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AVAILABILITY OF IRON TO RATS FROM SPIRULINA, A BLUE-GREEN ALGA

Phyllis E. Johnson, Ph.D.[1] and L. Elliot Shubert, Ph.D.[2]

[1]United States Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota. [2]Department of Biology, University of North Dakota, Grand Forks, North Dakota

ABSTRACT

Both cultured Spirulina platensis (Nordst.) Geitl, a blue-green alga, and commercially available dried Spirulina contained high levels of iron, 300-400 ppm on a dry weight basis. Iron availability to rats from cultured S. platensis and from commercial Spirulina equaled that of $FeSO_4$. Ingestion of the daily dose of Spirulina (10 g) recommended for human consumption by the commercial source would provide up to 1.5-2 mg absorbed iron. However, both cultured and commercial Spirulina contained approximately 9.5 ppm Hg, so that chronic use may lead to mercury intakes above prudent levels.

KEY WORDS: algae, iron availability, mercury, Spirulina

INTRODUCTION

The bioavailability of essential minerals for utilization has been a long-standing problem in human nutrition. In particular, there is a high incidence of iron deficiency in the United States and throughout the world (1) which is a major public health problem (2) affecting primarily infants and young women. The availability of iron for intestinal absorption is a crucial factor in the adequcy of the diet as a source for this element.

The purpose of this study was to investigate the bioavailability of iron from a blue-green alga, Spirulina platensis, which grows ubiquitously throughout the world. Spirulina is particularly predominant in alkaline lakes in Africa, Central America and Mexico, where it is collected by the natives and has been used as a food supplement for centuries (3). Spirulina is very high in protein (50%-70% of dry weight) (4) and also in iron (300-400 ppm). Because Spirulina lacks a cellulose wall, it is easily digestible. Currently, Spirulina is being sold commercially in the United States as a food supplement, iron and vitamin source,

and as an aid for weight loss under various trade names. Because Spirulina contains very high levels of iron, we decided to measure the bioavailability of iron from Spirulina to rats.

MATERIALS & METHODS

Algal Cultures

A xenic culture of Spirulina platensis (Nordst.) Geitl. (UTEX 2340) was obtained from the Culture Collection of Algae, The University of Texas at Austin. The algae was cultured in a 57 L vat aerated with a submersible pump. A modified Spirulina medium was used (Table 1). Cultures were maintained at 16 hr light: 9 hr dark cycle, 24 C and $2x10^3$ microeinsteins $m^{-2}sec^{-1}$ (400 watt high pressure sodium light). Approximately every 48 hr, 4 L of spent media was withdrawn and 4 L fresh sterile media was added.

TABLE 1
Modified Spirulina Medium (5)

Solution I:		Solution II	
$NaHCO_3$	13.61 g	$NaNO_3$	2.50 g
Na_2CO_3	4.03 g	K_2SO_4	1.00 g
K_2HPO_4	0.50 g	NaCl	1.00 g
Distilled water	500.00 ml	$MgSO_4 \cdot 7H_2O$	0.20 g
		Trace element mix	(see below)
		Distilled water	500.00 ml

Trace element mix (6) concentrations in mgL^{-1}; $CaCl_2$, 20,000; $FeCl_3$, 3,000; $MnCl_2$, 180; $ZnSO_4$, 22; Na_2MoO_4, 15; $CoCl_2$, 11; $CuSO_4$, 10 and 18 µM Na_2EDTA.

Solutions I and II were autoclaved separately, combined after cooling, and 5×10^{-6} g sterile vitamin B_{12} per liter added.

Non-living dried preparations of Spirulina were obtained from a local store.

Cell Harvesting and Analysis

Cells were harvested from live cultures by filtering a one liter aliquot through coarse filter paper with suction. The cells on the filter were rinsed twice with distilled deionized water and concentrated. The cells were freeze dried and stored at 20°C.

Commercially prepared Spirulina and cultured S. platensis were analyzed on a model 1140 Jarrell Ash inductively coupled argon plasma-emission spectrometer (ICAP) (Jarrell Ash division of Fisher Scientific Co., Waltham, MA) for major minerals and trace elements after digestion with nitric acid and hydrogen peroxide. The instrument was calibrated with standards made from Puratronic elements and compounds (Johnson Matthey, London, England) and commercially prepared standards (Jarrell Ash) in dilute (2-5%) nitric acid. Precision of duplicate analyses was 2%. Tests for matrix interferences by high levels of Ca, Na, K, Mg, Fe, and P on

Hg analysis showed no effect of these minerals on measured mercury levels.

