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Binding of Mineral Elements by Some Dietary Fibre Components—*In vitro* (II)*

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ABSTRACT

The ability of some fibre preparations (soluble fibre fractions of wheat bran and whole-grain wheat bread-dough, a bulk-laxative—Inolaxol—and cellulose) to bind copper(II), zinc(II) and cadmium(II) ions has been studied using a potentiometric method. Soluble fibre fractions of wheat bran and whole-grain wheat bread-dough, in which most of the phytate was present, interacted strongly with the metal ions. Incubation of these fractions with phytase reduced the complexing ability, indicating the active ligand to be phytate. Inolaxol (a bulk laxative containing about 80% Sterculia gum) also bound a considerable amount of the three metals, which could not be explained by the Sterculia gum itself. The complexing ability of cellulose was negligible.

INTRODUCTION

Fibre components, such as uronic acids, or substances associated with the fibre; for example, phytic acid, interact with cations and may therefore inhibit mineral absorption in man (James *et al.*, 1978; Harland & Morris,

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1985; Nävert *et al.*, 1985). Thus, there is a risk that the absorption of essential trace elements; for example, zinc and copper, could be reduced to a critical level if the intake of these minerals is low. On the other hand, toxic metals; for example, cadmium ions, might be excreted together with the fibre complex. Little is known, however, about the nature of the ligands responsible for metal binding to the fibre complex and the variation of binding strength to different metal ions.

Whole-grain cereals are some of the best sources of dietary fibre, minerals and trace elements. Increased consumption of unrefined cereals is therefore recommended in many countries. It has, however, been claimed that mineral bioavailability is reduced in diets rich in whole-grain flour products (Frølich, 1986). This has mainly been attributed to the phytic acid but also, in part, to the dietary fibre components themselves. The phytate level in the outer layers of wheat is high and Lolas *et al.* (1976) reported a mean value of about 5% in different wheat bran varieties.

When using the method of Asp *et al.* (1983), including an acid pepsin step to isolate the fibre most of the ash was recovered in the soluble fraction (Frølich & Asp, 1980). It was further shown that up to 75% of the phytic acid in cereals was recovered in the soluble fibre fraction (Frølich *et al.*, 1984). However, there are reports showing that the phytate content is reduced considerably at fermentation during baking (Frølich & Asp, 1985).

Bulk laxatives are often used in Western societies to increase faecal weight and avoid constipation. The increased excretion is mainly due to fibre but also to an increased excretion of protein, fat and minerals. When using balance experiments on rats it has been shown that, after feeding a bulk-laxative based on Sterculia gum (Inolaxol), a considerable amount of minerals was excreted in faeces, compared with a basal, fibre-free diet (Nyman & Asp, 1985). However, only a moderate binding to the raw material, Sterculia gum, could be observed *in vitro* by using the potentiometric method (Nair *et al.*, 1986).

In a previous paper we have studied the binding of copper, cadmium and zinc ions to some gel-forming and soluble types of dietary fibre (low- and high-methoxylated pectin, Sterculia gum and guar gum) (Nair *et al.*, 1986). In that study we found a considerable binding to the uronic acids in low-methoxylated pectin, but only a moderate binding to high-methoxylated pectin and Sterculia gum, although those polysaccharides also contain uronic acids. The metal binding to guar gum was negligible.

In the present study, the binding of copper, cadmium and zinc ions to different fibre preparations was investigated. Soluble fibre fractions of wheat bran and whole-grain wheat bread-dough were isolated and the influence of phytase treatment on metal binding was studied. Furthermore, the metal binding to Inolaxol and cellulose was studied. As a control we measured the

binding to a protein hydrolysate, similar to that present in the fibre preparations of bran and bread-dough due to the enzymatic fibre preparation method.

MATERIALS AND METHODS

Materials

The binding of copper, cadmium and zinc was studied in the following fibre preparations: Cellulose powder (Kebo, Arlöv, Sweden), Inolaxol (a bulk laxative containing 80% Sterculia gum, i.e. a highly branched polysaccharide containing approximately 20% rhamnose and galactose and 50% uronic acids), soluble fibre fractions from wheat bran and whole-grain wheat bread-dough, both preparations with and without treatment with phytase. The binding of copper, cadmium and zinc to a protein hydrolysate was also studied.

