Dietary fibre, physicochemical properties and their relationship to health

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Abstract

Dietary carbohydrates that escape digestion and absorption in the small intestine include non-digestible oligosaccharides (carbohydrates with a degree of polymerisation between three and ten), resistant starch and non-starch polysaccharides. The physiological effects of this heterogeneous mixture of substrates are partly predictable on the basis of their physicochemical properties. Monosaccharide composition and chain conformation influence the rate and extent of fermentation. Water-holding capacity affects stool weight and intestinal transit time. Viscous polysaccharides can cause delayed gastric emptying and slower transit through the small bowel, resulting in the reduced rate of nutrient absorption. Polysaccharides with large hydrophobic surface areas have potentially important roles in the binding of bile acids, carcinogens and mutagens. Ispaghula is capable of binding bile acids through a large number of weak binding sites on the polysaccharide structure, and having greatest effect on the potentially more harmful secondary bile acids deoxycholic acid and lithocholic acid.

Introduction

Dietary fibre is generally accepted as having protective effects against a range of diseases predominant in Western developed countries including colorectal cancer, coronary heart disease, diabetes, obesity and diverticular disease. The term 'dietary fibre' is commonly defined as plant material that resists digestion by the enzymes of the human alimentary tract. Exactly what type of plant materials constitute dietary fibre is still a matter of some debate (Prosky and Lee, 1997) but undoubtedly more emphasis needs to be placed on the source of the fibre and its potential, if any, health benefits. Increased fibre consumption has been associated with lowering total serum cholesterol and LDL cholesterol (Anderson *et al*, 1990; Lairon, 1996), modifying the glycaemic and insulinaemic response (Jenkins *et al*, 1976; Ellis, 1994) and protecting the large intestine from disease (Hill, 1997; Faivre and Giacosa, 1998; La Vecchia and Chatenoud, 1998). While the physiological properties of a polysaccharide are difficult to predict on the basis of structure alone, they are partly predictable on the basis of physicochemical properties such as fermentation, water-holding capacity, viscosity and bile acid binding.

The principal components of dietary fibre are non-starch polysaccharides and lignin. Non-starch polysaccharides are composed of various structural polysaccharides including cellulose (long chain β-glucans found in all plant cell walls), hemicellulose (composed of a variety of heteropolysaccharides including arabinoxylans found in cereals and xyloglucans found in fruits and vegetables) and pectins (galacturonans found mainly in fruits and vegetables). A number of non-structural polysaccharides composed of gums and mucilages from a variety of seeds and fruits are also included in the current definition of dietary fibre. Other components of plant foods that escape absorption and digestion in the small intestine and behave, at least physiologically, as dietary fibre include a variety of non-digestible oligosaccharides and resistant starch. Non-digestible oligosaccharides include the trisaccharide raffinose, the tetrasaccharide stachyose, fructo- and galactooligosaccharides, polydextrose and pyrodextrins. A number of non-digestible oligosaccharides, particularly fructooligosaccharides, have aroused significant interest in recent years due to their ability to stimulate growth of potentially beneficial bacteria such as *Bifidobacteria* in the gut

(Hidaka *et al*, 1986). A significant proportion of starch in the normal diet escapes degradation in the small bowel (Stephen *et al*, 1983) and is labelled resistant starch, but this portion is difficult to measure and will depend on a number of factors including the form of starch and the method of cooking prior to consumption (Wursch *et al*, 1986; Read *et al*, 1986). Nevertheless resistant starch serves as a primary source of substrate for the colonic microflora and may have several important physiological roles (Stephen, 1991; Cassidy *et al*, 1994).

The physiological properties of dietary carbohydrates are dependent on the site, rate and extent to which they are absorbed or fermented in the intestine. Consumption of dietary fibre has been found to: increase stool weight; alter gut transit time; alter activity of the colonic microflora; modify absorption of fats, sugars, minerals and bile acids; influence appetite; and absorb toxins. The extent to which specific dietary fibres exert their physiological effects will be dependent on a complex mixture of structural, chemical and physical properties (Table 1).

