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On the Possibility of Acid Hydrolysis of Inulin in the Rat Stomach

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ABSTRACT

The possibility of gastric acid hydrolysis of inulin was investigated in feeding experiments with rats receiving diets containing 5% inulin (dahlia tubers). An antibiotic drug, Nebacitin, was administered in the diet to suppress the hind-gut microflora. The rats were divided into two groups—one reference group, and one test group which, in addition to the Nebacitin treatment, was given daily doses of Omeprazol to inhibit gastric acid secretion. The pure inulin and preparations of faeces from the reference and test group, respectively, were chromatographed using a Sephadex G-15 column. The elution pattern indicated that the inulin recovered in faeces from the reference group was significantly depolymerized, whereas only minor fragmentation occurred during passage through the gastro-intestinal tract of animals with experimental achlorhydria. These results show that inulin was hydrolyzed under the acidic conditions prevailing in the rat stomach. Liberation of fructose through gastric hydrolysis is suggested as a mechanism by which a small fraction of inulin may be rendered available for digestion and absorption in the rat small intestine.

INTRODUCTION

During recent years, several papers dealing with the availability and physiological effects of fructans and fructo-oligosaccharides have been published (Karimzadegan *et al.*, 1979; Oku *et al.*, 1984; Tokunga *et al.*, 1986;

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Hidaka *et al.*, 1986; Stone-Dorshow & Levitt, 1987; Ziesenitz & Siebert, 1987). From these studies it has been concluded that the digestibility of these compounds is very limited.

Chemically, fructans and fructo-oligosaccharides consist of one α-glucopyranosyl unit linked to a chain of polymerized β-fructofuranosyl units. Studies of hydrolysis of fructans and fructo-oligosaccharides in small intestinal homogenates from rat and humans, and of absorption in segments from the rat small intestine, have been performed (Oku *et al.*, 1984; Nilsson *et al.*, 1986). No indications of any hydrolytic activity are reported. This is consistent with the fact that no β-fructofuranosidase has been identified in the small intestine of either humans or rats. However, very few studies have been conducted of gastric acid hydrolysis of β-fructofuranosyl linkages *in vivo*.

In a recent paper by Nilsson & Björck (1988), an investigation in rats fed a diet containing inulin or fructans from wheat flour was presented. The data indicated an apparent digestibility close to 100% in normal animals with an intact microbial activity in the hind-gut. Another group of rats received an antibiotic drug to reduce the fermentative activity of the intestinal microflora to obtain a measure of true digestibility in the upper gastro-intestinal tract. Approximately 80% of the ingested fructans were recovered in the faeces of antibiotic-treated animals, suggesting a small intestinal digestibility of 20%. Hence, the most important cause of a high apparent digestibility in normal animals was microbial fermentation of fructans in the hind-gut. Stone-Dorshaw & Levitt (1987) also reported extensive fermentation of fructans by colonic bacteria. They based their conclusions on the gaseous response following ingestion of fructo-oligosaccharides.

The finding that the true digestibility of inulin was not nil in the previous study (Nilsson & Björck, 1988) focused our interest on the fate of fructo-oligosaccharides in the upper gastro-intestinal tract. Minor amounts might have been fermented by remaining bacteria in the hind-gut despite the antibiotic treatment. However, in view of the acid-labile nature of fructo-furanosidic linkages, it is possible that fructans are hydrolyzed to some extent by hydrochloric acid in the stomach. If free fructose is liberated, this may explain a partial availability in the small intestine. Such a possibility has been suggested in a study of inulin digestibility in the pig (Graham & Åman, 1986).

Several attempts have been made to simulate the acidic conditions in the stomach to evaluate the possibility of fructan hydrolysis (Dahlqvist & Nilsson, 1984; Graham & Åman, 1986; Nilsson *et al.*, 1988). Although a depolymerization does occur under certain 'physiological' in-vitro conditions, no definite answer concerning the in-vivo situation has been reported. When fructans were incubated with gastric juice, a dramatic

decline in the rate of hydrolysis was seen when increasing pH from 1 to 2 (Nilsson *et al.*, 1988), suggesting that the physiological relevance of the pH chosen is critical. In the in-vivo situation, the hydrolytic capacity in the stomach is likely to be affected also by other parameters such as the buffering effects of other food components and the rate of gastric emptying.

