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Purification of Neurospora myo-inositol-1-phosphate synthase by affinity chromatography.

We have published a simple method for purification of myo-inositol-1-phosphate synthase (MIPS, EC 5.5.1.4) from Neurospora crassa (Zsindely et al. 1977 Neurospora Newsl. 24: 8; Zsindely et al. 1977 Acta biol. Acad. Sci. hung. 28: 281). The enzyme prepared by this method has a high specific activity (about 5000 U/mg) but is always seriously contaminated with glucose-6-phosphate dehydragenase (EC 1.1.1.49). We have employed affinity chromatography to separate these two enzymes.

5'AMP-Sepharose 4B and Blue Sepharose CL-6B were purchased from Pharmacia. NAD^+-Sepharose 4B was prepared from CH-Sepharose 4B by the method of Mosbach et al. (1972 Biochem. J. 127: 625). The concentration of immobilized NAO+ was 3.5 µmol/ml gel, estimated on the basis of phosphorus content. Glucose-6-phosphate and inositol were attached to Epoxy-activated Sepharose 6B at 37°C, pH 11.2, for 40 h. The capacities of these gels were 0.46 µmol G-6-P/ml and 0.52 µmol inositol/ml.

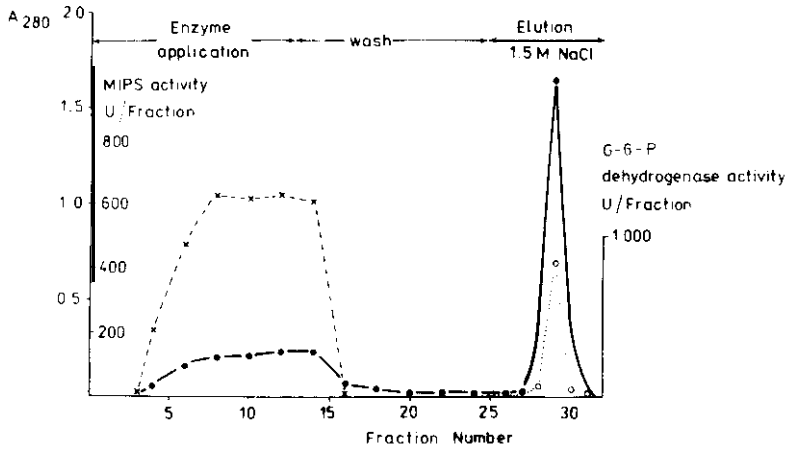

Figure 1. -- Elution profile of MIPS (12 mg protein) contaminated with G-6-P dehydrogenase. Equilibrating buffer: 20 mM NH_4Cl, 1 mM $MgCl_2$, 0.25 mM EDTA, 1 mM mercapto-ethanol, 25 mM Tris-HCl, pH 7.7; column: 8 x 95 mm Blue Sepharose CL-6B; fraction volume: 2.5 ml; flow rate: IO ml/h; protein absorbance at 280 nm (●—●); MIPS activity (x---x); G-6-P dehydrogenase activity(o···o).

The activity of MIPS was determined by the method of Barnett and co-workers (1970 Biochem. J. 119: 183). G-6-P dehydrogenase activity was measured with the same reaction mixture as that used for MIPS, with $NADP^+$ substituted for NAD^+. The final volume of the reaction mixture was 1 ml and contained 10-30 µl of enzyme; A change in absorbance of 0.1 at 30°C/min was taken as one unit of G-6-P dehydrogenase activity.

The binding of MIPS and G-6-P dehydragenase by the affinity adsorbents is summarized in Table 1. After protein application, the 1 ml columns were washed with 1.8 ml of equilibrating buffer to remove any unbound protein. The activity of the enzymes was measured in the effluent. The two enzymes can be separated both with 5'AMP-Sepharose 4B and with Blue Sepharose CL-68 (see Table 1). Partially purified MIPS was chromatographed on a Blue Sepharose CL-6B column with which MIPS was completely separated from G-6-P dehydrogenase (Fig 1).

In the starting preparation (Fraction 5), the specific activity of MIPS was 7450 U/mg, and that of G-6-P dehydrogenase was 310 U/mg. By passing it through Blue Sepharose CL-6B, the specific activity of MIPS was increased to 16,387 U/mg (with good yield), and it was completely free of G-6-P dehydrogenase activity. The enzyme was examined by SDS-polyacrylamide gel electrophoresis. Electrophoresis of 30 µg of MIPS gave a homogenous band of molecular weight 65,000.

TABLE 1

Adsorption of MIPS and G-6-P dehydrogenase on various affinity adsorbents

Adsorbent	Amount of unbound MIPS* %	Amount of unbound G-6-P dehydrogenase** %
G-6-P-Sepharose 6B	93	98
Inositol-Sepharose 6B	97	102
NAD^+-Sepharose 4B	40	76
Blue Sepharose CL-6B	101	0
5'AMP-Sepharose	81	3

● 100% = 1000-1500 U MIPS
**100% = 83 U G-6-P dehydrogenase

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