Pseudovitamin B_{12} Is the Predominant Cobamide of an Algal Health Food, Spirulina Tablets

Fumio Watanabe,*,† Hiromi Katsura,† Shigeo Takenaka,‡ Tomoyuki Fujita,§ Katsuo Abe,† Yoshiyuki Tamura,‡ Toshiyuki Nakatsuka,⊥ and Yoshihisa Nakano§

Department of Health Science, Kochi Women's University, Kochi 780-8515, Japan; Laboratory of Nutrition and Food Science, Hagoromo-gakuen College, Sakai 592-8344, Japan; Department of Applied Biological Chemistry, Osaka Prefecture University, Sakai 599-8531, Japan; and Department of Food Science, Shimane Women's College, Matsue 690-0044, Japan

The vitamin B_{12} concentration of an algal health food, spirulina (*Spirulina* sp.) tablets, was determined by both *Lactobacillus leichmannii* ATCC 7830 microbiological and intrinsic factor chemiluminescence methods. The values determined with the microbiological method were ∼6−9-fold greater in the spirulina tablets than the values determined with the chemiluminescence method. Although most of the vitamin B_{12} determined with the microbiological method was derived from various vitamin B_{12} substitutive compounds and/or inactive vitamin B_{12} analogues, the spirulina contained a small amount of vitamin B_{12} active in the binding of the intrinsic factor. Two intrinsic factor active vitamin B_{12} analogues (major and minor) were purified from the spirulina tablets and partially characterized. The major (83%) and minor (17%) analogues were identified as pseudovitamin B_{12} and vitamin B_{12}, respectively, as judged from data of TLC, reversed-phase HPLC, ^{1}H NMR spectroscopy, ultraviolet−visible spectroscopy, and biological activity using *L. leichmannii* as a test organism and the binding of vitamin B_{12} to the intrinsic factor.

Keywords: *Vitamin B_{12}; pseudovitamin B_{12}; algal health food; spirulina tablet; intrinsic factor; Lactobacillus leichmannii*

INTRODUCTION

A health food fad involves tablets of *Spirulina* sp., blue-green algae. Many preclinical studies suggest that *Spirulina* cells have therapeutic properties such as hepatoprotective (Vadiraja et al., 1998), immunological (Yang et al., 1997), and antiviral (Hayashi et al., 1996) activities. Spirulina (*Spirulina* sp.) tablets also contain large amounts of vitamin B_{12} (B_{12} or CN-B_{12}) and can contribute to human B_{12} needs, especially for vegetarians. Herbert and Drivas (1982), however, have reported that most of the B_{12} compounds found in the spirulina tablets are biologically inactive B_{12} analogues, probably cobinamide (α-ligand-free B_{12})-like compounds. Several studies (Dagnelie et al., 1991; Herbert and Drivas, 1982; van den Berg et al., 1988) have shown that spirulina B_{12} may not be bioavailable in mammals. Dagnelie et al. (1991) have also reported that inclusion of spirulina in B_{12}-deficient children's diet results in a further deterioration of mean corpuscular volume despite an increase in B_{12} plasma level, suggesting that spirulina contains some compounds interfering with B_{12} metabolism and/or biologically inactive B_{12} analogues. Although an extract of spirulina tablets has been reported to contain B_{12} analogues that can block B_{12} metabolism (Herbert, 1988), there is no information available on details of the spirulina B_{12} analogues. If the spirulina B_{12} analouges are analyzed in details and then identified, their bioavailability and/or toxicity will be shown more clearly.

In the present paper, we subject three brands of the spirulina tablets to both *Lactobacillus leichmannii* ATCC 7830 microbiological B_{12} assay method and chemiluminescence B_{12} analyzer using hog intrinsic factor (IF), the most specific B_{12}-binding protein. We also describe the purification and characterization of two B_{12} analogues active in the binding of IF from the spirulina tablets and discuss the bioavailability of the analogues in mammals.