Test Diet

A purified diet based on the AIN-76 formulation (7), but modified to contain only 20 ppm added iron, was fed throughout all experiments.

Iron Retention Experiments

Three groups of seven 21-day old Long-Evans rats were used. The rats were fed the basal diet for seven days. After fasting for 18 hours, the rats were fed their test meals. Group 1, the controls, were fed basal diet without Fe + 100 µg Fe (as $FeSO_4$) + 0.5 µCi $^{59}FeCl_3$; Group 2, commercial Spirulina, were fed basal diet without Fe + 200 mg Spirulina + 0.5 µCi $^{59}FeCl_3$; and group 3, cultured S. platensis, were fed basal diet with Fe + 300 mg S. platensis + 0.5 µCi $^{59}FeCl_3$. Dextrose was added to the test meals for the first two groups (300 mg/meal, group 1 and 100 mg/meal, group 2) so that the total weight of dextrose + algae was 300 mg in each 2.5 g test meal. Test meals for groups 2 and 3 each contained approximately 100 µg Fe from Spirulina. Iron concentration in the test meals was approximately 40 ppm. The rats were offered their respective test meals for four hours. After four hours the test meals were removed, and the rats were immediately measured for radioactivity in the whole body counter. Rats were fed the basal diet for the remainder of the experiment. The test meal remaining was weighed and the amount eaten by each rats was calculated. The rats were counted every two days for four weeks. This experiment was performed twice.

Whole Body Counter

All rats in both experiments were measured for radioactivity using a small-animal whole body counter which was constructed in this laboratory. The small-animal whole body counter consists of a supporting frame mounted on four pneumatic tires, two matched 10.1 cm x 10.1 cm x 40.6 cm single crystal NaI(T1) detectors (resolution for ^{137}Cs of 7.7%), two high voltage power supplies, and two spectroscopy amplifiers. The detectors lie on a horizontal shelf (top) and are positioned 10 cm apart with a plexiglass counting chamber (inside dimensions 8.9 cm x 8.9 cm x 38.1 cm) in the intervening region. The detectors and counting chamber are shielded with approximately 2.5 cm of nuclear grade lead. Access to the counting chamber is made by moving a 5.1 cm x 5.12 cm x 20.3 cm lead brick which covers one end of the counting chamber. The output of each spectroscopy amplifier is combined in a scanning amplifier and routed to a 1024 channel multichannel pulse height analyzer where the two combined spectra can be analyzed for specific gamma ray activities.

In addition to calibration with ^{59}Fe, day-to-day instrument fluctuations were corrected with reference to ^{137}Cs. Duplicate background counts were taken using an unlabeled rat to correct for

^{40}K contribution to the ^{59}Fe window counts. Geometry of the counting chamber was held consistent by using a standard sized block to control the placement of the plexiglass holder in the whole body counter. The rat was placed so that it was being measured at about midline of the body.

Data Analysis

For both experiments, the observed whole body counts for all groups were corrected for counter deadtime, background (including ^{40}K), instrument fluctuations and nuclide decay. The cpm obtained on day 0 represented 100 percent of the dose and all succeeding data were calculated as a fraction of the day 0 value.

The percent retention values for each animal were plotted against time using a log-linear scale. Absorption was defined to be the y-intercept of the least-squares regression line that was fitted to the linear portion of the retention curve. Data for each experiment were compared using one-way analysis of variance followed by Scheffe contrasts (8).

RESULTS

Analysis of commercial Spirulina and cultured S. platensis cells revealed high concentrations of major minerals and trace elements (Table 2). Iron was consistently high in both preparations. However, some other trace elements were also very high (Hg, Mn, Se, Zn). Some were less concentrated in spent media and thus concentrated in the algae (Ca, Fe, Hg, K, Mg, Mn, Se).