Sample preparation

Soluble fibre fraction of wheat bran

Soluble fibre components were recovered from wheat bran using the enzymatic method of Asp *et al.* (1983) with some modifications. This method includes incubations with a heat-stable amylase (Termamyl), pepsin and pancreatin.

To avoid coprecipitation of phosphorus, Tris-buffer was used instead of phosphate-buffer throughout the isolation procedure. Termamyl was also excluded from the assay, due to the rather high mineral content of this enzyme preparation. Further, to avoid the use of Celite, which contains minerals, the enzyme digest was centrifuged instead of filtered, in a Beckman centrifuge (J2-21) at 4°C for 30 min and 1600 × *g*.

Soluble fibre components were recovered by dialysing the supernatant obtained after centrifuging the enzyme digest. The solution was dialysed against 4 litres of double distilled water for 48 h at 4°C in Spectapor sacks (Spectrum Medical Industries Inc., Los Angeles, USA) with an exclusion limit of 6000–8000 daltons. The sack content was then lyophilised. The dialysis procedure was used to avoid possible structural alterations of soluble fibre or phytate due to alcohol precipitation which has been pointed out in a previous study Frølich *et al.* (1984).

Soluble fibre fractions from whole-grain bread-dough

Bread-dough was made from whole-grain flour and bran. The recipe of the

dough was as follows: water, 700 ml; whole-grain wheat flour, 700 g; wheat bran, 300 g; salt, 17·5 g; yeast, 35 g.

Samples were taken immediately after mixing the ingredients and dried in a microwave oven (Philips HN 1104 A) for 20 s on full power, and in a traditional oven for an additional hour at 105°C. The samples were then ground in a laboratory mill (Cyclotec, Tecator AB, Höganäs, Sweden) to a particle size of less than 0·45 mm and thereafter kept in closed containers. Soluble fibre components from the bread-dough were then recovered as described above for wheat bran.

Soluble fibre fractions treated with phytase

A 0·1-g portion of the lyophilized soluble fibre fractions was redissolved in 30 ml distilled water and the pH was adjusted to 4·5. The solution was incubated for 20 h with 5 ml of a phytase solution at 37°C, prepared from wheat according to Peers (1953). The enzyme reaction was stopped with 0·1 M trichloroacetic acid (TCA) and neutralised to pH 4·5 with NaOH. The incubate was then dialysed as described above.

Whole grain sour dough and wheat bran contained 1·8 and 3·7% (dry weight basis) phytate, respectively, determined by the method of Holt (1955). After treatment with phytase no phytate could be detected.

Protein hydrolysate

Casein (lactic acid casein), 5 g, was dissolved in 75 ml 0·1 M HCl. Pepsin, 500 mg (Merck 7190, Darmstadt, West Germany), was added to the solution which was then incubated for 3 h at 37°C in a water-bath with agitation.

Methods

The interaction between the fibre preparations and copper, zinc and cadmium ions, respectively, was studied using a potentiometric technique elaborated by Persson (1970) and Norberg & Persson (1984) and described previously (Nair *et al.*, 1986). By emf-measurements at 25°C with amalgam electrodes in solutions containing known total concentrations of metal ion (C_M) and fibre, the concentration of free metal ion $[M^{2+}]$, was determined. Thus, the fraction of metal bound to the fibre or complexed otherwise $(C_M - [M^{2+}])/C_M$ could be calculated. The interaction between metal ion and fibre was studied as a function of pH. From the total amount of metal bound at a certain pH, the experimentally determined fraction existing as hydroxo complexes was subtracted, and the net metal binding to fibre components obtained (Nair *et al.*, 1986). Every titration series was repeated at least once.

RESULTS AND DISCUSSION

The amount of copper, cadmium and zinc bound to the different fibre preparations, or existing as hydroxo complexes, is shown as functions of pH in Figs. 1–3. The starting total concentration of fibre was generally 0·6 g dry weight litre^{-1} and of metal ion, 2·6 mM. However, titration series with higher concentrations of fibre were also performed. The corresponding data, corrected for hydroxo complexes, are collected in Table 1.

