ELSEVIER

Food Chemistry 77 (2002) 213–218

Food Chemistry

www.elsevier.com/locate/foodchem

Magnesium bioavailability from magnesium-fortified spirulina in cultured human intestinal Caco-2 cells

Perrine Planes[a], Jean-Max Rouanet[a,*], Caroline Laurent[a], Jean-Claude Baccou[b], Pierre Besançon[a], Bertrand Caporiccio[a]

[a]*Unité Nutrition, Laboratoire Génie Biologique et Sciences des Aliments, CC 023, Université Montpellier II, Place Eugène Bataillon, 34095 Montpellier Cédex 05, France*

[b]*Unité de Physiologie et Technologie Végétales, Laboratoire Génie Biologique et Sciences des Aliments, Université Montpellier II, Montpellier, France*

Received 22 May 2001; received in revised form 3 September 2001; accepted 3 September 2001

Abstract

The role of magnesium (Mg) for health promotion and disease prevention is generally accepted world wide. The aim of this study was to examine magnesium availability from Mg-fortified spirulina. We have used an in vitro digestion/Caco-2 cell culture system to measure magnesium spirulina availability. Magnesium concentration measurements were performed by atomic absorption spectrometry in digests containing the same amount of magnesium and at the basal side of Caco-2 cells exposed to these digests. Comparison was made with crude spirulina, Banania, Kellogs all bran and Magnogène (magnesium chloride) as a reference. Our results show that Mg-fortification of spirulina does not improve Mg availability and that crude spirulina represents an adequate source of Mg as efficient as All bran and Banania.

Keywords: Magnesium bioavailability; Spirulina; In vitro digestion; Caco-2 cells

1. Introduction

Magnesium (Mg) is implicated in protein synthesis, lipid and carbohydrate metabolism. This essential micronutrient is also required for phosphorylation reactions and energy transfer (Wilkinson, Welch, Mayland, & Grunes, 1990). Magnesium is involved in neuromuscular, cardiovascular, immune, and hormonal function (Aikawa, 1981; Durlach, 1988; Ebel & Günther, 1980) and, to maintain these functions, adequate amounts of this micronutrient are needed in the diet. In developed countries, the recommended dietary allowances have been first set at 4.5 mg/kg per day (Shils & Rude, 1996), and further it was suggested that 6 mg/kg per d would ensure adequate Mg status (Marx & Neutra, 1997). In these countries marginal Mg intake may increase the prevalence of Mg deficiency (Galan et al., 1997; Marx & Neutra, 1997). Advance in food technology and processing have led to a deterioration of Mg level in dietary vegetables and meat. Flour sieving level, rice polishing and sugar refining still further reduce Mg amounts in these foodstuffs. All in all, Mg is an usually malabsorbed ion: only about 30% of dietary Mg is absorbed by human intestine (Roth & Werner, 1979). Since Mg entering the gastrointestinal tract is not completely absorbed, its bioavailability is expected to be weak. Some plant-source proteins (Turnlund, Betschart, Liebman, Kretsh, & Sauberlich, 1992), saturated fats, fructose (Kenney & Mac Coy, 2000), dietary fibres (Durlach, 1978), phytic acid (Brink, Dekker, van Beresteijn & Beyen, 1991), increased intakes of P (Rodder, Mize, Forman & Uauy, 1992) or Ca (Shils, 1997) or both Ca and P (Brink, Beyen, Dekker, van Beresteijn, & van der Meer, 1992; Brink, Dekker, van Beresteijn, & Beyen, 1992), as well as alcohol (Tokmak et al., 1999) inhibit Mg absorption.

The aim of this work was to examine the bioavailability of Mg from magnesium-fortified spirulina. The cyanobacterium *Spirulina platensis* (blue-green algae), commercially available for human consumption, is used as a health food source for humans (Kay, 1991). Spirulina represent one of the richest protein sources of plant origin (60–70%) and are a good source of vitamins and minerals (Cases et al., 1999; Dillon, Phuc, &

* Corresponding author. Tel.: +33-4-67-143521; fax: +33-4-67-633649.

E-mail address: rouanet@arpb.univ-montp2.fr (J.-M. Rouanet).

