Effects of Cellulose, Agar and Their Mixture on Colonic Mucin Degradation in Rats

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Summary A feeding study was conducted to elucidate the role of two different fibers, cellulose and agar, and mixture of the two fibers on fecal mucinase activity in rats. Fiber-free basal control diet was mixed with either 15% cellulose, 15% agar or half cellulose (7.5%) and half agar (7.5%). These diets were fed for 5 weeks to groups of 6 male Sprague-Dawley rats. Mucinase activity was assayed in fresh rat feces. Body weight gain of rats fed different diet groups did not show significant difference (p > 0.05). Specific and total mucinase activity was highest in rats fed fiber-free control diet and 15% agar diet, intermediate in rats fed the fiber mixture group and lowest in rats fed 15% cellulose diet. The differences among the three groups were significant (p < 0.05).

Key Words dietary fiber, colon mucin degradation, mixed fiber

Dietary fiber has long been considered an inert and insignificant part of man's diet, mainly because it was believed to contribute little nutritionally. However, recent epidemiological observations have suggested important roles of dietary fiber in maintaining man's health. For example, prolonged lack of dietary fiber has been associated with a broad spectrum of diseases such as colon cancer (1-8) and atherosclerosis (9, 10). The mechanism behind this is still not clear. A number of explanatory hypotheses have been formulated. These include that some kinds of dietary fiber may act simply by shortening fecal transit time, reducing the exposure of the gut to toxins or carcinogens in the fecal stream; fiber may also act by increasing fecal bulk and volume, thus lowering the concentration, if not the amount, of offensive chemicals.

In our previous study, we proposed a hypothesis based on the relationship among dietary fiber, colonic bacteria, mucin degradation and permeability (11, 12), since colonic permeability may be an important factor in the etiology of colon cancer, chemicals probably need to reach the colonic mucosa before they can induce tumor formation, and they may do so by penetrating from the lumen. Some of the barrier properties of the colon are attributable to mucin and it may be altered
by the presence of dietary fiber. Fecal mucinase activity may serve as an indication of the colonic mucin degradation (11).

Most studies that were dealing with the possible mechanism of fiber have focused on studying the effect of only one type of fiber at a time. Different source and composition of fiber may not have the same physiological effect. Moreover, there is lack of information as to the physiological impact of mixing different fibers.

The purpose of this study was to evaluate two very different dietary fibers—water-soluble fiber, agar; water-insoluble fiber, cellulose—and the mixture of these two fibers on colonic mucin degradation in rats.

MATERIALS AND METHODS

Diets and animal feeding. The composition of the basal diet is shown in Table 1. Dietary fiber was added at 15% of either cellulose or agar and 7.5% of each cellulose and agar (wt/wt). Cellulose is a polysaccharide with the glucose units linked as in celllobiose; it is practically insoluble but dispensable in water. Agar is a complex range of polysaccharide having alternating \( \alpha-(1 \rightarrow 3) \) and \( \beta-(1 \rightarrow 4) \) linkages and varying in total charge content; it is insoluble in cold water but slowly soluble in hot water to a viscous solution. Both cellulose and agar were bought from Sigma Co. (St. Louis, MO, USA).

Twenty-eight-day-old male weanling Sprague-Dawley rats (Animal Center, National Taiwan University, Medical School, Taipei, Taiwan, ROC) with weight ranging from 50–60 g were randomly assigned to four experimental diet groups of 6 animals each. Animals were housed individually, given water and food ad libitum, and kept on a 12-h light/dark cycle, the lights remaining on from 06:30 to 18:30. Ambient temperature was 25°C. Rats were fed their respective diets for 5 weeks before fecal samples were collected. Animal weights and food consumption

| Ingredient          | %   |
|---------------------|-----|
| Casein              | 20  |
| Corn oil            | 5   |
| Corn starch         | 70  |
| Vitamin mixture\(^1\) | 1   |
| Salt mixture\(^2\)  | 4   |
| Total               | 100 |

\(^1\) Vitamin mixture: (mg/100 g of diet), D-biotin 1%, 20.0; vitamin D (200 IU/g), 0.25; folic acid, 1.0; thiamin HCl, 1.5; riboflavin, 1.5; menadione, 1.5; vitamin A concentrate (500,000 IU/g), 2.0; niacin, 5.0; pyridoxine HCl, 1.0; D-Ca-pantothenate, 5.0; dl-\( \alpha \)-tocopheryl acetate, 20.0; choline bitartrate, 100.0; vitamin B\(_2\), 0.002. \(^2\) Salt mixture: (g/100 g of mixture), CaCO\(_3\), 19.56; CaHPO\(_4\), 38.60; Na\(_2\)HPO\(_4\), 17.58; KCl, 19.75; MgSO\(_4\), 11.60; MnSO\(_4\)·H\(_2\)O, 0.402; CuSO\(_4\), 0.071; ZnSO\(_4\)·7H\(_2\)O, 0.084; KIO\(_3\), 0.028; FeSO\(_4\)·7H\(_2\)O, 0.473.