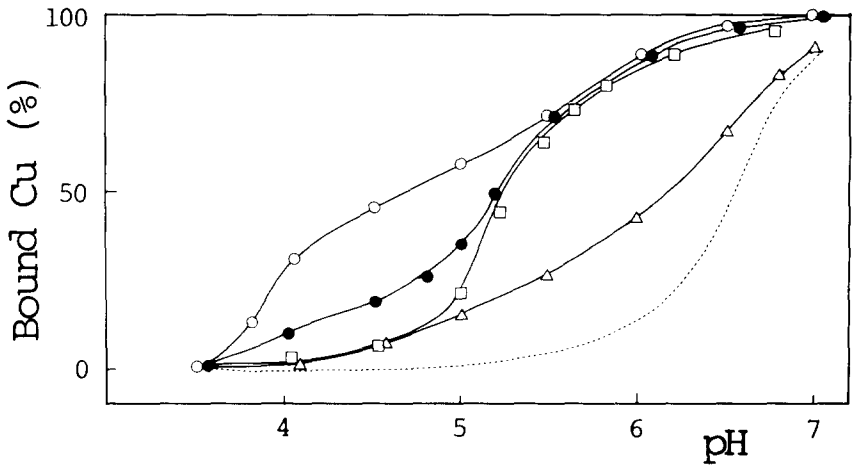

Fig. 1. Bound copper as a function of pH at addition of different dietary fibre preparations: (○) soluble fibre fraction from whole-grain wheat bread-dough; (●) Phytase-treated soluble fibre fraction from whole-grain wheat bread-dough; (□) Inolaxol; (△) protein hydrolysate; (- - -) without fibre. The starting concentration of fibre was 0·6 g/litre and of Cu, 2·6 mM.

The present study shows that the interaction, fibre–metal, is strongly pH-dependent, starting at pH = 3·5–4 and generally completed at about pH = 5·5. At increasing pH values copper(II) ions become extensively hydrolysed, and at pH about 6·5 the hydroxo complexes start to dominate over the fibre complexes. Zinc(II) and cadmium(II) ions are considerably less hydrolysed and at pH 7 less than 5% of the zinc ions and less than 2% of the cadmium ions exist as hydroxo complexes.

Cellulose

The binding to cellulose was negligible both for copper and cadmium (Table 1).

Fibre fractions from wheat bran and bread-dough

Soluble dietary fibre isolated from whole-grain bread-dough was found to bind both copper and cadmium ions strongly.

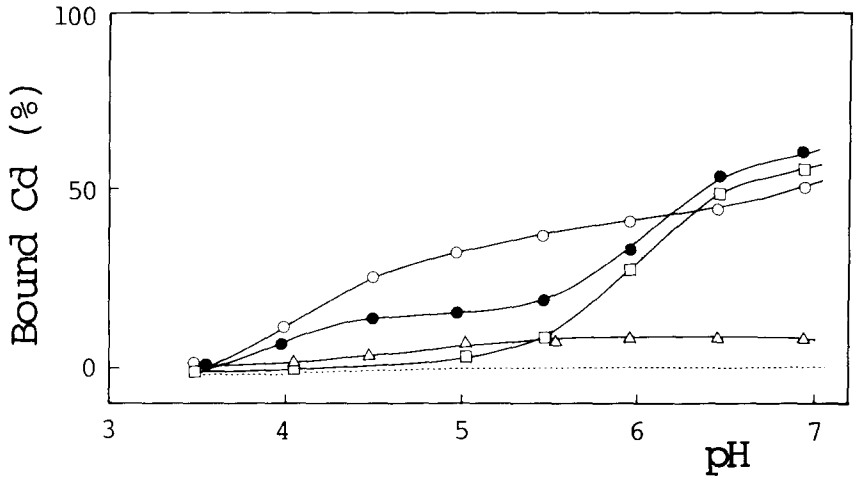

Fig. 2. Bound cadmium as a function of pH at addition of different dietary fibre components: (○) soluble fibre fraction from whole-grain wheat bread-dough; (●) Phytase-treated soluble fibre fraction from whole-grain wheat bread-dough; (□) Inolaxol; (△) protein hydrolysate; (- - -) without fibre. The starting concentration of fibre was 0·6 g/litre and of Cd, 2·6 mM.

Figure 1 indicates that the copper complex formation takes place in two steps, one at pH = 3·5–4·5 and another one at pH = 5–6. For cadmium, the first step also occurs at pH = 3·5–4·5 but the second step starts at about pH = 6 (Fig. 2).