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00341-7

Durbach, 1995). The simple cultivation technology and the good quality of their protein, as well as the absence of any toxic side effects (Chamarro, Herrera, Salazar, Salazar, & Ulloa, 1988; Yoshino, Hirai, Takahashi, Yamamoto, & Yamazaki, 1980), favour their large-scale production. Their chemical composition can be changed by varying cultivation conditions: this microalga is an easily Mg-supplementable vegetable through the aquatic environment. The algal magnesium availability was compared with that of magnesium chloride (in the form of the therapeutic agent Magnogène) which is considered as well absorbed, and that of foodstuffs such as All-Bran (Kellogs) and Banania (an instant breakfast containing cocoa, cereals, banana and honey).

A most promising technique to predict the mineral availability is the human intestinal epithelial (Caco-2) cell line system (Alvarez-Hernandez, Nichols, & Glass, 1991; Fairweather-Tait, 1998). We recently applied this model to the study of iron availability from spirulina (Puyfoulhoux, Rouanet, Besançon, Baroux, Baccou, & Caporiccio, 2001). Cultivation of these cells under standard culture conditions resulted in a sponteanous structural and functional differentiation and polarization (Pinto et al., 1983) after reaching confluency, forming a well-defined brush border on the apical surface and tight cellular junctions (Hidalgo, Raub, & Borchardt, 1985; Pinto et al., 1983). Moreover, they represent an alternative method which reduces or can replace the use of laboratory animals (Balls & Fentem, 1999).

2. Materials and methods

2.1. Spirulina magnesium-fortification

This step was performed at Aquamer S.A. (Mèze, France): algae (Spirulina *platensis*) were grown in a 130 l photobioreactor under continuous lighting on Zarouk's medium at 22 °C and pH 10.5 in the presence of $Mg(NO_3)_2$. This medium contained $NaHCO_3$ 16.8 g/l, K_2HPO_4 0.5 g/l, $NaNO_3$ 2.5 g/l, K_2SO_4 1.0 g/l, NaCl 1.0 g/l, $MgSO_4,7H_2O$ 0.2 g/l, $CaCl_2$ 0.04 g/l, $FeSO_4,7H_2O$ 0.01 g/l, EDTA 0.08 g/l, H_3BO_3 2.86 mg/l, $MnCl_2,4H_2O$ 1.81 mg/l, $ZnSO_4,7H_2O$ 220 μg/l, $CuSO_4,5H_2O$ 79 μg/l, MoO_3 15 μg /l, Na_2MoO_4 21 μg/l and was supplied with a light aeration (30 l/min) and with addition of 0.03% CO_2. At the end of the growth, the biomass was recovered and filtered through a 20-μm membrane, thoroughly washed with distilled water, frozen and lyophilized.

2.2. Cell culture

Caco-2 cells, originating from human colorectal carcinoma, were obtained from the American Type Culture Collection (ATCC, Rockville, USA) at passage 22, and used in experiments at passage 35. Caco-2 cells were maintained and expanded in 75-cm^2 flasks at 37 °C in an atmosphere of 5% CO_2-95% air at constant humidity and in a Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% heat-inactivated fetal calf serum, 1% nonessential amino acids and 4 mM L-glutamine, and it was changed every other day. When the cells were 80–90% confluent, they were harvested by treatment with a solution containing 0.25% trypsin and 1 mM EDTA, thoroughly washed and resuspended in supplemented growth medium. For transport experiments, Caco-2 cells were seeded at a density of 3×10^5 cells per 24 mm (Ekmekcioglu, Ekmekcioglu, & Marktl, 2000) of collagen-coated permeable polycarbonate Transwell® cell culture inserts (0.4 μm pore size; Corning Costar Science Products, Brumath, France). The filters were located in 6-well plates separating an upper from a lower compartment. The cells were used in the magnesium transport experiments at 28 days postseeding. During this time the development of the monolayer was monitored by determining the transepithelial passage of phenolred which is only transported paracellularly. In this way 1.0 ml complete culture medium containing 45 μM phenol red was added to the upper compartment and 2.0 ml phenol red-free medium to the lower compartment. The diffusion of phenol red across the monolayer was determined after 120 min by measuring absorbance at 558 nm in the lower solution. A transport rate lower than 0.5 per cent per hour, as compared to the transport without cells, indicated the intactness of the monolayer (Ekmekcioglu et al., 2000). Integrity of the confluent polarized monolayer was checked prior to and after the experiments.

