Phytase activity in the human and rat small intestine

T H Iqbal, K O Lewis, B T Cooper

Abstract
Phytase is the major storage form of phos-
phorus in seeds and so is a common
dietary constituent. Excessive ingestion
of undegraded phytates can cause mineral
deficiencies in humans. In addition,
phytic acid is antineoplastic in animal
models of both colon and breast
carcinoma. There have been no previous
studies quantifying phytase activity in
the human small intestine although it is
present in animals. Small intestinal
phytase and alkaline phosphatase activity
and distribution was measured in vitro in
mucosal homogenates from two human
small intestinal specimens obtained from
transplant donors. Rat intestine was also
studied for comparison. Phytase activity
was found in human small intestine at
low values (30 times less than that in rat
tissue and 1000-fold lower than alkaline
phosphatase in the same tissue). The
activity was greatest in the duodenum
and lowest in the ileum. In conclusion,
the normal human small intestine has
very limited ability to digest undegraded
phytates. Although this may have adverse
nutritional consequences with respect
to metabolic cation imbalances, the
presence of undigested phytate in the
colon may protect against the develop-
ment of colonic carcinoma.

(Gut 1994; 35: 1233–1236)

Phytate (myo-inositol hexaphosphate) is the
major storage form of phosphorus in seeds and
as such forms a variable large component of
the diet of many animals and humans.1 Phytic
acid is a strong chelator of cations and has been
shown to cause deficiencies in minerals such as
calcium, iron, zinc, and magnesium when
present in excess in the human diet.2

Phytase (E.C.3.1.3.8.) is present in the small
intestines of animals such as rats, calves,
chickens, and pigs.3–5 Dietary phytate intake
and the ability of the intestine to break
down phytic acid have been extensively
studied in animals because of the importance
of phytic acid in mineral balance and the poten-
tially deleterious effect of high concentra-
tions of undegraded phosphates in livestock excreta.6 7

Despite the fact that humans consume large
amounts of phytates, which can cause mineral
deficiencies,8 published works on the ability
of the human intestine to degrade phytic acid are
surprisingly sparse. There has only been one
previous study on phytase activity in the
human small intestine, which found it to be
present but made no mention of the activity of
the enzyme or its distribution in the intestine.5

Metabolic balance studies in human volunteers
have suggested that the human intestine is
poor at metabolising phytic acid.9

Our aim was to measure human intestinal
phytase activity in vitro on fresh small intesti-
nal material and to compare this with the
activity in the rat. As there has been con-
troversy in the past as to whether phytase
activity actually results from alkaline phos-
phatase,10 we planned to measure alkaline
phosphatase activity in the same tissues and to
use phenylalanine, which is known to inhibit
alkaline phosphatase but not phytase.11

Methods
Phytase activity is determined by measuring the
liberation of inorganic phosphate when inositol
hexaphosphate is broken down. Most methods
for measuring phosphate are based on the
colour formed by the reduction of phospha-
mylobate complexes.12 Various reducing
agents have been used. The application of this
principle to the determination of phytase
activity has been complicated by the finding
that phytase itself interferes in these colorimetric
determinations of inorganic phosphate.13 A
published method for the determination of
phytase in duodenal biopsy specimens entails
the reduction of phosphomolybdate complexes
with ascorbic acid after the extraction of these
complexes from remaining phytates using
isosobuty/lethane.14

In a preliminary trial of this method,
however, we found that the variability at the
extraction and reduction phases made the
procedure too imprecise for the determination
of the very small amounts of phosphate
released by the human tissue.

