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Binding of Mineral Elements by Dietary Fibre Components in Cereals—*In vitro* (III)†

Hans Persson

Department of Physical Chemistry I, Chemical Centre, University of Lund, PO Box 124, S-221 00 Lund, Sweden

Margareta Nyman

Department of Applied Nutrition and Food Chemistry, Chemical Centre, University of Lund, PO Box 124, S-221 00 Lund, Sweden

Helena Liljeberg, Gunilla Önning

Institute for Nutrition Research, University of Oslo, Box 1046, 0316 Oslo 3, Norway

&

Wenche Frølich

Norwegian Food Research Institute, Osloveien 1, N-1430 Aas, Norway

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ABSTRACT

The ability of soluble fibre fractions isolated from three different cereals (barley flour, whole grain rye flour and oat bran) to bind copper(II), cadmium(II) and zinc(II) ions has been studied using a potentiometric method. Considerable association was found between all fibre fractions and metals investigated. The ability of the metal ions to form complexes was found

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to be in the order Cu(II) > Zn(II) ≈ Cd(II) for all cereal fibres. The order of interaction for the fibres with metal ions was barley > oats ≈ rye. Between pH 3·5 and 5 phytic acid was an important complexing agent, especially for oats. No metal complexing ability was found for pure β-glucans, isolated from barley.

INTRODUCTION

The ability of dietary fibre to interact with mineral elements is of interest from a nutritional point of view (Kelsay, 1982). A consequence of this could be a decreased absorption of various minerals. Thus, for toxic mineral elements the interaction could be considered positive, as the metal ions, bound to the fibre components, may be excreted in faeces. On the other hand, the availability of essential trace elements could be reduced. Cereals are one of the best sources for both dietary fibre and minerals, especially the outer parts of the grain (Pedersen & Eggum, 1983*a*, *b*, *c*; Nyman *et al.*, 1984; Frølich & Nyman, 1988).

Knowledge about the binding strength of different types of dietary fibres to different metal ions is limited, as well as which ligands in the fibre are responsible for the metal binding (Davies, 1982; Kelsay, 1982). The minerals associated with dietary fibres are largely found in the soluble fibre fraction (Frølich & Asp, 1981; Frølich & Nyman, 1988).

Pectic substances, containing appreciable amounts of uronic acids, have been shown to bind metal ions strongly (Nair *et al.*, 1987; Persson *et al.*, 1987). The metal complexing ability of the uronic acids was found to depend on the proportion of free carboxyl groups. Considerable binding to low-methoxylated pectin could be observed, while the binding to Sterculia gum and high-methoxylated pectin was less pronounced (Nair *et al.*, 1987).

The metal binding capacity of cellulose and guar gum, i.e. fibres containing minute amounts of charged ions, turned out to be rather small (Nair *et al.*, 1987; Persson *et al.*, 1987). An association between iron and the soluble fibre components present in the oat kernel has been reported (Frølich & Nyman, 1988). This was considerably higher than that obtained between iron and the soluble fibre components in wheat (Frølich & Asp, 1985).

The proportion of soluble fibre as well as the dietary fibre composition vary in different types of cereals. In whole grain barley (dehusked), oats (dehusked) and rye, approximately one-third of the total fibre content is soluble, whereas only one-tenth in whole grain wheat is soluble (Nyman *et al.*, 1984; Nyman & Asp, 1988). The soluble fibre in oats contains a large proportion of β-glucans, which is also the fact for barley, whereas the soluble

polymers of rye and wheat consist of arabinose and xylose (Nyman *et al.*, 1984; Nyman & Asp, 1988). As the dietary fibre composition and proportion of soluble fibre in the different cereals vary to a great extent, the degree of the association, fibre–metal ion, can also be expected to vary.

The mineral binding in cereals may be influenced by the presence of substances associated to the fibre, e.g. phytic acid. Phytic acid, which is present in significant amounts in most cereals, is known to form complexes with divalent cations (Reinhold *et al.*, 1981). The binding capacity of the soluble fibre in wheat bran and in wheat-bread dough to copper, cadmium and zinc was shown to depend on the presence of phytic acid (Persson *et al.*, 1987). Like the dietary fibre and the minerals, most of the phytic acid is found in the bran (aleurone) fraction (O'Dell *et al.*, 1972). When isolating dietary fibre according to Asp *et al.* (1983), most of the phytic acid in cereals has been reported to be recovered in the soluble fibre fraction (Frølich *et al.*, 1984).

