Enterokinase in normal intestinal biopsies and those from patients with untreated coeliac disease

J. F. WOODLEY AND ROSALEEN KEANE

From the Department of Biochemistry, University College, Galway, Ireland

SUMMARY Enterokinase was measured in peroral intestinal biopsies showing normal histology and in those from untreated coeliac patients which showed gross villous atrophy. There was no significant difference in the specific activity of enterokinase between these two groups. These results do not support the recent hypothesis that enterokinase is a brush border enzyme, but would be consistent with the idea that it is adsorbed to the cell membrane following secretion.

Enterokinase (enteropeptidase, E.C.3.4.4.8) is the key enzyme in the activation of the pancreatic proteolytic enzymes. It converts trypsinogen into trypsin, which then activates the other zymogens—chymotrypsinogen, procarboxypeptidase, and proelastase. The commonly held belief has been that enterokinase was secreted into the intestinal lumen from the epithelium, although the exact source remained unknown. Recently, however, this view has been challenged by reports that enterokinase is in fact a brush border enzyme, ie, located in or on the membrane of the microvilli of the epithelial cells, along with other brush border enzymes, such as sucrase, isomaltase, lactase, etc. In 1970, Holmes and Lobley reported that enterokinase was localized to the brush border membrane of guinea pigs, and subsequently Nordström and Dahlqvist (1970, 1971) have confirmed this observation with rats.

It is well established (Booth, 1970) that in the mucosa of untreated coeliac patients, which shows villous atrophy, there is a marked reduction of brush border enzymes. The levels of these enzymes return towards normal on the institution of a strict gluten-free diet. This is accompanied by a slow return to normal of the villous architecture. If enterokinase is a ‘true’ brush border enzyme then it would be expected to show a similar effect.

Methods and Materials

The coeliac patients were biopsied for diagnostic purposes. The controls were normal healthy volunteers, symptomatic patients, and non-symptomatic relatives of coeliac patients who were being investigated as part of a familial study. Biopsies were obtained using a modified Crosby capsule, and the position of the tube was checked fluoroscopically. The biopsies were examined macroscopically as soon as they were obtained, and a small piece was retained and fixed for histology. The remainder was frozen and stored at −20°C until the enzyme assays were performed.

For analysis, the biopsies were weighed and homogenized in distilled water at a concentration of approximately 20 mg/ml. Disaccharidases were measured by the method of Dahlqvist (1968) and enterokinase by the method of Nordstrom and Dahlqvist (1971). Protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as standard. The results of the enzyme assays were expressed as mIU/mg protein.

Results

The biopsies were graded histologically according to the system of McNicholl and Egan (1968). In this system the biopsies were graded from 0 to III. Grade 0 represents normal histological appearance of the mucosa, and grade III total villous atrophy or the typical ‘flat’ mucosa as seen in untreated coeliac patients. Grades I and II are intermediate stages of mucosal damage, but in this study only biopsies which were clearly grade 0 (normal) or grade III (total villous atrophy) were included. The control group consisted of biopsies which appeared histologically normal (grade 0), regardless of whether they were obtained from normal volunteers, symptomatic controls, or relatives of coeliac patients.

The results are shown in Table I, duodenal samples,
Enterokinase in normal intestinal biopsies and those from patients with untreated coeliac disease

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Enterokinase</th>
<th>Sucrase</th>
<th>Lactase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0 (normal)</td>
<td>2.77 ± 1.31</td>
<td>60.2 ± 25.1</td>
<td>26.8 ± 17.8</td>
</tr>
<tr>
<td>Grade III (flat)</td>
<td>2.61 ± 1.28</td>
<td>14.9 ± 12.6</td>
<td>4.8 ± 5.0</td>
</tr>
</tbody>
</table>

Table I Activities of enterokinase, sucrase, and lactase in duodenal biopsies

1Activities are specific activities expressed as mIU/mg protein. Ranges are shown in parentheses

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>Enterokinase</th>
<th>Sucrase</th>
<th>Lactase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0 (normal)</td>
<td>1.49 ± 0.82</td>
<td>76.5 ± 32.0</td>
<td>50.5 ± 23.1</td>
</tr>
<tr>
<td>Grade III (flat)</td>
<td>1.28 ± 0.48</td>
<td>119 ± 8.0</td>
<td>3.4 ± 2.5</td>
</tr>
</tbody>
</table>

Table II Activities of enterokinase, sucrase, and lactase in jejunal biopsies

1Activities are specific activities expressed as mIU/mg protein. Ranges are shown in parentheses

and Table II, jejunal samples. The distinction was made between the two as it is known that enterokinase levels are higher in the duodenum than in the jejunum (Nordström and Dahlqvist, 1971). Table I shows that in the duodenal samples there is a considerable reduction of the levels of sucrase and lactase in the grade III biopsies with no concomitant significant reduction in enterokinase levels. Results from the jejunal samples (Table II) show a similar trend, again a dramatic reduction of the disaccharidases in the grade III biopsies, and although there is some reduction of enterokinase, it is not statistically significant. Table III shows the enzyme levels of the grade III biopsies as a percentage of the normals. The normal values agree well with those of Dahlqvist (1970).