MATERIALS AND METHODS

Materials. Cyano-B_{12} (CN-B_{12}) and a reversed-phase high-pressure liquid chromatography (HPLC) column (Wakosil-II 5C18RS, ∅ 4.6 × 150 mm; particle size = 5 μm) were obtained from Wako Pure Chemical Industries (Osaka, Japan). A filter paper (type 50) for chromatography was obtained from Toyo Roshi (Tokyo, Japan). A B_{12} assay medium for *L. leichmannii* was obtained from Nissui (Tokyo, Japan). Silica gel 60 thin-layer chromatography (TLC) aluminum sheets were obtained from Merck (Darmstadt, Germany). Amberlite XAD-4 was obtained from Japan Organo Co. (Tokyo, Japan). Sephadex G-15 was purchased from Pharmacia-LKB Biotechnology (Uppsala, Sweden). Cosmosil 140C18-OPN was obtained from Nacalai Tesque Inc. (Kyoto, Japan). Cyanocobamides (5-hydroxybenzimidazolylcobamide, benzimidazolylcobamide, *p*-cresolylcobamide, and pseudovitamin B_{12}) isolated from bacteria were kindly provided by Dr. E. Stupperich, Ulm University, Germany. All other reagents used were of the highest purity commercially available. The spirulina tablets tested were purchased from a local market in Kochi-city, Japan.

* Author to whom correspondence should be addressed (telephone and fax +81-88-873-2483; e-mail watanabe@cc.kochi-wu.ac.jp).
† Kochi Women's University.
‡ Hagoromo-gakuen College.
§ Osaka Prefecture University.
⊥ Shimane Women's College.

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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 CN-B_{12} and its analogues in paper chromatography, respectively. A fully automated chemiluminescence B_{12} analyzer ACS 180 (Chiron Diagnostics, East Walpole, MA) was used for B_{12} assay.

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) according to the manufacturer's instructions. 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 (%T) of *L. leichmannii* test culture was measured at 600 nm with a Hitachi spectrophotometer (U-1000).

Extraction of Vitamin B_{12} in Spirulina Tablets. Each (1 g) of the spirulina tablets was powdered by the use of a food mill (MX-X51, National, Osaka, Japan) and added to 50 mL of 0.05 mol/L acetate buffer, pH 4.8. Total B_{12} was extracted from the cell suspension by boiling with KCN at acidic pH (Frenkel et al., 1980); specifically, 20 mg of KCN was added to the suspension, which was boiled for 30 min at 98 °C in a glass flask capped with aluminum foil under dark conditions. The extraction procedures were done in a Dalton (Tokyo, Japan) draft chamber. The extract was centrifuged at 10000*g* for 10 min. The supernatant was used for the B_{12} assay.

Paper Chromatography. The extract of the spirulina tablets was spotted quantitatively (4000 pg for the chemiluminescence method) on the filter paper (type 50) and developed with 1-butanol/2-propanol/water (10:7:10) as a solvent in the dark at room temperature. The filter paper was dried and cut into small pieces (5 mm) by scissors. B_{12} was extracted with 1 mL of distilled water from the pieces of the filter paper for 24 h at 4 °C in the dark and used as samples. Authentic CN-B_{12} (40 μg) was analyzed under the same conditions, extracted from the filter papers, and determined with a Shimadzu UV−visible spectrophotometer (UV-1600) by measuring absorbance at 361 nm.

Sephadex G-15 Gel Filtration. The extract of the spirulina tablet was put on a column (25 × 50 mm) of Amberlite XAD-4 equilibrated with 1% (v/v) acetic acid solution and washed with 200 mL of the same solution. The spirulina B_{12} analogues were eluted with 150 mL of 80% (v/v) ethanol solution, evaporated to dryness under reduced pressure, and dissolved in 2 mL of distilled water. This solution was put on a column (14 × 900 mm) of Sephadex G-15 equilibrated with 0.1 mol/L NaCl solution and eluted with the same solution. The column eluate was collected at 2 mL with a Bio-Rad Laboratories (Richmond, CA) fraction collector (Model 2110). B_{12} was assayed in the fractions by both microbiological and chemiluminescence methods. All procedures were done in the dark.