The aim of the present investigation was to study the interaction between soluble dietary fibre fractions of different cereals and metal ions and especially to determine the complexing role of phytic acid. The binding of the soluble fibre, isolated from oat bran, barley flour and whole grain rye flour, to copper(II), cadmium(II) and zinc(II), was studied by using a potentiometric technique (Persson, 1970; Norberg & Persson, 1984).

MATERIALS AND METHODS

Materials

Soluble dietary fibre preparations isolated from barley flour, oat bran and whole grain rye were investigated, before and after treatment with phytase.

The barley flour, commercially available (extraction rate = 90%, Nord Mills, Malmö, Sweden), was a mixture of different types of barley grown in the south of Sweden. Before grinding, the husk was removed from the kernel.

The oat bran was obtained by grinding and sieving the dehusked oat kernel (of the variety Selma) by particle size in a laboratory mill (Quadrumat Senior Brabender GmbH) to four botanical fractions (Frølich & Nyman, 1988). The fraction $> 1050\,\mu m$ was defined as coarse bran and used in the present study. The content of phytic acid (inositol phosphates) in coarse bran was 1·00 g/100 g (dwb, dry weight basis) measured by the method of Holt (1955), (Frølich & Nyman, 1988).

Whole grain rye of the variety Petkus was milled by a laboratory mill (Kamas Mill Machines, Malmö, Sweden) to obtain a flour with 100% extraction rate.

Sample preparation

Isolation of soluble fibre

Soluble fibre components from barley, oats and rye were recovered by using the enzymic gravimetric method of Asp *et al.* (1983) with some modifications. Since the filtering aid Celite contains minerals that might disturb the potentiometric measurements of minerals, this step was excluded. To separate soluble fibre components from insoluble, the enzyme digest was centrifuged (1600 g, 30 min, MSE High Speed 25) instead of filtered. The soluble fibre was precipitated with ethanol, lyophilised and stored in a freezer, at −20°C, until analysis (Nair *et al.*, 1987).

Treatment with phytase

Approximately 3 g of the soluble fibre fraction was redissolved in distilled water (60 ml) and pH was adjusted to 5·1 with HCl. To degrade the phytic acid (inositol hexa-phosphate) to lower inositol phosphates and free phosphate, the solution was treated with 50 mg of phytase per g of soluble fibre fraction and incubated for 22 h at 37°C (Persson *et al.*, 1987). The phytase was isolated from wheat, 0·04 Units/mg solid (Sigma, St Louis, Minnesota, USA). Wheat phytase can be used to degrade the phytate in rye, barley and oats, as phytase is not specific for any cereal (Frølich *et al.*, 1988).

For barley and rye this procedure was efficient, and no inositol phosphates could be detected after phytase treatment. However, in the soluble fibre fraction of oats, appreciable amounts of different inositol phosphates could still be detected after this phytase treatment (Tables 1 and 2). When soluble dietary fibre components are isolated according to Asp and coworkers, the fibres are subjected to heat treatment (100°C,

TABLE 1

Contents of Different Inositol Phosphates in the Soluble Fibre Fraction Isolated from Coarse Bran of Oats (μmol/g dwb)[a,b]

	IP_6	IP_5	IP_4	IP_3	IP_3–IP_6
−Phytase[c]	150 (9·9)	8 (0·5)	1 (0·05)	3 (0·1)	162 (10·6)
+Phytase[d]	53 (3·5)	19 (1·1)	73 (3·6)	59 (2·5)	204 (10·7)
+Phytase[e]	9 (0·6)	8 (0·5)	22 (1·1)	82 (3·4)	121 (5·6)

[a] Values within parentheses give the amount of inositol phosphate in g/100 g on dry weight basis.

[b] Measured by HPLC according to Sandberg & Ahderinne (1986).

[c] Soluble fibre isolated without any treatment with phytase.

[d] Soluble fibre first isolated and then treated with phytase.

[e] Oat bran treated with phytase followed by a recovery of soluble fibre.

15 min), phytic acid might partly be modified to unavailable complexes. The extent of such a change may be due to the composition of the actual cereal. Therefore, in order to hydrolyse more phytic acid in oats, another procedure was tried, where oat bran was first treated with phytase before the soluble fibre was recovered. To 80 g oat bran, 450 ml distilled water and 200 mg phytase (0·04 Units/mg solid, Sigma, St Louis, Minnesota, USA) were added. The suspension was adjusted to pH 5·1 and incubated for 22 h at 37°C. This procedure turned out to be considerably more efficient (Tables 1 and 2).

