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Binding of Mineral Elements by Some Dietary Fibre Components—*in vitro* (I)

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ABSTRACT

The binding of copper, cadmium and zinc ions to some soluble and gelforming types of dietary fibre (guar gum, low and high methoxylated pectin and sterculia gum) has been investigated potentiometrically. Considerable binding was found to low methoxylated pectin, but the binding to sterculia gum and high methoxylated pectin was less pronounced. The binding to guar gum was negligible. Thus, the formation of complexes seemed to be due to the proportion of free carboxyl groups. The amount of added metal bound to sterculia gum was proportional to the fibre concentration, whereas the pectins showed increased binding at higher fibre concentrations. This was possibly due to intermolecular interactions between the polymers and metal ions. The order of complex formation ability to the fibres investigated was different for different metals. To low methoxylated pectin the order Cu > Zn ≫ Cd was observed.

INTRODUCTION

Dietary fibre is plant material not digested by the enzymes of the alimentary canal. A number of clinical experiments as well as a considerable amount of epidemiological data suggest that the dietary fibre in food affects the incidence of certain diseases prevalent in western societies (Burkitt & Trowell, 1975; Kelsay 1978; Vahouny & Kritchevsky,

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1982). Increased intake of dietary fibre increases the amounts of intestinal contents. As a result the passage of the food residue through the intestinal canal becomes faster, giving rise to a larger quantity of faeces having a smoother consistency. Regulation of diabetes, low frequency of different intestinal diseases and low levels of plasma cholesterol through increased excretion of bile salts in the faeces are implied to be some of the beneficial effects of increased intake of dietary fibre.

Furthermore, dietary fibres have the ability to bind certain carcinogenic substances (Sjödin *et al.*, 1985) and also a number of mineral elements (Reinhold *et al.*, 1976). As a consequence, the absorption of these substances from the intestinal tract is reduced. Thus, many toxic substances may be excreted through faeces bound to the dietary fibre components. At the same time some risk arises that the availability of essential trace elements could be reduced to a critical level.

The physiological effects of the dietary fibre components depend very much on their chemical composition. Wheat bran has been shown to reduce the availability of minerals in several investigations, whereas other studies have shown no such effect (Frølich, 1986). Phytic acid, which is present in wheat bran, can form insoluble complexes with many mineral elements such as calcium, zinc and iron and a negative balance of calcium, magnesium, zinc and phosphorus was observed earlier by Reinhold *et al.* (1981). Kies *et al.* (1979), Ranhotra *et al.* (1979) and McHale *et al.* (1979) reported increased levels of some mineral elements in the faeces of rats as well as humans as a result of increased intake of dietary fibre. On the other hand, bran has a high content of minerals, and reduced percentage absorption does not necessarily mean reduced uptake of minerals.

Frølich & Asp (1980) showed data indicating that the water-soluble fraction of dietary fibre is mainly responsible for binding minerals. Furda (1979) has discussed some aspects of the mechanism and chemistry in interactions between metals and polysaccharide molecules. Although the nature of the interaction between metals and protein has been studied extensively, interest in the binding of metals by dietary polysaccharide is comparatively recent. Rendleman (1978*a*; 1978*b*) has reviewed the existing literature on metal polysaccharide complexes and also suggested possible explanations for the formation of these complexes.

One method for studying the binding of ions is the use of cation exchange resins in solution in the presence and absence of polysaccharides. In the present investigation a potentiometric technique elaborated by Norberg & Persson (1984), for studies of the complex formation between metal ions and polysaccharides produced by *Zoogloea ramigera*, was used. The concentration of the free metal ions in the solution was measured under various conditions of polysaccharide concentration and pH using amalgam electrodes.

MATERIALS AND METHODS

Materials

Soluble fibre fractions isolated from guar gum and pectin with low (37%, LM) and high (74%, HM) extent of methoxylation (Copenhagen Pectin Factory Ltd, Skensved, Denmark), were investigated. Furthermore, sterculia gum (fibre content 87%, dry weight basis) a highly gelforming but rather insoluble type of polysaccharide was obtained from Selena AB, Solna, Sweden. The composition of the total fibre, measured by gas-liquid chromatography (Nyman & Asp, 1982; 1985) is described in Table 1.