We therefore used a method for phosphate
measurement using malachite green as the
chromogen.15 This improved method does
not entail phytate extraction before colour
development. It also has the added advantage
of not requiring protein precipitation as
deproteinisation has been shown to release
phosphate groups from cellular constituents.15

REAGENTS
The following reagents were used: sodium
phytate (30 mmol/l), hydrochloric acid
(5 mol/l); malachite green made up in acidi-
fied molybate (340 mg/l); aqueous TWEEN
solution (15 ml/l); ammonium molybdate
in hydrochloric acid (34 mmol/l); aqueous
urea solution (360 g/l), aqueous zinc chloride
(4 mmol/l); aqueous magnesium chloride
(40 mmol/l).
SPECIMENS
Complete human small intestinal specimens were obtained from two previously fit subjects during organ harvesting for transplantation purposes. One was a 50 year old woman who died suddenly from a subarachnoid haemorrhage; the other a 40 year old man who died suddenly in a road traffic accident.

Rat small intestine was harvested from two Wistar male rats killed by cervical dislocation. All tissue samples were immediately frozen in liquid nitrogen. Subsequently the rat and human donor specimens were kept at −70°C.

PHOTOMETER
A unidex (410 nm) photometer.

Precipitation and then found that Unicam PU86 samples the allowing 10 inorganic mixture gave phosphatase activity, homogenised in HC1 buffer for three minutes at 37°C, added to the tubes and water added to the blanks. Reagent blanks consisting of buffer with or without phytate were included. The tubes were then incubated at 37°C for two hours after which an aliquot (100–500 μl) was taken for determination of inorganic phosphate content. Urea solution (1 ml) was added to this to prevent protein precipitation and then malachite green reagent (4 ml) containing acidified molybdate. After allowing 10 minutes for colour development, the samples were read at 640 nm on a Pye Unicam PU8610 UV/Visible spectrophotometer.

For the measurements on rat tissue we found that 100 μl aliquots from the incubation mixture gave good colour formation. The much smaller phytase activity in the human intestine, however, required 500 μl of incubate and at this concentration there is a contribution from undigested phytate to the final colour. This could be compensated for by reagent blanks consisting of buffer with and without phytate.

The concentrations of phosphate in the incubates and blanks were derived from the standard curve.

The phytase activity expressed as μmolP/min/mg tissue was calculated as:

\[ \text{Phytase activity} = \frac{(\text{T} - \text{T}) - (\text{B} - \text{B})}{(\text{protein conc} \times \text{time})} \]

where Tp: tissue + phytate, T: tissue blank, Bp: buffer + phytate, B: reagent blank.

RESULTS
WORKING RANGE OF MALACHITE GREEN METHOD
By plotting the absorbance of varying concentrations of phosphate standards (Fig 1) we derived a linear working range of between 1 and 5 mmol/l.

FREEDOM FROM INTERFERENCE BY PHYTATE
Phosphate standards with added phytate up to 400 times that used in the final colour reaction product had negligible effect on the absorbance (Fig 2).

FREEDOM FROM SPONTANEOUS HYDROLYSIS OF PHYTATE
Reagent blanks comprising buffer with and without phytate showed negligible effects on the final absorbance from phytate showing that under the experimental conditions there was no spontaneous hydrolysis of phytic acid.

Figure 1: Working range for malachite green method.

Figure 2: Effect of phytate on malachite green method for phosphate.

Figure 3: Rate of phosphate liberation during incubation of rat duodenal homogenate with phytate.
Phytase activity in the human and rat small intestine

<table>
<thead>
<tr>
<th>TABLE I Rat duodenal phytase and alkaline phosphatase activity and kinetics. Mean (SD) on six replicates shown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity</td>
</tr>
<tr>
<td>Phytase (umolP/mg/min)</td>
</tr>
<tr>
<td>Alkaline phosphatase (umolP/mg/min)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE II Human phytase and alkaline phosphatase. Mean (SD) on six replicates shown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum:</td>
</tr>
<tr>
<td>Phytase (umolP/mg/min)</td>
</tr>
<tr>
<td>Subject 1</td>
</tr>
<tr>
<td>9.33 (0.8)×10&lt;sup&gt;-4&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.966 (0.07)</td>
</tr>
<tr>
<td>1083</td>
</tr>
</tbody>
</table>

**RECOVERY EXPERIMENT**

Known concentrations of phosphate standards (1–6 mmol/l) were added to rat tissue homogenates in the absence of phytate substrate. Recoveries ranged from 90% at a concentration of 1 mmol/l to 83% at 6 mmol/l.