Methods

Complex formation

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.*, 1987). By emf-measurements at 25°C with amalgam electrodes in solutions containing known total concentrations of free metal ions (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

TABLE 2

Contents of Inositol Phosphates in the Soluble Fibre Fraction Isolated from Oat Bran, Barley Flour and Whole Grain Rye (g/100 g dwb)[a]

	Oats	*Barley*	*Rye*
−Phytase[b]	9·2	5·5	4·3
+Phytase[c]	6·8	0·0	0·0
+Phytase[d]	3·4	—[e]	—[e]

[a] Measured with the method of Holt (1955). By this method a sum of inositol phosphates is determined.
[b] Soluble fibre isolated without any treatment with phytase.
[c] Soluble fibre first isolated and then treated with phytase.
[d] Oat bran treated with phytase followed by a recovery of soluble fibre.
[e] Not determined.

fibre components obtained (Nair *et al.*, 1987). Every titration series was repeated at least once.

Dietary fibre composition

The chemical composition of the soluble fibre was determined by gas–liquid chromatography (GLC) of the neutral sugars and by a spectrophotometric determination of the uronic acids (Theander & Åman, 1979; Englyst & Cummings, 1984). Approximately 30 mg fibre was suspended in 1 ml of sulphuric acid (12M) and hydrolysed. The hydrolysate was diluted to 100 ml. To 10 ml of the diluted hydrolysate, 0·5 mg internal standard (allose) was then added and the monosaccharides were reduced and acetylated according to Theander & Åman (1979). The composition of the soluble fibres in oat bran, barley flour and whole grain rye flour, respectively is shown in Table 3.

Phytic acid

The content of inositol phosphates was determined by spectrophotometry according to the method of Holt (1955) (Table 2). However, as this is an iron precipitation method, not only phytic acid (inositol hexa-phosphate), but also other inositol phosphates are precipitated (Frølich *et al.*, 1986). Tetra- and penta-inositol phosphates have been shown to be quantitatively precipitated in such methods, whereas mono-inositol phosphates are completely soluble (Sandberg & Ahderinne, 1986). With this method the sum of inositol phosphates, at least including tetra- to hexa-phosphates, is obtained. In the case of oats, where the phytase treatment seemed to be less

TABLE 3
Distribution of the Soluble Dietary Fibre Saccharides in the Isolated Fraction (%)

	Oats	*Barley*	*Rye*
Rhamnose	tr[a]	6	7
Arabinose	6	5	21
Xylose	5	5	24
Mannose	1	3	4
Galactose	3	2	3
Glucose	84[b]	79	41[c]
Uronic acids	1	0	0

[a] Traces <0·05%.
[b] 2% of which was starch.
[c] All of this was starch since it was totally degraded by amyloglucosidase.

efficient, different inositol phosphates (tri- to hexa-) were therefore separated and analysed with HPLC (High Performance Liquid Chromatography) (Sandberg & Ahderinne, 1986). The results are collected in Table 1.

RESULTS AND DISCUSSION

The amount of copper, cadmium and zinc bound to the different soluble fibre preparations isolated from the various cereals or existing as hydroxo complexes, are shown as functions of pH in Figs 1–3. The starting total concentration of fibre preparation was here 1·32 g/litre of solution and of metal ion 2·6 mM.

The figures show results from potentiometric titration series performed without and with phytase treatment. To check the influence of fibre concentration, corresponding measurement series with a lower starting concentration of fibre (0·66 g/litre) and the same concentration of metal ion (2·6 mM) were also performed. All experimental data from the titration series, corrected for hydroxo complexes, have been collected in Table 4.

Complex formation

The present investigation indicates considerable interaction between all metals and solubie fibre preparations studied. The amount of metal bound was roughly proportional to the concentration of fibre. This interaction was strongly pH-dependent, starting generally between pH 3·5 and 4·0.

At increasing pH values, metal–hydroxo complexes were formed as well. Copper(II)–hydroxo complexes started to dominate over the fibre

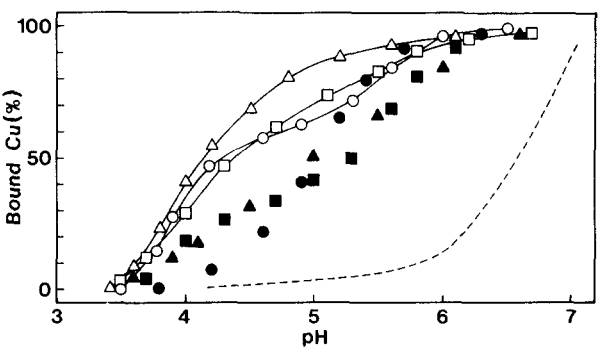

Fig. 1. Bound copper as a function of pH at addition of different soluble dietary fibre fractions: (△) barley; (○) oats; (□) rye; (– – –) without fibre. Filled symbols denote fibre fractions treated with phytase: (▲) barley; (●) oats; (■) rye. The starting concentration of fibre preparation was 1·32 g/litre and of copper(II) 2·6 mM. In the case of oats, the fibre preparation where the soluble fibre was isolated after treatment with phytase, was used.