Sample preparation

Soluble fibre from guar gum and pectin was recovered by using the method of Asp *et al.* (1983) with some modifications. The use of Celite was avoided, as it contains minerals that could disturb the potentiometric determinations of minerals. Thus the enzyme digest was centrifuged (MSE High Speed 25) for 30 min at 1600 *g* instead of filtered to separate soluble fibre. This was then precipitated with ethanol and lyophilised. Sterculia gum was used without further preparation.

In vitro potentiometric measurements of binding of various ions to the dietary fibre components

The interaction between fibre components and copper, zinc and cadmium ions respectively at different pH values was studied using a potentiometric technique (Persson, 1970; Norberg & Persson, 1984). The emf, E of galvanic cells of the following composition was measured:

$$M(Hg)|S + T_1 + T_2||10{\cdot}0\text{ mM NaCl}|Ag(s), AgCl(s)$$

where M is Cu, Zn or Cd respectively. The solution in the left-hand half cell was prepared in the following way. To 20·0 ml of a solution S (3·2 mM $M(ClO_4)_2$), 5·0 ml of a suspension of fibre components containing about 3–12 g dry weight litre^{-1} was added. By adding a solution of perchloric acid (T_1), pH was adjusted to such a low value that the interaction between metal ions and the polymers could be neglected, i.e. pH 2·5–3·0. Subsequently, the pH of the solution was raised by titrating with a solution of sodium hydroxide (T_2). After every addition of a titrant, pH and E were measured. Every titration series was repeated at least once.

All the chemicals were of analytical grade. A two-phase copper amalgam (2% by weight) was prepared according to Fronaeus (1948) by electrolysing a copper sulphate solution between a platinum anode and a mercury

TABLE 1
Dietary Fibre Content and Relative Composition of the Fibre Preparations

Fibre	*Total fibre* ($g\,100\,g^{-1}$)	*Relative composition* (%)							
		Rhamnose	*Arabinose*	*Xylose*	*Mannose*	*Galactose*	*Glucose*	*Uronic acids*	*Klason lignin*
Guar gum	91	1	2	1	58	34	3	1	0
LM pectin	92	1	0·5	0·5	0·5	2	0·5	95	0
HM pectin	89	1	4	0·5	0·5	4	1	89	0
Sterculia gum	87	19	1	0	0	22	3	50	5

cathode. A two-phase cadmium amalgam (8% by weight) was prepared by dissolving cadmium in mercury. The amalgams were stored under dilute sulphuric acid. The two-phase zinc amalgam (2% by weight), which is extremely sensitive to air oxidation, was prepared in vacuum and stored under nitrogen as described by Persson (1970). Stirring of the solution was provided by leading a stream of nitrogen gas into the titration vessel (Ingold 605, slightly modified to keep a little pool of amalgam at the bottom). In this way oxidation of the amalgam was avoided. The pH was measured with Radiometer pHM 52 equipped with a Radiometer glass electrode GK 2402C. The temperature was $25{\cdot}0 \pm 0{\cdot}1$°C. Equilibrium was attained within a few minutes after the addition of titrant. For the galvanic cell described above the relation:

$$E = E^0 - 29{\cdot}58 \lg [M^{2+}] \quad (1)$$

was proven experimentally to be valid to within $\pm 0{\cdot}2$ mV for metal ion concentrations between 0·5 and 5 mM. In the series E^0 was determined from measurements at low values of pH at which the concentration of free metal ion $[M^{2+}]$ approximately equals the total metal concentration C_M. From the measured values of E, the free metal concentration could then be calculated from eq. 1 and thereby the total amount of metal bound to the polymers (or complexed otherwise predominantly as hydroxo complexes in the actual pH region) could be calculated. Thus, the total fraction of metal bound $(C_M - [M^{2+}])/C_M$ is obtained as a function of pH. To correct the measured results with respect to the formation of metal/hydroxo complexes, identical titration series were performed without polymers, and so the fraction of metal existing as hydroxo complexes was obtained as a function of pH as well. The fraction of metal bound to the fibre components was then obtained as the difference between the two values. To check how varying amounts of fibre affect the metal binding, further series with increased concentrations of fibre were performed.

RESULTS AND DISCUSSION

The amounts of copper, cadmium and zinc ions bound to the various dietary fibre components are shown as functions of pH in Figs 1 to 3. In the measurements, a fibre concentration of about 0·63 g dry weight litre^{-1} was used.

