A comparative study of lactase and sucrase–isomaltase activities and immunoreactivities in jejunal biopsies of patients suffering from the malabsorption syndrome

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Recently, there has been a trend towards detecting enzymes as antigens using immunohistochemical methods instead of as catalytically active substances. The reason for this is the increasing use of immunohistochemical methods in diagnostic pathology. However, differences arise with the two approaches whenever one demonstrates an enzyme as a marker for a certain cell type (e.g. prostatic acid phosphatase for the recognition of cancer cells of prostatic origin) or whenever an enzyme deficiency is to be assessed (Lojda, 1981). It is true that one cannot demonstrate enzyme activity when the respective enzyme protein is absent. On the other hand a positive immunoreaction does not prove that the enzyme is catalytically active. This is not surprising in the case of enzymes which exist as inactive pro-enzymes (e.g. gastric and pancreatic proteases). However, for the overwhelming majority of other enzymes, this fact is not taken into consideration.

In this communication this fact is elucidated on the basis of a comparative study of catalytic activities and immunoreactivities of lactase and sucrase–isomaltase in jejunal biopsies of patients sent to our Laboratory for diagnostic examination. Biopsies were subjected to the battery of reactions recommended previously for the diagnosis of the malabsorption syndrome (Lojda, 1976, 1983). For this study, biopsies from 9 patients with normal findings, 3 patients with a minimal lesion, 20 patients with isolated lactase deficiency, 2 patients with isolated sucrase–isomaltase deficiency, 25 patients with overt coeliac disease and 9 patients with coeliac disease in remission were chosen.

The immunohistochemical demonstration of lactase and sucrase–isomaltase was performed on unfixed cryostat sections with indirect immunofluorescence methods. Guinea-pig antisera raised against purified lactase isolated from the brush border fraction of the small intestine of suckling rats and against purified sucrase–isomaltase of the brush border fraction of the small intestine of adult rats (cf.
Kraml, 1970) were used in the first reaction. Rabbit anti-guinea-pig gamma-globulin conjugated with Fluorescein isothiocyanate (Sevac, Prague) was used in the subsequent step. Antisera against rat lactase and sucrase–isomaltase cross-react with the respective human enzymes. Non-immune guinea-pig sera (NIS), antisera pre-absorbed with lactase or sucrase–isomaltase used for immunization (LPA, SPA), and antisera pre-absorbed with the material released from the brush border fraction of suckling rats with papain from which lactase was removed (BBPS; used in connection with lactase antiserum only) were employed in control experiments. The results were compared with those obtained in serial sections with indigogenic and azo-dye methods and with the glucose oxidase–peroxidase-diaminobenzidine method (cf. Lojda et al., 1979; Lojda, 1983) for demonstrating lactase and sucrase–isomaltase activities.

In biopsies with normal findings or with a minimal lesion, a positive immunoreactivity of both lactase and sucrase was evident in the brush border of crypt enterocytes (Fig. 1b). It was stronger at crypt openings and very strong in the brush border of enterocytes covering the villi (Fig. 1b,e). With NIS, a negative reaction was found. Using LPS or SPS, no immunoreactivity, or only a trace, was apparent. No significant decrease of immunofluorescence was obtained with BBPS. No lactase activity was seen in crypts. However, lactase appeared in the brush border of enterocytes at the crypt openings (Fig. 1a). Sucrase activity was demonstratable in the brush border of all crypt enterocytes. The highest activities of both enzymes resided in the brush border of enterocytes covering the sides of villi. In 3 cases with a minimal lesion, lactase activity was very weak or not demonstrable in enterocytes of the top quarter of villi (Fig. 1a). In 2 out of 3 cases, a strong immunoreactivity was still present in this site (Fig. 1b). Patients with isolated lactase deficiency displayed a normal morphological pattern. No lactase activity was detected in them, even after a prolonged incubation. In 6 out of 20 cases, a positive lactase immunoreactivity of weak to medium intensity was demonstrated in crypts and villi. In patients with overt coeliac disease, no lactase activity was evident (Fig. 1c). In 12 out of 25 patients, a fairly strong lactase immunoreactivity was present in the brush border of crypt enterocytes (Fig. 1d). In patients with coeliac disease in remission, the lactase activity was weak. In these patients, medium or strong lactase immunoreactivity was found.

In 2 patients with isolated sucrase deficiency, the morphological pattern was normal. No sucrase activity was demonstrated when sucrose was used as substrate in the glucose oxidase–peroxidase-diaminobenzidine method. When 6-bromo-2-naphthyl-α-D-glucoside with hexazonium-p-rosaniline was used, a trace activity was detectable in both cases. The immunoreactivity of sucrase–isomaltase was different, however. In one case, a trace of immunoreactivity was found (Fig. 1f) but the other patient displayed a much higher immunoreactivity in the brush border (Fig. 1g). Its intensity was similar to that in patients with normal findings (compare Figs. 1 e,g). In patients with overt coeliac disease, the sucrase–isomaltase activity was significantly lower. In the majority of these patients a quite strong sucrase–isomaltase immunoreactivity was present (Fig. 1h).

From these results, it is apparent that there are the following discrepancies between