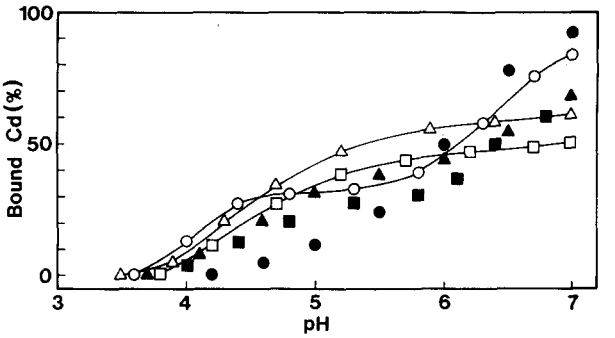

Fig. 2. Bound cadmium as a function of pH at addition of different soluble dietary fibre fractions: (△) barley; (○) oats; (□) rye. Filled symbols denote fibre fractions treated with phytase: (▲) barley; (●) oats; (■) rye. The starting concentration of fibre preparation was 1·32 g/litre and of cadmium(II) 2·6 mM. Without fibre no cadmium complexation was found in the pH interval 3·0–7·0. In the case of oats, the fibre preparation where the soluble fibre was isolated after treatment with phytase, was used.

complexes already at about pH 6·5. On the other hand, zinc(II) and cadmium(II) ions were considerably less hydrolysed, and at pH 7·0, less than 5% of the zinc ions and less than 2% of the cadmium ions existed as hydroxo complexes.

For all fibre fractions studied, the following order of interaction with metal ions was found: Cu(II) > Zn(II) ≈ Cd(II) i.e. the same order obtained for most fibres in earlier investigations (Nair *et al.*, 1987, Persson *et al.*, 1987). When comparing the complexing ability of the fibres the order barley > oats ≈ rye was found (Figs 1–3). When including also results from an earlier investigation we found that wheat bran had a considerably higher complexing ability for zinc(II) than the cereals here studied (Persson *et al.*, 1987).

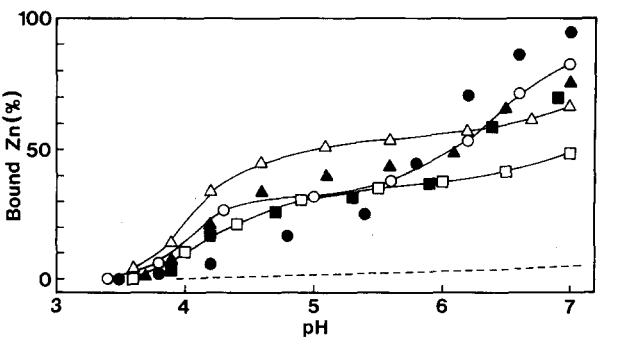

Fig. 3. Bound zinc as a function of pH at addition of different soluble dietary fibre fractions: (△) barley; (○) oats; (□) rye; (----) without fibre. Filled symbols denote fibre fractions treated with phytase: (▲) barley; (●) oats; (■) rye. The starting concentration of fibre preparation was 1·32 g/litre and of zinc(II) 2·6 mM. In the case of oats, the fibre preparation where the soluble fibre was isolated after treatment with phytase, was used.