Purification of Vitamin B_{12} Analogues from Spirulina Tablets. Four hundred grams of the spirulina tablets was powdered by the use of the food mill and added to 4 L of 0.063 mol/L acetate buffer, pH 4.8. KCN was added to the cell suspension at the final concentration of 10 mmol/L. Total B_{12} was extracted for 30 min at 98 °C from the cell suspension in a loosely capped container. Amberlite XAD-4 resin (1 kg), which was washed with 10 L of methanol and equilibrated with distilled water, was added to the supernatant fraction of the boiled cell suspension centrifuged at 10000*g* for 10 min and stirred for 3 h at room temperature in the dark. The resin suspension was passed through a glass funnel with frittered disk (type 25G1, Iwaki, Tokyo, Japan), and the resin was washed with 5 L of distilled water. The washed resin was added to 2 L of an 80% (v/v) methanol solution and stirred for 3 h at room temperature in the dark. The resin suspension was passed through the glass funnel. The eluant containing B_{12} analogues was pooled, evaporated to dryness under reduced pressure, and dissolved in 30 mL of distilled water. The solution was put on a column (24 × 70 mm) of Cosmosil 140C18-OPN (Nacalai Tesque, Inc.), which was washed with 75% (v/v) ethanol solution and equilibrated with distilled water, and eluted with a linear gradient of 0−25% (v/v) ethanol. The B_{12}-active fractions were assayed by the chemiluminescence B_{12} analyzer, pooled, evaporated to dryness under reduced pressure, and dissolved with a small amount of distilled water. The solution was further twice purified with the Cosmosil column chromatography under the same conditions. The concentrated solution was purified by HPLC using a Shimadzu HPLC apparatus (LC-6A pump, SPD-6A spectrophotometer, CTO-6A column oven, C-R6A Chromatopac). The sample (100 μL) was put on a reversed-phase HPLC column (Wakosil-II 5C18RS, ∅ 4.6 × 150 mm; particle size = 5 μm) equilibrated with a 20% (v/v) methanol solution containing 1% (v/v) acetic acid at 35 °C. The flow rate was 1 mL/min. The spirulina B_{12} analogues were isocratically eluted with the same solution, monitored by measuring absorbance at 278 nm, and collected at 1 mL with a Bio-Rad Laboratories fraction collector (Model 2110). The B_{12}-active fractions were pooled, evaporated to dryness under reduced pressure, and dissolved in a small amount of distilled water. The concentrated solution was put on a silica gel 60 TLC sheet and developed with 1-butanol/2-propanol/water (10:7:10 v/v) as a solvent in the dark at room temperature. Two red spots were separated by the TLC, collected, extracted with the solvent, evaporated to dryness under reduced pressure, and dissolved in 50 μL of distilled water. Each concentrated solution was further purified with the TLC under the same conditions. Each final red spot was re-extracted with the solvent and dissolved in 20 μL of distilled water. Each concentrated solution was further purified with the reversed-phased HPLC under the same conditions. Each final red fraction was collected, evaporated to dryness under reduced pressure, dissolved in 0.5 mL of distilled water, and used as a purified spirulina B_{12} analogue. The B_{12} analogues were purified from total amounts of 2 kg of the spirulina tablets.

Analytical TLC and HPLC. The concentrated solutions (2 μL) of the purified spirulina B_{12} analogues, CN-B_{12}, and cyanocobamides (benzimidazolyl, 5-hydroxybenzimidazolyl, *p*-cresolyl cyanocobamides, and pseudovitamin B_{12}) were spotted on the silica gel 60 TLC sheets and developed with 1-butanol/2-propanol/water (10:7:10 v/v) and 2-propanol/NH_4OH (28%)/water (7:1:2 v/v) as solvents in the dark at room temperature. The TLC sheets were dried, and R_f values of the red spots of the corrinoids were determined.

