Influence of High Dietary Cellulose on Fecal Glycosidases in Experimental Rat Colon Carcinogenesis

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ABSTRACT

The effect of either high-cellulose (15%) or regular-cellulose (5%) diets on fecal bacterial glycosidases was assessed in two groups of ten Wistar rats, each given an injection of 1,2-dimethylhydrazine, and in two groups of six control rats. During a 4-month period, fecal activities of bacterial β-galactosidase and β-acetylgalactosaminidase were reduced markedly in control rats maintained on the high-cellulose diet. Enzyme differences were even more significant in rats fed high- or regular-cellulose diets and given injections of the carcinogen. This decrease in fecal bacterial enzymes induced by a high-cellulose diet was observed as early as 20 days after initiation of the diet. Lowering of bacterial β-glycosidases by a high-cellulose diet may preserve the glycoprotein integrity of colonic cells. It may also reduce the luminal production of potential mutagens from dietary β-glycosides in the colon. The latter has been postulated as an important mechanism in colonic tumor development.

INTRODUCTION

Certain dietary elements, such as flavonoids, may influence mutagenesis in the intestinal lumen. Brown (3) has shown that flavonoids may reverse Salmonella mammalian-microsome assay. Rat cecal extracts may further increase the rate of Salmonella reversion by activating potential mutagens (4). Tamura et al. (28) have noted that fecal glycosidases can release potential mutagens (e.g., quercetin) from glycosides found in dietary substances, such as tea or red wine. Nondietary mutagen glycosides, such as amylgladin, have also been shown to be activated by bacterial or intestinal glycosidases (19). Thus, high levels of mutagens could potentially occur in the intestinal lumen as end products of bacterial degradation of dietary or nondietary substances.

Colon carcinoma is less prevalent in rural populations of Africa and Asia than in Western societies (1, 32). This lower prevalence has been postulated to be due to high-fiber, low-protein, and low-fat intake (5, 23). In experimental animals, bran and cellulose have been used commonly as a source of dietary fiber for protection against injected or ingested carcinogens. With both fibers, results have been variable (6, 9, 31). The lack of success in some studies was partly due to the high dosages of carcinogen injection (29), which made any protective effects of fiber unobservable, and partly due to the different caloric intake of regular- and high-fiber-fed animals (31). Two studies have revealed that significantly fewer rats given injections developed colonic tumors when fed a cellulose diet (10, 11). The cause of this phenomenon has not been elucidated. Goldin and Gorbach (12) have shown a marked decrease in fecal bacterial β-glucuronidase, nitroreductase, and azoreductase in animals maintained on a high-grain diet. Conceivably, reduced activity of bacterial glycosidases could result in decreased levels of free mutagens in the intestine. We were interested to see whether high cellulose alone or associated with an exogenous carcinogen like DMH2 may result in changes of fecal glycosidase activity of the kind known to affect the mutagenic potential of dietary or nondietary components.

MATERIALS AND METHODS

Thirty-two male 130- to 150-g Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) were caged individually and assigned randomly to one of 4 groups. Two groups of 6 and 10 animals were fed a powdered regular diet (Table 1) containing 5% fiber as ground cellulose (Alphacel; ICN Nutritional Biochemicals, Cleveland, OH). The other 2 groups of 6 and 10 animals received a similar diet modified by an increase of cellulose to 15% and a concomitant 10% decrease in sucrose. Animals received drinking water ad libitum. The 2 groups of 6 animals each given 10 weekly i.p. injections DMH (25 mg/kg) in a 0.5% solution of 1 mM EDTA at pH 6.5. Each week, feces were collected for a 24-hr period from each animal, weighed, and frozen at −25°C until analyzed. Preliminary studies showed that freezing did not significantly alter enzyme activity. The other 2 groups of 6 animals each served as controls and were given injections for 10 weeks of buffered EDTA. After thawing, samples were homogenized in cold distilled water, centrifuged at 500 x g to sediment debris, and recentrifuged at 25,000 x g for 30 min to obtain a clear bacteria-free supernatant for protein and enzyme analysis. Fecal supernatants were assayed for glycosidase activity against p-nitrophenyl-β-p-glycosides as described previously (21). Protein was determined by the method of Lowry et al. (17) using bovine serum albumin as standard.