TABLE 4

Fraction of Metal Ion Bound to Different Soluble 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)*[a]	*Metal ion*[b]	*Bound metal (%) at various pH values*								
			3·0	*3·5*	*4·0*	*4·5*	*5·0*	*5·5*	*6·0*	*6·5*	*7·0*
Barley	0·66	Cu	0	1	14	27	32	38	47	44	8
	0·66	Cd	0	0	0	13	22	26	28	30	32
	0·66	Zn	0	2	13	22	26	29	30	32	36
	1·32	Cu	0	2	41	69	83	86	81	54	10
	1·32	Cd	0	0	9	28	41	51	57	59	61
	1·32	Zn	0	2	21	42	47	51	53	59	67
Barley, phytase treated	0·66	Cu	0	1	20	26	29	39	51	43	9
	0·66	Cd	0	0	3	12	20	25	28	36	43
	0·66	Zn	0	4	14	25	28	30	32	36	42
	1·32	Cu	0	3	15	30	47	60	70	54	10
	1·32	Cd	0	0	5	19	32	38	44	55	68
	1·32	Zn	0	0	12	30	38	43	44	63	70
Oats	0·66	Cu	0	2	14	19	28	46	57	44	9
	0·66	Cd	0	0	4	11	12	13	20	41	48
	0·66	Zn	0	0	13	16	19	22	30	50	57
	1·32	Cu	0	1	36	53	62	74	81	54	10
	1·32	Cd	0	0	13	29	32	34	44	67	84
	1·32	Zn	0	1	14	29	31	35	43	63	76
Oats, phytase treated	0·66	Cu	0	0	7	17	36	59	70	52	10
	0·66	Cd	0	0	0	3	9	19	32	53	61
	0·66	Zn	0	0	5	14	19	22	31	62	69
	1·32	Cu	0	0	5	16	45	77	81	52	10
	1·32	Cd	0	0	0	3	12	14	50	78	82
	1·32	Zn	0	0	3	10	19	26	55	79	89
Rye	0·66	Cu	0	2	10	23	33	42	50	44	9
	0·66	Cd	0	0	3	12	21	25	28	28	29
	0·66	Zn	0	0	4	11	16	21	24	28	33
	1·32	Cu	0	3	29	52	66	77	77	53	10
	1·32	Cd	0	0	6	20	32	42	46	48	51
	1·32	Zn	0	0	10	24	30	33	35	38	43
Rye, phytase treated	0·66	Cu	0	1	7	12	16	32	46	38	6
	0·66	Cd	0	0	1	9	15	20	24	36	42
	0·66	Zn	0	0	3	12	15	18	23	35	40
	1·32	Cu	0	2	18	30	38	58	74	54	10
	1·32	Cd	0	0	4	15	23	30	34	53	63
	1·32	Zn	0	0	9	22	28	32	38	59	66
Wheat bran[c]	0·66	Zn	0	4	34	47	53	54	54	57	63
No fibre	0·00	Cu	0	0	0	1	2	6	14	44	89
	0·00	Cd	0	0	0	0	0	0	0	0	1
	0·00	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 2·6 mM.

[c] From Persson *et al.* (1987).

The complex formation curves for the different fibres exhibited an interesting difference (Figs 1–3). For the soluble fibre of oats, two distinct steps of complex formation could be observed. The first step occurred between pH 3·5 and 4·6 for all the metals studied and the second step between pH 5·0 and 6·0 for copper and between pH 5·5 and 7·0 for cadmium and zinc. For barley and rye, however, only one complex formation step could be noticed, approximately between pH 3·5 and 6·0.

Phytase treatment

In the pH interval 3·5–6·0 less interaction between metals ions and fibre fractions was found after phytase treatment than before (Figs 1–3). For oats the most pronounced reduction took place between pH 4·0 and 5·0 and for barley and rye between pH 4·5 and 5·5. While the phytase treatment of the soluble fibre fractions was very efficient in the case of barley and rye, this method appeared to reduce the phytic acid content in oats by only about 30%, when using the spectrophotometric method of Holt (1955) for analysis (Table 2). On the other hand, if phytic acid was hydrolysed with phytase before the soluble oat fibre was recovered, the content of inositol phosphates was reduced to a greater extent, approximately 60% (Table 2). However, a disadvantage of Holt's spectrophotometric method (1955) is that not only phytic acid (inositol hexa-phosphate), but also other inositol phosphates (especially tetra- and penta-phosphates) are precipitated, giving a too high value of the content of phytic acid (Frølich *et al.*, 1986; Sandberg & Ahderinne, 1986).

Therefore, to make a more accurate analysis of the hydrolysis products from the two procedures of phytase treatment, an HPLC method where the different inositol phosphates could be separated, was also used (Sandberg & Ahderinne, 1986). By using this method of analysis the amount of inositol hexa-phosphate (IP_6, phytic acid) was found to be reduced by 65% when using the first hydrolysis procedure and by 95% with the second one (Table 1). Further, at the first procedure, most of the IP_6 was hydrolysed to tetra- (IP_4) and tri- (IP_3) phosphates, whereas further hydrolysis could be observed at the second procedure, where mainly IP_3 was found (Table 1). A large part of the phytic acid has also probably been degraded to mono- and di-phosphates at the second procedure, as the sum of IP_3–IP_6 was lower in this case.