In the case of HPLC, the concentrated solutions (2 μL) of the purified spirulina B_{12} analogues, CN-B_{12}, and these cyanocobamides were analyzed with the reversed-phase HPLC column (Wakosil-II 5C18RS, ∅ 4.6 × 150 mm; particle size = 5 μm). The corrinoids were eluted with a 20% (v/v) methanol solution containing 1% (v/v) acetic acid and with a linear gradient of 5−70% (v/v) methanol in 1% (v/v) acetic acid solution at 35 °C and monitored by measuring the absorbance at 278 nm. The flow rate was 1 mL/min.

Ultraviolet−Visible Spectra. The spectra were measured with a Shimadzu spectrophotometer (UV-1600) at room temperature. Quartz cuvettes (d = 1 cm) were used. The purified spirulina B_{12} analogues were dissolved in 1 mL of CH_3OH. The ultraviolet−visible spectrum of the purified compound **I** (28.4 μmol/L) had λ_{max} (absorbance) at 545.5 (0.236), 517.5 (0.234), 409 (0.127), 360 (0.789), 321 (0.282), and 277 (0.521) nm. That of the purified compound **II** (5.2 μmol/L) had λ_{max} (absorbance) at 549.5 (0.045), 520 (0.04), 361 (0.153), 322 (0.059), and 278.5 (0.100) nm.

^{1}H NMR Spectra. ^{1}H NMR spectra were measured with a JEOL JNM α-500 spectrometer in D_2O. Chemical shifts were given on as δ (ppm) with 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP) as an internal standard. ^{1}H NMR spectral data of the compound **I**: δ 8.13 (1H, s), 7.20 (1H, s), 6.53 (1H, J = 3.7 Hz), 6.08 (1H, s), 2.56 (3H, s), 2.44 (3H, s), 1.79 (3H, s), 1.50 (3H, s), 1.41 (3H, s), 1.37 (3H, s), 1.25 (3H, J = 6.4 Hz), 1.18 (3H, s), 0.45 (3H, s). ^{1}H NMR spectral data of compound **II**: δ 7.28 (1H, s), 7.09 (1H, s), 6.51 (1H, s), 6.35 (1H, d, J = 3.5 Hz), 6.09 (1H, s), 2.57 (3H, s), 2.54 (3H, s),

Table 1. Vitamin B_{12} Concentration of Spirulina Tablets

	vitamin B_{12} concn[a] (μg/100 g of dry wt)		
	claim on bottle[b]	microbiological assay	chemiluminescence assay
A	233	244.3 ± 3.3	8.3 ± 0.1
B	100−250	127.2 ± 6.6	6.2 ± 0.1
C	none	147.5 ± 19.3	17.4 ± 0.2