The present investigation is to be seen as an extension of the above-mentioned rat balance study (Nilsson & Björck, 1988). It was designed to qualitatively evaluate the possibility of gastric acid hydrolysis of inulin in the rat stomach.

MATERIAL AND METHODS

Materials

Inulin from dahlia tubers, with a degree of polymerization (DP) of about 30, was obtained from Sigma Biochemical Company (St. Louis, USA) and sucrose, glucose and fructose preparations of analytical grade were from Kebo Grave (Malmö, Sweden).

Omperazole was obtained from AB Hässle (Mölndal, Sweden) and Nebacitin from Konglige Veterinaer og Landbohöjskoles Apotek (Copenhagen, Denmark).

Analysis and gel-permeation chromatography of inulin

Gel-permeation chromatography (Sephadex G-15, 200 × 5 cm, void volume 1300 ml, eluted with water, flow rate 45 ml h^{-1}) was performed on the inulin preparation as previously described (Nilsson *et al.*, 1986). The hexose content of eluted fractions was analysed by the anthrone reagent as described by Scott & Melvin (1953).

The monomeric composition of the inulin was analysed enzymatically following hydrolysis in 0·75M HCl (Nilsson & Björck, 1988). The contents of fructose and glucose (polymer weight) were 89·2 and 3·9%, respectively, on a dry weight basis (dwb). No sucrose, free fructose or glucose could be detected in the inulin preparation.

Animal experiments

The composition of the test diets was identical to that in the previous paper (Nilsson & Björck, 1988). A casein/DL-methionine (99:1, w/w) mixture was used as the protein source, supplying 15 g N kg^{-1} (dwb). All diets contained (dwb) 40 g kg^{-1} mineral mixture, 16 g kg^{-1} vitamin mixture, and 15 g kg^{-1} soy

bean oil. Corn starch was used to adjust dry matter content. Inulin was added at a level corresponding to 50 g kg^{-1} in the diets (dwb). The vitamin and mineral mixtures were as described by Björck *et al.* (1986).

The feeding experiments were performed in male Wistar rats according to a commonly used technique for evaluation of protein nutritional value (Eggum, 1973) as well as for studies of fermentative degradation of dietary fibre in the hind-gut (Nyman & Asp, 1982). The exact procedure in the animal experiments was as described in our previous quantitative evaluation of the true digestibility of inulin (Nilsson & Björck, 1988). Hence, an antibiotic drug, Nebacitin, was included in the test diets to suppress hind-gut fermentation (7 g kg^{-1}, dwb). This treatment has been shown to reduce microbial activity to approximately 1/10 of that present in untreated animals (Eggum *et al.*, 1982).

The rats (101·8 g ± 2·6 SD) were divided into two groups—one reference group treated only with Nebacitin and one test group, which, in addition to this antibiotic, was given Omeprazol by oro-gastric intubation. Omeprazol is a potent inhibitor of gastric acid secretion and acts by blocking the parietal cell proton pump (Larsson *et al.*, 1983). The drug was dissolved in Methocel solution (27·6 mg ml^{-1}). A volume of 0·5 ml was administered daily corresponding to approximately 400 μmol Omeprazol per kg body weight. This dose totally inhibits gastric secretion for 18 h and reduces secretion by 80% up to 24 h after administration (Björn Wallmark, Hässle, pers. comm.). Intubation was performed at 2 pm, 1 h before the animals were given access to the daily portion of feed. As most of the feed is consumed during the night, gastric acid secretion could be expected to have negligible influence under these experimental conditions.

