The dried green laver contained four known types of biologically active vitamin B_{12} analogues (approximately OH-B_{12}, 45%; CN-B_{12}, 35%; AdoB$_{12}$, 6%; and MeB$_{12}$, 0.2%; determined by the chemiluminescence method), and the non-coenzyme forms (OH and CN forms) of B_{12} predominated; the identical result was also obtained by using the microbiological B_{12} assay method (Figure 1A,B). Most B_{12} (∼80%) determined by both B_{12} assay methods in the dried purple laver was recovered in the OH-B_{12} fraction (Figure 1C,D). No B_{12} compounds were found in the fractions with R_f of 0.5−1.0 by either B_{12} assay method. Yamada et al. (1996) have reported that MeB$_{12}$ is predominantly found in a raw purple laver. Even if raw lavers contain substantial amounts of light-labile MeB$_{12}$, it would be converted to OH-B_{12} during the drying process (probably sunlight exposure).

Only two or three sheets of nori [one sheet (20.0 × 20.0 cm), ∼3 g] are sufficient to satisfy the demands of daily intake for adult human (2 μg/day) (National Research Council, 1989). Accurate bioavailability of vitamin B_{12} from the dried lavers should be evaluated in B_{12}-deficient mammals that significantly excrete methylmalonic acid in the urine as an index of B_{12} deficiency. van den Berg et al. (1991) have shown that B_{12} from nori (*Porphyra tenera*) is absorbed by B_{12}-depleted rats. Rauma et al. (1995) have reported that the concentration of B_{12} in the serum of a strict vegetarian subject consuming nori (5 g/day) is significantly higher than the mean value of the strict vegetarians group tested. Takenaka et al. (1997) have shown that when B_{12}-deficient rats that excrete substantial amounts of methylmalonic acid (71.7 ± 20.2 μmol/day) in the urine are fed a nori (*Porphyra yezoensis*) (10 g/kg diet) supplemented diet for 20 days, the urinary methylmalonic acid excretion becomes undetectable and the hepatic B_{12} level is significantly increased in the rats.

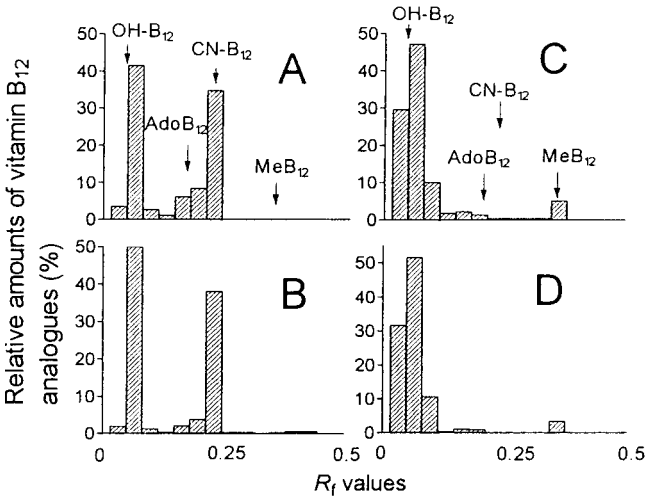

Figure 1. Silica gel TLC analysis of vitamin B_{12} analogues of dried green and purple lavers. The green laver B_{12} analogues were determined according to the chemiluminescence B_{12} assay (A) and microbiological B_{12} assay (B) methods. The purple laver B_{12} analogues were determined according to the chemiluminescence B_{12} assay (C) and microbiological B_{12} assay (D) methods. The detailed procedures are described in the text. The R_f values of authentic OH-B_{12}, AdoB_{12}, CN-B_{12}, and MeB_{12} on this TLC system were 0.03, 0.20, 0.22, and 0.36, respectively. Data present a typical migration pattern of vitamin B_{12} on the TLC from three experiments.

Table 2. Iodine Concentration of Dried Green and Purple Lavers

laver	iodine concn[a] (mg/100 g of dry wt)
green	6.35 ± 1.22
purple	4.28 ± 0.81

[a] All values obtained represent mean ± SEM (n = 4). The detailed procedures were described in the text.

These results indicate that B_{12} from the dried lavers (nori) is bioavailable in mammals.

The excessive feeding of seaweeds, however, has been reported to concomitantly lead to harmful amounts of dietary iodine (Rauma et al., 1995). The concentration of iodine was assayed in the dried green and purple lavers (Table 2), which contained 6.35 ± 1.22 and 4.28 ± 0.81 mg of iodine/100 g of dry weight, respectively. Katsura and Nakamichi (1959) have reported that kelp (kombu) contains substantial amounts of iodine (166 mg/100 g of dry weight), but wakame and nori do not (6−8 mg/100 g of dry weight). The consumption of a considerably larger amount (5 g/day) of the dried green and purple lavers (~0.2−0.3 mg of iodine/day) is sufficient to satisfy the demands of daily intake for an adult (0.15 mg of iodine/day) (National Research Council, 1989) but will not lead to the excessive intake of dietary iodine.

The results presented here indicate that the dried green and purple lavers (nori) are the most excellent source of vitamin B_{12} among edible seaweeds, especially for strict vegetarians.

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