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

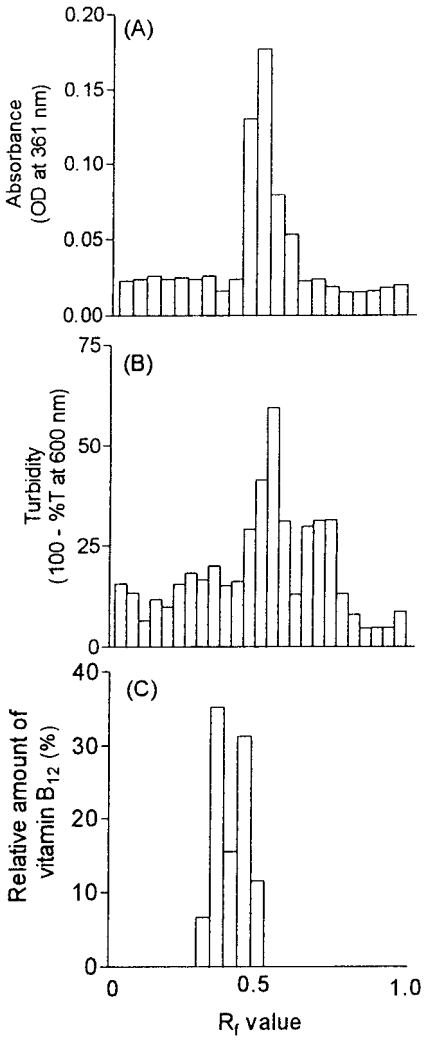


Figure 1. Paper chromatographic analysis of vitamin B_{12} of spirulina tablets. Migration patterns of authentic CN-B_{12} (A) and spirulina B_{12} were determined according to microbiological (B) and chemiluminescence methods (C). Data present a typical migration pattern of B_{12} on paper chromatography from three experiments.

2.26 (6H, s), 1.87 (3H, s), 1.45 (3H, s), 1.40 (3H, s), 1.39 (3H, s), 1.25 (3H, d, J = 6.1 Hz), 1.20 (3H, s), 0.45 (3H, s).

RESULTS

Vitamin B_{12} Contents Determined with the Microbiological Method and IF Chemiluminescence B_{12} Analyzer in Spirulina Tablets. Table 1 shows the B_{12} contents determined with the two methods in the algal tablets. The values determined by using the microbiological method are significantly different from those determined by using the chemiluminescence method in three spirulina tablets (A−C). Remarkably,

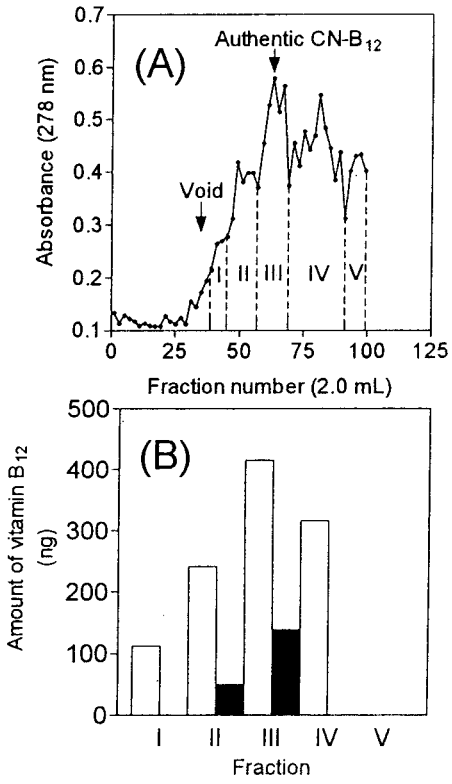


Figure 2. Elution profile of spirulina vitamin B_{12} during Sephadex G-15 gel filtration: (A) absorbance peaks at 278 nm during Sephadex G-15 of spirulina B_{12} partially purified by Amberlite XAD-4; (B) amount of spirulina B_{12} determined by using the microbiological (□) and chemiluminescence methods (■) in the fractions I−V. Data present a typical elution pattern of B_{12} on the column chromatography from three experiments.

in all spirulina tablets, B_{12} contents determined with the microbiological method were ~6−9-fold greater than the values determined with the chemiluminescence method.

Paper and Sephadex G-15 Chromatography of a Vitamin B_{12} Extract of the Spirulina Tablets. To clarify why there are such differences between the values determined with the two methods in the spirulina tablets, an extract of the spirulina tablets was analyzed by paper chromatography. Authentic CN-B_{12} gave a single red spot with an R_f of 0.5 (Figure 1). The spirulina B_{12} was separated as about three peaks with R_f values of 0.33 (17.4%), 0.53 (52.3%), and 0.75 (30.3%) with the microbiological method but as two peaks of R_f values of 0.35 (31.2%) and 0.53 (35.2%) with the chemiluminescence method. Only a few percentage points of the sum of B_{12} determined with the microbiological method was recovered in the B_{12}-active fractions with R_f values of 0.35 and 0.53 by using the chemiluminescence method. R_f values of authentic cyanocobamides, pseudo-B_{12} and 5-hydroxybenzimidazolyl, benzimidazolyl, and *p*-cresolyl cyanocobamides, were 0.34, 0.40, 0.42, and 0.68, respectively.

