Ultrastructural localisation of alkaline phosphatase in adult human large intestine

C BENJAWATANAPON,* L E BELL, AND L WILLIAMS

From the Department of Anatomy, University of Queensland, St. Lucia, Queensland, Australia

SUMMARY The localisation of alkaline phosphatase in human large intestine was investigated at the ultrastructural level. Alkaline phosphatase was found in the mature absorptive cells of the surface, upper, and middle crypt epithelium; the reaction product was localised in the glycocalyx coat of the microvilli, the Golgi apparatus, and the rough endoplasmic reticulum. Slight activity was found in the immature absorptive cells of the middle and lower regions of the crypts. The undifferentiated cells and the enterochromaffin cells were negative. In the lamina propria, alkaline phosphatase was localised in the plasma membrane of the endothelial cells of the venules, in the rough endoplasmic reticulum of the plasma cells, and the cell membrane of macrophages. These results are consistent with the role of alkaline phosphatase in membrane transport.

Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1.) is the collective name for a heterogeneous group of enzymes that catalyse the cleavage of orthophosphate from monophosphate esters at alkaline pH. Alkaline phosphatase activity has been identified in a wide range of human tissues including bone, liver, placenta, small intestine, and kidney. It has also been demonstrated in large intestine using starch gel electrophoresis¹ and by light microscopy using low temperature plastic embedding.² Activity appeared to be localised on the surface of the absorptive cells in the upper crypts and on the luminal surface, in some lamina propria cells, and on the luminal surface of some blood vessels.

The present ultracellular study was undertaken to examine the intracellular localisation of the enzyme in order to obtain some understanding of its role in the metabolic processes of the colon.

Methods

MATERIAL

The material consisted of partially resected human colons removed for carcinomata. Because the colonic alkaline phosphatase is heat labile, the most reliable method of demonstration of the enzyme at

the light microscope level was found to be by freeze-drying unfixed tissue, and subsequent low temperature plastic embedding.² It is not possible to examine unfixed material at the ultrastructural level. During the present study the tissues were maintained at 4°C except during incubation. Blocks of tissue were taken from macroscopically normal areas at least 10 cm from the tumour and fixed for 1½ hours in 4% paraformaldehyde in 3% distilled glutaraldehyde in 0.2 M cacodylate buffer pH 7.2 at 4°C. The Gomori calcium cobalt method³ and the method of Hugon and Borgers⁴ were used to visualise the enzyme. Control sections were incubated in a substrate-free medium. After incubation the tissues were post-fixed in osmium, dehydrated, and embedded in epon. Thin sections were cut with a glass knife on an LKB ultramicrotome and viewed in a Zeiss EM9S electron microscope. Sections were stained with lead citrate and uranyl acetate.

Results

LOCALISATION OF ALKALINE PHOSPHATASE IN COLONIC EPITHELIAL CELLS

Alkaline phosphatase was localised mainly in the intermicrovillous glycocalyx of the mature absorptive cells (Fig. 1) both on the luminal surface and in the upper and middle portions of the colonic crypts. Some activity was also observed in the apical glycocalyx. The Golgi was also positive. The lateral cell membranes of both mature absorptive and goblet cells showed some activity but this was not a

*Present address: Prince of Songkla University, Songkla, Thailand.

†Address for reprint requests: L E Bell, Department of Anatomy, University of Queensland, St. Lucia, Q. 4067, Australia.

consistent result. Control sections gave negative results.

In the lower and middle crypts the intermediate cells displayed enzyme activity on the microvillous borders and occasionally in the Golgi apparatus lysosomes and lateral cell membranes. No enzyme activity was detected in the immature goblet cells.

Fig. 1 Microvilli of mature absorptive cells on luminal surface. Alkaline phosphatase reaction products are localised in the intervillous glycocalyx. Some enzyme activity is seen in the apical glycocalyx. Gomori, $\times 24000$ (original magnification).

Fig. 2 Immature absorptive cell (A) and undifferentiated cells (U) of lower crypt. Alkaline phosphatase is localised on the lateral cell membranes (arrows). Gomori, $\times 5200$ (original magnification).

In the lower crypt, the immature absorptive cells displayed alkaline phosphatase activity on the Golgi apparatus, free ribosomes, secretory granules, lysosomes, and the lateral membranes (Fig. 2). The undifferentiated cells exhibited only slight or no enzyme activity in free ribosomes, lysosomes, and lateral membranes. The enterochromaffin cells were negative.

LOCALISATION OF ALKALINE PHOSPHATASE IN LAMINA PROPRIA

In the plasma cells of the lamina propria, the rough endoplasmic reticulum displayed a positive reaction, as did the cell membrane of macrophages.