The animals, five per diet, were allowed free access to water, but their feed intake was restricted to 10 g per day, dwb. After a 4 day adaptation period, feed residues and faeces were collected during a 5 day experimental period. The faeces were collected dry, lyophilized, ground in a mortar, and stored frozen until analysed. Feed residues were negligible in the balance period (< 0·4 g dwb) and no significant difference was observed in feed intake between groups. The faecal weight was 3·66 g ± 0·74 in the reference group versus 4·19 g ± 0·65 in the test group with experimental achlorhydria. The somewhat higher faecal dry matter content in the test group was not statistically significant.

Gel-permeation chromatography of inulin recovered in faeces

Faeces from each group were pooled. A suitable amount of pooled faeces from the test group and reference group, respectively, was dispersed in distilled water and applied to the Sephadex column. The eluate was sampled

in 40 ml fractions and analysed for hexose content with an anthrone reagent (Scott & Melvin, 1953).

RESULTS AND DISCUSSION

The results from gel-permeation chromatography of ingested inulin and inulin recovered in the faeces from the reference group and test group, respectively, are shown in Figs 1–3. The inulin preparation gave a well defined peak at an elution volume of about 1380 ml (Fig. 1). However, in faeces from rats treated only with the antibiotic Nebacitin, the saccharide peak appears at 1600–1700 ml (Fig. 2). In addition, there were also noticeable peaks eluted at 2100–2500 ml, indicating an important reduction in the degree of polymerization (DP). When compared to earlier separations of fructans using an identical column (Nilsson *et al.*, 1986), the elution volume of the lower-molecular-weight components recovered in faeces corresponds to a DP of about 3 or less. The fragmentation of inulin in Nebacitin-treated animals (Fig. 2) is interpreted in terms of a partial hydrolysis of inulin during passage through the upper gastro intestinal tract. As judged from the heterogeneous appearance on the chromatogram, with one group of fructo-oligosaccharides with a comparatively high DP, and a second group of low-molecular weight, acid hydrolysis occurs preferably near end groups.

In contrast, in faeces of rats receiving both Nebacitin and Omeprazole, the main peak corresponding to high-molecular weight oligo-fructans was only slightly shifted to about 1490 ml (Fig. 3), compared with 1380 ml in the

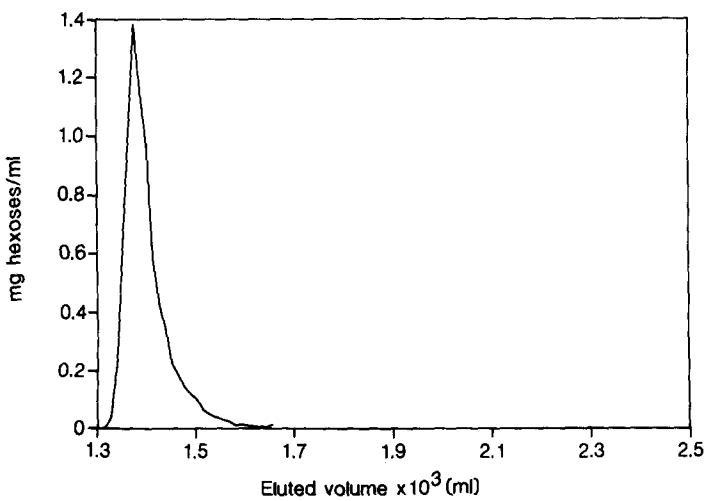

Fig. 1. Gel-permeation chromatogram of the inulin preparation included in the test diets.

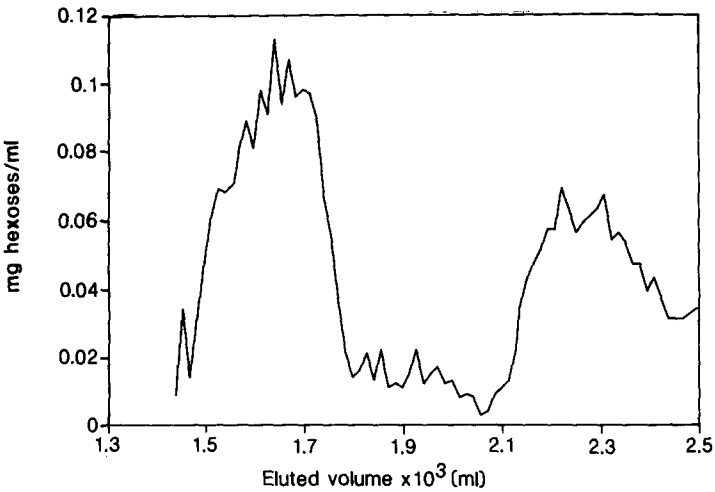

Fig. 2. Gel-permeation chromatogram of inulin recovered in faeces from Nebacitin-treated rats (reference group).