After B_{12} was partially purified from the extract of the spirulina tablets by an Amberlite XAD-4 column chromatography, the partially purified B_{12} was analyzed with Sephadex G-15 column chromatography (Figure 2). B_{12} was detected in fraction III (authentic CN-B_{12} fraction) with the chemiluminescence method but in fractions I−IV with the microbiological method. These results indicate that in the spirulina tablets, most of the B_{12} determined by using the microbiological method

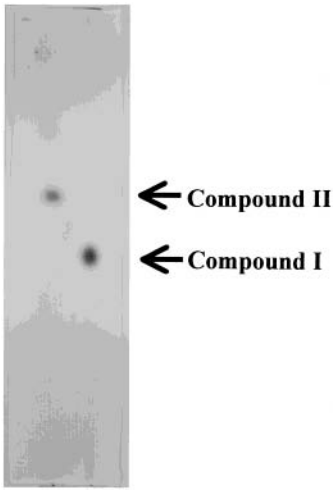


Figure 3. Silica gel 60 TLC pattern of the purified spirulina B_{12} analogues. Each final purified preparation (2 μL) was spotted on the silica gel 60 TLC sheet and developed with 2-propanol/NH_4OH (28%)/water (7: 1: 2 v/v) at room temperature in the dark. Data present a typical migration pattern of the purified B_{12} analogues on the TLC from three experiments.

was derived from various B_{12} substitutive compounds and/or B_{12} analogues inactive for the binding of IF.

Purification of Vitamin B_{12} Analogues Active in the Binding of IF from the Spirulina Tablets. The IF-active B_{12} analogues were purified from the extract of the spirulina tablets. The B_{12} analogues were separated as two (major and minor) red spots by the preparative TLC. Analytical silica gel 60 TLC and C_{18} reversed-phase HPLC for the final preparation of the major compound **I** and minor compound **II** gave a single red spot with R_f values of 0.42 and 0.56, respectively (Figure 3), and a single peak with a retention times of 12.8 and 15.6 min, respectively. These results indicate that the B_{12} analogues, compounds **I** and **II**, are purified to homogeneity.

Characterization of the Purified Spirulina Vitamin B_{12} Analogues. The ultraviolet−visible spectrum of each purified compound **I** and **II** showed a typical absorption spectrum of cobalt-containing corrinoids. The purified spirulina B_{12} analogues, authentic CN-B_{12}, and cyanocobamides (pseudo-B_{12} and 5-hydroxybenzimidazolyl, benzimidazolyl, and *p*-cresolyl cyanocobamides), which occur in bacteria, were analyzed by using analytical silica gel 60 TLC and C_{18} reversed-phase HPLC (Table 2). The R_f values (0.14 and 0.42 in solvents I and II, respectively, by TLC) of compound **I** were identical to the values of pseudo-B_{12}, of which the retention times (12.8 and 18.4 min with the isocratic and gradient elution, respectively, by HPLC) were also identical to those of compound **I**. The R_f values (0.23 and 0.56) of compound **II** were identical to the values of authentic CN-B_{12}, of which the retention times (15.6 and 19.0 min) were also identical to those of compound **II**.

In the ^{1}H NMR spectrum of compound **I**, two protons (δ 8.13 and 7.20) due to adenyl moiety, one anomeric proton [δ 6.53 (d, J = 3.7 Hz)] of ribose, and an olefinic proton (δ 6.08, H-10) were observed in the low-field region. These data suggest the presence of the adenyl and ribosyl moieties in compound **I**. Eight singlet methyl signals at δ 0.45, 1.18, 1.37, 1.41, 1.50, 1.79, 2.44, and 2.56 derived from the corrin skeleton and one doublet methyl signal at δ 1.25 (d, J = 6.4 Hz) on the propyl moiety were also observed in the high-field region. These spectral data were identified with those of pseudo-B_{12} cited in the literature (Stupperich and Kräutler, 1988). Therefore, compound **I** was identified as pseudo-B_{12}. On the other hand, compound **II** was identified as B_{12}, as the ^{1}H NMR spectrum of compound **II** completely agreed with that of authentic CN-B_{12}.