TABLE 2
ICAP Analysis of Commercially Prepared Spirulina and Cultured Spirulina Platensis UTEX 2340

	Commercial Spirulina	S. platensis
	(dry weight basis)	
Ca (ppm)	1840.[a]	422.[a]
Cd (ppm)	ND[b]	ND[b]
Cr (ppm)	1.42	1.55
Cu (ppm)	5.19	2.72
Fe (ppm)	372	302
Hg (ppm)	9.79	9.29
K (ppm)	7970	15,000
Mg (ppm)	2260	1940
Mn (ppm)	34.2	16.9
Mo (ppm)	0.45	1.09
Na (ppm)	1840	54,300
Ni (ppm)	1.61	1.85
P (ppm)	7170	7490
Pb (ppm)	2.5	1.26
Se (ppm)	1.13	2.52
V (ppm)	ND[b]	0.16
Zn (ppm)	24.7	7.44

[a]mean of duplicate analyses [b]ND not detectable

In both experiments, iron from cultured Spirulina platensis was absorbed as well as iron from ferrous sulfate (Table 3). In the

TABLE 3
Iron absorption from cultured and commercial Spirulina compared to absorption from ferrous sulfate

	% Absorption (mean ± SD)			
	First Experiment %	Relative to $FeSO_4$	Second Experiment %	Relative to $FeSO_4$
Iron sulfate control	55.2 ± 11.3[a]	1.00	74.4 ± 4.6	1.00
Commercial Spirulina	83.4 ± 8.5[b]	1.51	66.3 ± 8.4	0.89
Spirulina platensis (cultured)	59.7 ± 12.0[a]	1.08	74.5 ± 4.8	1.00

In the first experiment, means with different superscripts are significantly different, ab: $p < 0.001$.
For the second experiment there were no significant differences between the groups, $p > 0.05$.

first experiment, iron from commercial Spirulina was absorbed significantly better than iron from ferrous sulfate. In the second experiment, iron absorption from the commercial Spirulina was lower than but not significantly different than from ferrous sulfate ($p=.078$). Rat weights were 60.3 ± 4.1 and 87.8 ± 8.5 on Day 0 (test meal) and 227 ± 18, 211 ± 28 on Day 28 for the first and 2nd experiments, respectively.

DISCUSSION

Iron from cultured S. platensis was absorbed equally as well as ferrous sulfate in both experiments. The cultured material was prepared within a month of the feeding experiment in both cases. The lower absorption of iron from the cultured Spirulina compared to the commercial material may be due to the presence of excessive salts such as calcium and phosphates from the culture medium in the freeze-dried preparation, even though the cells were rinsed with distilled water. Calcium (9) or calcium and phosphate combinations (10) have been shown to decrease iron absorption. Nevertheless, the data demonstrated that the cultured Spirulina was as good a source of iron as iron sulfate. Our harvesting technique was crude compared to the commercial process which involved screening, washing, pasteurization and spray drying (11). The commercial process ensured better preservation of the cells.

The much lower absorption of iron from commercial Spirulina in the second experiment, relative to ferrous sulfate, than in the first experiment may be due to a limited shelf-life of the commercial product. The two experiments were done approximately twelve months apart, using Spirulina from the same bottle. An earlier, preliminary experiment done approximately five months before the first experiment, showed iron absorption from the commercial product almost six times higher than from ferrous sulfate. It appears that breakdown or decomposition may be occurring in the opened bottle on the shelf. This could be either the decomposition of an organic ligand for the iron or oxidation of the iron itself.

Spirulina contains a highly available form of iron. The complexity of a cell makes it difficult to speculate on the chemical form of the iron. About 35% of the total leaf iron in higher plants is estimated to be in the form of ferritin (12). The role of phytoferritin as a cellular buffer has been established, but little is known about other functions of ferritin in plants (12). Plant and animal ferritins are believed to be much the same in structure, and in plants ferritin seems to be formed as a result of high iron levels within the plants (13). However, iron from animal ferritin is generally not well absorbed (14-16), so it seems unlikely that the highly available iron in Spirulina is ferritin.

Dietary iron consists of two pools, heme and nonheme iron. The use of extrinsic tracers of $^{59}FeCl_3$ or $^{55}FeCl_3$ to assess absorption of nonheme iron intrinsic to foods has been studied extensively, and the ratio of extrinsic to intrinsic iron absorption has been found not to differ significantly from unity (17,18). Thus one may conclude that, in the absence of contaminating iron oxides or other highly insoluble forms of iron, exchange between the biological nonheme iron and the added tracer is essentially complete. In this study iron was added as an extrinsic label in the form of $^{59}FeCl_3$ and the absorption of the ^{59}Fe may be considered equivalent to absorption of nonheme iron from the algae.

