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Identification of individual enzymes in crude extracts by acrylamide gel electrophoresis

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Abstract

Identification of enzymes by acrylamide gel electrophoresis

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By use of acrylamide gel electmphoresis it has been possible to identify protein bands (Steward and Barber 1964 Ann. N. Y. Acad. Sci. 121525) or to identify individual enzymes by specific staining techniques (Laycocl Thurman and Boulter 1965 Clin. Chim. Acta 11: 98). This communication describes methods for identifying specific enzymes among non-specifically stained proteins on the same acrylamide gel. Electrophoresis was carried out at toom temperature and 2 1/2 milliamperes per gel.

In one method the gel was split lengthwise with a razor blade and one half was stained with amido black while specific enzymes we&identified in the other half. Amido black was made up as a 1% solution in 7% acetic acid and this caused some shrinkage of the gel. In order to allow exact matching of the halves, the specifically stained half was also transferred to a 7% acetic acid solution after the reduced MTT-tetrazolium dye became apparent. Exact division of the gel into halves was difficult, but irregularities were on aid in rematching the halves after staining.

In a second method the entire gel was stained specifically for the desired enzyme. After the MTT-tetrazolium dye became apparent, the gel was transferred to a dilute solution of amido black (0.01% in 7% acetic acid). This method is easier but can be used only when bands surrounding the specifically stained band give a very pronounced amido black stain. If conditions ore good, this method gives a clear demonstration of the location of certain enzyme activities since the MTT-tetrazolium dye is a different color than the amido black stain.

Wild type Neurospora crassa (74-OR8-1a) and a variety of mutants were grown in Vogel's minimal medium (supplemented where necessary) for 48 hours. The frozen mycelium was ground in a Waring blendor in 0. 1 M phosphate buffer pH 7.0 containing 25% sucrose and centrifuged at 20,000 x g for 30 min. at 0°C. The precipitate was refrozen and pursed through a modified Roper-Hyatt press at 10,000 lbs p.s.i. The supernatant and precipitate were then remixed and again centrifuged at 20,000 x g for 30 min. at 0°C. The supernatant was finally centrifuged at 120,000 x g for 90 min. at 0°C. Protein concentrations of the final supernatant were generally in the range of 10-20 mg/ml. Approximately 0.20-0.25 mg protein were used far each gel.

A number of dehydrogenase enzymes were observed by incubation in the following mixture: 100 mM Na_2HPO_4; appropriate substrate (15 mM L-malic acid or IO mM DL-lactic acid or IO mM Na-succinate.$6H_2O$ or 5 mM L-glutamic acid or 5 mM DL-isocitric acid - the pH was adjusted to 6.50); 0.325 mM phenazine methosulfate and 3.0 mM MTT-tetrazolium (Sigma Chemical Co.). The enzyme reaction was initiated by the addition of 0.75 mM NAD in the assays for malic acid dehydrogenase, lactic acid dehydrogenase, succinic acid dehydrogenase, isocitric acid dehydrogemse and NAD-specific glutamic acid dehydrogenase (total volume 5 ml). The identification of isocitric acid dehydrogenase required the addition of Mg^{++} and both lactic acid dehydmgenase and succinic acid dehydrogenase were active in the absence of added co-enzyme. However, the reactions were more intense when coenzyme was added to the reaction mixture.

The NADP-specific glutamic acid dehydmgenase reaction was initiated by the addition of 0.60 mM NADP to the reaction mixture. Use of these reaction mixtures has permitted identification of a number of the twenty bands found with amido block staining. We have identified a single band corresponding to the NADP-specific glutamic acid dehydmgenose, two bands corresponding to lactic acid dehydrogenases and two bands corresponding to wccinic acid dehydrogenases. Use of isocitric acid and malic acid as substrates indicated two species of isocitric acid dehydrogenase and three species of malic acid dehydrogenase but these enzymes were not correlated unequivocally with the bands stained by amido black. Four species of malic acid dehydmgenase out of twenty amido black-stained bands have been reported previously (Laycock, Kolmark and Boulter 1963 Neurospora Newsl. 4:20).

The enzyme activities and the amido black band patterns of a number of amination deficient mutants (am) were also studied. am_2 (47305) and am_3 (52949) produce an NADP-specific glutamic acid dehydrogemse which is apparently inactive in vivo but shows in vitro activity when the substrate concentration is high (Finch am 1962 J. Mol. Biol. 4: 257). When stained in amido black, gels of am2 and am3 extracts had bands corresponding to the normal NADP-glutamic acid dehydrogemse of wild type (74-OR&la) and these bands showed enzyme activity by reduction of the MTT-tetrazolium dye. am_1 (32213) has not been shown to have any latent activity under any tested condition in vitro (Fincham ibid.). Correspondingly, the normal band was present in amido biack stained gels of am_1 but the protein in this band showed no ability to reduce MTT-tetrazolium. In on extract of am4 (1381) the NADP-specific glutamic acid dehydmgenase band was missing from its normal position in the amido black stained gels and no band developed by the reduction of MTT-tetrazolium. No new bands were detected in the amido black stained gels. The indications are, therefore, that the altered NADP-specific glutamic acid dehydmgenase of am_4 has an altered electrophoretic mobility and that its new position was obscured by one of the other bands and also that this altered protein is enzymatically inactive (see Fincham and Stadler 1965 Genet. Res. 6: 121).

When gels were incubated in the mixture specific for the NAD-specific glutamic acid dehydrogenase, no reduction of the MTT-tetrazolium was observed even when the extracts indicated a high activity prior to electrophoresis as measured by oxidation of NADH at 3400 Å. Presumably the NAD-pecific glutamic acid dehydrogenase was inactivated during electrophoresis. It was therefore not possible to correlate this enzyme with any of the amido black-stained bands. - - - Department of Molecular and Genetic Biology, University of Utah, Salt Lake City, Utah 84112.