Activity in the post-capillary venules was localised on the cell membranes of the endothelium. Micro-pinocytotic vesicles at the cell surface also showed enzyme activity.

Discussion

It is generally believed that absorption of water and production of mucus are the functions of the large intestine.⁵ One must therefore question the role of alkaline phosphatase in the colon.

Most investigators believe that membrane localisation of an enzyme involves that enzyme in active transport.^{6,7,8,9}

Haussler and colleagues¹⁰ working with rachitic chickens obtained significant increase in both intestinal calcium absorption and intestinal alkaline phosphatase activity after a single physiological dose of vitamin D₃. Kowarski and Schachter¹¹ confirmed that vitamin D increases alkaline phosphatase in the brush border. Also Stumpf and co-workers¹² found in rats that vitamin D₃-dependent calcium and phosphate absorption occurred in all segments of the intestinal tract including the colon. By using tritium-labelled 1, 25-dihydroxy-vitamin D₃ they showed that the target cells for vitamin D₃ in the intestine were the absorptive cells of the small intestine and the colon.

It seems possible, therefore, that, in addition to absorption of water, some absorption of vitamin D₃-dependent calcium and phosphate may occur in the human colon and that this involves alkaline phosphatase. A similar role can be assumed for the alkaline phosphatase demonstrated in the walls of the post-capillary venules in the lamina propria.

Glutaraldehyde is the universal fixative for ultrastructural studies; however, any fixative inhibits enzyme activity. Glutaraldehyde-containing solutions are more inhibitory than formaldehyde alone but the latter is an inadequate fixative at the ultrastructural level; thus a certain inhibition of the enzyme is inevitable. The inhibitor effects of

fixation could therefore result in some low activity sites not being detected. Demonstration of these awaits more refined techniques.

Fishman and colleagues¹³ found that, in fasting patients, alkaline phosphatase was localised on the microvillous border of the small intestine but, after infusion of the intestine for two hours with long chain fatty acids, activity was located mainly in the lamina propria. In the present study, as tissue was removed at operation from fasting patients, it is interesting to speculate whether during absorption a change in activity site would also occur in the large intestine, possibly revealing the absorption pathway.

We thank Dr M G Neely of the Mater Misericordiae Hospital, South Brisbane, for supplying the surgical specimens.

References

- 1 Williams LM. A new alkaline phosphatase in human caecum. *J Anat* 1977; 124:516.
- 2 Bell L, Williams L. Histochemical demonstration of alkaline phosphatase in human large intestine, normal and diseased. *Histochemistry* 1979; 60:85-9.
- 3 Pearse AGE. In: *Histochemistry—theoretical and applied*. London: Churchill, 1968.
- 4 Hugon J, Borgers M. Fine structural localization of acid and alkaline phosphatase activities in the absorbing cells of the duodenum of rodents. *Histochemie* 1968; 12:42-66.
- 5 Ham A, Cormack D. In: *Histology*. Philadelphia and Toronto: Lippincott, 1979.
- 6 Kaplan MM. Progress in hepatology. Alkaline phosphatase. *Gastroenterology* 1972; 62:452-68.
- 7 Fishman WH. Perspectives on alkaline phosphatase isoenzymes. *Am J Med* 1974; 56:617-50.
- 8 Gawlik Z, Najberg G, Aleksandrowicz R, Wisniewska I. The activity of alkaline phosphatase in the process of regeneration of rat liver. *Folia Histochem Cytochem (Krakow)* 1976; 14(2): 91-8.
- 9 Ugolev AM, De Laey P, Iezuitova NN, Rakhimov KR, Timofeeva NM, Stepanova AT. Membrane digestion and nutrient assimilation in early development. In: *Development of mammalian absorptive processes*. Ciba Foundation Symposium 70 (new series). Amsterdam: Excerpta Medica, 1979.
- 10 Haussler MR, Nagode LA, Rasmussen H. Induction of intestinal brush border alkaline phosphatase by vitamin D and identity with CA-ATPase. *Nature* 1970; 228:1199-201.
- 11 Kowarski S, Schachter D. Vitamin D and adenosine triphosphatase dependent on divalent cations in rat intestinal mucosa. *J Clin Invest* 1973; 52:2765-73.
- 12 Stumpf WE, Sar M, Reid FA, Tanaka Y, De Luca HF. Target cells for 1,25-dihydroxyvitamin D₃ in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. *Science* 1979; 206:1188-90.
- 13 Linscheer WG, Malagelada J, Stolback LL, Fishman WH. The effect of fatty acid perfusion on intestinal alkaline phosphatase. 1. Studies on man. *Am J Dig Dis* 1977; 22:509-15.