Copper formed considerably stronger complexes than cadmium with all

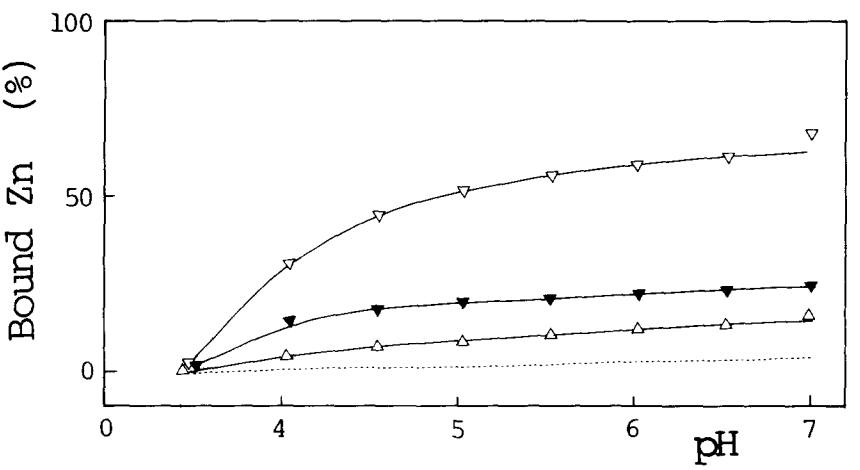

Fig. 3. Bound zinc as a function of pH at addition of different dietary fibre components: (▽) soluble fibre from wheat bran; (▼) phytase-treated soluble fibre from wheat bran; (△) protein hydrolysate; (- - -) without fibre. The starting concentration of fibre was 0·6 g/litre and of Zn, 2·7 mM.

TABLE 1

Fraction of Metal Ion Bound to Different Fibre Components

(The fibre concentration is expressed as g dry weight/litre. The fraction of metal ion, bound as hydroxo complexes, has been subtracted)

Fibre	*Initial concentration of fibre (g litre^{-1})*[a]	*Metal ion*[b]	*Bound metal (%) at various pH*								
			3·0	*3·5*	*4·0*	*4·5*	*5·0*	*5·5*	*6·0*	*6·5*	*7·0*
Cellulose	0·63	Cu	0	0	0	0	0	0	2	5	0
	0·63	Cd	0	0	0	0	0	0	0	0	0
Soluble fibre	0·64	Cu	0	1	30	46	56	67	75	53	10
from wholegrain	0·64	Cd	0	0	12	27	32	35	38	45	54
wheat bread-dough	1·06	Cd	0	0	16	45	57	61	64	68	76
	0·69	Zn	0	0	20	25	28	30	35	57	66
Phytase treated	0·77	Cu	0	0	9	18	30	67	75	53	10
	0·77	Cd	0	2	8	14	16	19	33	54	60
	1·53	Cd	2	10	20	27	32	37	52	79	91
Soluble fibre	0·66	Zn	0	4	34	47	53	54	54	57	63
from wheat bran	1·08	Zn	0	7	45	73	83	86	89	90	90
Phytase treated	0·66	Zn	0	4	14	17	17	18	18	18	19
	1·08	Zn	0	9	31	37	38	38	39	41	43
Inolaxol	0·61	Cu	0	0	2	6	20	62	71	51	10
	1·22	Cu	0	1	3	8	37	77	83	56	11
	0·62	Cd	0	0	0	1	4	8	30	48	54
	1·22	Cd	0	0	2	3	8	12	50	74	85
Hydrolysed protein	0·67	Cu	0	0	1	6	13	20	26	22	1
	0·67	Cd	0	0	2	4	7	9	10	11	12
	0·67	Zn	0	1	4	6	7	8	9	10	11
No fibre	0	Cu	0	0	0	1	2	6	14	44	89
	0	Cd	0	0	0	0	0	0	0	0	0
	0	Zn	0	0	1	2	2	3	3	4	5

[a] Before the titrants T_1 and T_2 were added.

[b] The starting concentration of metal ion before the titrants T_1 and T_2 were added was 2·56 mM.

the fibre preparations (Table 1). Similar results were obtained with the fibre fractions studied earlier (Nair *et al.*, 1986).

A reduction in binding due to phytase treatment could be observed for copper and cadmium up to pH = 5. At higher pH no reduction in metal binding was obtained by phytase treatment.