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were measured weekly.

**Mucinase activity determination.** Collection and homogenization of fecal pellets were performed according to the method previously described (11). Fresh fecal pellets were placed in test tubes in an ice bucket. The fecal pellets were weighed and then mixed with 0.01 M sodium phosphate buffer (pH 7.5) at a ratio of 1:100 (wt/wt) and allowed to sit in test tubes on ice for approximately 20 min to promote softening of the pellet. The pellets were then homogenized (Polytron PT 10/35, Brinkmann Instruments, Westbury, NY). Enzyme assays were performed within 3 h of fecal collection.

Fecal homogenate (0.9 ml) was placed into tubes and incubated at 30°C for 2 min. Then 0.1 ml of 0.5% (wt/wt) porcine gastric mucin (Sigma Co., St. Louis, MO, USA) was added. Control incubations consisted of fecal homogenates to which 0.1 ml of distilled water was added. Substrate controls consisted of 0.1 ml porcine gastric mucin and 0.9 ml distilled water. All tubes were incubated at 30°C for 25 min and then placed into boiling water for 3 min to stop enzymatic action. Reducing sugar release was measured by the Nelson-Somogyi method (13, 14). Fecal N content was determined by Kjeldahl procedure (15).

**Statistical analysis.** Statistical significance was determined by using analysis of variance. Multiple comparisons among means were calculated by Duncan’s New Multiple Range test (16). Significance level was set at 5% (p < 0.05).

### RESULTS

Food intake and weight gain after the 5-week experiment period are summarized in Table 2. Total food intake was significantly higher (p < 0.05) in rats on fiber-containing diets, with the lowest intake of 797.6 g, compared with consumption of fiber-free diets where intake was 711.8 g. When calculated on the basis of the fiber-free portion, food intake in the different dietary groups was comparable (p > 0.05). Rats fed with all the fiber-containing diets had identical weight gain and

| Diet           | Food intake (g) | Weight gain (g) |
|---------------|-----------------|-----------------|
|               | Total intake    | Fiber-free portion |               |
| Fiber free    | 711.8±53.1a     | 711.8±53.1a     | 271.9±18.6a   |
| 15% Agar      | 797.6±19.7b     | 688.0±16.7a     | 258.6±12.2a   |
| 15% Cellulose | 810.0±49.0b     | 688.5±41.7a     | 253.9±28.7a   |
| 7.5% Agar     | +               | 815.7±24.6b     | 693.4±20.9a   | 256.8±16.4a |
| 7.5% Cellulose|                 |                 |                |

1 Data are mean ± SD (n = 6). 2 Figures in the same column having the same superscript are not significantly different (p > 0.05).
Table 3. Mucinase activity of rats fed various diets.\(^{1,2}\)

| Diet            | Specific activity\(^3\) | Total activity\(^4\) |
|-----------------|-------------------------|----------------------|
| Fiber free      | 48.41 ± 3.12\(^a\)       | 502.27 ± 20.14\(^a\) |
| 15% Agar        | 46.92 ± 1.91\(^a\)       | 511.21 ± 31.20\(^a\) |
| 15% Cellulose   | 23.17 ± 1.82\(^c\)       | 342.83 ± 21.62\(^c\) |
| 7.5% Agar +     | 30.37 ± 2.11\(^b\)       | 418.71 ± 18.53\(^b\) |
| 7.5% Cellulose  |                          |                      |

\(^{1,2}\)Same as Table 2. \(^3\)Nanomol reducing sugar/min•mg protein. \(^4\)Nanomol reducing sugar/min•day.

were not significantly different from the rats fed fiber-free control diet \((p > 0.05)\).

Fecal mucinase activities are given in Table 3. Specific and total activities in rats fell into three groups. They were high in the fiber-free and 15% agar diet groups followed by the 7.5% agar, 7.5% cellulose diet group and then by the 15% cellulose diet group. The differences among the three groups were significant \((p < 0.05)\).