<table>
<thead>
<tr>
<th>Enterokinase</th>
<th>Sucrase</th>
<th>Lactase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>94.2</td>
<td>24.8</td>
</tr>
<tr>
<td>Jejunum</td>
<td>86.0</td>
<td>15.6</td>
</tr>
</tbody>
</table>

Table III Enzyme activities of flat mucosa as percentages of normal

Discussion

Reports suggesting that enterokinase is a brush border enzyme such as those by Lobley and Holmes (1970) and Nordström and Dahlqvist (1971) are based on the technique of preparing brush border fractions from mucosal homogenates, and showing that there is an enrichment of both enterokinase and other well established brush border enzymes. In some cases (Holmes and Lobley, 1970; Hadorn, Steiner, Sumida, and Peters, 1971) the enrichment of enterokinase activity is considerably greater than that of sucrase. These methods do not preclude the possibility that enterokinase is binding to the membranes of the microvilli, following secretion from some other locus in the intestine. Certainly the association of enterokinase with the brush border membrane is much more superficial than the other enzymes. Enterokinase is much more easily released from the membranes by the action of proteolytic enzymes (Nordström, 1972). Bile salts release enterokinase from the membranes (Hadorn et al, 1971) but also release other enzymes such as the disaccharidases (Nordström, 1972). Our results would not support the idea that enterokinase is a true brush border enzyme. If it were a true brush border enzyme, the level would be expected to be drastically reduced in biopsies of coeliac patients as is demonstrable with the disaccharidases. Our results, however, would be consistent with the binding of enterokinase to the microvillous membrane, following secretion from cells or tissues which are unaffected by coeliac disease.

By using an immunohistochemical technique, Takano, Suzuki, and Yasuda (1971) demonstrated that in the porcine intestine, there was a concentration of enterokinase in the goblet cells, as well as a diffuse distribution on the epithelial cell surface. These authors suggest that enterokinase is secreted by the goblet cells, and our results would not contradict this view. While the mucosa of coeliac patients shows severe villous atrophy, there is no evidence that there is a reduction in the concentration of goblet cells.

While undoubtedly enterokinase is found in brush border preparations, it may be premature to describe it as a 'brush border' enzyme comparable with the disaccharidases. The results also indicate that enterokinase cannot be implicated in the aetiology of coeliac disease.

We wish to thank the following from Galway...
Regional Hospital for obtaining the biopsies studied: Professor C. McCarthy, Dr M. Mylotte, and Dr F. Stevens, Department of Gastroenterology, and Professor B. McNicholl and Dr B. Mitchell, Department of Paediatrics.

We also thank the Medical Research Council of Ireland and the Wellcome Trust for financial support.

References


The October 1972 Issue

THE OCTOBER 1972 ISSUE CONTAINS THE FOLLOWING PAPERS

The stimulant effect of drugs on indocyanine green clearance by the liver
V. Melikian, J. D. Eddy, and A. Paton

The composition of hepatic and gallbladder bile in patients with gallstones
C. MacKay, J. N. Crook, D. C. Smith, and R. A. McAllister

Renal and intrarenal blood flow in non-cirrhotic portal hypertension
M. C. KeW, Carol Limbrick, R. R. Varma, and Sheila Sherlock

Chronic gastritis, alcohol, and non-ulcer dyspepsia
D. M. Roberts

Functional and morphological response of the dog colon to ischaemia
J. W. L. Robinson, C. Rausis, P. Basset, and V. Mirkovitch

The recovery of function and microcirculation in small intestinal loops following ischaemia
J. W. L. Robinson and V. Mirkovitch

Noradrenaline concentration and turnover in different regions of the gastrointestinal tract of the rat: an approach to the evaluation of sympathetic activity in the gut
H. L. Taubin, B. Djahanguiri, and L. Landsberg

Circulating antibodies to cow's milk proteins in ulcerative colitis
D. P. Jewell and S. C. Truelove

An interim report on the production of colonic diverticula in the rabbit
W. J. B. Hodgson

Effect of eating on motility of the pelvic colon in constipation or diarrhoea
Sheila L. Waller, J. J. Misiewicz, and Nancy Kiley

Technique
A comparison of stable and 14C-labelled polyethylene glycol as volume indicators in the human jejunum
David L. Wingate, Rodney J. Sandberg, and Sidney F. Phillips

Progress report Carbenoxolone sodium
W. Sircus

Progress report Immunoglobulins and the gut
E. A. Jones

The British Society of Gastroenterology

Notes and activities

Copies are still available and may be obtained from the Publishing Manager, British Medical Association, Tavistock Square, London WC1H 9JR, price 87p
Enterokinase in normal intestinal biopsies and those from patients with untreated coeliac disease

J. F. Woodley and Rosaleen Keane

Gut 1972 13: 900-902
doi: 10.1136/gut.13.11.900

Updated information and services can be found at:
http://gut.bmj.com/content/13/11/900

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.

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/