In the measurement series, represented in Figs 1–3, the soluble fibre fractions of oats, treated with phytase have been produced by this latter procedure. Thus, oat bran was first treated with phytase before the soluble fibre fraction was recovered. The binding of copper(II) ions to untreated soluble fibre fractions of oats as well as to oat fibre fractions incubated with

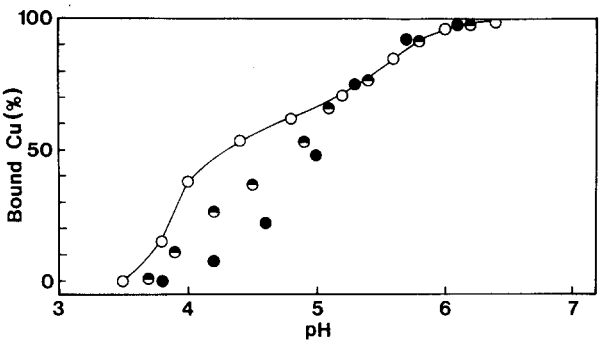

Fig. 4. Bound copper as a function of pH at addition of soluble dietary fibre fractions from oat bran, before and after treatment with phytase: (○) before treatment with phytase; (◓) soluble fibre first isolated and then treated with phytase; (●) oat bran treated with phytase followed by a recovery of soluble fibre. The starting concentration of fibre was 1·32 g/litre and of copper(II) 2·6 mM.

phytase in these two different ways, is shown in Fig. 4. There was a striking connection between the phytic acid content of the oat fibre fraction and the binding of copper(II) ion in the pH interval 3·5 to 5·0. If we take into consideration that most (95%) of the phytic acid (inositol hexa-phosphate) was hydrolysed after our most efficient procedure of phytase treatment, it is obvious that the first step of metal complexation can be largely ascribed to phytic acid (inositol hexa-phosphate). However, there also seem to be other ligands. As the content of inositol tri-phosphate was high, it cannot be excluded that this species was responsible for some of the remaining binding capacity. However, any conclusions about the binding capacity of the lower inositol phosphates (tri- to penta-) cannot be drawn here. This needs further investigation. Calcium and zinc uptake by rats have been found to be inhibited by the higher inositol phosphates (IP_5 and IP_6), whereas the lower ones (IP_3 and IP_4) had no effect (Lönnerdahl *et al.*, 1989).

Analogous measurements of the zinc(II)- and cadmium(II)-interaction with oat fibre showed a similar pattern (Figs 2 and 3). For barley and rye, however, the results were different. The very efficient phytase treatment at most reduced the metal ion association to these fibres by 30–50%. Binding to lower inositol phosphates (tri-, tetra- and penta-) can probably be excluded as no phytic acid could be detected with the method of Holt (1955) after phytase treatment. Thus, it is obvious that, in the case of barley and rye, phytic acid (inositol hexa-phosphate) was not the only ligand responsible for binding metals at low pH values.

Within reasonable experimental error limits, the binding curves for untreated fibres and phytase-treated fibres began to coincide at pH values about 6·0 for each metal. Thus, at high pH values ligands other than phytic acid are responsible for the metal binding. However, at high pH values

(pH > 6·0) an unexpected effect was obtained for zinc(II) and cadmium(II). In this pH region the metal binding to phytase-treated fibre fractions was somewhat higher than to untreated fibre fractions. The interpretation of this fact is not self-evident. One possible explanation might be that new ligand groups were formed during the phytase treatment (Table 1). In the case of oats, a high content of inositol tri-, tetra- and penta-phosphates could be detected and a high content of mono- and di-phosphates cannot be excluded. Further, phosphate groups are released during the phytase treatment, mainly existing as HPO_4^{2-} at pH > 6·5. The monohydrogen-phosphate ion is a reasonably strong complexing agent for several metal ions.

To study the metal binding capacity of β-glucans, potentiometric measurement series, with a pure β-glucan isolated from barley (Grindstedt A/S, Brabrand, Denmark) and cadmium(II), were performed. The concentration of Cd(II) was 2·6 mM and of β-glucan 1·32 g/litre. No complex formation was found in the pH interval studied (pH 3·0–7·0). In an earlier investigation it was shown that copper(II), cadmium(II) and zinc(II) ions were not bound to cellulose (Persson *et al.*, 1987). Our results therefore indicate that pure polymers of β-glucose are poor complexing agents for these metal ions; either they have β-1,4-bonds (cellulose) or both β-1,3- and β-1,4-bonds (β-glucans).