**RAT DUODENUM**

*Linearity of reaction with time*

Rat homogenate (2 ml) was incubated with phytic acid for two hours and 100 μl samples were taken every 15 minutes for immediately assay of phosphate content. Figure 3 shows the rate of phosphate production obeys first order kinetics.

**Phytase activity, Km, and V<sub>max</sub> for rat duodenum**

Table 1 shows the results of phytase activity, alkaline phosphatase activity, and kinetics for phytase. The results are in good agreement with those found by other workers.11

**HUMAN INTESTINE**

**Human intestinal phytase activity**

Table II shows the activity of phytase and alkaline phosphatase at different sites of the human small intestine.

It can be seen that there is much less phytase activity in the human small intestine compared with the rat and that this activity diminishes lower down the intestine.

Figure 4 shows the distribution of phytase and alkaline phosphatase activities in the two human intestines investigated. Determination of the kinetics of human phytase was beyond the sensitivity of the method because of the low phytase activity found in the human tissue.

**Phenylalanine inhibition**

We estimated phytase activity in three human duodenal homogenate replicates with and without the addition of phenylalanine (50 mmol/l). Alkaline phosphatase was estimated on the same homogenate with and without the addition of phenylalanine in the same concentration. Figure 5 shows that while the alkaline phosphatase activity was reduced by 95% by phenylalanine, there was no effect on the phytase activity.

**Discussion**

This is the first investigation to quantify the activity of human small intestinal phytase. In our study the proximal human small intestine was found to contain phytase activity at very low values, which declined distally along the intestine. Human duodenal phytase activity was roughly 1000 times lower than that of alkaline phosphatase and 30 times less than that seen in the rat duodenum. This measured activity did not result from alkaline phosphatase as shown by the lack of inhibition of phytase activity under conditions where alkaline phosphatase is almost completely inhibited. Other workers have found that phytase itself inhibits alkaline phosphatase.18 19 We found phytase activity in the rat small intestine comparable with that found by other workers using methods requiring phytate extraction.11 The only previous study on human phytase activity showed the enzyme in the human small intestine but did not report the levels of activity.1 The authors implied that they saw activity similar to that seen in the rat.
This is at variance with the much lower activity found in this study. Nutritionists have estimated human phytase activity indirectly by studying the breakdown of various foodstuffs by the whole gut in normal controls and in patients with ileostomies and have suggested that intestinal phytase activity is very low.9,20 Our direct measurements of human intestinal phytase activity in vitro would support these findings.

The ability of the small intestine to degrade phytic acid is of considerable nutritional importance as evidenced by the mineral deficiencies seen in human populations consuming large quantities of undegraded phytates.20-23 Phytates in the diet are probably hydrolysed mainly by endogenous phytase in food (for example, yeast phytase) and this breakdown probably occurs largely in the acid environment of the stomach.9 This would explain why most people taking diets containing modest amounts of phytates do not become deficient in minerals.

An additional recent development in phytate metabolism has been the hypothesis that phytate may be protective against colonic carcinogenesis.24 Phytic acid has recently been found to be anti-neoplastic in both mammary and colon carcinogenesis models in vivo in the rat.25 26 The low level of phytase activity in the intestine dictates that, from the nutritional point of view, foodstuffs comprising high concentrations of phytic acid should undergo processing before human consumption.27 In view of the recent evidence, however, that phytic acid is anti-neoplastic26 it may be that consumption of undegraded phytates should be encouraged notwithstanding the nutritional implications.

Phytase activity in the human and rat small intestine.

T H Iqbal, K O Lewis and B T Cooper

Gut 1994 35: 1233-1236
doi: 10.1136/gut.35.9.1233

Updated information and services can be found at:
http://gut.bmj.com/content/35/9/1233

Email alerting service

These include:
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections

Articles on similar topics can be found in the following collections
Colon cancer (1547)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/