Table 1

Structure-function relationships of dietary carbohydrates in the gut

Physicochemical property	Principal dietary sources	Physiological effect
Fermentation	Resistant oligosaccharides Non-starch polysaccharides Resistant starch	Energy source • increase in biomass Short chain fatty acid production • reduction in pH of colon (inhibition of 7-α-dehydroxylase) • anti-neoplastic activity of butyrate
Water-holding capacity	Non-fermentable portion of polysaccharides (e.g. cellulose, ispaghula husk, wheat bran, lignin)	Increased stool bulk • shorter gut transit times
Viscosity **Gel formation**	Pectins, ispaghula husk, gums, mucilages (e.g. guar, locust bean gum, xanthan etc.)	Delayed gastric emptying and slower transit time through small bowel • reduced rate of nutrient absorption (e.g. glucose, bile acids)
Binding of organic molecules	Polysaccharides with large hydrophobic surface area (e.g. lignin, ispaghula husk, oat bran)	Binding of bile acids, carcinogens and mutagens

Structural characteristics

Carbohydrates are classified on the basis of their main monosaccharide components and the sequences and linkages between them, as well as the anomeric configuration of linkages, the ring size (furanose or pyranose), the absolute configuration (D or L) and any other substituents present. Certain structural characteristics such as chain conformation and intermolecular associations will influence the physicochemical properties of polysaccharides. The most stable arrangement of atoms in a polysaccharide will be that which satisfies both the intra and intermolecular forces. Regular ordered polysaccharides in general are capable of assuming only a limited number of conformations due to severe steric restrictions on the freedom of rotation of sugar units about the interunit glycosidic bonds. There is also a clear correlation between allowed conformations and linkage structure. The structural non-starch polysaccharides such as cellulose, xylan and mannan have preferred orientations that automatically support extended conformations (Rao *et al*, 1998). Storage polysaccharides such as amylose tend to adopt wide helical conformations. The degree of stiffness and regularity of polysaccharide chains is likely to affect the rate and extent of their fermentation. Pentose sugars such as arabinose and xylose can adopt one of two specific conformations, furanose rings (often formed by arabinose) that can oscillate and are more flexible and pyranose rings (usually formed by xylose and glucose) which are less flexible. Cereal arabinoxylans are composed of β–linked xylan chains and are relatively stiff molecules with extended conformations. Arabinoxylan solubility and degree of flexibility is decreased with increasing arabinosylation, but the key parameter is likely to be the distribution of these side-chains along the backbone since this will have the most direct effect on conformation. Also, due to their extended conformation, arabinoxylans exhibit a very high viscosity in aqueous solution. Pectins, containing galacturonic acid residues, form more flexible extended conformations and also have regular 'hairy' regions with pendant arabinogalactans. Pectins are probably completely degraded in the large intestine by the colonic flora.

Carbohydrates, especially those containing large numbers of hydroxyl groups, are often thought of as being hydrophilic, but they are also capable of generating apolar surfaces depending on the monomer ring conformation, the epimeric structure and the stereochemistry of the glycosidic linkages. Apolarity has been demonstrated for dextrin, α–(1a→4e) linked glucans, while dextran, α–(1→6) glucans and cellulose, β–(1e→4e) glucans, are much less hydrophobic and unable to project an apolar surface (Balasubramanian *et al*, 1993). Hydrophobicity will also be affected by the degree of polysaccharide hydration, particularly the amount of intra-molecular hydrogen bonding. Hydrophobicity will affect their availability for fermentation in the gut and their binding to bile acids.

Fermentation

The rate, site and extent of carbohydrate fermentation in the gut is dependent on a number of factors including solubility, chemical structure, availability of other more readily fermentable substrates and the composition of the colonic microflora. Since dietary fibre is normally consumed in

the diet as whole grains and not isolated fibres, the extent of pre-processing, particle size and the hydration state of the polysaccharide is also important. Soluble fibres are more readily fermented and earlier in the colon, than insoluble fibres. Terminal residues are fermented first and carbohydrates containing α–arabinose or α–galacturonic acid residues are generally more susceptible to fermentation (Clayton *et al*, 1990). Of the major components of dietary fibre xylans, pectins and gums are significantly fermented in the gut, cellulose is only partly broken down and lignin is essentially an inert material. Resistant starch is completely degraded in the large bowel, forms a major part of the substrate available for colonic fermentation and probably has a significant role in the protection systems associated with carbohydrate fermentation (Macfarlane and Cummings, 1991). The main end products of colonic fermentation are the short chain fatty acids (SCFA) acetic, propionic and butyric and the gases carbon dioxide, hydrogen and methane.

SCFA are an important energy source for anaerobic bacteria and may play a role in the prevention of colorectal cancer (Scheppach *et al*, 1995). Production of SCFA lowers the intestinal pH (Alberts *et al*, 1996) resulting in enzymatic inhibition of the 7-α–dehydroxylase (Reddy, 1993) that catalyses secondary bile acid formation and the reduction of secondary bile acid concentrations due to precipitation (Alder *et al*, 1993). Lower colonic pH may also change the composition of the gut flora to one less prone to produce carcinogens. Butyrate has been proposed to have a direct role in colorectal cancer prevention, due to its ability to inhibit colon carcinoma cell growth *in vivo* (Archer *et al*, 1998).