2.3. In vitro digestion

This was performed as previously reported (Glahn, Lee, Yeung, Goldman, & Miller, 1998; Puyfoulhoux et al., 2001). Briefly, porcine pepsin (800–2500 units/mg protein), pancreatin (activity, 4×USP specification) and bile extract (glycine and taurine conjugates of hyodeoxycholic and other bile salts) were purchased from Sigma (Saint Quentin Fallavier, France). Peptic and intestinal digestions were conducted on a rocking platform shaker (Rotomix, Bioblock, Illkirch, France) in an incubator at 37 °C with a 5% CO_2–95% air atmosphere maintained at constant humidity. To start the peptic digestion, the pH of each sample was adjusted to pH 2.0 with 5.0 mol/l HCl. The sample was transferred to a 50 ml screw-cap culture tube, 0.5 ml of the pepsin solution was added per 10 ml of sample and the tube was capped, placed horizontally, and incubated in the rocking shaker for 60 min. For the intestinal digestion step, the pH of the digest was raised to 6.0 by adding 1 mol/l

$NaHCO_3$ dropwise. Then 2.5 ml of pancreatin-bile extract mixture was added per 10 ml of original sample, the pH was adjusted to pH 7.4 with NaOH, and the volume was brought to 15 ml with 120 mmol/l NaCl and 5 mmol/l KCl. The digestion was carried out in the upper chamber insert with the cell monolayer cultured on the polycarbonate surface.

2.4. Experimental design

In preliminary experiments, it was verified that magnesium secretion from the cells into the upper or lower chamber was negligible during 120 min of intestinal digestion. Just before the intestinal digestion step, the growth medium was removed from each well and the cell monolayer was washed twice with Minimum Essential Medium (MEM, Gibco) containing no added magnesium and at 37 °C and pH 7.4. This medium was supplemented with 10 mmol/l PIPES (piperazine-N,N′-*bis*-[2-ethanesulfonic acid]), 1% antibiotic-antimicotic solution, hydrocortisone (4 mg/l), insulin (5 mg/l), selenium as sodium selenite (5 μg/l), triiodothyronine (34 μg/l) and epidermal growth factor (20 μg/l). All culture medium components were from Sigma (Saint Quentin Fallavier, France). During the experiment, the cells were covered with 1.0 ml of MEM. Then a representative 1.5 ml aliquot of the peptic digest was carefully poured into the upper chamber and the plate was covered and incubated on the rocking shaker for 120 min. At the end of the intestinal digestion, the medium from the basal compartment was collected and transferred to plastic tubes for the determination of the magnesium amount transported across the monolayer.

2.5. Experimental procedure and food sampling

In our experiments we compared samples of magnesium chloride (Magnogène, Novartis Santé Famille S.A., Rueil-Malmaison, France), Banania (Bestfood France, Antony, France), All-Bran (Kellogg's Produits Alimentaires, Rosny-sous-Bois, France) and crude spirulina with magnesium-fortified spirulina that was prepared by Aquamer S.A. (Mèze, France). They contained 0.79, 1.35, 1.28, 2.68 and 4.88 mg Mg/g sample, respectively. Digests of the above samples contained 170 μg Mg (per 1.5 ml) from each sample. Additionally, a blank digest system only containing pepsin, pancreatin and bile extract, without addition of Mg or food, was used.

2.6. Chemical analyses

Analyses of the magnesium content of the solutions and food samples were performed by atomic absorption spectrometry using a Varian Vista spectrometer (Varian, Les Ulis, France) after samples were digested in nitric acid and hydrogen peroxide.

2.7. Statistical analysis

For each food sample tested, two 6-well plates were run and this experiment was repeated twice; data from each food sample were averaged ($n = 24$) and this average value was the data point used in the statistical analysis. Data were subjected to logarithmic transformation where necessary to achieve homogeneity of variances. Data are given as mean ± S.E.M. and they were analyzed by one way analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) method for comparing groups, using Stat View 4.5 (Abacus Concepts, Inc, Berkeley, CA). A significance level of $P < 0.05$ was adopted for all comparisons.