To ascertain the activity of fecal glycosidases against complex natural glycosides, an axenic male rat was given an i.p. injection of 15 μCi of [U-14C]glucosamine as glycoprotein precursor. Four hr later, the animal was sacrificed, the cecum was excised aseptically and washed thoroughly with sterile distilled water, and the cecal content was placed in sterile centrifuge tubes. The content was centrifuged at 9000 x g for 30 min and lyophilized to obtain powdered 14C-labeled mucin glycoproteins. The labeled mucin glycoprotein was then treated for 24 hr with a commercially purchased α-galactosidase purified from Aspergillus niger (Sigma Chemical Co., St. Louis, MO). The treatment removed the terminal α-galactose and rendered an α-agalacto-14C-glycoprotein. This substrate was then incubated with enzyme from feces of control and DMH-treated groups obtained 90 days post-carcinogen injection. Glycosidase activity was assayed by measuring the release of radio-labeled sugars from oligosaccharides. Reactions were terminated by boiling, and glycoproteins were precipitated with 60% ethanol. After centrifugation at 39,000 x g for 30 min, supernatants containing released sugars were chromatographed on Whatman no. 1 filter paper in a pyridine:ethyl acetate:acetic acid:water (5:5:1:5) buffer system (26). Chromatograms were cut into 1-cm strips and placed in scintillation vials for counting in...
a Packard TriCarb scintillation counter. Alternatively, chromatograms were stained for amino sugars with 0.2% ninhydrin in methanol or for simple sugars and amino sugars with p-dimethylaminobenzaldehyde (Sigma). The Mann-Whitney U test for nonparametric data (24) was used to statistically compare the groups.

Enzyme separation was achieved by electrophoresis of fecal protein (18). Acrylamide gels were stained for protein with Coomasie blue or sliced horizontally at 1-mm intervals. Each slice was extracted with 0.9% NaCl solution and assayed for activity against both synthetic substrates and labeled colonic mucin glycoproteins. To assess the hydrolyzing capacity of the separated fecal β-galactosidase on the labeled natural glycoprotein, the substrate was incubated with the α-agalacto-14C-glycoprotein. To test the activity of the β-N-acetylgalcosaminidase band, the α-agalacto-14C-glycoprotein was further treated with a purified β-galactosidase obtained from Escherichia coli (Sigma) prior to incubation with the fecal enzyme. This step produced an α,β-agalacto-14C-glycoprotein for use as substrate.

RESULTS

Mean food consumption and fecal weight for each group are shown in Table 2. Similar to weight, water content was 10 to 20% higher in feces from high-cellulose rats.

Fecal β-glycosidase activities, when expressed in milliunits/mg of fecal protein from control animals fed a high-cellulose diet, were greatly reduced as compared to β-glycosidase activities of animals fed regular-cellulose concentration. Mean fecal protein from high-cellulose feces, e.g., 10.4 ± 2.0 mg/g stool, was not different from the mean fecal protein, 10.5 ± 1.3 mg/g stool from regular-cellulose feces. In 3 of 5 collection periods, activities of both β-N-acetylgalcosaminidase and β-galactosidase were significantly lower in controls eating high-cellulose diets (Chart 1, A and B). The decrease in fecal glycosidase activities was noted as early as 20 days after initiating the diet.

The differences became even more apparent when animals were given injections of the carcinogen DMH. Hence, in almost every collection period, β-glycosidase activities of fecal extracts from rats fed high cellulose were 50 to 70% lower than from rats given the regular diet (Chart 1, C and D). As noted in control groups, a marked decrease in activity of β-galactosidase was found after 20 days of the high cellulose. Activities of β-glycosidases were similarly lower in the high-cellulose group whether expressed per g of feces or in milliunits per 24-hr fecal weight (Table 3). To rule out any possible enzyme-inactivating action of dietary cellulose (33), we preincubated for 30 min 0.2 to 2.0% cellulose with fecal glycosidases obtained from DMH-injected rats given a regular diet. After the incubation period, the enzymes were assayed with p-nitrophenylglycosides. No change in enzyme activity was observed.

The above findings described using synthetic substrates were also observed when the 14C-labeled cecal glycoproteins were used as natural substrates. In normal rats, β-N-acetylgalcosaminidase was decreased significantly in the high-cellulose group. The differences in activity upon the oligosaccharide chain β-linkages were more marked in DMH-treated rats. Fecal extracts containing glycosidases from DMH-treated rats fed high-cellulose diets consistently and significantly released less radiolabeled galactose and acetylgalcosamine than did extracts from DMH-treated rats on regular diets (Chart 2).

