Fungal Genetics Reports

Volume 14 Article 27

Purification of glutomine synthetase

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Abstract

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Kapoor, M. and D. Bray. Purification procedure for glutamine synthetase.

Neurospora crassa strain pe Y8743m (FGSC#37) was used as a source of glutamine synthetase. Lyophilized mycelial powders were prepared as described for phosphofructokinase above. Extraction and purification were carried out at 3°C in a cold room. Twenty-five grams of the powder was extracted with 500 ml of 0.05 M phosphate buffer (5×10^{-4} M in EDTA and 10^{-4} M in ß-mercaptoethanol), pH 7.5 for 30 min. The mixture was strained through four layers of cheese-cloth and the supernatant was centrifuged at 15,000 rpm for 15 min in a Sorvall RC-2 refrigerated centrifuge. The supernatant from this step was treated with a saturated solution of ammonium sulfate and the precipitate formed dissolved in the original buffer and further fractionated by adsorption on alumina Cy gel, as described in a recent publication (Kappor and Bray 1968 Biochemistry 7:3583). Elution from the gel was carried out according to our published procedure with the modification that the elution buffer was mode 3×10^{-3} M with regard to $MgCl_2$. The addition of Mg^{++} leads to a much more efficient elution and in addition to stabilization of the enzyme. The gel eluate con be stored in the form of lyophilized powder for several weeks without any loss of activity. The powder is dissolved in a small quantity of water and passed through a column of Sephadex G25 equilibrated against the original buffer containing Mg^{++}. The enzyme obtained at this stage shows an increase of 20-fold in specific activity over that of the crude extracts.

In the next step, the enzyme preparation is purified by ion exchange chromatography on a DEAE-sephodex column (2.5 x 31 cm) equilibrated with 0.02 M phosphate buffer with ß-mercaptoethanol 5×10^{-4} M, EDTA 5×10^{-4} M, rorbitol 0.1 M, $MgCl_2$ 10^{-3} M (pH 7.7). Approximately 60 mg of protein is applied on the column and washed down with buffer. Elution is then carried out by means of a linear gradient of O-O.6 M NaCl prepared in the above-mentioned buffer. Five ml froctionr are collected with an effluent flow rote of 1.0 ml per minute. The enzyme appears towards the end of the gradient, preceded by a major non-enzymatic protein peak. Without rorbitol and Mg^{++} in the eluting buffer, all enzyme activity is lost within a few hours of collection of the froctionr, but rorbitol stabilizes the enzyme considerably. The fractions containing enzyme activity are pooled and concentrated by ultrafiltration, using Amicon Diaflo Ultrafilter with XM-50 membrane. This giver an enzyme preparation with an increase in specific activity of ca. 100 to 150-fold over that of the crude extract. The peak fractions show a much higher specific activity but, again, a loss occurs during concentration of the enzyme preparation. The addition of sorbitol has proved very useful in this care also. - - - Department of Biology, University of Calgary, Calgary, Alberta, Canada.