3. Results

Growing spirulina on Zarouk's medium led to a 1.8-fold Mg-fortification. In our experiment, food sample amounts were prepared in order to supply 1700 μg Mg in each digest, i.e. about one third of the recommended dietary allowances; in this way 1260 mg Banania, 1320 mg All Bran, 630 mg crude spirulina, 348 mg Mg-fortified spirulina and 2.16 mg Magnogène were used in each digest. The amount of magnesium measured in 1.5 ml of each digest placed in the upper chamber was consistent with the expected magnesium content of the digests (only 170 μg of magnesium was loaded in the upper chamber of each culture well) and no significant difference appeared among the digests, as shown in Fig. 1. The fractional transport values for magnesium, measured from the different digests 120 min after the start of intestinal digestion, are summarized in Fig. 2: the fractional transport was about 1.7-fold higher for Magnogène than for spirulina, either

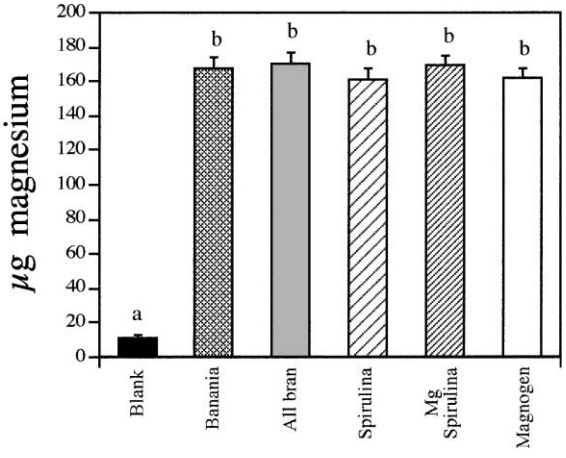

Fig. 1. Total magnesium in 1.5 ml digest loaded in the upper chamber. Blank indicates that only enzymes (pepsin and pancreatin) plus bile extract are introduced in the digest system, with no added magnesium or food. Values are means ± S.E.M. ($n = 6$). Bars with identical letters are not significantly different ($P < 0.05$).

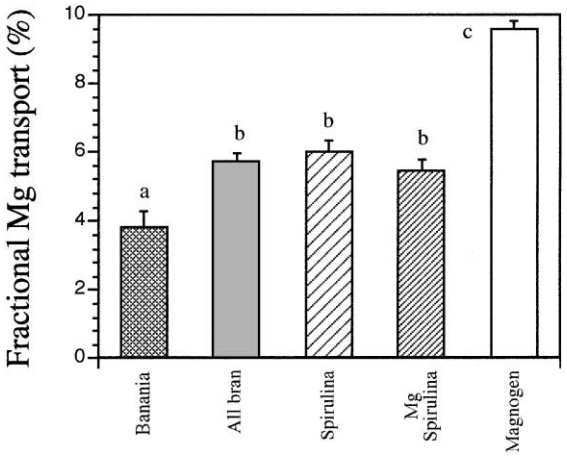

Fig. 2. Fractional Mg transport from the apical to basal compartment across Caco-2 cell monolayer from sample digests. Values are means ± S.E.M. ($n = 24$). Bars with identical letters are not significantly different ($P < 0.05$).

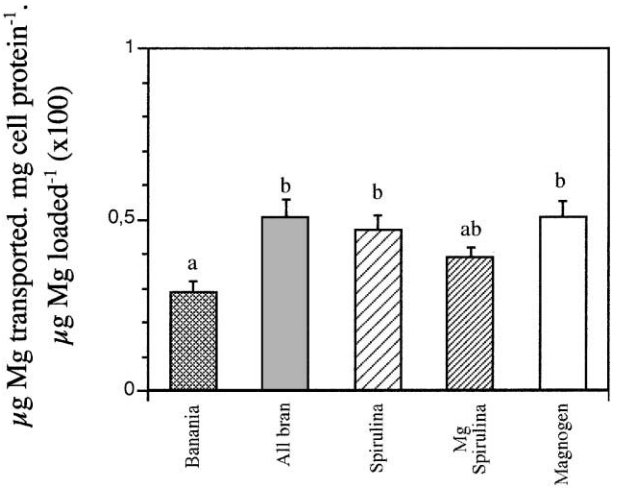

Fig. 3. Magnesium transported from the apical to basal compartment across Caco-2 cell monolayer 120 min after in vitro digestion and expressed per mg cell protein and per µg magnesium loaded in each upper chamber. Values are means ± S.E.M. ($n = 24$). Bars with identical letters are not significantly different ($P < 0.05$).

crude or Mg-fortified. No significant difference appeared for Mg transport between spirulina and All Bran. Magnesium from Banania exhibited a significantly lower transport (about 1.5-fold in comparison with spirulina and All Bran). When Mg transport is expressed per mg cell protein per µg Mg loaded in each upper chamber (Fig. 3), there are no significant differences among the digests except for Banania which led to the lowest value.