From Fig. 1 it is obvious that low methoxylated pectin binds by far the most copper ions per gram of fibre, followed by sterculia, high methoxylated pectin and guar gum. The same order of binding to the fibre was shown with cadmium and zinc ions (Figs 2, 3). The Figures also show that practically no complexes were formed at pH below 3 and that the

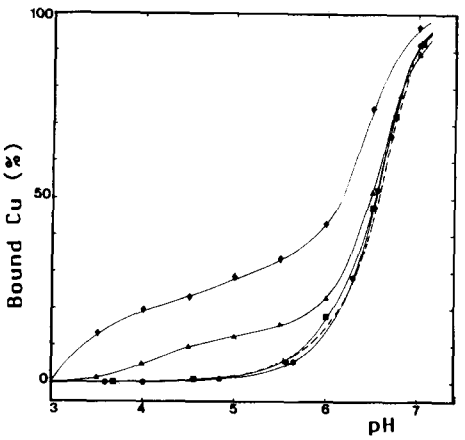

Fig. 1. Bound copper $(C_{Cu} - [Cu^{2+}])/C_{Cu}$ as a function of pH at addition of different dietary fibre components. ● = guar gum, ■ HM pectin, ◆ LM pectin, and ▲ sterculia gum; – – – no dietary fibre added. The starting concentration of fibre was 0·63 g dry weight litre^{-1} in all cases and of C_{Cu} 3·22 mM. The plots show the total amount of metal complexed, i.e. no correction has been made for hydroxo complexes.

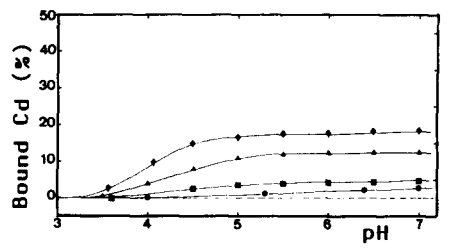

Fig. 2. Bound cadmium $(C_{Cd} - [Cd^{2+}])/C_{Cd}$ as a function of pH at addition of different dietary fibre components. ● = guar gum, ■ HM pectin, ◆ LM pectin, and ▲ sterculia gum; – – – no dietary fibre added. The starting concentration of fibre was 0·63 g dry weight litre^{-1} in all cases and of C_{Cd} 3·25 mM. The plots show the total amount of metal complexed, i.e. no correction has been made for hydroxo complexes.

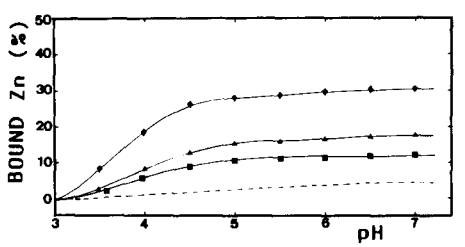

Fig. 3. Bound zinc $(C_{Zn} - [Zn^{2+}])/C_{Zn}$ as a function of pH at addition of different dietary fibre components. ■ HM pectin, ◆ LM pectin, and ▲ sterculia gum; – – – no dietary fibre added. The starting concentration of fibre was 0·63 dry weight litre^{-1} in all cases and of C_{Zn} 3·35 mM. The plots show the total amount of metal complexed, i.e. no correction has been made for hydroxo complexes.

complex formation between fibre and metal ion became independent of pH at about pH = 5.

From the reproducibility of the measurement series the random errors were estimated. Thus, over the pH range 3–7, fibre complexes with cadmium and zinc can be determined with an accuracy of about ±1%. Also for copper an accuracy of about ±1% can be accomplished between pH3 and 5·5. However, at higher pH values the accuracy is decreased due to the rapid formation of copper hydroxo complexes and less reproducibility of the amalgam electrode. A reasonable estimation of the largest error limits is about ±5% occurring at pH about 6·5.

In any study of complex formation it is essential to consider the formation of hydroxo complexes. Table 2 also includes measurements without fibre, showing the amount of metal prevailing as hydroxo complexes at different pH values. Thus, it can be concluded that copper is extensively hydrolysed at pH > 5. Appreciable zinc hydrolysis is observed at about pH = 7 but hydroxo complexes of cadmium can be neglected at pH < 8.