GENERAL DISCUSSION

The soluble fibre fractions from the cereals investigated have a pronounced binding capacity between pH 5 and 7. In the small and large intestine pH is around 6 and mineral interaction can thus be important from a nutritional point of view. Our experiments *in vitro* show, however, that phytase treatment decreased the binding capacity of the fibre fractions considerably at pH < 6. This effect may also be of nutritional significance. Still, above pH 6 appreciable binding capacity remains. A matter of discussion is which of the other components in the soluble fibre fraction are responsible for the remaining binding.

The remaining binding could be due either to the soluble fibre polymers, to lower inositol phosphates which were formed during the phytase treatment, to inorganic phosphate or to other components associated with the soluble fibre fraction. However, any considerable binding to the pure soluble fibre polymers can probably be excluded. The soluble fibre mainly consisted of neutral sugars (oats, β-glucans—82%; barley, β-glucans—79% and rye, arabinoxylans—45%). Only minute amounts of polymers containing charged groups (uronic acids) could be detected and previous

investigations have indicated that metal binding is primarily caused by protolytic groups (Nair *et al.*, 1987; Persson *et al.*, 1987). Our experiments also indicate that pure and neutral polymers of glucose, either if the polymer chain consists of β-1,4 linkages (cellulose) or β-1,4 and β-1,3 linkages (β-glucans), bind minerals (copper, cadmium and zinc) to a minor extent. On the other hand, even if pure polymers *per se* do not show any binding capacity, this does not necessarily mean that these polymers in association with other undigestible components in the soluble fibre preparation are unable to bind minerals. Of course one should also be cautious to transfer conclusions to other minerals than those studied here.

The metal binding capacity of lower inositol phosphates needs further investigation. A large proportion of lower inositol phosphates has, e.g. been detected in ileostomy patients (Sandberg *et al.*, 1987). Therefore, it would be interesting to study to what extent these lower inositol phosphates can bind minerals *in vitro*.

In the case of barley and rye practically no lower inositol phosphates (IP_5–IP_3) could be discovered after phytase treatment. Thus, for these cereals, such species are of minor interest as presumptive complexing agents. Another factor of nutritional importance, when discussing the binding between minerals and fibre, is the fermentability of the fibre in the large intestine. Soluble dietary fibre components are often fermented to a large extent. It has been shown that dietary fibre in Mother's Oat Bran (Quaker Oats Company, Chicago, Illinois, USA) and barley flour (extraction rate 69%), both containing a large proportion of soluble fibre (mainly β-glucans), are largely fermented (approximately 85%) while whole grain rye flour is considerably less fermented (Nyman *et al.*, 1985; Nyman & Asp, 1988). If fermentability is taken into consideration, the chelating capacity *in vivo* of the soluble fibre fraction might be considerably less than that found *in vitro*, and thus more mineral will be available for absorption. The experiments of James *et al.* (1978) on calcium and pectin are a good illustration of this, indicating that calcium, bound to pectin could be released and absorbed from the colon after it has been fermented in the large intestine. Soluble dietary fibres are, on the other hand, seldom completely fermented and the remaining dietary fibre fraction may, even if it is small, form complexes with minerals, making them unavailable for absorption. This is also the case with insoluble dietary fibre components, which are considerably more resistant to fermentation. Thus, our observations of chelating properties of mineral elements to dietary fibre *in vitro*, may indicate binding effects *in vivo* as well. However, it is important to perform *in vivo* experiments before making definite conclusions. Very limited knowledge is available concerning mineral absorption from the colon both in animals and humans, and much research is needed in this complicated field.

REFERENCES

Asp, N.-G., Johansson, C.-G., Hallmer, H. & Siljeström, M. (1983). Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem.*, **31**, 476–82.

Davies, N. T. (1982). Effects of phytic acid on mineral availability. In *Dietary Fiber in Health and Disease*, ed. G. V. Vahouny & D. Kritchevsky. Plenum Press, New York and London, pp. 105–16.

Englyst, H. N. & Cummings, J. H. (1984). Simplified method for the measurement of total non-starch polysaccharides by gas–liquid chromatography of constituent sugars as alditol acetates. *Analyst*, **109**, 937–42.

Frølich, W. & Asp, N.-G. (1981). Dietary fiber content in cereals in Norway. *Cereal Chem.*, **58**, 524–7.

Frølich, W. & Asp, N.-G. (1985). Minerals and phytate in the analysis of dietary fiber from cereals. III. *Cereal Chem.*, **62**, 238–42.

Frølich, W. & Nyman, M. (1988). Minerals, phytate and dietary fibre in different fractions of oat-grain. *J. Cereal Sci.*, **7**, 73–82.

Frølich, W., Schweizer, T. F. & Asp, N.-G. (1984). Minerals and phytate in the analysis of dietary fiber from cereals. II. *Cereal Chem.*, **61**, 357–9.

