Journal of Chromatography, 445 (1988) 282–284
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 469

Note

Activity staining of inulin fructotransferase II on polyacrylamide gels

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(Received March 4th, 1988)

Fructotransferases II (E.C. 2.4.1.93) are enzymes that produce non-reducing difructose anhydrides from fructans. Three different fructotransferases II have been described, producing either difructose anhydride I (β-2, 1′; β-2′, 1)[1], difructose anhydride III (β-2,1′; α-2′,3), difructose anhydride IV (β-2, 6′; β-2′, 6)[6–10] from inulin and levan respectively.

Common purification procedures[11] for fructotransferases II use fractional precipitation, ion exchange, gel permeation chromatography or sodium dodecyl sulphate (SDS)-electrophoresis. The purified enzymes have been characterized by their molecular weight, pH and temperature optima, substrate specificity and inhibitors[6,8,9,11].

However no data about the appearance of isoenzoymes in crude preparations have been reported so far. The most elegant way of determining isoenzymes in a crude enzyme preparation is the use of a non-denaturating electrophoresis in combination with a detection technique that makes use of the reactivity of the enzyme. Recently the determination of inulase isoenzymes using an activity staining method was published[12]. The analogous application of this technique to fructotransferases II should permit determination of any isoenzymes contained in a crude preparation.

MATERIAL AND METHODS

Non-denaturating polyacryl amide gel electrophoresis was carried out as described elsewhere. In a block gel apparatus a separation gel with 10% acrylamide (Merck) cross-linked 1:100 with bis(methyleneacrylamide) (Merck), pH 8.9 [tris(hydroxymethyl)aminomethane–citric acid] containing 6% inulin (Laevosan) was used[12].

The electrophoresis buffer was a tris(hydroxymethyl)aminomethane–glycine system. For an experiment lasting 2 h a voltage of 300 V and a starting current of

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0021-9673/88/$03.50

120 mA were used. The apparatus was cooled with a flow of tap-water to protect the enzyme from heat denaturation.

A 20-μl volume of the crude inulin fructotransferase II preparation from *Arthrobacter ureafaciens* (acetone precipitate of the culture supernatant) was separated; the concentration of the enzyme solution was *ca.* 1% in acetate buffer pH 5.0.

After electrophoresis the gel buffer was removed by rinsing with tap-water. To induce enzymatic activity, the gels were immersed in a 0.1 *M* acetate buffer pH 5.0 and kept at 37°C. Gel strips were removed after a certain incubation time and immersed overnight in methanol–acetic acid–water (5:1:5) in the refrigerator to visualize the zones of enzymatic activity. The opalescent gels, due to the precipitation of inulin in the methanol–acetic acid–water, show transparent bands where the enzyme is located.

RESULTS AND DISCUSSION

The inulin fructotransferase II that was available for this study was that from *Arthrobacter ureafaciens*[2–5,11]. This enzyme is known to act on inulin, producing difructose anhydride III as the sole reaction product. In Fig. 1 the electrophoretic separation of the acetone powder of the culture supernatant is shown. There is only one transparent band which becomes more intense with longer incubation times. Also a broadening of the band is observed with much longer incubation times. This is due to diffusion of the enzyme in the gel. Obviously there is only one zone of isoenzyme present in the preparation. Incubation times longer than 16 h resulted only in a broadening of the original band; no other bands were revealed.

The sensitivity of this activity staining method compared with the non-selective protein staining methods is about one to two magnitudes higher. Even the highly sensitive silver stain for proteins failed to give bands when activity staining is quite intense. In addition to sensitivity, the great selectivity of this method is a big advantage compared with overall protein staining.

This newly applied method might serve as the basis of further studied on fructotransferases II, *e.g.*, intra- and extracellular forms, membrane-bond enzymes and

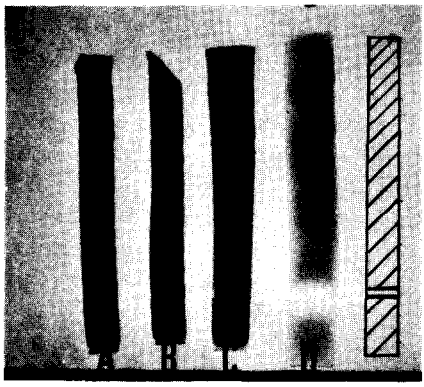

Fig. 1. Electrophoretic separation of fructotransferase II activity staining performed as described in Material and methods. Incubation times: (A) 1; (B) 2; (C) 4 and (D) 16 h.

the change in structure during secernation. Its ease and the short time needed to conduct the test, its high selectivity and sensitivity justify further investigation of activity staining methods for enzyme research.

ACKNOWLEDGEMENT

A grant for one of the authors (R.H.F.B.) by the Fonds zur Förderung der wissenschaftlichen Forschung is acknowledged.

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