Specific fibres may vary in both the composition and total concentration of SCFA produced during colonic fermentation (May *et al*, 1994). In a recent study hydrolysed guar gum produced the highest levels of total SCFA compared with other dietary fibre sources when incubated with human faecal microflora, while cellulose and ispaghula produced significantly higher levels of propionate and butyrate respectively (Velazquez *et al*, 2000). Fructooligosaccharides, oligomers and polymers of fructose, have been proposed to alter the bacterial composition of the colon in a beneficial way by stimulating growth of bacteria from the genera *Bifidobacteria* in preference to the potentially more harmful anaerobic bacteria such as *Clostridium* spp (Yazawa *et al*, 1978; Wang and Gibson, 1993). Resistant starch, however, has been reported to stimulate growth of the more anaerobic *Clostridia* species (Jaskari *et al*, 1998).

Water-holding capacity

Dietary fibre can retain water, due to the hydrophilic nature of the saccharide residues. This has an important effect on stool bulking and consequently on gut transit times. Increased stool weight can cause dilution of the intraluminal contents limiting the exposure of the gut to secondary bile acids (Faivre *et al*, 1991) and other toxins (Reddy *et al*, 1989). The water-holding capacity (WHC) of a fibre is related to the primary chemical structure, the hydrophobic/hydrophilic balance and the particle size. Insoluble fibres, such as those present in the aleurone cells of bran, retain water in a network of pores and energy is required to remove it. WHC increases with particle size, due to the greater number of pores and voids in the sponge-like cells. Wheat bran and cellulose both have relatively low water contents and a low WHC but as they are only partly fermented in the gut they appear to increase stool weights due to their ability to retain water. Conversely pectins, guar gum and most other soluble fibres are almost completely fermented in the gut and, despite high initial WHC, have little effect on transit time (Lampe *et al*, 1992).

The high water-holding capacity of soluble fibres has an affect on their viscosity and their interactions with other molecules in the gut. Ispaghula husk (psyllium), obtained from the seeds of *Plantago ovata*, is a particularly effective stool-bulking agent and is widely used in the treatment of both constipation (Lennard-Jones, 1993) and diarrhoea (Eherer *et al*, 1993), where its water-holding properties improve the consistency of liquid stools. The laxative effect is a direct consequence of its ability to form a gel and hold many times its own weight in water. A rich source of arabinoxylans, ispaghula is only partly fermented in the gut and may possess several apparently unique properties.

Viscosity

Dietary fibres are often categorised as either soluble (e.g. pectins, guar and ispaghula) or insoluble (cellulose and lignin), though this labelling is not always helpful in predicting their physiological effects. Soluble fibres are noted for their effect on the stomach and the small intestine whereas insoluble fibres are noted for their effect on the large intestine, though some carbohydrates (e.g. ispaghula) have an effect on both. The extent of pre-processing, including cooking, affects the rate of solubilisation in the intestine, as does particle size (smaller particles have larger exposed surface areas). Also the type and regularity of branching present affects both solubility and the extent of the exposed hydrophobic surface. Carbohydrates with a greater degree of conformational flexibility are also more soluble.

Many water-soluble carbohydrates develop a high viscosity that is linked to, among other factors, molecular weight, conformation and the hydrodynamic volume. In general, as the molecular weight increases so does the viscosity. Some polysaccharides exist in aqueous solution as random coils with polymer entanglement between adjacent chains leading to increased resistance to flow. The high viscosity associated with soluble fibres is often given as the cause of their effect on glucose and lipid metabolism (Leclere *et al*, 1994). Soluble fibres such as ispaghula increase the viscosity of the intestinal contents, reducing the rate of absorption of bile acids, glucose and nutrients (Jenkins *et al*, 1995) so allowing their absorption along a greater length of the small intestine.