Frølich, W., Drakenberg, T. & Asp, N.-G. (1986). Enzymic degradation of phytate (myo-inositol hexa phosphate) in whole grain flour suspension and dough. A comparison between ^{31}P-NMR spectroscopy and a ferric ion method. *J. Cereal Sci.*, **4**, 325–34.

Frølich, W., Wahlgren, M. & Drakenberg, T. (1988). Studies on phytase activity in oats and wheat using ^{31}P-NMR spectroscopy. *J. Cereal Sci.*, **8**, 47–53.

Holt, R. J. (1955). Studies on dried peas. 1. The determination of phytate phosphorus. *J. Sci. Food Agric.*, **6**, 136–42.

James, W. P. T., Branch, W. J. & Southgate, D. A. T. (1978). Calcium binding by dietary fibre. *Lancet*, **i**, 638–9.

Kelsay, J. (1982). Effects of fiber on mineral and vitamin bioavailability. In *Dietary Fiber in Health and Disease*, ed. G. V. Vahouny & D. Kritchevsky. Plenum Press, New York and London, pp. 91–103.

Lönnerdahl, B., Sandberg, A.-S., Sandström, B.-M. & Kunz, C. (1989). Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr.*, **119**, 211–14.

Nair, B. M., Asp, N.-G., Nyman, M. & Persson, H. (1987). Binding of mineral elements by some dietary fibre components—*in vitro* (I). *Food Chem.*, **23**, 295–303.

Norberg, A. & Persson, H. (1984). Accumulation of heavy-metal ions by *Zoogloea ramigera*. *Biotechnology and Bioengineering*, **26**, 239–46.

Nyman, M. & Asp, N.-G. (1988). Fermentation of oatfiber in the rat intestinal tract: A study of different cellular areas. *Am. J. Clin. Nutr.*, **48**, 274–9.

Nyman, M., Siljeström, M., Pedersen, B., Bach Knudsen, K.-E., Asp, N.-G., Johansson, C.-G. & Eggum, B. O. (1984). Dietary fiber content and composition in six cereals at different extraction rates. *Cereal Chem.*, **61**, 14–19.

Nyman, M., Asp, N.-G., Pedersen, B. & Eggum, B. O. (1985). Fermentation of dietary fibre in the intestinal tract of rats—a comparison of flours with different extraction rates from six cereals. *J. Cereal Sci.*, **3**, 207–19.

O'Dell, B. L., de Boland, A. R. & Koirtyohann, S. R. (1972). Distribution of phytate

and nutritionally important elements among the morphological components of cereal grains. *J. Agric. Food Chem.*, **20**, 718–21.

Pedersen, B. & Eggum, B. O. (1983*a*). The influence of milling on the nutritive value of flour from cereal grains. 1. Rye. *Qual. Plant. Plant Foods Hum. Nutr.*, **32**, 185–96.

Pedersen, B. & Eggum, B. O. (1983*b*). The influence of milling on the nutritive value of flour from cereal grains. 2. Wheat. *Qual. Plant. Plant Foods Hum. Nutr.*, **33**, 51–61.

Pedersen, B. & Eggum, B. O. (1983*c*). The influence of milling on the nutritive value of flour from cereal grains. 3. Barley. *Qual. Plant. Plant Foods Hum. Nutr.*, **33**, 99–112.

Persson, H. (1970). The complex formation in the zinc thiosulfate and the cadmium thiosulfate systems. *Acta Chem. Scand.*, **24**, 3739–50.

Persson, H., Nair, B. M., Frølich, W., Nyman, M. & Asp, N.-G. (1987). Binding of mineral elements by some dietary fibre components—In vitro (II). *Food Chem.*, **26**, 139–48.

Reinhold, J. G., Garcia, J. S. & Garzon, P. (1981). Binding of iron by fiber of wheat and maize. *Am. J. Clin. Nutr.*, **34**, 1384–91.

Sandberg, A.-S. & Ahderinne, R. (1986). HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *J. Food Sci.*, **51**, 547–50.

Sandberg, A.-S., Andersson, H., Carlsson, N.-G. & Sandström, B. (1987). Degradation products of bran phytate formed during digestion in the human small intestine: Effect of extrusion cooking on digestibility. *J. Nutr.*, **117**, 2061–5.

Theander, O. & Åman, P. (1979). Studies on dietary fibres. 1. Analysis and chemical characterization of water-soluble and water-insoluble dietary fibres. *Swed. J. Agric. Res.*, **9**, 97–106.