Soluble fibre from wheat bran and whole-grain wheat bread-dough interacted strongly with zinc ions (Table 1). The complex formation started at pH 3·5 and was almost completed at pH 5 (Fig. 3). When the soluble fraction of wheat bran fibre was treated with phytase, the binding of zinc ions was substantially reduced (> 50%), which is in accordance with the results of Frølich & Asp (1985). In contrast to the finding for copper and cadmium, phytase treatment reduced zinc binding substantially at all pH values tested (up to pH = 7·0).

The fraction of metal ions bound was closely proportional to the concentration of fibre. At pH 5–6 about 80–90% of the zinc was bound in a solution containing 1 g fibre/litre versus only 50–60% in a solution containing 0·6 g fibre/litre (Table 1). Similar results were obtained for cadmium and the soluble fibre from whole-grain wheat bread-dough.

At lower pH (<5), phytic acid seems to be an important chelator of all metals studied here, since the complex formation decreased considerably when the soluble fibre fraction was hydrolysed with phytase. At pH >6, hydrolysis with phytase did influence complex formation of zinc but not of copper and cadmium. This indicates that other components than phytic acid present in the soluble fibre fraction could be of importance for the metal binding at physiological pH, especially for copper and cadmium.

During hydrolysis of phytate (inositol-hexaphosphate) with phytase, the molecule will be broken down to lower inositol phosphates and finally to totally dephosphorylated inositol and free phosphates. This hydrolysis has a very distinct pattern, proceeding in a stepwise manner (Frølich *et al.*, 1986).

It is not certain what effect the lower inositol phosphates have on the bioavailability of nutrients, but it has been claimed that lower inositol phosphates bind minerals poorly under physiological conditions (Frølich *et al.*, 1986). Our results indicate that lower inositol phosphates or dephosphorylated inositol, present here after 20 h of incubation, do not complex with zinc ions to any great extent.

Great care is needed when extrapolating *in vitro* studies to *in vivo* conditions, because the chelating properties of minerals may be different in a mixed meal than in isolated *in vitro* systems. Further, the structure could be altered during isolation of different components, giving different binding properties (Frølich & Asp, 1985). The measurements from *in vitro* studies, however, can give valuable information as to what extent a certain fibre component may associate certain minerals at various pH values.

Inolaxol

Inolaxol exhibited strong binding to both copper and cadmium—the complex formation starting at pH 4·5. Inolaxol, which is a bulk laxative, contains about 80% Sterculia. The contents of Sterculia, however, cannot account for the major metal binding. In a previous investigation (Nair *et al.*, 1986) we determined that, at maximum, about 10% of copper, cadmium and zinc, respectively, were bound to Sterculia at pH = 6 at a fibre concentration of 0·6 g litre^{-1}. The binding effects of Sterculia may be due to its high content of uronic acids.

Table 1 shows that Inolaxol, at pH = 6, and the same fibre concentration binds about 70% copper and 30% cadmium. The difference in total amount of metal bound to Inolaxol and Sterculia and the selectivity of Inolaxol to

bind copper and cadmium show that the major metal binding of Inolaxol cannot be caused by the Sterculia polysaccharide. In addition to Sterculia, Inolaxol contains kaolin which might be responsible for the main part of metal binding.

However, even here great care is needed when transferring *in vitro* results to *in vivo*. Dietary fibre is not digested by the enzymes in the human alimentary tract, but bacterial enzymes in the large intestine can partially degrade dietary fibre as discussed, for example, by Nyman (1985). The degree of fermentation can have an effect on the mineral absorption and minerals that are obviously bound to the fibre *in vitro*, might be released from the fibre during fermentation and then absorbed. Thus it has been shown that calcium bound to fibre can be absorbed in the colon during fermentation James *et al.* (1978).

Protein-hydrolysate

Interestingly, hydrolysed protein also showed a moderate binding to copper and cadmium (Figs 1 and 2). The complex formation began at pH 4 and was completed at pH 5·5. However, the protein hydrolysate present in the fibre preparation would make a minor contribution to the total binding capacity.

CONCLUSION

The strongest binding to metal ions was exhibited by soluble wheat fibre including phytate and Inolaxol. The binding to cellulose was negligible.

The results with wheat bran and wheat bread-dough, where the soluble fibre fractions were treated with phytase, indicate that phytic acid is a strong chelator of all the minerals investigated at low pH, and of zinc also at neutral pH. The binding to Inolaxol is too strong to be caused by the Sterculia polysaccharide.

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