4. Discussion

Magnesium bioavailability depends on the digestion and absorption of nutrients in the gut. In humans, Mg is more absorbed in small intestine than in colon (Coudray, Bousset, Tressol, Bellanger, Pepin, & Rayssiguier, 1996; Hardwick, Jones, Brautbar, & Lee, 1990; Kayne & Lee, 1993). Ileum and colon are the major sites of Mg homeostasis regulation (Karbach & Felmeier, 1991). Mechanisms for Mg absorption depend on metabolic energy supply (Partridge, Davie, & Birch, 1987); some points are still open relative to the saturability of this transport (Ebel & Günther, 1980; Jüttner & Ebel, 1998) and interactions with numerous dietary components (Brink, Beynen et al., 1992; Brink et al., 1991; Brink, Dekker et al., 1992; Durlach, 1978; Kenney & Mac Coy, 2000; Rodder et al., 1992; Shils, 1997; Tokmak et al., 1999). Magnesium bioavailability can be defined as the amount of ingested Mg that enters the body beyond the intestinal cells and is incorporated into physiologically-functional pools. Due to these above-mentioned points Mg availability is generally weak (Roth & Werner, 1979). Nevertheless there are few studies devoted to dietary Mg bioavailability (Brink, Beynen, et al., 1992; Brink, Dekker, et al., 1992; Ekmekcioglu et al., 2000; Rimbach & Pallauf, 1999; Wrobel, 1999) but data on the evaluation of Mg bioavailability in humans have been reported (Benech & Grognet, 1995). We have used a human intestinal epithelial cell line system (Caco-2 cells), which represents a most promising technique at present for determining micronutrient availability (Alvarez-Hernandez et al., 1991; Fairweather-Tait, 1998). In this study, experimental conditions were selected in order to obtain a low cell Mg contamination and the MEM was chosen with no added Mg in its composition. Moreover as recommended, pH of digests and MEM were adjusted to 7.4, in order to mimick the human intraluminal pH in the middle to distal part of the ileum (Ekmekcioglu et al., 2000). Here we have used magnesium chloride (Magnogène) as a reference for Mg availability. As expected, Mg transport from Magnogène was higher than that from the other Mg sources (from 42 to 58%; Fig. 2). This discrepancy disappeared when Mg transported was expressed per mg cell protein per µg Mg loaded in each upper chamber (Fig. 3). There were no significant difference among the Mg sources utilized excepted for Banania which contains polyphenolic compounds and lipids. Consequently, Mg-enrichment of spirulina (about two-fold) is not as efficient as selenium or iron fortification of the alga (Cases et al., 1999; Puyfoulhoux et al., 2001). In fact, the % Mg transport from the enriched and non-enriched spirulina is comparable. However, the enriched algae contained about twice as much Mg. Therefore, per unit weight, the enriched algae would provide about twice as much Mg. An hypothesis would be that at least two Mg pools could coexist in spirulina after fortification, one of them being not or less rapidly releasable. Spirulina fractionation studies are in progress to test this hypothesis. There is evidence from epidemiological studies that Mg intake,

in a large proportion (about 20%) of the population in industrialized countries, is below the recommended daily allowances (Durlach, Bac, Durlach, Rayssiguier, Bara, & Guiet-Bara, 1998). Moreover, it has been reported that hypomagnesemia was found in 12% of German children younger than one year and an incidence of 30.5% in adolescents aged 16–18 years with neurological disorders (Schimatschek & Classen, 1993). Additionally, according to Wong, Rude, Singer, and Shaw (1983), approximatively 10% of patients admitted to large city hospitals are hypomagnesemic. Our results showed that crude spirulina was also an interesting source of magnesium, suggesting that intake of spirulina caps (either crude or Mg-fortified) might provide another means of Mg supplementation that could help to cope with low intakes of Mg for long periods, which may be responsible for the appearance of symptoms that have thus far remained unexplained. Examples include the relationship between low erythrocyte concentrations of Mg and sleep alteration (Depoorter, Francon, & Llopis, 1991), chronic fatigue syndrome (Cox, Campbell, & Dowson, 1991), depression (Widmer, Henrotte, Raffin, Bovier, Hilleret, & Gaillard, 1995) and increased levels of free radicals (Günther & Höllriegl, 1989).

Acknowledgements

We are grateful to Bruno Baroux (Aquamer S.A., Mèze, France) for his fruitful help and spirulina culture.

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