inulin preparation. Further, there were no pronounced peaks in the low-molecular weight area. Consequently, the achlorhydria introduced with Omeprazole significantly reduced the extent of hydrolysis in the test group. The similarity in elution profile of pure inulin and inulin recovered in faeces from the test group, suggests that the fermentative activity following Nebacitin-treatment was reduced to low levels. A high faecal recovery of easily fermentable undigestible polysaccharides in Nebacitin-treated rats is consistent with recent findings with beet-fibre (Nyman & Björck, 1989).

The present data do not provide direct evidence for liberation of

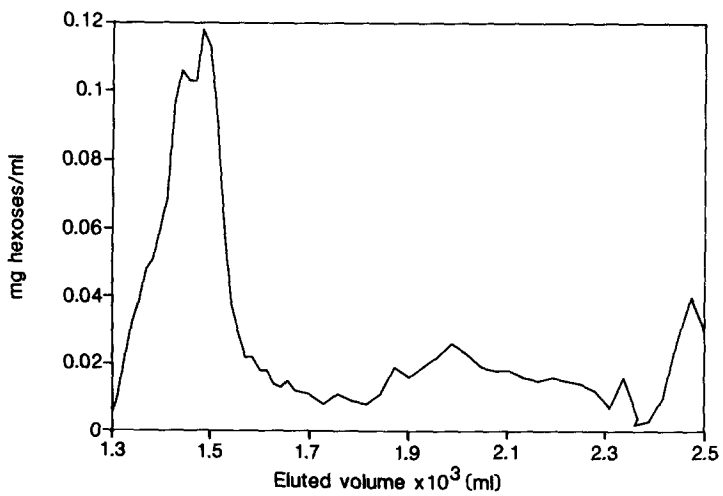

Fig. 3. Gel-permeation chromatogram of inulin recovered in faeces from Nebacitin-treated rats with experimental achlorhydria (test group).

absorbable units from inulin since, e.g. fructose can be expected to be almost completely absorbed in the small intestine. However, the results show that acid hydrolysis of inulin to lower-molecular weight fractions does take place in the rat stomach. In view of the presence of a comparatively large saccharide fraction with a DP ~ 3 in faeces from the reference group, formation of small amounts of fructose appears likely.

Previous studies *in vitro* have demonstrated that significant amounts of free fructose (about 8%) may be formed during incubation of inulin with hydrochloric acid at 'physiological conditions' (0·05M HCl) (Nilsson & Björck, 1988). However, incubation of inulin with human gastric juice indicated that, although a prominent hydrolysis occurred at pH ~ 1, the extent of hydrolysis already decreased to very low levels when approaching a pH of about 2 (Nilsson *et al.*, 1988). These data imply that the catalytic effect of the gastric mucosa may exceed that predicted from the luminal pH. In view of the low pH of the juice produced by human parietal cells, about 0·8, and the large mucosal area, this is probably the case also in man.

We conclude that a partial hydrolysis of inulin occurs in the upper gastro-intestinal tract. The extent of hydrolysis was negligible in rats with experimental achlorhydria, suggesting the involvement of the gastric juice. Gastric hydrolysis to absorbable units, preferably fructose, may explain an apparent digestibility of 20% which was reported in our previous rat balance study (Nilsson & Björck, 1988). It should be noted that efficient absorption only can be expected if monosaccharides or sucrose are produced. Disaccharides, apart from sucrose, and larger fructan fragments are only taken up very slowly by passive diffusion (Menzies, 1974). Further studies are needed to elucidate the impact of gastric acid hydrolysis on inulin bioavailability in man.

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