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Note

Localization of glycosidases on polyacrylamide gel

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A number of methods for the localization of enzymatic activities on polyacrylamide gels have previously been described[1]. The "autochromic method" for the identification of glycosidic enzymes involves the incubation of the enzyme-containing gels in buffered solutions of chromogenic substrates such as *p*-nitrophenylglycosides. For the localization of amylase the use of soluble starch as a substrate, added prior to polymerization of the acrylamide, has been successfully employed[2]. This paper describes a sensitive, specific and inexpensive procedure for the identification of two glycosidases, α-mannosidase (EC 3.2.1.24) and invertase (α-fructofuranoside fructohydrolase, EC 3.2.1.16), a method in which advantage is taken of the Prussian blue reaction after the release of reducing sugar by the enzyme.

EXPERIMENTAL

Polyacrylamide gel electrophoresis of purified α-mannosidase from jack beans[3,4] and invertase from baker's yeast[5] was carried out in an apparatus for vertical electrophoresis according to the method of Raymond[6], with slight modifications. The acrylamide concentration in the gel was 10% (for α-mannosidase) and 7.5% (for invertase), respectively. Three grams of yeast mannan[7] per 100 ml of acrylamide solution were added as a substrate for α-mannosidase prior to polymerization. After running the samples (pH 8.5) at 450 V and 100 mA for 3 h, both gels were rinsed with distilled water. That with α-mannosidase was incubated in 0.05 *M* acetate buffer (pH 5.0) that was 0.1 m*M* in zinc sulphate, for 1 h at 37°. The gel that contained invertase was incubated in a solution containing 5 g of sucrose per 100 ml of the same buffer, without the addition of zinc sulphate, for 30 min at 37°. After incubation, both gels were washed with distilled water and then treated with a 4.8% solution (w/v) of potassium hexacyanoferrate(III) for 10 min at room temperature. After rinsing the gels again with water, they were further treated with a 2.5% aqueous solution (w/v) of iron(III) chloride for another 10 min. Dark blue bands indicated the release of mannose and invert sugar, respectively. The gels were finally stored in the mixture methanol–acetic acid–water (5:1:5).

RESULTS AND DISCUSSION

Both α-mannosidase and invertase were easily localized on polyacrylamide gel

by the Prussian blue reaction, which produced blue bands in those sections of the gels in which reducing sugar had been liberated by the enzymatic action. The bands coincided with the protein staining of the pure enzymes in parallel samples.

This method seems to be ideally suited for the identification of hydrolytic enzymes on polyacrylamide gel, which produce detectable amounts of reducing sugar on being incubated with an appropriate substrate. Although only two enzymes were investigated in our studies, the procedure can be used analogously for the localization of other glycosidases. As the dark blue bands contrast very well with the background, this method is more sensitive than the "autochromic method", which involves the release of *p*-nitrophenol by the enzymes from the corresponding glycosides. It may be considered that the detection of a reducing property is an unspecific reaction that does not exclude the presence of interfering substances other than enzymatically released monosaccharides. It should therefore be pointed out that (a), no band was visible after the staining procedure when the addition of the appropriate substrate had been omitted; (b), both the protein and reducing sugar bands were identical, thus indicating the breakdown of substrate into monosaccharides by the enzymes; and (c), in none of our experiments was interference with other reducing substances observed.

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