Binding to bile acids

In vitro binding of bile acids by certain components of dietary fibre has been well documented (Eastwood and Hamilton, 1968; Story and Kritchevsky, 1976; Agte and Joshi, 1991). Bile acids are a group of related amphiphilic steroids, possessing both a hydrophilic and a hydrophobic face. The primary bile acids, cholic acid and chenodeoxycholic acid, are synthesised from cholesterol in the liver and released into the bile conjugated to glycine or taurine in order to solubilise fats and cholesterol for uptake in the small intestine (Kritchevsky, 1991). In the colon, bile acids that are not absorbed and recycled by the enterohepatic circulation are mostly deconjugated and 7-dehydroxylated to give the secondary bile acids, deoxycholic and lithocholic acid. Conjugation and the presence of hydroxyl groups gives the primary bile acids more

hydrophilic character whereas deoxycholic acid and lithocholic acid are more lipophilic in nature. The effect of pH, available surface area and hydrophobicity of the sterol on the adsorption of bile acids to certain cereal type fibres has been demonstrated (Huang and Dural, 1995). The adsorption capacity of bile acids to wheat, corn, oat, barley and rice fibres was favoured by an acidic pH environment, large hydrophobic surface area and greater hydrophobicity of the bile acid.

Adsorption of bile acids by dietary fibre is one of the proposed mechanisms for the hypocholesteraemic effect of dietary fibre. Increased faecal excretion of bile acids leads to the increased metabolism of cholesterol in the liver, thus lowering serum cholesterol levels (Story and Kritchevsky, 1976). Evidence in human subjects suggests that soluble fibres such as pectin, guar, oat bran and ispaghula (Anderson *et al*, 1988; Blake *et al*, 1997; Haskell *et al*, 1992) are effective serum cholesterol-lowering agents.

Bile acids have been implicated in the aetiology of colon cancer (Hill, 1991; Chaplin, 1998). A number of studies have shown that colorectal cancer patients have higher levels of secondary bile acids both entering (Kishida *et al*, 1997) and leaving the colon (Owen *et al*, 1986). In a recent review of the epidemiological literature, Hill (1997) suggested a strong relationship between cereal fibre consumption and the prevention of colorectal cancer. Many of the most effective dietary fibres contain arabinoxylans. Cereal fibres are particularly effective at binding or partitioning putative co-carcinogens such as fecapentaenes, heterocyclic amines and secondary bile acids into the matrix of the fibre, thus reducing their cytotoxic effect. These carcinogens/promoters are then carried out of the body by undigested insoluble fibre thus lowering their effective concentrations in the intestinal tract.

The exact nature of the interaction between bile acids and dietary fibre has been unclear to date. Some authors have claimed that the driving force for bile acid adsorption to polysaccharides is hydrophobic in nature (Eastwood and Hamilton, 1968). It has also been claimed that the presence of lignin may be important (Kay *et al*, 1979).

Do dietary fibres bind bile acids at a limited number of highly selective points or are the bile acids simply 'partitioned' between the dietary fibre matrix and the bulk aqueous phase? We report here new data probing the nature of bile acid binding by the viscous polysaccharide ispaghula husk.

Binding of bile acids by ispaghula husk

Materials and methods

Extraction of ispaghula husk

Ispaghula husk, batch DS8617, from *Plantago ovata* Forsk was donated by Reckitt and Colman Products Ltd (Hull, UK). The bile acids and salts used in this study were obtained from Sigma (St Louis, USA). The husk was prepared by stirring 1.0 g of ispaghula in 100 ml of deionised water for 2 h at room temperature. This solution was then centrifuged at 4,000 g for 2 h at room temperature. This yielded three distinct fractions: the top solution was decanted, the bottom husky layer cut away and the middle clear gel-like layer was retained. This gel-like layer was subsequently dialysed against distilled water and then lyophilised. The dialysed gel weighed 30.36 g wet giving a water content of 98.13% and the dry weight yield from the starting husk was 56.5 %.

Equilibrium dialysis experiments

Binding of bile acids to ispaghula was measured using equilibrium dialysis. Five Teflon dialysis cell pairs, incubated in a water bath, were simultaneously rotated at 12 rpm for the duration of each experiment. Each cell was matched for volume, ionic strength and pH on both sides of the membrane. The membranes were prepared from 15 kDa cut-off dialysis tubing. The extracted ispaghula preparation was weighed out for a 1% w/v solution in 100 mM NaCl and the pH adjusted to 8.0. Ispaghula was then dialysed against free bile acids (200 or 400 µM concentration) or ispaghula and bile acids could be dialysed against 100 mM NaCl. All but the lithocholic acid experiments were carried out at 37°C for 24 h. Lithocholic acid experiments (300 µM concentration) were carried out at 80°C for 8 h due to problems with the low water-solubility of this bile acid.