DISCUSSION

In the present study, cellulose and agar were used as dietary fiber source. Cellulose is a simple polymer of glucose that has a very low solubility and no functional moieties other than the hydroxyl group. Unlike cellulose, agar is a hydrophilic polymer; it can form a gel-matrix that is viscous in nature. The fiber-containing diets of the study were made by mixing a fiber-free basal diet with a purified source of cellulose and agar, thus preserving the balance of protein, starch, fat and micronutrients. Although the fiber-fed group ingested a greater weight of food, they still achieved the same total nutrient intake as the control diet since the fiber-containing diets had a lower caloric density. This can be seen when calculated on the basis of fiber-free portion (Table 2). Fiber with different water solubility did not affect the food intake of rats. Rats that received either the cellulose-containing, the agar-containing diet or mixture of the two fell in a same cluster (Table 2).

Dietary fiber is by definition (17) resistant to the digestive enzymes in the gastrointestinal tract. The microflora of the colon, however, is able to ferment partly the dietary constituents. The extent of fermentation is quite variable between various types of dietary fiber and thus may be important for its physiological effects. The human colon contains one of the most complex bacterial ecosystems known. Some bacteria can digest either host products or the fiber ingested; it is possible that these bacteria utilize host products such as mucin whenever there is not enough fermentable dietary fiber. It has been reported that activities of mucin-degrading enzyme (mucinase) in the colon are influenced by the diet of host. Mucinase
activity of rat fecal bacteria is high when fiber-free diets are fed and is much lower when diets containing more easily degradable fiber such as pectin or guar gum are fed (11). If the secretory activity of the goblet cells fails to compensate for changes in degradation, then changes in mucinase activity may result in changes in intestinal permeability. Therefore, dietary fiber may influence colon carcinogenesis by changing mucin degradation and colon permeability (12).

In the present study, fecal mucinase activity in rats fed dietary agar diet was comparable to that of rats fed the fiber-free diet and both were significantly \( p < 0.05 \) higher than that of rats fed the cellulose diet (Table 3). If this change in enzyme activity is accompanied by a change in the rate of mucin degradation and a shift in the balance between mucin secretion and degradation, then it may also be accompanied by a change in the susceptibility of the intestinal mucosa to toxins or carcinogens. This may provide an explanation for the facts that the degree of tumorigenesis induced by 1,2-dimethylhydrazine (DMH) increased when agar was ingested (18), whereas the degree of tumorigenicity induced by DMH decreased when cellulose was ingested (19, 20).

Whether it is better to use specific activities or total enzyme output to express the enzyme activity is an open issue. If the total metabolic capability of the entire colonic microflora is of interest, the total daily output of enzyme activity is the appropriate parameter to use. However, if only a small portion of colonic bacteria is actually exposed to fresh substance then specific activity may be more appropriate. The choice between specific activity and total enzyme output may be made only by comparing them with the physiological consequences of their activities for the host. In the present study, both enzyme activity expressions were presented (Table 3) and the same trend of the results was observed.

The high colonic bacterial mucinase activity found in rats ingesting the water-soluble fiber, agar, is quite interesting. Other water-soluble fiber such as pectin and guar gum has been reported to decrease mucinase activity (11). The reason for this opposite physiological influence on colonic mucin degradation by agar or by other easily degradable fiber is not known. It has been suggested that agar may perform different physiological roles in the gut as compared to other fibers. For example, agar caused deep, coarse folds that appeared as discontinuities in the epithelial lining (21); developed colonic mucosal hyperplasia in both the distal and proximal colon (22); increased colonic permeability (23) and decreased protein digestibility (24). Therefore, besides the differences of the water solubility or the degree of fermentation with different fiber in the colon, other factor(s) may also be involved. One interesting question remaining would be the location of the mucin degrader. They may locate mainly in the mucin layer and adjacent to the mucosa or they may stay as free-living in the lumen. Bacteria that are lodged in the mucin layer may not normally encounter dietary fiber because the mucin itself prevents diffusion of large molecules. Thus, it would be important to elucidate in the future whether dietary fiber or even the different type of fiber in the diet affects the location of the bacteria.
In the present study, the fecal mucinase activity of rats fed a diet containing a blend of agar and cellulose, was in between the two enzyme activities in rats fed the individual fiber, suggesting that mixture of different types of fiber in the diet alters the mucin degradation of the colon. To our knowledge, no such data based on the mixture of different fibers had yet been studied. We feel the data obtained from the present study may be valuable since in our normal meal consumption, fiber ingestion could hardly be restricted to just one single fiber source.

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