The influence of increasing fibre concentration on metal binding is shown in Table 2. All measurements are corrected for hydroxo complex formation. A quantitative comparison of copper, cadmium and zinc with respect to the fraction of metal bound at a certain pH reveals different patterns for the different fibres. Sterculia gum bound all three metals to similar extents at the same fibre concentration. Furthermore, the amount of metal bound to sterculia gum was approximately proportional to the concentration of fibre, indicating that the same type of complex is formed over the fibre concentration range investigated.

For low methoxylated pectin the order of metal binding was Cu > Zn ≫ Cd and high methoxylated pectin exhibits the order Zn > Cu > Cd. For both types of pectin, however, a doubling of the fibre concentration increased the portion of metal bound with a factor 2 to 4, indicating the formation of different kinds of complexes. The large difference in binding ability between low and high methoxylated pectin is interesting and might be explained by the degree of methoxylation. Both pectins here investigated are rather pure uronic acid polymers (Table 1), but with different extents of methoxylation (74 and 37% respectively). The carboxyl groups of the uronic acids are usually considered to be responsible for metal binding (James *et al.*, 1978). As the carboxyl groups are blocked at methoxylation, high methoxylated pectin contains fewer units available as ligands.

Our measurements thus reflect the complicated nature of the interactions between metals and polysaccharides discussed, e.g. by Rendleman (1978*b*). The large variations between the metals, with respect to complex formation,

TABLE 2

% Metal Bound at Different Values of pH, and at Different Fibre Concentrations for Copper, Cadmium and Zinc Respectively (the values for the different fibres include corrections for hydroxo complexes)

Fibre	*Metal ion*[a] *(g fibre litre*$^{-1}$*)*[b]	*Bound metal* (%) *at pH* =								
		3·0	3·5	4·0	4·5	5·0	5·5	6·0	6·5	7·0
Sterculia	Cu (0·63)	0	1	4	9	11	10	10	10	0
	Cu (1·26)	0	1	10	17	20	18	17	14	0
	Cd (0·63)	0	0	4	8	11	12	12	12	12
	Cd (1·26)	0	2	10	17	21	22	22	23	23
	Zn (0·63)	0	1	6	9	12	12	12	12	12
	Zn (1·26)	0	2	11	17	19	20	20	20	20
LM pectin	Cu (0·63)	1	12	19	24	27	27	28	29	7
	Cu (1·26)	24	48	62	73	80	82	79	53	9
	Cd (0·63)	0	1	9	16	16	17	17	18	18
	Cd (1·26)	1	10	22	33	38	39	38	38	37
	Cd (2·44)	19	55	79	88	90	90	91	92	95
	Zn (0·63)	0	7	16	23	24	25	25	25	25
	Zn (1·26)	5	26	46	58	62	63	62	62	63
HM pectin	Cu (0·63)	0	0	0	0	0	0	1	2	0
	Cu (1·26)	0	9	14	17	17	14	16	14	0
	Cu (2·50)	5	34	39	40	45	47	43	30	0
	Cd (0·63)	0	0	0	2	3	3	3	3	2
	Cd (1·26)	0	1	4	7	9	10	10	10	11
	Cd (2·52)	0	3	10	15	17	18	19	20	20
	Zn (0·63)	0	0	3	6	7	7	7	7	7
	Zn (1·26)	0	7	20	26	27	27	27	27	27
No fibre	Cu (0)	0	0	0	1	2	6	14	44	89
	Cd (0)	0	0	0	0	0	0	0	0	1
	Zn (0)	0	0	1	2	2	3	3	4	5

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

[b] Starting concentration of fibre before the titrants T_1 and T_2 were added.

point to localised binding. A possible explanation for the enhanced pectin complex formation at large fibre concentrations can be intermolecular interactions between metal and ligand groups on different fibre chains as proposed by Furda (1979) for Ca^{2+} ions.

The complex binding between fibre and metals obtained in this investigation might be of interest in considerations of trace metal balance in the human body. However, great care is needed when transferring *in vitro* results to *in vivo*. Pectins are known to be easily degraded by bacterial enzymes in the colon (Nyman & Asp, 1982), whereas sterculia gum is

resistant to degradation (Nyman & Asp, 1985). Thus, metals bound to the fibre *in vitro* might be released during bacterial degradation in the colon and absorbed.

To provide a broader base for discussion in this field we have started further investigations of other common dietary fibre components.

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