The masses of the transferred solutions were checked by weighed difference. After dialysis for 24 h, the samples were removed and 300 mg aliquots taken by weight. The samples were acidified (200 µl 0.1 M HCl), mixed thoroughly and lyophilised to constant weight. 500 µl methanol was added, any lumps broken, mixed thoroughly and centrifuged. 400 µl supernatant was removed and evaporated at 60°C. The residue, except for lithocholic acid experiments, was analysed by a modified version of the colorimetric Pettenkofer reaction (1.5 ml 70% v/v H_2SO_4 plus 300 µl 0.25% v/v furfural, left for 1 h and the absorbance read at 510 nm) adapted from Boyd *et al* (1966). Standards were assayed both free and after addition of ispaghula, in a similar fashion.

Table 2

Structure-function relationships of dietary carbohydrates in the gut

Bile acid	µmol bound/g Ispaghula*	Solubility µM**	Hydrophobicity***	CMC mM****	t-test*
Cholic acid	1.1	235	0.13	13	< 0.001
Chenodeoxycholic acid	1.6	27	0.59	9	< 0.001
Deoxycholic acid	16.7	28	0.72	10	< 0.001
Lithocholic acid**	11.4	0.05	1.13	0.9	< 0.001

* The amount of ispaghula bound by each bile acid is the average of numerous replicates; 187 equilibrium dialysis experiments were carried out in total

** (Roda and Fini, 1984)

*** (Heuman, 1989)

**** CMC is the critical micelle concentration (Hofmann and Roda, 1984)

* t-test indicates that there is a significant difference ($p < 0.001$) between acid concentrations on the free and bound side of the membrane after dialysis

** lithocholic acid experiment (300 µM bile acid) used the (Sigma) enzyme assay rather than the Pettenkofer reaction, which had low sensitivity with this bile acid

Table 3

Effect of ispaghula and bile acid concentration on binding

Ispaghula	Deoxycholic acid	μmol bound/g Ispaghula*	t-test*
1%	200	8.5	< 0.001
1%	400	16.7	< 0.001
2%	400	5.8	< 0.001

* The amount of ispaghula bound by each bile acid is the average of numerous replicates; 143 equilibrium dialysis experiments were carried out in total

* t-test indicates that there is a significant difference ($p < 0.001$) between acid concentrations on the free and bound side of the membrane after dialysis

Ispaghula was found to interfere with the Pettenkofer assay in the presence of lithocholic acid, again probably due to the insolubility of this bile acid and these experiments were assayed with an enzymatic bile acids assay kit (Sigma, UK).

Results

The ability of ispaghula to adsorb a series of common bile acids was demonstrated using equilibrium dialysis, a technique for measuring the binding of small molecules to macromolecules. Ispaghula (1% w/v in 10 mM NaCl, pH 8) was dialysed against free bile acids (400 μM, pH 8) at 37°C for 24 h and then assayed using the Pettenkofer reaction to determine the equilibrium concentration of bile acid on each side of the membrane. Maximum effect was found on the potentially more harmful secondary bile acids, deoxycholic acid and lithocholic acid (Table 2). Bile acids have detergent properties due to their amphiphilic nature and above a certain concentration, termed the critical micelle concentration (CMC), aggregate to form micelles. The hydrophobic effect is responsible for the ability of amphiphilic molecules to form micelles; however it would appear that there was no simple correlation between either CMC, solubility or hydrophobicity and the capacity for bile acid binding by ispaghula (Table 2).

The effect of ispaghula and bile acid concentration on equilibrium binding is shown in Table 3. Doubling the deoxycholic acid concentration results in an increase in the amount of bound bile acid by a factor of two. This would indicate that there are a large number of very weak binding sites rather than a small number of high affinity binding sites. If, as seems likely, the association of the bile acids with the ispaghula is essentially by partition (bile acids and salts are distributed between the fibre and the bulk intestinal aqueous phase), then the amount bound will depend on the volume of the phases present. Because of the feedback effect, even a small loss of bile acid in this way can affect the lithocholic acid/deoxycholic acid ratio (Chaplin, 1998). The lower amount (Table 3) apparently bound at the higher concentration of ispaghula (2%) may be due to the gel having less water than its equilibrium value requires (2 g requires 107 ml). This may have relevance to ispaghula treatments, suggesting a possible limitation on useful dosage.

Conclusion

The precise relationship between the structural, chemical and physical properties of dietary fibre and its physiological function in the gut is undoubtedly a complex one and simple chemical definitions based on 'soluble' or 'insoluble' fibre, for example, should be avoided. However there is enough evidence to suggest that the physicochemical properties of carbohydrates can be related to their effect on the intestine and specific information on the type and source of fibre present in foods should be given on food labels to help consumers determine the potential health benefits of eating certain foods.

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