L. leichmannii ATCC 7830 could utilize both pseudo-B_{12} and compound **I** as well as authentic CN-B_{12} (Table 3). Although both pseudo-B_{12} and compound **I** showed only ~32−36% of the activity of authentic CN-B_{12} in the IF chemiluminescence assay method, the relative amount of compound **II** was identical to that of CN-B_{12} in both B_{12} assay methods. These results indicate that the purified spirulina B_{12} analogues, compounds **I** and **II**, are pseudo-B_{12} and B_{12}, respectively.

DISCUSSION

B_{12} contents of foods have been determined by bioassay with B_{12}-requiring microorganisms; *L. leichmannii* ATCC7830 has been used widely. Herbert and Drivas (1982) have reported that in some spirulina tablets >80% of the B_{12} determined with the microbiological method is B_{12} analogues (cobinamide-like compounds), which cannot be assayed by a radioisotope dilution assay method with hog IF. The B_{12} contents determined by using the *L. leichmannii* microbiological method have been also shown to be 8-fold greater than the values determined with the radioisotope dilution assay method (van den Berg et al., 1988) or the chemiluminescence method (Watanabe et al., 1998). Our previous study (Watanabe et al., 1998) has indicated that except for foods containing substantial amounts of inactive B_{12} and/or B_{12} substitutive compounds, the observed correlation coefficient between the microbiological and chemiluminescence methods in foods is excellent. These observations support our results in Table 1.

It has been reported that *L. leichmannii* ATCC 7830 can utilize the B_{12} analogues inactive for human as well as B_{12}, but not cobinamide, and that both deoxyribosides and deoxynucleotides may substitute B_{12} (Schneider, 1987). Both the findings reported by Herbert and Drivas

Table 2. R_f Values and Retention Times of the Purified Spirulina B_{12} Analogues, CN-B_{12}, and Cyanocobamides on TLC and HPLC

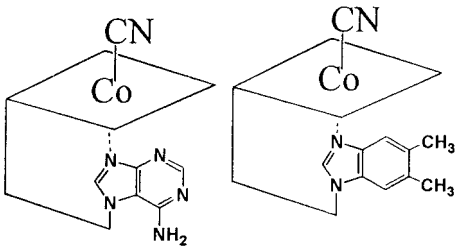
	spirulina compound **I**	spirulina compound **II**	B_{12}	benzimidazolyl cobamide	5-hydroxybenzimidazolyl cobamide	pseudovitamin B_{12}	*p*-cresolyl cobamide
silica gel 60 TLC[a]							
solvent I	0.14	0.23	0.23	0.18	0.20	0.14	0.38
solvent II	0.42	0.56	0.56	0.52	0.47	0.42	0.62
C_{18} revered-phase HPLC[b]							
isocratic	12.8	15.6	15.6	12.2	11.8	12.8	>30
gradient	18.4	19.0	19.0	18.3	18.2	18.4	26.1

[a] Solvent I, 1-butanol/2-propanol/water (10:7:10); solvent II, 2-propanol/NH_4OH (28%)/water (7: 1: 2). [b] Isocratic, 20% (v/v) methanol solution containing 1% (v/v) acetic acid; gradient, a linear gradient of methanol (5−70%, v/v) in 1% (v/v) acetic acid solution.