Electrophoretic separation of fecal extracts revealed one band for β-N-acetylgalcosaminidase and another band for β-galactosidase, with activity against both the synthetic and natural substrates. As in previous experiments, activities of fecal extracts from DMH-treated, high-cellulose animals were markedly lower than those from DMH-treated rats on the regular diet (Chart 3).

DISCUSSION

Tamura et al. (28) proposed the mixture of fecal bacterial glycosidases named "fecalase" as a preparation useful for the production of mutagens from dietary glycosides. Our study provides experimental evidence that these bacterial glycosidases which act as activators of mutagens in the colonic lumen are decreased significantly by high dietary cellulose, at least in the rat. This decrease in fecal glycosidase activity is likely to represent an alteration in the bacterial flora metabolism (32), induced by high dietary cellulose. This appears to be substantiated by the lack of inactivating action of dietary cellulose upon the fecal glycosidase activity.

Since these β-glycosidases are not significantly recovered from germ-free rat feces, they probably originate from intestinal microflora (13). Moreover, in this study, electrophoresis of fecal extracts showed the presence of enzymes of similar mobility to those described previously in contents from ceca of conventional rats (21). Finally, like the bacterial β-glycosidases described in a previous report (12), in the present study, enzymes were able to hydrolyze colonic mucin glycoproteins. In contrast, mammalian lysosomal glycosidases, as opposed to bacterial glycosidases, are unable to degrade natural colonic glycoproteins (14).

The question that originates from these findings is whether or not the decrease in fecal bacterial glycosidase activity induced by high dietary cellulose bears any relationship to the inhibitory effect of the high-cellulose diet on the incidence of DMH-induced tumors (22). A possible explanation is that lower bacterial glycosidase activity may protect host cell glycoproteins from DMH-induced changes (8). Analyses of colonic mucosa from DMH-induced tumors have shown that the concentration of sugars in glycoproteins from tumor homogenates is lower than in glycoproteins from normal colonic mucosa (15). Although soluble
Cellulose, Glycosidases, and Colon Carcinogenesis

Chart 1. Activities of β-acetylglucosaminidase and β-galactosidase in fecal extracts from rats fed a regular-cellulose (□) and high-cellulose (□) dietary concentration. A and B data are from normal control rats; C and D results are from DMH-injected rats. Bars, S.E. of 6 experiments in A and B and of 10 experiments in C and D. *, statistically significant between $p < 0.02$ and $p < 0.005$ values.

Colon mucins were used in our experimental design, it is possible that the decreased bacterial glycosidase activity could also result in less degradation of insoluble membrane glycoproteins (20). Alternatively, quantitative or qualitative changes in luminal bacterial flora, or in their metabolism, induced by high dietary cellulose, could lower the concentration of dietary cocarcinogens acting in conjunction with DMH within the lumen (7, 2, 16). Finally, the cellulose effect on bacterial flora may abolish or diminish a possible mutagenic effect of cycasin (25) or its derivative DMH on bacteria.

Regardless of the protective mechanisms, the changes in fecal glycosidase activity occurred as early as 20 days after initiating the high-cellulose diet. Hence, enzyme changes in the lumen were manifested long before the known development of colonic neoplasms (30) and even prior to changes in the synthesis of nuclear proteins, changes which are not detectable until at least...
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Table 3

<table>
<thead>
<tr>
<th>Dietary cellulose</th>
<th>β-Acetylglucosaminidase</th>
<th>β-Galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>milliunits/mg protein</td>
<td>milliunits/g stool</td>
</tr>
<tr>
<td>High</td>
<td>18 ± 3.3</td>
<td>330 ± 75</td>
</tr>
<tr>
<td>Regular</td>
<td>44 ± 4.0</td>
<td>741 ± 89</td>
</tr>
</tbody>
</table>

Significance of differences

- p < 0.005
- p < 0.02
- p < 0.005
- p < 0.002
- NS

Mean ± S.E.

NS, not significant.

30 days after carcinogen injection (2). This earlier effect of cellulose in reducing bacterial glycosidase activity in the colon lumen may shed light on the possible protection offered by this type of diet in defined human populations. In this context, the early inhibition of bacterial β-glucuronidase, an enzyme known to release carcinogens, has been shown to result in a decrease of carcinogen-induced colonic tumors (27). It is then possible that the early introduction of this type of fiber among dietary components may diminish bacterial activation of dietary procarcinogens, thus preventing the exposure of colonic cells to luminal mutagens.

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