It is unusual to find plant-derived iron that is highly available, since nonheme iron from plant sources is not considered to be well absorbed by animals. Not more than 10% of the nonheme iron from plant sources is usually absorbed by humans (19-21). Absorption of iron by rats and humans has been found to be highly correlated ($r=0.94$); the average human response is 68% of that by rats (22). Another comparison of in-vivo rat and human absorption values yielded a correlation coefficient $n=0.9250$ for "standard" meals and $r=0.8418$ ($p<0.05$) for semisynthetic meals (23). These experiments demonstrated a high bioavailability of iron from Spirulina to rats, since the absorption of iron from the algae was equal to that from iron sulfate. In comparison, absorption of iron from potatoes (24) and from whole wheat bread (25) by rats was about half that from ferrous sulfate. While absorption of iron from Spirulina by humans will likely be less than by rats, it nevertheless appears that Spirulina is a fairly concentrated source of available iron. Of course, its availability will also depend on the composition of other foods eaten in conjuction with Spirulina.

Spirulina is high in iron and it is also high in trace metals such as Cr, Cu, Hg, Mn, Ni, Se and Zn. The high concentration of Group I and Group II elements was not surprising, since these algae thrive in aquatic alkaline sodic environments. The values found for Ca, P, Fe, Na, Mg, Mn and Zn were similar to those reported previously for spray-dried Spirulina (species unspecified) (11) and much lower than for Phormidium tenue, another edible blue-green alga from Lake Texcoco (26). The trace element content of commercial Spirulina and cultured S. platensis were similar to each other and to the product information available from the manufacturer. However, we found significantly more mercury (9 ppm) than the commercial analysis (<0.05 ppm). Analysis of other brands of Spirulina also served a high (> 10 ppm) Hg content (L.E.S. and P.E.J., unpublished data). Mercury levels in some fruits, vegetables, eggs and meat have been reported to be 0.05-0.1 ppm (27). The average for 34 commonly consumed seafoods was 0.13 ppm (28) and the average for all foods was 0.02 ppm (29). Daily human dietary intake of mercury estimated from analysis of duplicate diets was 6.5 µg/day (30). The enforcement of the U.S. Food and Drug Administration action level has been revised from 1 ppm total mercury to 1 ppm methyl mercury in fish (31,32), but the FDA has also suggested a "prudent" limit for inorganic mercury in fish of 10 ppm (31). U.S. per capita daily fish consumption is 18 g (28). A maximum "prudent" level of mercury consumption is thus 180µg/day. Foods other than fish are not regulated, but considered on a case-by-case basis as questions arise.

Metal accumulation by algae and aquatic plants is a well-known but poorly understood phenomenon (34,34). Our analysis of fresh and spent media confirmed this metal accumulation to be true for Spirulina also. Some metals, such as Ca, Fe, K, Mg, Mn, and Se were concentrated by the algae, and thus were 40-60% less concentrated in spent than fresh medium. Many algae can accumulate metals to potentially toxic levels, although it has been demonstrated that some of the metals can be removed with an 0.01M EDTA or 0.1N HNO_3 rinse (33). It is not known if a EDTA rinse reduces the mercury level. The ability of algae to accumulate metals is generally in the following rank order: mercury > lead > cadmium (33).

A dose of 10 g per day, as recommended by the commercial source, of Spirulina would contain 3-4 mg of iron, of which as much as 1-2 mg might be absorbed. Thus, Spirulina is probably a good source of iron, although more expensive than inorganic iron tablets (35). However, according to our analysis, this dose would contain 90 µg Hg (the FDA's "prudent" limit). This is half of the intake which would occur from ingestion of 18 g of fish containing 10 ppm Hg, but fourteen times greater than the normal 6.5 µg Hg/d intake cited above (29). Evaluation of the chemical form of this mercury is needed.

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PROPRIETARY STATEMENT

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture, and does not imply its approval to the exclusion of other products that may also be suitable.

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