Table 3. Biological Activities of the Purified Spirulina B_{12} Analogues in the B_{12}-Requiring Microorganism and Mammalian B_{12}-Binding Protein

	relative B_{12} activity (% against the activity of CN-B_{12})			
	CN-B_{12}	pseudo-B_{12}	spirulina compound **I**	spirulina compound **II**
L. leichmannii	100	96.1	98.1	98.2
intrinsic factor	100	36.1	32.1	98.5

[a] The B_{12} concentrations of the purified spirulina B_{12} analogues, CN-B_{12}, and pseudo-B_{12} (identical amount; 10 ng/mL) were assayed by both *L. leichmannii* bioassay and IF chemiluminescence methods. The experiment was performed in triplicate. The relative B_{12} activity was defined as relative amount (percent) of the purified spirulina B_{12} analogues and pseudo-B_{12} against that of CN-B_{12} determined with both methods.



Compound I **Compound II**

Figure 4. Structures of the purified compounds **I** and **II** from spirulina tablets.

(1982) and our results in Figures 1 and 2 indicate that the spirulina tablets contain substantial amounts of various B_{12} substitutive compounds and/or inactive B_{12} analogues (both cobinamide-like and/or -dislike compounds) and a small amount of B_{12} analogues active in the binding to IF. The two IF-active B_{12} analogues [major compound **I** (~83%) and minor compound **II** (~17%)] were isolated from spirulina tablets and identified as pseudo-B_{12} and B_{12}, respectively (Figure 4), indicating that pseudo-B_{12} is the predominate cobamide in spirulina tablets.

Various anaerobic bacteria can synthesize substantial amounts (600−670 nmol/g of dry cells) of pseudo-B_{12} de novo (Stupperich et al., 1990). Some green algae can take up and then accumulate exogenous B_{12}, which is not essential for cell growth (Watanabe et al., 1991, 1997). It is not, however, clear whether *Spirulina* cells have the ability to synthesize pseudo-B_{12} de novo or to take up the exogenous one (or both).

Several studies have shown that spirulina B_{12} may not be bioavailable in mammals (Dagnelie et al., 1991; Herbert and Drivas, 1982; van den Berg et al., 1988). IF involved in the intestinal absorption of B_{12} (Kolhouse and Allen, 1977) strictly recognizes the structure of the B_{12} molecule. Pseudo-B_{12} has been reported to reveal moderate affinity to IF among various B_{12} analogues (Stupperich and Nexø, 1991). Intestinal absorption and ileal content of pseudo-B_{12} 24 h after oral adiministration of radiolabeled pseudo-B_{12} to rabbits have been shown to be ~13−21% of those of authentic B_{12} (Kolhouse and Allen, 1977). An intravenous injection of transcobalamin II−pseudo-B_{12} complex has shown that plasma clearance and tissue distribution of pseudo-B_{12} are very similar to those of authentic B_{12} but that urinary corrinoid excretion is slightly greater in the pseudo-B_{12}-injected rabbits than in the authentic B_{12}-injected rabbits (Kolhouse and Allen, 1977). Although the adenosyl coenzyme form of pseudo-B_{12} has a 1000-fold higher K_m for adenosyl-B_{12}-depedent mammalian methylmalonyl-CoA mutase than does adenosyl-B_{12} (Lengyet et al., 1960), pseudo-B_{12} can be fully active in human methyl-B_{12}-dependent apomethionine synthase under the experimental conditions used by Kolhouse et al. (1991). These observations suggest that pseudo-B_{12} does not have the ability to act as a B_{12} antagonist in mammals. Herbert (1988) has reported that an extract of spirulina contains two B_{12} analogues which can block B_{12} metabolism. We could not find another B_{12}-like compound capable of binding to IF in the extract of spirulina tablets. The B_{12} analogues inactive for the binding of IF would be hardly absorbed in mammalian intestine by the IF-mediated system. van den Berg et al. (1991) have demonstrated that a spirulina-supplemented diet does not induce in rats a severe B_{12} deficiency, implying that feeding of spirulina may not interfere with B_{12} metabolism. To clarify the B_{12} analogues and/or other compounds that have the ability to block B_{12} metabolism, further biochemical studies are needed.

The results presented here strongly suggest that spirulina tablet algal health food is not suitable for use as a B_{12} source, especially in vegetarians.

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