SOME REMARKS CONCERNING THE HISTOCHEMICAL DETECTION OF DISACCHARIDASES AND GLUCOSIDASES

ZDENĚK LOJDA

With 12 Figures in the Text, of which 1 in Colour

Summary. In the histochemical detection of the disaccharidases and glucosidases the reliability of methods with coupled oxidation of glucose (with various buffers, tetrazolium salts and concentrations of substrates, tetrazolium salts and PMS) and azo-dye methods with the postincubation as well as simultaneous azo-coupling in cryostat sections (unfixed, fixed with Baker's formol and acetone) and frozen sections after fixation in cold Baker's formol and glutaraldehyde was tested. Various rat organs and human enterobiopsies were used. The methods were modified.

Despite the fact that glutaraldehyde and formol fixation does not completely destroy enzyme activities splitting maltose, sucrose, trehalose and lactose (as it could be shown by a simple Glukophan test) the use of the fixed sections is not recommended. Activity of these enzymes is not completely structurally bound and a part of them escapes from the unfixed cryostat sections into the solutions used for rinsing or for incubation. Activities of these enzymes were demonstrated in the content of the rat jejunum as well. The results of the detection of disaccharidases with a coupled oxidation of glucose are dependent on buffer (type and pH), on the tetrazolium salt (type and concentration), on the concentration of phenazine methosulfate and of disaccharides, on the conditions during the incubation (temperature, anaerobic or aerobic conditions, aqueous or gel media) and on the type of sections.

With all the substrates used (maltose, sucrose, trehalose and lactose) a positive reaction in the enterocytes (both of rat and human) and in the cells of convoluted tubules in rat kidney was obtained. With lactose the reaction was weak and irregular and could be obtained under anaerobic conditions only. A proximodistal gradient in the rat intestine was revealed. In the detection of lactose the use of galactose oxidase in combination with glucose oxidase decreased the intensity of the staining. In evaluating the validity of the localization the artifacts caused by the diffusion of disaccharidases and by the method with coupled oxidation of glucose were considered, the latter being their main source. By no means such artifacts could be avoided. The positive staining is revealed in the sites of the bound tetrazolium salt where it is contacted by the reduced PMS. No reaction can be obtained in sites lacking affinity for the tetrazolium salts even if they contained an active enzyme. The technique allows at the most the localization on the cellular but not intracellular level. The "disaccharidase granules" of DAHLQUIST and BERN are artifacts.

When the sections are incubated individually with the described gel media or in the incubation chambers the amount of produced formazan may serve as a measure of the activity of the respective disaccharidase. Such technique proved to be of value in investigating the changes of activities of disaccharidases in the jejunum of patients with primary malabsorption syndrome. These activities were reduced in comparison with the normal jejunum.

The limitations in localization of the postincubation azo-coupling methods for the detection of glucosidases and galactosidases are much the same as those of the methods with coupled oxidation of glucose. In addition to it the relative substrate specificity of the intestinal disaccharidases has to be considered, because identical enzymes may not be detected with synthetic and natural substrates. Using our new method with hexazo-p-rosaniline in the simultaneous azo-coupling an improved localization of 6-Br-2-naphthyl-α-D-glucosidase was achieved. In the enterocytes the enzyme was localized in the microvillous zone and apical part of the cytoplasm.

Recently an increasing interest has been devoted to intestinal disaccharidases—enzymes belonging to the family of glucosidases (glucoside hydrolases, E. C. 3.2.1) and splitting disaccharides of the food. It has been shown that the defect of the
intestinal mucosa in these enzyme activities which causes the malabsorption of disaccharides occurs either as an inborn error or as an acquired disturbance (Dahlquist 1962, Dahlquist et al. 1963, Klotz 1964, Sonntag et al. 1964, Sunshine and Kretchmer 1964, Auricchio et al. 1965, Haemmerli et al. 1965, Littman and Hammond 1965 etc.). It was recognized that the bulk of activities of intestinal disaccharidases is confined to enterocytes (Borgström et al. 1957, Dahlquist and Borgström 1957, Miller and Crane 1961 etc.). Conflicting reports exist as to the intracellular localization of these enzymes. On the basis of the biochemical studies they are claimed to be localized in the region of the microvillous zone of the enterocytes (Miller and Crane 1961) or on its outer surface only (Ugolev et al. 1964). On the other hand Dahlquist and Brun (1962) suppose their localization in so called “disaccharidase granules“ which they believe to demonstrate by their histochemical method. This localization might be a correlate to the microsomal localization claimed by Doell and Kretchmer (1962). De Duve (1963) lists glucosidases and galactosidases among the lysosomal enzymes. It is not known whether and to what extent the lysosomal glucosidases and galactosidases can split the natural disaccharides.

The glucosidases and disaccharidases have been investigated mostly biochemically. The histochemical technique uses three types of methods:

1. Methods with postincubation azo-coupling using α- and β-D-glucosides or α- and β-D-galactosides of 6-Br-2-naphthol as substrates (Coenen et al. 1952a, b, Rutenburg et al. 1958, 1960, Monis et al. 1963). These methods are limited in their localization due to the binding of the released 6-Br-2-naphthol. The other shortcoming is due to the fact that 6-Br-2-naphthyl-α-D-glucoside is split by 2 out of the 3 intestinal maltases whereas 6-Br-2-naphthyl-β-D-galactoside and 6-Br-2-naphthyl-β-D-glucoside are split by other enzymes than the natural substrates (Dahlquist et al. 1965a, b).

2. Indigogenic methods (Pearson et al. 1962, 1963) using as substrates β-D-glucoside or β-D-galactoside of various substituted indoxyls (5-Br-, 5-Br-4-Cl-, 5-Br-6-Cl-). Concerning the localization these methods might be better than the postincubation azo-coupling methods. The question of being split by the enzymes is probably similar to the 6-Br-2-naphthyl derivatives.

3. The method originated by Dahlquist and Brun (1962) which uses natural disaccharides as substrates. The released glucose is detected by the coupled oxidation by means of the exogenous glucose oxidase which reduces phenazine methosulfate (PMS) and this in turn a tetrazolium salt (nitroBT) in the incubation mixture. Dahlquist and Brun succeeded in localizing the invertase in the stomach, small and large intestine and kidney of rats, and trehalase in small intestine of rats. Using other disaccharides as substrates either a decomposition in the incubation medium was observed (with maltose) or no reaction occurred (with lactose). Dahlquist and Brun consider the localization correct even on the intracellular level. They claim the existence of so called “disaccharidase granules” and argue on the basis of these findings against the localization of disaccharidases in the microvillous zone of enterocytes as demonstrated by Miller and Crane (1961).

Besides these methods the immunohistochemical demonstration of intestinal disaccharidases was attempted by Rosen and Kretchmer (1964). The problems